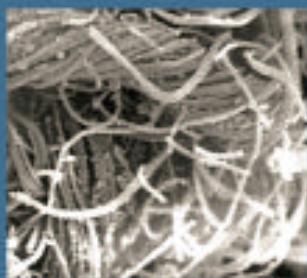
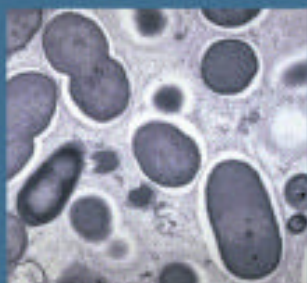
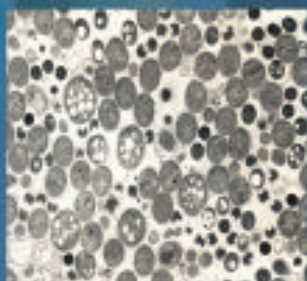




Anaerobic Biotechnology

Environmental
Protection and
Resource Recovery

Herbert H. P. Fang
Tong Zhang



Imperial College Press

Anaerobic Biotechnology

Environmental
Protection and
Resource Recovery

Notes on Book Cover Photos

1. The Minhe plant at Shandong Province, China, anaerobically treating chicken manure collected from 23 communal farms, producing electricity and organic fertilizers as by-products (Chapters 13 and 14). (photo provided by Kaijun Wang)
2. Transmission electron microscopic (TEM) image of microbial syntrophy in an anaerobic reactor treating fatty acid-rich wastewater (Chapter 2). (photo provided by Herbert HP Fang)
3. Transmission electron microscopic (TEM) image of microbial syntrophy in anaerobic reactor treating petro-chemical wastewater (Chapters 2 and 3). (photo provided by Herbert HP Fang)
4. Scanning electron microscopic (SEM) image of *Methanosaeta*, the key methane producer in anaerobic degradation of organic pollutants (photo provided by Herbert HP Fang)

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Preface

Anaerobic biotechnology plays a significant role for the two issues crucial to our society in the 21st century: environmental protection and resource recovery. Human history has repeatedly shown that societies have vanished when people either were ignorant of or chose to neglect the significance of these two issues*. Today, 7.3 billion of us are living in this global village, sharing an increasingly polluted environment and rapidly depleting resources. The survival of our global village is further threatened by the continued increase of the human population, which may reach more than 12 billion by the end of the 21st century. Anaerobic technology, after decades of development, has been demonstrated as an effective treatment process for various kinds of wastes and wastewater. However, this biotechnology is still often overlooked by many today despite its intrinsic advantages and demonstrated effectiveness. One of our motives in publishing this book is to awaken the awareness of those who have overlooked this green and sustainable technology as a means to clean up our environment and to recover valuable resources at the same time.

Under anaerobic conditions, microbes degrade complex organic matters into simple molecules, gaining carbon and energy for their own growth and reproduction in the process. The first unicellular organism appeared on Earth over three billion years ago. The early forms of life were all anaerobes as molecular oxygen was not present on the Earth's surface until one billion years later. Hence, the anaerobes today are survivors of three billion years of evolution and natural selection. They are highly effective and competitive in environments absent of oxygen. For example, anaerobes are abundant in our guts, breaking proteins, lipids and carbohydrates in food into smaller organics that are absorbed through the intestines into the bloodstream for use by our body. For centuries, we have used anaerobes to ferment carbohydrates in grapes and excess crops into alcohol. More recently, we have also used anaerobes in the pharmaceutical industry to produce valuable drugs.

One may also apply the same anaerobic process to degrade pollutants in wastes and wastewater. For centuries, farmers have digested livestock and human manures in cesspits and used the residues as fertilizers. Later, sanitary engineers used similar processes to digest waste sludge generated from the aerobic wastewater treatment process. Although anaerobic digestion could reduce sludge mass by half and convert organic matters into methane, the process often required lengthy retention due to primitive reactor design and the lack of understanding of the degradation mechanism.

* Jared Diamond, *Collapse: How Societies Choose to Fail or Succeed*, 2005, Viking Press.

Anaerobic digestion was treated as a black box; as a consequence, the process had been perceived for years by many as inefficient and had been overlooked by practitioners and policy decision-makers. Fortunately, through the relentless effort of more than one generation of scientists and engineers, anaerobic processes have now developed into matured technology for wastes and wastewater treatment with much better understanding of the related microbiology and biochemistry as well as more effective process and reactor designs.

Compared to the more conventional but costly aerobic process, the anaerobic process does not require the energy-intense aeration, produces only 10% of waste sludge, converts pollutants into readily usable biofuel in the form of methane or hydrogen, and retains most of the nutrients in the sludge, which may be used as organic fertilizer. Although there are certain drawbacks and limitations, anaerobic processes remain the technology of choice for most wastes and wastewater treatment when one looks at the overall picture holistically from the perspectives of both environment and natural resources. Today, tens of thousands of full-scale facilities have been installed worldwide to treat various wastes and wastewaters, including sewage and wastewaters from food, beverage, distillery and petrochemical industries, as well as livestock manures, sewage sludge and crop stalks. Many of these technological advancements and a number of case studies are presented and discussed in this book.

As the sequel to the well-received *Environmental Anaerobic Technology: Applications and New Developments* (Fang, H.H.P. 2010, Imperial College Press, London), this new book compiles the most updated information on anaerobic biotechnology developed in the past five years and groups it into three categories: fundamentals, applications and challenges towards sustainability. It consists of 16 chapters contributed by 48 renowned experts from 13 countries/regions. It covers the wide range of significant and interesting subjects listed below:

- the latest developments and challenges of the anaerobic biotechnology (Chapters 1 and 5),
- anaerobic microbial communities and syntrophic associations (Chapters 2, 3 and 4),
- the Anammox process treating N-rich wastewaters (Chapter 4),
- applications of metagenomics, including identifying crucial anaerobes at low concentrations and the potential findings of industrial biomolecules, enzymes and biodegradation genes (Chapter 5),
- chemical analysis on the impacts of NH_3 and H_2S (Chapter 6),
- modelling of anaerobic processes (Chapter 7),
- microbial fuel cell (Chapter 8),
- applications of anaerobic membrane bioreactors (Chapters 9 and 10),
- treatment of sewage (Chapters 10, 12 and 13),
- treatment of farm wastes (Chapters 13, 14 and 15),
- treatment of petrochemical wastewater (Chapter 11),
- applications of fluidized-bed reactor (Chapters 10 and 11),
- applications of anaerobic filter, upflow anaerobic sludge blanket (UASB) reactor and the like (Chapters 11, 12, 13 and 15),

- full-scale operational experiences in East Asia, particularly in Japan (Chapter 9), Taiwan (Chapter 11) and China (Chapter 13),
- full-scale operational experiences in Latin America, mainly in Brazil, Mexico and Colombia (Chapters 12 and 15),
- commercialization of an enzyme from a bacterium found in anaerobic digester (Chapter 14),
- proposal of the concept of holistic farming (Chapter 14),
- holistic energy analysis of bioethanol production from sugarcane (Chapter 15) and
- achieving a more sustainable society from anaerobic biotechnology (Chapter 16).

This book is targeted not only for engineers and scientists, but also for practitioners as well as decision-makers on energy and environmental policies. We wish that this book may help in the broadening applications of anaerobic biotechnology to environmental protection and resource recovery and, as a consequence, help in the sustainable development of our society in the 21st century.

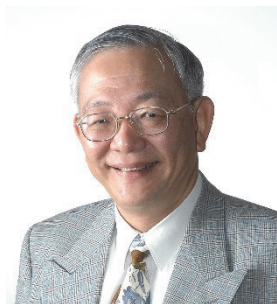
Lastly, we wish to express our gratitude to all the authors for their efforts and contributions, as well as to the continual generous support from the University of Hong Kong, the Croucher Foundation and the Hong Kong General Research Fund over the past decades. We also wish to thank all of our past and present collaborators and research students whose dedications and cheerful attitude have made our research journey joyful, inspiring and fulfilling. Special thanks are also given to Dr. Ying Yang and Ms. Amanda Yun for their assistances in the preparation of this book.

February 20, 2015

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Editors



Herbert H.P. Fang, Emeritus Professor at the University of Hong Kong, received his PhD from the University of Rochester and BSc from National Taiwan University, both in chemical engineering. After three years of post-doctoral research at the University of Illinois at Urbana-Champaign and 12 years of industrial research at Stauffer Chemical (now Akzo Nobel) in New York, he joined the University of Hong Kong in 1987, where he retired in 2014 as Chair of Environmental Engineering in the Department of Civil

Engineering. His research interests have been in applying biotechnology for environmental protection and resource recovery. He is the recipient of China's State Scientific and Technological Progress Award (2008) and the Senior Research Fellowship of Croucher Foundation (1999). Prior to his retirement, Professor Fang served as visiting professor of 11 universities in China and the Distinguished Visiting Chair Professor of Feng Chia University (Taiwan). He is the editor of the well received 2010 book, *Environmental Anaerobic Technology: Applications and New Developments* (Imperial College Press).



Tong Zhang is an Associate Professor presently in charge of the Environmental Biotechnology Laboratory in the Department of Civil Engineering at the University of Hong Kong. He obtained his BSc and MPhil degrees in Environmental Science and Engineering from Nanjing University, China, and his PhD degree from the University of Hong Kong. His research areas include anaerobic digestion and bioenergy production from wastes/wastewater (cellulosic biomass, sludge, kitchen waste, and wastewater), biological

wastewater treatment (N removal and P recovery), bio-degradation of emerging pollutants (antibiotics, PPCP, and EDCs), and antibiotic and heavy metal resistance genes. He is the editorial board member of several international journals, and served as advisor for the Beijing Genomics Institute (BGI) on *Environmental Microbiology and Biotechnology* (2011–2014), and the American Society of Microbiology (ASM) Country Liaison to China (Hong Kong) (2012–2014).

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Contributors



Jaeho Bae, a professor at Inha University (Korea), has more than 20 years of experience in environmental engineering and research. His interests are in the anaerobic treatment of waste and wastewater, biological nutrient removal, and treatment of landfill leachates. He has published over 50 international papers and patents related to anaerobic treatment of wastewater. He serves as leader of the World Class University research team for “Reduction of greenhouse gas production and energy consumption in wastewater treatment plants” funded by Korean Research Foundation. His team

together with Perry L. McCarty at Stanford developed a novel Anaerobic Fluidized Bed Membrane Bioreactor, which requires less energy for fouling control and has high potential to remove micropollutants. He serves as vice president of the Korean Society of Water and Wastewater.



Damien J. Batstone is currently professor and leader of resource recovery and anaerobic biotechnology at the Advanced Water Management Centre, The University of Queensland. He has worked extensively across the area, with a focus on interfacing process modelling, understanding of microbial fundamentals, and translation to practical technology and novel processes and concepts in wastewater treatment. This has led to high-impact international activities, including lead author of the IWA Anaerobic Digestion Model

No. 1, and current chair of the IWA Generalized Physicochemical Modelling Task Group, which is focused on identifying the way in which biological and chemical processes interact across the whole treatment plant. He works extensively on industrial projects, and has directly translated much of the fundamental modelling research into applied outcomes, as well as over 100 journal citations, and six books or book chapters with over 3,000 combined citations.



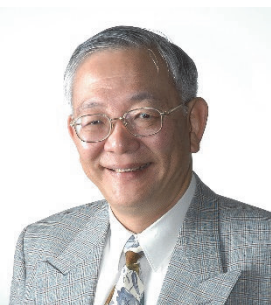
Sheng-Shung Cheng is Professor Emeritus at the National Cheng Kung University (Taiwan), where he had served for 42 years before his retirement in 2014. Prior to receiving his PhD (1984) from the Georgia Institute of Technology with the dissertation entitled *Two-stage Anaerobic Filter Process Treating Phenolic Wastewater*, he obtained his bachelor's degree from NCKU and MSc from the University of Illinois, Urbana-Champaign, all in civil engineering. His research has been focused on the applications of biotechnology

(especially anaerobic and nitrification–denitrification processes) for wastes and wastewater treatment in Taiwan. Among his major contributions is the development of an anaerobic fluidized bed process for the treatment of petrochemical wastewater. This process has been successfully applied to dozens of full-scale applications in Taiwan.



Carlos Chernicharo got his PhD from the University of Newcastle-upon-Tyne in 1990 and since then, has been teaching and developing basic and applied research at the Federal University of Minas Gerais - Brazil, in the field of anaerobic treatment of domestic sewage. His book *Anaerobic Reactors*, written originally in Portuguese, was later translated to English and Spanish and has been used by many students and engineers, especially in developing countries.

His current topics of research are mainly directed to challenge the still-remaining limitations of UASB technology for treating domestic sewage, and therefore include operational issues, post-treatment, control of gaseous emissions, and valuation of the by-products of the treatment aiming at energy recovery.



Herbert H.P. Fang, Emeritus Professor at the University of Hong Kong, received his PhD from the University of Rochester and BSc from National Taiwan University, both in chemical engineering. After three years of post-doctoral research at the University of Illinois at Urbana-Champaign and 12 years of industrial research at Stauffer Chemical (now Akzo Nobel) in New York, he joined the University of Hong Kong in 1987, where he retired in 2014 as Chair of Environmental Engineering in the Department of Civil

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Environmental Anaerobic Technology: Applications and New Developments (Imperial College Press).



Yoichi Kamagata is the Senior Scientist and Director General of the Hokkaido Center at Japan's National Institute of Advanced Industrial Science and Technology (AIST), and Professor at Hokkaido University. He has been working at AIST since 1986 after receiving his PhD in Microbiology at Hokkaido University. He has been extensively studying ecology, physiology, and genetics of anaerobic microorganisms including methanogens and syntrophs that play important roles in oxygen-free environments, such as deep subsurface, rice paddies, and methane-fermenting processes. He has also been

interested in isolation and cultivation of microorganisms that are yet-to-be cultivated. He has published over 200 peer-reviewed papers that has fascinated broad audiences in microbiology. He is an editorial board member and *ad hoc* reviewer of a number of prestigious journals. He has been the Editor-in-Chief of *Microbes and Environments* published by the Japanese Society of Microbial Ecology since 2011.



Po-Heng (Henry) Lee is an Assistant Professor in the Department of Civil and Environmental Engineering at Hong Kong Polytechnic University, where he joined in 2012. Prior to that, he served for two years as a full-time lecturer in the Department of Environmental Engineering at the Inha University (South Korea) after receiving his PhD from the Department of Civil, Construction, and Environmental Engineering at Iowa State University (2010), MSc from National Chiao Tung University (Taiwan) and BSc from National Ilan University (Taiwan), both in environmental

engineering. His general research interest is to apply thermodynamics principles for the analysis of chemical and biological processes in resource recovery from wastes and wastewater. Specifically, his efforts emphasize on interaction mechanisms in adsorption and electrochemical processes and syntrophic energetics in fermentation, anaerobic digestion, and anaerobic ammonium oxidation (Anammox).



After his PhD work on radioactive wastewater treatment at Delft University, in 1970 at Wageningen University, **Gatzke Lettinga** started his pioneer work on anaerobic treatment and supplementary micro-aerobic and physical-chemical treatment, comprising tackles directed to Resource Recovery Reuse and optimal decentralization. It resulted in the development and worldwide application of innovative UASB and EGSB reactor systems. More than 50 PhD students and 100 MSc students contributed to the work; it resulted in a significantly improved

Sustainability of Environmental Protection. Lettinga and his group were honored with prestigious awards, viz. the 1992 Karl Imhoff IWA-Award, the 2000 Royal Dutch Shell Prize, the 2007 Tyler Prize for Environmental Achievement, and the 2009 Lee Quan Yew Water Prize, and he received Honoris Causa Dr degrees from the universities of Valladolid (2001), Santiago de Compostella (2013), and Xanthi (2009).



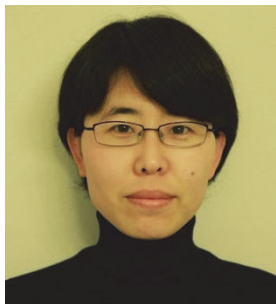
Yu-You Li is a Full Professor in the Department of Civil and Environmental Engineering at Tohoku University (Japan) responsible for the Laboratory of Environment Protection Engineering. He received his BSc (1982) from Xian University of Architecture & Technology (China), MSc (1985) from Tianjin University (China), and PhD (1990) from Tohoku University (Japan). His research interests have been in applying biological technologies, especially anaerobic biotechnology, for environmental protection and resource

recovery. He has published over 260 journal papers and 18 books. He received many research prizes from the Japan Society of Civil Engineers and the Japan Society on Water Environment. He is currently serving as the Chairman of the Anaerobic Biotechnology Committee in the Japan Society on Water Environment, a visiting researcher in the National Research Institute of Environment (Japan), and Visiting Professor at the Xian University of Architecture & Technology (China).



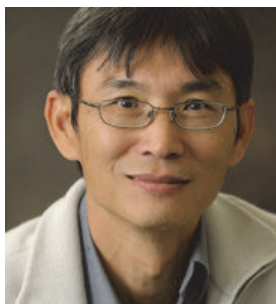
Jih-Gaw Lin has been a professor in the Institute of Environmental Engineering at National Chiao Tung University, Taiwan since 2000 and served as the Director of the Institute since August 2012. He obtained his Master in Civil Engineering from Tennessee Technological University and PhD in Engineering Science from Southern Illinois University at Carbondale in 1982 and 1989, respectively. He worked as an engineer from 1976 to 1980 at the Taiwan Water Supply Corporation after obtaining his BSc in Civil

Engineering from National Cheng Kung University, Taiwan in 1976. His research interests have been in anaerobic ammonium oxidation (Anammox) and aerobic membrane bioreactors for hi-tech industrial wastewater treatment, bio-degradation of PAHs, heavy metal removal by bioleaching, and high-rate composting processes. He has published more than 100 international journals articles and conference proceedings and holds several patents in Taiwan, China, U.S., and EU. His recent innovation of simultaneous partial nitrification, Anammox, and denitrification (SNAD) has been used in two full-scale applications for the treatment of landfill leachate.



Hong Liu, Associate Professor of Oregon State University, received her PhD in Environmental Engineering in 2003 from the University of Hong Kong. Prior to joining the faculty at Oregon State in 2005, she was a postdoctoral researcher at Pennsylvania State University. Her research interests have been in developing biological processes to recover energy/products from waste streams. She and her co-workers the pioneered development of several microbial electrochemical technologies to generate electricity and

hydrogen from wastewater. She has authored and co-authored over 80 publications with over 9,500 citations by March 2015. She is ranked among the top 1% most cited for her subject field (Thomson Reuters Highly Cited Researchers) for 2014. She is also one of the recipients of the NSF CAREER award in 2010 and one of the recipients of the Popular Mechanics Break-through Award in 2005.



Wen-Tso Liu is an Arthur C. Nauman Endowed Professor and the chair of the Environmental Engineering & Science program in the Department of Civil and Environmental Engineering at the University of Illinois at Urbana-Champaign (UIUC). He received his PhD from the University of Tokyo in 1995. His research interests and efforts focus on the microbial ecology and molecular microbiology aspects of water and wastewater treatment processes. To better design, improve, and optimize

treatment processes in the long run, he studies the aspects of microbial diversity, community structure, function, and interaction using advanced molecular tools, including the development of the method Terminal Restriction Fragment Length Polymorphism. He has served as a member of the editorial board for several leading journals in Environmental Microbiology, and has published more than 100 articles in peer-reviewed journals.



Perry L. McCarty, Silas H. Palmer Professor Emeritus of Stanford University, joined Stanford in 1962 when he came to develop the environmental engineering and science program. He served as Chairman of Stanford's Department of Civil and Environmental Engineering (1980–1985), and later as Director of the Western Region Hazardous Substance Research Center (1989–2002). His research interests have been in biological processes for the control of environmental contaminants. He was elected to membership in the National

Academy of Engineering in 1977 and the American Academy of Arts and Sciences in 1996. He received the John and Alice Tyler Prize for Environmental Achievement in 1992 and the Athalie Richardson Irvine Clarke Prize for Outstanding Achievements in

Water Science and Technology in 1997. His pioneering research on anaerobic treatment of wastewater laid the foundations of today's anaerobic biotechnology.



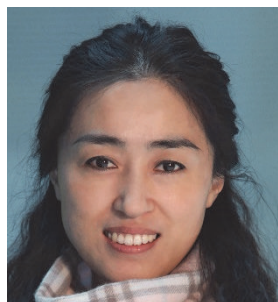
Adalberto Noyola is an environmental engineer with a PhD in wastewater treatment engineering (INSA Toulouse, France). He is a senior researcher and Head of the Institute of Engineering at the National Autonomous University of Mexico (UNAM). He has worked on anaerobic treatment of organic wastes, mainly wastewater and sludge, for more than 30 years. At present, his research projects deal with anaerobic wastewater treatment, sludge digestion, and membrane bioreactors. An additional academic activity covers

the determination of methane emission factors from municipal wastewater treatment facilities and the application of life cycle assessment for wastewater treatment.



Jason C.H. Shih, Professor Emeritus of North Carolina State University, Founder and Advisor of BioResource International, Inc., was born in China, educated in Taiwan, and conferred his doctoral degree at Cornell University, and then his career developed at NC State University. His research interests covered broadly in microbiology, nutrition, agricultural biotechnology, and environmental and energy technologies. He pioneered the study of thermophilic anaerobic digestion of poultry waste from the laboratory to

commercial farms. Through this, he discovered a feather-degrading bacterium and its keratinase enzyme. As a feed additive, the enzyme improved protein digestibility and thus significantly saved the feed cost. In 2000, he and his son, Dr. Giles Shih, co-founded BioResource International (BRI) to produce keratinase in industrial scales and to market the enzyme product worldwide. He has received numerous national and international awards, including the World Poultry Congress Scholarship and the PSA Evonik Degussa Research Award, the two most prestigious awards in poultry science. He is the only scholar who has been presented with both honors.



Aijie Wang, Professor of the Harbin Institute of Technology (HIT), P.R. China, joined HIT in 1997. Her research interests have been in bio-based technology for heavily polluted industrial wastewater treatment and resources recovery from waste (water)/biosolids. She was awarded *Distinguished Professor of Yangtze River Scholar* by the Ministry of Education in 2011. She received *the National Outstanding Youth Science Fund Award* in 2012 and *the Youth Science and Technology Innovation Talent Award* in 2013. Her

pioneering research work on anaerobic treatment of wastewater is elemental sulfur reclamation in an innovative desulfurization and denitrification process system. She also

developed multi-function bioreactors by intimate coupling of electrolysis and bio-degradation to deeply remove recalcitrant compounds.



Kaijun Wang has been the Professor of School of Environment at Tsinghua University since 2008. He got his doctoral degree from the Environmental Technology Department of the Wageningen Agricultural University (the Netherlands) and served as the Chief Engineer of Beijing Municipal Environmental Protection Research Institute prior to joining Tsinghua. His research interests have been in anaerobic and aerobic treatment of sewage and industrial wastewater, sewage sludge treatment, and disposal technology.

He has promoted the application of UASB and EGSB reactor systems in China for two decades and has built more than 300 treatment systems in co-operation with companies. More recently, he has promoted the development of anaerobic technology in kitchen waste treatment and disposal, as well as the implementation of the State Bio-CNG Program. He has established a number of biogas refinery demonstration projects.



Adrianus van Haandel has been a professor at the University of Campina Grande in Brazil since 1971. His main research interest is in biological waste water treatment under tropical conditions, with a focus on anaerobic digestion and activated sludge applications. He has also been a consulting engineer for the design of sewage treatment plants and industrial wastewater facilities and an expert for international agencies. He is the co-author of several textbooks on anaerobic and aerobic wastewater treatment plants with

emphasis on design and optimization.



Jules van Lier is full professor “Wastewater treatment/environmental Engineering” at the section Sanitary Engineering of Delft University of Technology, with a 0.2 fte posted position at Unesco-IHE. From 1988–2008, he was working at Wageningen University in close cooperation with professor Lettinga. In the period 1997–2005, he was director of the Lettinga Associates Foundation (LeAF) and in 2005, he obtained an appointment as part-time professor in Anaerobic Treatment Technology at Wageningen University. Jules van

Lier chaired the IWA Anaerobic Digestion Specialist group between 2001 and 2009. In 2011, he became a nominated member of the IWA Fellow program and he is an associate editor of *Water Science & Technology*. He promoted 12, co-promoted five, and is currently supervising 15 PhD students. At present, he has 165 publications in peer-reviewed journals. He received the 2014 Open MOOC Award of Excellence (ACE) for the course *Introduction to Water Treatment* with EdX.



Willy Verstraete, Emeritus Professor of Gent University (Belgium), received his bachelor's degree (1968) in bioengineering from Gent University and PhD degree (1971) in Microbiology at Cornell University (U.S.). He joined Gent University in 1971, and became Professor in 1979, and then head of the Laboratory of Microbial Ecology and Technology (LabMET - Faculty of Bioscience Engineering). The central theme of his R&D has been Microbial Resource Management, i.e., the design, operation, and control of processes mediated

by mixed microbial cultures. He has served at the editorial board of *Microbial Biotechnology*, and in various international review panels, including the DELFT-Cluster and SENSE research schools in the Netherlands, the EPFL environmental study center in Lausanne, Switzerland, the Helmholtz research institute in Leipzig, Germany, and the Environmental Biotechnology Cooperative Research Center in Brisbane, Australia. During 2008–2012, he was a member of the European Research Council (ERC) in the domain of Life Sciences. In 2014, he became chairman of the Cluster on Resource Recovery of the International Water Association.



Tong Zhang is an Associate Professor presently in charge of the Environmental Biotechnology Laboratory in the Department of Civil Engineering at the University of Hong Kong. He obtained his BSc and MPhil degrees in Environmental Science and Engineering from Nanjing University, China, and his PhD degree from the University of Hong Kong. His research areas include anaerobic digestion and bioenergy production from wastes/wastewater (cellulosic biomass, sludge, kitchen waste, and wastewater), biological

wastewater treatment (N removal and P recovery), bio-degradation of emerging pollutants (antibiotics, PPCP, and EDCs), and antibiotic and heavy metal resistance genes. He is the editorial board member of several international journals, and served as advisor for the Beijing Genomics Institute (BGI) on *Environmental Microbiology and Biotechnology* (2011–2014), and the American Society of Microbiology (ASM) Country Liaison to China (Hong Kong) (2012–2014).

Contents

I. Fundamentals

Chapter 1:	Anaerobic Digestion: About Beauty and Consolation <i>Willy Verstraete and Jo De Vrieze</i>	3
Chapter 2:	Syntrophy in Anaerobic Digestion <i>Yoichi Kamagata</i>	13
Chapter 3:	Microbial Community Involved in Anaerobic Purified Terephthalic Acid Treatment Process <i>Takashi Narihiro, Masaru K. Nobu, Ran Mei and Wen-Tso Liu</i>	31
Chapter 4:	State-of-the-Art Anaerobic Ammonium Oxidation (Anammox) Technology <i>Xiaoming Ji, Yu-Tzu Huang, Qian Wang, Giin Yu Amy Tan, Jih-Gaw Lin and Po-Heng Lee</i>	49
Chapter 5:	Application of Metagenomics in Environmental Anaerobic Technology <i>Feng Ju, Herbert H.P. Fang and Tong Zhang</i>	73
Chapter 6:	Transformations and Impacts of Ammonia and Hydrogen Sulfide in Anaerobic Reactors <i>Yu-You Li and Wei Qiao</i>	109
Chapter 7:	Modelling Anaerobic Digestion Processes <i>Damien J. Batstone and Jorge Rodríguez</i>	133

II. Applications

Chapter 8:	Microbial Fuel Cells: From Fundamentals to Wastewater Treatment Applications <i>Ningshengjie Gao, Keaton Larson Lesnik, Hakan Bermek and Hong Liu</i>	163
------------	---	-----

Chapter 9:	Development and Applications of Anaerobic Membrane Bioreactor in Japan <i>Yu-You Li, Takuro Kobayashi and Shinichiro Wakahara</i>	191
Chapter 10:	Anaerobic Fluidized Bed Membrane Bioreactor for the Treatment of Domestic Wastewater <i>Perry L. McCarty, Jeonghwan Kim, Chungheon Shin, Po-Heng Lee and Jaeho Bae</i>	211
Chapter 11:	Development and Application of Anaerobic Technology for the Treatment of Chemical Effluents in Taiwan <i>Sheng-Shung Cheng, Teh-Ming Liang, Ryninta Anatrya and Wen-Tso Liu</i>	243
Chapter 12:	Anaerobic Sewage Treatment in Latin America <i>Carlos A.L. Chernicharo, Jules B. Van Lier, Adalberto Noyola and Thiago B. Ribeiro</i>	263
Chapter 13:	Applications and the Development of Anaerobic Technology in China <i>K.J. Wang, C.P. Wang, A.J. Wang, H. Gong, B.C. Dong, H. Xu, L.W. Deng and C. Li</i>	297
III. Challenges Towards Sustainability		
Chapter 14:	Development of Anaerobic Digestion of Animal Waste: From Laboratory, Research and Commercial Farms to a Value-Added New Product <i>Jason C.H. Shih</i>	339
Chapter 15:	Role of Anaerobic Digestion in Increasing the Energy Efficiency and Energy Output of Sugar Cane Distilleries <i>Adrianus van Haandel and Jules B. van Lier</i>	353
Chapter 16:	With AnWT and AnDi Systems towards a more Sustainable Society <i>Gatze Lettinga</i>	375
Index		389

I. Fundamentals

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Chapter 1

Anaerobic Digestion: About Beauty and Consolation

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There is intrinsic beauty in things that cannot be ignored. Since the beginning of the eighties, anaerobic digestion has become of central importance in the field of environmental technology, because it can be considered the dominant process determining the outcome of anaerobic microbial metabolism. But there is more to its beauty. The overall sequential microbial reactions are so intrinsically related and regulated that it is a constant challenge to further unravel and comprehend them. This constitutes a continuous source of intellectual joy for scientists. Nevertheless, the fact that still so many “old” questions remain unresolved and that the anaerobic microbiome is so difficult to manipulate, let alone manage, is often a cause of frustration. In that respect, the practitioner of anaerobic digestion regularly faces moments of despair and is in need of consolation.

1. The Anaerobic Digestion (AD) Microbiome

At present, the mindsets in science and society are tuned to the themes of reductionism, wholeness and wellness. The climax of these developments can be found in the current worldwide attention for the gastro-intestinal human microbiome (David *et al.* 2013). Indeed, while about five years ago few scientists showed interest in the complex microbiology of human fecal matter, now a stampede of laboratories are heavily competing to bring out new revelations concerning our gut companions. The remarkable thing hereby is that not just numerous new species are discovered, but also new concepts with high intrinsic value, such as the evolutionary stable strategy driver concept (Blaser and Kirschner 2007). According to this concept, the mixed community of microorganisms forms teams in which a few star players take the major share of the activity, while the bench players wait for their chance (Van den Abbeele *et al.* 2011). Hence, “winning” microbial teams evolve and because these are based on strong communication within, but also with the host, only a restricted number of those so-called enterotypes come to existence (Arumugam *et al.* 2011).

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Thus far, the microbiomes that are active in anaerobic digestion systems have not received as much attention as the human gut microbiome, although several attempts have been undertaken (Koch *et al.* 2014; Riviere *et al.* 2009; Sundberg *et al.* 2013; Werner *et al.* 2011). Nevertheless, if we want to obtain the capability to increase the performance of the AD microbiomes and make them more robust towards stress, we need to unravel the beauty of their complexity.

At this point, it is known that several key anaerobic species are highly conductive in terms of electron transfer. Several *Methanosarcina* sp. growing on acetate contain specific electron transport shuttles, such as ferredoxin, cytochrome c and methanophenazine (Wang *et al.* 2011). *Methanococcus* sp. on the other hand are able to corrode Fe(0) granules (Uchiyama *et al.* 2010). *Geobacter* sp. (typically electrochemically very active) can grow in syntrophy with the acetoclastic genera *Methanosaeta* and *Methanosarcina* (Morita *et al.* 2011; Wang *et al.* 2011). It has also been demonstrated that *Methanosaeta*, the world's most prodigious methanogen, up till now considered only to be able to produce methane via the acetoclastic pathway, can accept electrons via direct interspecies electron transfer for the reduction of carbon dioxide (Rotaru *et al.* 2014). Anaerobic granules have been shown to be highly conductive, which can even be improved by the addition of granular activated carbon, which may serve as a nucleation core (Liu *et al.* 2012; Morita *et al.* 2011). Finally, it is well known that conductivity above 30 mS cm⁻¹ in the liquid phase of the digester negatively influences functioning of the AD process (Chen *et al.* 2008; De Vrieze *et al.* 2012).

Altogether, these findings suggest that bio-electrochemical communication is of fundamental importance in the organization of the AD microbiome. These electron currents can regulate cross-feeding between partner cells, but can also be important in terms of organizational positioning of the different partners within the consortium. Indeed, like in other chain production processes, the correct position of the subsequent actors is of crucial importance to ensure a stable flow and coordination of the intermediate materials, as these undergo transformations from A to B to C, etc. Hence, the three-dimensional (3-D) configuration of the actors in an anaerobic chain process must be of crucial importance (Fig. 1.1). The dimensions of scale (from micrometer to millimeter) at which the operational microbial unit is organized, is however still not determined. Next to that, there is the need to understand how a successful three-dimensionally organized AD team reproduces itself. The question is whether a fully

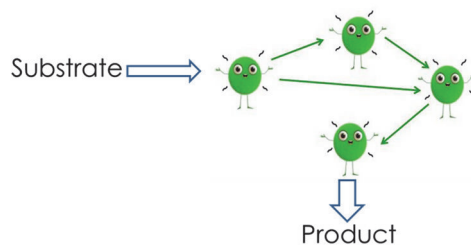


Fig. 1.1. Anaerobic digestion and system biology. It is postulated that the AD microbiome exchanges signals to come to an optimal t organized team to ensure a stable flow of intermediary metabolites between the different functional entities.

established and successful team can serve as a template to reproduce itself or whether the different partners send information to the next site, where analog species may make good use of this information to form an equally effective 3-D constellation. In this framework, it is important to note that methanogens have been reported to exhibit hydrogenotaxis (direct swimming to H_2) (Brileya *et al.* 2013). Clearly, just as for the human gut microbiome, there is a wealth of great discoveries waiting in the domain of anaerobic digestion system biology.

2. The AD Evolutionary Extravaganzas

The continuous emerging of reports on the new potentials to produce methane under extreme conditions or from new substrates, which in the past were considered unsuitable for methane production, is quite astonishing. For example, under conditions of high salt concentrations or other forms of stress, acetate is no longer converted to methane by acetoclastic methanogens, but is metabolized by syntrophic acetate oxidizing bacteria and hydrogenotrophic methanogens, in which the changes in free energy over the overall reaction chain are divided among different species (Hattori 2008; Nettmann *et al.* 2010; Schnurer and Nordberg, 2008). It raises the question on the “economics of living together in a microbiome” and how the latter is regulated in order to make sure that all partners keep functioning. The anaerobic degradation of terephthalate via a “meandering metabolism” in which not the direct short degradation pathway, but a lengthy route allowing several species to participate, is used (Lykidis *et al.* 2011), demonstrates that a complex multi-species teamwork, although requiring thorough coordination, must have advantages over a set of straightforward actions by a few or only one dominant species. The reports on methanogenic consortia capable to gasify fossil fuel organics (Gray *et al.* 2011; Jones *et al.* 2008; Siddique *et al.* 2012) and on the biomethanation of the recalcitrant ether bonds present in xenobiotics, such as methyl tert-butyl ether (Somsamak *et al.* 2004), and natural polymers, such as lignin (Wu and He 2013), further indicate that, as we continue to explore, more and more successful AD teams will be discovered. Recently, it was shown that a single axenic culture of *E. coli*, that was transferred in plain mineral medium at regular intervals, is still evolving new traits that never could have been predicted from the initial genome even after 10 000 generations (Wiser *et al.* 2013). If a single species is surprisingly creative in responding to simple environmental conditions, it can be expected that under challenging environmental conditions, highly developed logistic complex microbial organizations, such as the AD microbiomes, will continue to generate metabolic surprises in terms of substrates converted, conditions tolerated and efficiencies obtained.

3. The AD Technological Upgrades

Gradually, several tools have become available to effectively manage the AD microbiome. Enzyme mixes to enhance cellulose decomposition are becoming commercially available (De Moor *et al.* 2013). The bio-augmentation of AD with specific strains that may enhance the cross-feeding in the microbiome, such as *Syntrophomonas zehnderi*, has been reported (Cavaleiro *et al.* 2010). Carriers, such as nylon particles, on

which the methanogenic archaea but not the sulfate-reducing bacteria can attach, are described (Ahmmed *et al.* 2013). Startling technological advances are also becoming available, such as the coupling of AD with (bio-)electrochemical systems to achieve, for example, selective recovery of ammonia and other cations from digestate (Desloover *et al.* 2012) or for biogas upgrading or polishing (Marshall *et al.* 2012; Tartakovsky *et al.* 2013; Zamalloa *et al.* 2013). Even nitrogen-rich substrates, such as slaughterhouse waste, are currently no longer a hurdle to be treated by AD, even as a single substrate (Bayr *et al.* 2012; Franke-Whittle and Insam, 2013). Also quite promising is the development of the AD of saline waters, as reported for the staged anaerobic fluidized MBR on sewage, explored in Korea (Yoo *et al.* 2012). Interestingly, the potential of autogenerative high pressure digestion systems that combine AD and biogas upgrading in one step, are also currently under investigation (Lindeboom *et al.* 2011). The key feature in practice now is that several installations attain a power capacity of 10 MW or even higher. Altogether, the energy production through AD, in the domains of domestic and industrial wastewater, agricultural crops and wastes and the organic fraction of municipal solid waste worldwide totals several 10,000 MW capacities. This makes the overall significance of AD in the domain of resource recovery and circular economy “incontournable” in relation to planning and decision-taking at the regional and governmental level.

4. Integration of AD in Different Technological Platforms

Biomethanation has the exceptional characteristic that it converts a multitude of molecules to the simplicity of two major metabolites, i.e. methane and carbon dioxide. Both molecules play a key role in the context of climate change. As such, AD will be in the center of different types of the oncoming climate change and biorefinery platforms. Indeed, by integrating AD in the conventional sugarcane-to-ethanol factory, the efficiency of the process can be increased from 40% in the conventional process to a situation that allows 60% of the initial crop energy to be harvested as ethanol, but moreover, 25% as biogas and 15% as biochar (Fig. 1.2).

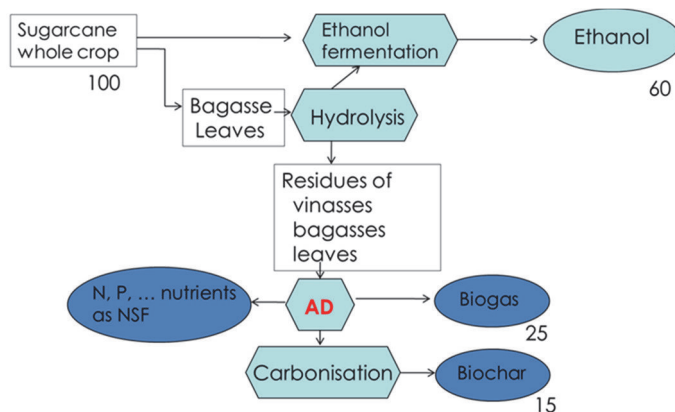


Fig. 1.2. AD as the central process in the biorefinery (adapted from Weiland *et al.* 2009).

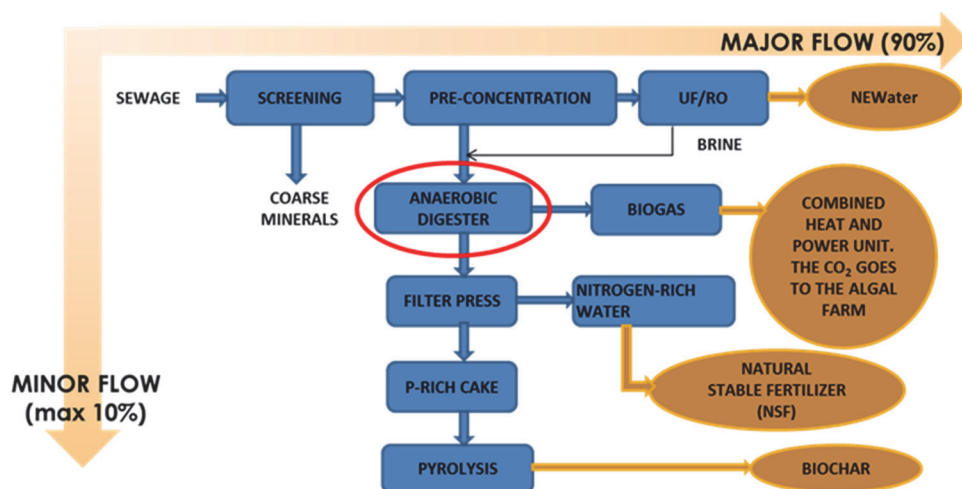


Fig. 1.3. Major and minor line process scheme based on concentration of wastewater in the beginning of the wastewater treatment plant, thus allowing maximal recovery of resources from domestic wastewater (adapted from Verstraete *et al.* 2009).

In the domain of conventional sewage treatment, AD can achieve partial recovery (20–30%) of the chemical energy present in the organics. However, the fact that the conventional activated sludge consumes high amounts of energy to produce first “poorly digestible sludge” and to nitrify and subsequently denitrify, brings about the assumption that this process, after 100 years of existence, is in absolute need for reconsideration in terms of sustainability (Verstraete and Vlaeminck 2011). The newly proposed concept that involves the direct separation of the organics at the entry of the sewage plant, and the consequent implementation of this so-called “Major and Minor” line technology offers, prospects to obtain a much better approach to handle wastewaters and related wastes for the cities of the future (Fig. 1.3).

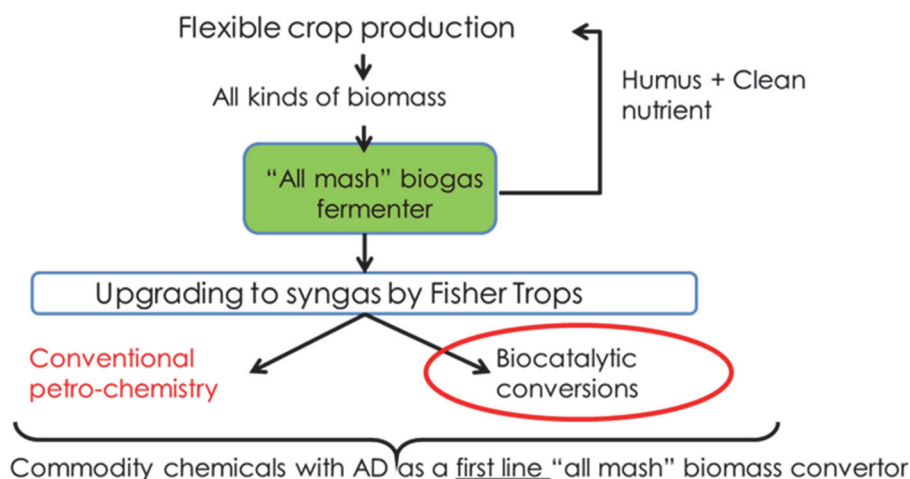


Fig. 1.4. AD biogas based sustainable organic chemistry.

Finally, it is clear that at present, the petrochemical sector, by the discoveries of deep gas reservoirs and the developments of fracking and tar sand extraction, has found new dynamics in terms of energy supply. Although the scarcity of fossil fuel supply no longer appears to be a vibrant issue, the problem of the abatement of climate change is at present even more prominent than ever before. In this respect, coupling of AD, e.g. as a supplier of bio-based methane, with the petrochemistry in order to make greener chemicals (Fig. 1.4), is a potential that deserves support (Datar *et al.* 2004; Younesi *et al.* 2005).

5. The AD Clearance Issue

Recovery of resources from waste materials is generally heralded as the new trend in our economy. Yet, the products that are being recovered must become “cleared” in terms of their composition and quality before they can be sold to and applied by potential users. In this respect, the output products of AD are generally situated in a difficult position. First and foremost, the biogas itself contains a variety of components next to methane and carbon dioxide (e.g. H_2S and NH_3). There is quite some progress in the cleanup of biogas by, for example, carbonation by means of bottom ash, additional fermentation to pure methane, physical membrane technology, chemical sorption or washing (Götz *et al.* 2011; Luo and Angelidaki 2013; Starr *et al.* 2012).

Second, the digestate forms a much worse problem in terms of applicability for reuse, as it contains a multitude of chemicals and very often matter of fecal origin. This makes its reuse from an ethical and sanitation point-of-view generally problematic. There are at present, to the best of our knowledge, no products that are derived from the digestate generated in full-scale AD installations that can be granted the “at spec” grade. Hence,

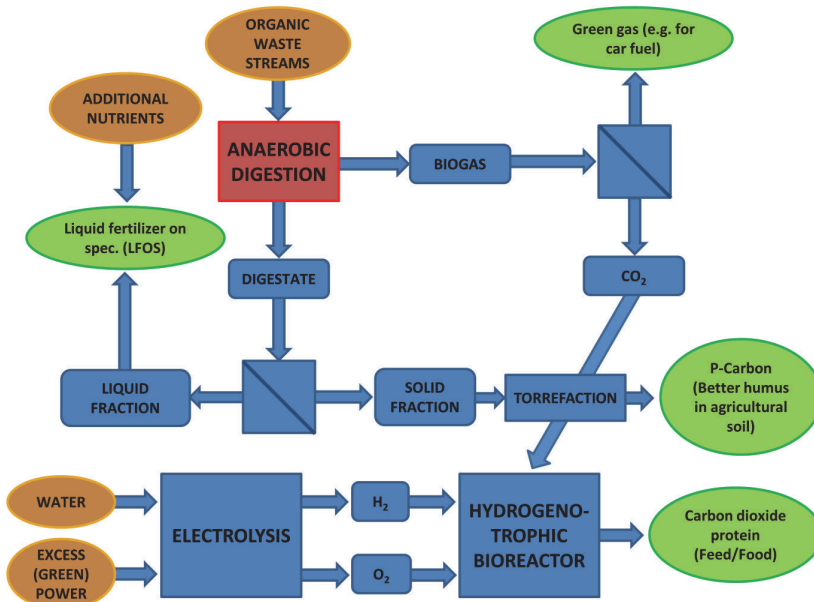


Fig. 1.5. The AD clearance concept.

there is a real need to develop suitable downstream processes in which the digestate is dealt with in a way that a qualified fertilizer (i.e. a fertilizer, accepted by the authorities, that is equivalent to a conventional chemical fertilizer) is produced. Incidentally, the sewage treatment industry has succeeded in closing the cycle and harvesting drinking water from plain sewage (Dewettinck *et al.* 2001). Therefore, the AD platforms must have a similar ambition to be able to upgrade their derivatives by means of short track process lines to feed and food level and, thus, effectively evolve to full-cycle configurations (Fig. 1.5). Treatment steps to extract the N, P and K and the organic carbon present in the digestate, clearing them from hygienic restrictions, should be made possible (Fig. 1.5). The real challenge for AD in the coming decades is to clear its outputs from the current cultural and legislative barriers, thus making sure that they are fully entering the resource recycle loops.

6. Conclusions

AD can be considered clean technology “par excellence”. It is a process that cannot be ignored in the biosphere because it is the central driver in all oxygen-free bioprocesses in nature. There lie, however, a multitude of challenging scientific questions ahead of us. The fact that several of them are long-lasting and hard to resolve may invoke a need of consolation. Yet, the very fact that much newer findings in for example the domain of electrochemical microbiology, open startling new insights and potentials, generates intellectual beauty in its terms, which constitutes an ever-renewing motivation for the scientist.

AD has top-notch potentials in the context of contributing to the abatement of climate change, becoming an integrated part of the bio- and also the petrorefinery and opening roads to new inorganic and organic chemicals and even feed and food commodities.

AD is just at the beginning of its implementations in the bio-economy. Happy are those who start to discover AD today and embark on exploring its potentials.

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Chapter 2

Syntrophy in Anaerobic Digestion

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Syntrophy is the closely associated relationship between at least two organisms based on electron (usually in the form of H_2 or formate) transfer from one to the other. Syntrophs are bacteria that decompose a variety of organic materials (usually fatty acids, alcohols and aromatic compounds) to produce acetate and H_2 , through thermodynamically endergonic reactions. To make this reaction energetically feasible, syntrophs, as the name suggests, depend very much on H_2 -consuming methanogens. The dependence on partner organisms is also characterized by their close physical proximity that allows for instantaneous interspecies H_2 transfer. Anaerobic ecosystems are based on such H_2 -producing and H_2 -consuming partnerships. Syntrophs are well-known as one of the most elusive organisms to cultivate, but efforts have been made to isolate them in the presence of partner organisms. Together with physiological and biochemical analyses of cultured syntrophs, recent high-throughput sequencing technologies are revealing the complex ecosystems in which syntrophs and methanogens drive the decomposition of organic materials.

1. Introduction

In order for researchers and engineers to have a closer look into anaerobic digestion (AD) processes, it is essential to understand that microorganisms form an intricate network system, in which tight interactions between organisms — referred to as syntrophy — greatly contributes to the degradation of organic matters to methane. Without understanding syntrophic associations, especially based on hydrogen (or formate or electron) transfer from one organism to the other, it would be impossible to know their entities, functions, and spatiotemporal change in community in strictly anaerobic methanogenic ecosystems. This chapter provides an overview of the biological and physicochemical significance of syntrophy, isolation and characterization of syntrophs, and the latest (meta)genomics that is unveiling the overall network in AD ecosystems.

2. Anaerobic Food Web and Syntrophy

A diverse array of microorganisms in AD processes are involved in the decomposition of organic materials to produce methane. The process of biodegradation and methanogenesis is characterized by its “food web” system. This can be exemplified by the conversion of glucose to methane. Glucose is an easily metabolizable substrate that most “aerobic” organisms completely oxidize into H_2O and CO_2 . In this process, plenty of energy can be gained in a single organism.



By contrast, in methanogenic ecosystems, glucose is converted to methane and CO_2 , unless other electron acceptors such as sulfate, nitrate or oxidized forms of metals (e.g. Fe^{3+}) are available.



The difference in energy yield between aerobic oxidation (complete mineralization) and methanogenesis is the energy that methane still retains. Thus, methane can be recovered as an energy resource for further use to generate electricity or heat. One of the characteristic features in this process, quite different from aerobic oxidation by single organisms, is that methanogenic degradation involves at least four different trophic groups, as shown in Fig. 2.1: (A) Heterotrophic organisms that anaerobically ferment glucose to produce fatty acids (volatile fatty acids such as butyrate, propionate and acetate), alcohols (butanol, ethanol), ketones (acetone) and H_2 . (B) Proton-reducing H_2 -producing substrate-oxidizing heterotrophs that degrade low molecules produced by trophic group A; this group oxidizes substrates to produce mainly acetate and H_2 . (C) H_2 -utilizing (hydrogenotrophic) methanogenic archaea. (D) Acetate-consuming (acetoclastic) methanogenic archaea. Each trophic group contains a wide array of organisms with different catabolic traits. Although one can easily assume that these

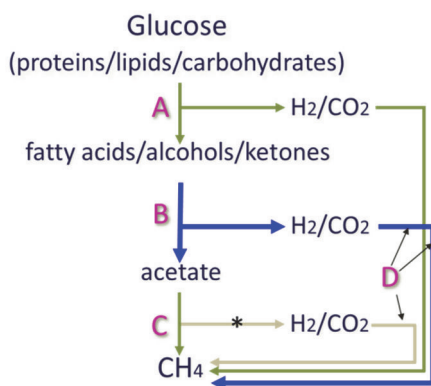


Fig. 2.1. Anaerobic digestion of organic matters by different trophic organisms (A to D). *Bacteria B* are syntrophs that rely on methanogenic H_2 -producing *archaea D*. *Bacteria A* are heterotrophic fermenters that sometimes produce H_2 , but most of them do not rely on *archaea D*. *Archaea C* are acetoclastic methanogens that directly produce CH_4 and usually do not produce H_2 . Some specific bacteria syntrophically oxidize acetate (*) relying on *Archaea D*.

trophic groups degrade substrates step-by-step and convey the products to the downstream microorganisms sequentially, this is actually not the case. Instead, the trophic groups closely associate and share energy with each other with each degradation step depending very much on the other trophic groups (Schink 1997; Schink and Stams 2006; Stams and Plugge 2009; Okabe and Kamagata 2009).

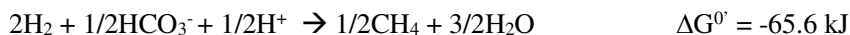
In particular, of the four trophic groups, proton-reducing H₂-producing substrate-oxidizing bacteria (group B mentioned above) are tightly associated with H₂-utilizing methanogens (group C) in terms of substrate oxidation and energy conservation. For instance, butyrate is oxidized by trophic group B as follows:

Group B (proton-reducing H₂-producing substrate-oxidizing bacteria)

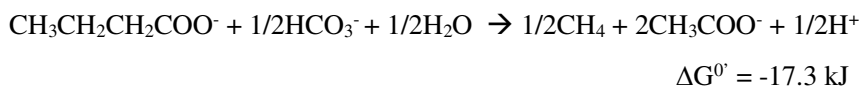


It is evident that this reaction is endergonic, but if it is coupled with the following reaction by H₂-utilizing methanogens (group C), the whole reaction (B + C) becomes exergonic.

Group C (H₂-utilizing methanogens)



Group B + Group C



The most important point in this process is that H₂ is the “key intermediate” and it has to be immediately consumed by H₂-utilizing methanogens since fatty acid oxidation concomitantly with H₂ production is an endergonic reaction, whereas H₂ consumption by methanogens is exergonic. Hence, H₂-utilizing methanogens play a crucial role to keep the H₂ partial pressure sufficiently low to make whole reactions energetically feasible. In other words, proton-reducing H₂-producing substrate-oxidizing bacteria are very much dependent on H₂-utilizing methanogens to act as “H₂-scavenging” partner, this is the primary reason why proton-reducing H₂-producing substrate-oxidizing bacteria are referred to as “syntrophs”. For this reason, H₂-utilizing methanogens make an essential contribution not only to methane production, but also to the oxidation of butyrate by syntrophs.

In this chapter, syntrophy is defined as the closely associated relationship between organisms based upon H₂ transfer from one to the other, and an H₂-producing organism that relies on H₂-consuming organisms for its growth is referred to as a syntroph (syntrophic organism).

3. Isolation and Cultivation of Syntrophs

AD processes harbor a large variety of microorganisms, most of which are yet-to-be cultured (Sekiguchi and Kamagata 2004; Lykidis *et al.* 2011). The advent of high-throughput genome sequencing technologies and genome informatics is now unveiling

the entire community structure inside AD. In other words, recent studies are trying to circumvent the limitation and laboriousness of isolation work, in particular, the isolation of syntrophs, one of the most difficult and time-consuming tasks. The state-of-the-art technologies have now proven that community structure and functional networks can be envisaged to a certain extent (Nobu *et al.* 2014b; Narihiro *et al.* 2014) and deciphering the byzantine community network using such approaches will progress rapidly over the next decade. Nonetheless, the most important point is that one can never circumvent isolation in order to comprehensively characterize a microorganism and unveil its true entity. Moreover, without isolates and their biochemical and genomic information, the network system could not be clarified in a convincing and compelling manner based solely on culture-independent advanced technologies.

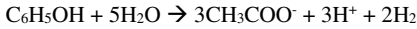
So far, many efforts have been made to cultivate and isolate syntrophs. Earliest successes in isolating syntrophs were the cultivation of propionate-oxidizing H₂-producing bacterium *Syntrophobacter wolinii* (Boone and Bryant 1980) and butyrate-oxidizing H₂-producing bacterium *Syntrophomonas wolfei* (McInerney *et al.* 1981). Without these landmark efforts, there would not have been a number of successful cultivations of syntrophs we now have in hand. The key to isolation of these syntrophs was that they were co-cultivated with *Desulfovibrio* sp., an H₂-consuming sulfate-reducing bacterium, or *Methanospirillum hungatei* (or *Methanobacterium* sp.), an H₂-consuming methanogen. Serial dilutions of enrichment cultures were made and those strains were eventually isolated from roll tube agar medium in which sufficient amounts of H₂-consuming organism cells were mixed in agar. This method of isolation is called “lawn culture”, meaning that an excess of H₂-consuming organism cells are used as background to scavenge H₂ generated by syntrophs.

Currently, a number of syntrophs are isolated in “pure culture” or “pure co-culture” with H₂-consuming partners (Table 2.1). As to “pure culture”, many syntrophs were found to grow in pure culture when appropriate substrates were provided. For instance, crotonate supports the growth of *S. wolfei*, *Syntrophus buswellii*, *Syntrophothermus lipocalidus* and *Pelotomaculum terephthalicum* in pure culture, and pyruvate supports the growth of *S. wolinii*, *Thermacetogenium phaeum* and *Pelotomaculum thermopropionicum* (for references, see Table 2.1). These results implicate that in many cases, syntrophs are not in a syntrophic association and thus are sometimes referred to as “facultative syntrophs”, whereas the syntrophs that are strictly dependent on H₂-consuming partners for their growth are referred to as “obligate syntrophs”.

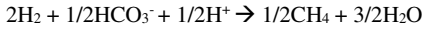
Isolation and cultivation of syntrophs have now allowed for the analysis of the physiological traits and biochemistry behind them. For instance, the genome of *S. wolfei* isolated in the earliest era (McInerney *et al.* 1981) was sequenced and we can now identify the central pathway that is coupled with H₂ generation (Sieber *et al.* 2010) (Fig. 2.2). With the rapid implementation of genome sequencing of isolates, the resulting outcomes facilitate the understanding of structure and function of methanogenic communities based on massive sequencing-dependent metagenomics and metatranscriptomics, which in turn, require the information on pure cultures to avoid making far-fetched speculations based solely on–omics analyses.

Table 2.1. Degradation of low-molecule organic matters by methanogenic syntrophic associations and well-known syntrophic bacteria and partner methanogens involved in respective metabolism via interspecies electron transfer.

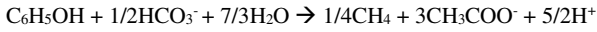
Butyrate		
$\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COO}^- + \text{H}^+ + 2\text{H}_2$	$\Delta G^{0'} = +48.3 \text{ kJ}$	
$2\text{H}_2 + 1/2\text{HCO}_3^- + 1/2\text{H}^+ \rightarrow 1/2\text{CH}_4 + 3/2\text{H}_2\text{O}$	$\Delta G^{0'} = -65.6 \text{ kJ}$	
$\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + 1/2\text{HCO}_3^- + 1/2\text{H}_2\text{O} \rightarrow 1/2\text{CH}_4 + 2\text{CH}_3\text{COO}^- + 1/2\text{H}^+$	$\Delta G^{0'} = -17.3 \text{ kJ}$	
<i>Syntrophomonas wolfei</i> (McInerney <i>et al.</i> 1981), <i>Syntrophospora bryantii</i> (Stieb and Schink 1985), <i>Syntrophothermus lipocalidus</i> (Sekiguchi <i>et al.</i> 2000)		
Propionate		
$\text{CH}_3\text{CH}_2\text{COO}^- + 3\text{H}_2\text{O} \rightarrow \text{CH}_3\text{COO}^- + \text{HCO}_3^- + \text{H}^+ + 3\text{H}_2$	$\Delta G^{0'} = +76.0 \text{ kJ}$	
$3\text{H}_2 + 3/4\text{HCO}_3^- + 3/4\text{H}^+ \rightarrow 3/4\text{CH}_4 + 9/4\text{H}_2\text{O}$	$\Delta G^{0'} = -98.4 \text{ kJ}$	
$\text{CH}_3\text{CH}_2\text{COO}^- + 3/4\text{H}_2\text{O} \rightarrow 3/4\text{CH}_4 + \text{CH}_3\text{COO}^- + 1/4\text{HCO}_3^- + 1/4\text{H}^+$	$\Delta G^{0'} = -22.4 \text{ kJ}$	
<i>Syntrophobacter wolinii</i> (Boone and Bryant 1980), <i>Syntrophobacter fumaroxidans</i> (Harmsen <i>et al.</i> 1998), <i>Pelotomaculum thermopropionicum</i> (Imachi <i>et al.</i> 2002), <i>Smithella propionica</i> (Liu <i>et al.</i> 1999)		
Acetate		
$\text{CH}_3\text{COO}^- + 4\text{H}_2\text{O} \rightarrow 2\text{HCO}_3^- + \text{H}^+ + 4\text{H}_2$	$\Delta G^{0'} = +94.9 \text{ kJ}$	
$4\text{H}_2 + \text{HCO}_3^- + \text{H}^+ \rightarrow \text{CH}_4 + 3\text{H}_2\text{O}$	$\Delta G^{0'} = -131.2 \text{ kJ}$	
$\text{CH}_3\text{COO}^- + \text{H}_2\text{O} \rightarrow \text{CH}_4 + \text{HCO}_3^-$	$\Delta G^{0'} = -36.3 \text{ kJ}$	
<i>Clostridium ultenense</i> (Schnürer <i>et al.</i> 1996), <i>Thermacetogenium phaeum</i> (Hattori <i>et al.</i> 2000), <i>Syntrophaceticus schinkii</i> (Westerholm <i>et al.</i> 2010), <i>Tepidanaerobacter acetatoxydans</i> (Westerholm <i>et al.</i> 2011)		
Ethanol		
$\text{C}_2\text{H}_5\text{OH} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{COO}^- + \text{H}^+ + 2\text{H}_2$	$\Delta G^{0'} = +9.6 \text{ kJ}$	
$2\text{H}_2 + 1/2\text{HCO}_3^- + 1/2\text{H}^+ \rightarrow 1/2\text{CH}_4 + 3/2\text{H}_2\text{O}$	$\Delta G^{0'} = -65.6 \text{ kJ}$	
$\text{C}_2\text{H}_5\text{OH} + 1/2\text{HCO}_3^- \rightarrow 1/4\text{CH}_4 + \text{CH}_3\text{COO}^- + 1/2\text{H}_2\text{O} + 1/2\text{H}^+$	$\Delta G^{0'} = -56.0 \text{ kJ}$	
<i>Pelobacter venetianus</i> (Schink and Stieb 1983), <i>Pelobacter venetianus</i> (Schink 1984), <i>Tepidanaerobacter syntrophicus</i> (Sekiguchi <i>et al.</i> 2006)		
1-Propanol		
$\text{C}_3\text{H}_7\text{OH} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{CH}_2\text{COO}^- + \text{H}^+ + 2\text{H}_2$	$\Delta G^{0'} = +3.0 \text{ kJ}$	
$2\text{H}_2 + 1/2\text{HCO}_3^- + 1/2\text{H}^+ \rightarrow 1/2\text{CH}_4 + 3/2\text{H}_2\text{O}$	$\Delta G^{0'} = -65.6 \text{ kJ}$	
$\text{C}_3\text{H}_7\text{OH} + 1/2\text{HCO}_3^- \rightarrow 1/2\text{CH}_4 + \text{CH}_3\text{CH}_2\text{COO}^- + 1/2\text{H}_2\text{O} + 1/2\text{H}^+$	$\Delta G^{0'} = -62.6 \text{ kJ}$	
<i>Pelobacter venetianus</i> (Schink and Stieb 1983), <i>Pelotomaculum thermopropionicum</i> (Imachi <i>et al.</i> 2002)		
Benzoate		
$\text{C}_6\text{H}_5\text{COO}^- + 7\text{H}_2\text{O} \rightarrow 3\text{CH}_3\text{COO}^- + 3\text{H}^+ + \text{HCO}_3^- + 3\text{H}_2$	$\Delta G^{0'} = +49.5 \text{ kJ}$	
$3\text{H}_2 + 3/4\text{HCO}_3^- + 3/4\text{H}^+ \rightarrow 3/4\text{CH}_4 + 9/4\text{H}_2\text{O}$	$\Delta G^{0'} = -98.4 \text{ kJ}$	
$\text{C}_6\text{H}_5\text{COO}^- + 19/4\text{H}_2\text{O} \rightarrow 3/4\text{CH}_4 + 3\text{CH}_3\text{COO}^- + 9/4\text{H}^+ + 1/4\text{HCO}_3^-$	$\Delta G^{0'} = -48.9 \text{ kJ}$	
<i>Syntrophus buswellii</i> (Mountfort and Bryant 1982), <i>Syntrophus gentianae</i> (Wallrabenstein <i>et al.</i> 1995), <i>Sporotomaculum syntrophicum</i> (Qiu <i>et al.</i> 2003), <i>Pelotomaculum terephthalicum</i> (Qiu <i>et al.</i> 2006), <i>Pelotomaculum isophthalicum</i> (Qiu <i>et al.</i> 2006), <i>Syntrophorhabdus aromaticivorans</i> (Qiu <i>et al.</i> 2008)		
Hydroxybenzoates		
$\text{C}_6\text{H}_5(\text{OH})\text{COO}^- + 6\text{H}_2\text{O} \rightarrow 3\text{CH}_3\text{COO}^- + \text{HCO}_3^- + 3\text{H}^+ + 2\text{H}_2$	$\Delta G^{0'} = +5.4 \text{ kJ}$	
$2\text{H}_2 + 1/2\text{HCO}_3^- + 1/2\text{H}^+ \rightarrow 1/2\text{CH}_4 + 3/2\text{H}_2\text{O}$	$\Delta G^{0'} = -65.6 \text{ kJ}$	
$\text{C}_6\text{H}_5(\text{OH})\text{COO}^- + 4.5\text{H}_2\text{O} \rightarrow 1/2\text{CH}_4 + 3\text{CH}_3\text{COO}^- + 1/2\text{HCO}_3^- + 5/2\text{H}^+$	$\Delta G^{0'} = -60.2 \text{ kJ}$	
<i>Pelotomaculum terephthalicum</i> (Qiu <i>et al.</i> 2006), <i>Pelotomaculum isophthalicum</i> (Qiu <i>et al.</i> 2006), <i>Syntrophorhabdus aromaticivorans</i> (Qiu <i>et al.</i> 2008)		

Phenol

$$\Delta G^{0'} = +10.2 \text{ kJ}$$

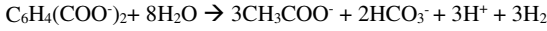


$$\Delta G^{0'} = -65.6 \text{ kJ}$$

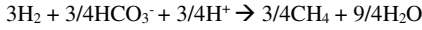


$$\Delta G^{0'} = -55. \text{ kJ}$$

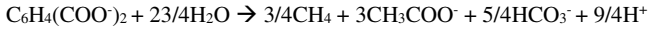
Syntrophorhabdus aromaticivorans (Qiu *et al.* 2008)

Phthalates

$$\Delta G^{0'} = +38.9 \text{ kJ}$$



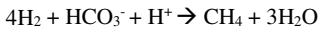
$$\Delta G^{0'} = -98.4 \text{ kJ}$$



$$\Delta G^{0'} = -59.5 \text{ kJ}$$

Pelotomaculum terephthalicum (Qiu *et al.* 2006), *Pelotomaculum isophthalicum* (Qiu *et al.* 2006),

Syntrophorhabdus aromaticivorans (Qiu *et al.* 2008)

Partner methanogens responsible for H₂ consumption and methanogenesis

$$\Delta G^{0'} = -131.2 \text{ kJ}$$

Please see articles describing hydrogenotrophic methanogens.

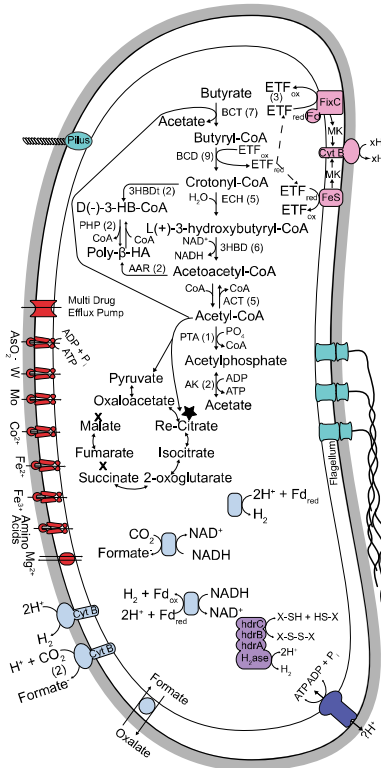


Fig. 2.2. Overview of the metabolism of *Syntrophomonas wolfei*. Primary metabolic reactions are black with the number of homolog in parentheses, cell wall is grey, hydrogenases and formate dehydrogenases are light blue, ATP synthase is dark blue, transport and efflux pumps are red, flagella and pili are aqua, heterodisulfide reductase is purple and genes potentially involved in reversed electron transfer are pink. Dashed lines indicate the potential routes for reverse electron transport. xH^+ and xH_2 could be either hydrogen or formate, in the oxidized or reduced states, respectively. ETF, electron transfer flavoprotein; Fd, ferredoxin; MK, menaquinone. Star denotes *re*-citrate synthase. Abbreviations for enzymes are based on the first letters of the enzyme name. The figure is taken from Sieber *et al.* (2010).

4. Visualization and Physicochemical Analyses of Syntrophs

The AD system has become very popular since the emergence of a new process in the 1980s (Lettinga 1995). The advent of the wastewater treatment system currently well known as the upflow anaerobic sludge blanket (UASB) reactor drastically improved the reactor performance compared with old-fashioned AD systems; it completely changed our view about methanogenic processes and it made methane fermentation economically feasible. The UASB process is now a prevailing technology for treating medium and high strength organic wastewaters and over 1,000 full-scale reactors are now in use worldwide. It can retain dense biomass by taking advantage of granule formation of microbial communities. This system showed us not only how a dense community contributes to the reactor performance, but also how a variety of microorganisms interact within a granule. The syntrophic association between organisms and overall structure within UASB granules were successfully visualized in thin sections of granules using 16S rRNA-targeted fluorescence in situ hybridization (FISH) (Sekiguchi *et al.* 1999; Rocheleau *et al.* 1999; Wu *et al.* 2001; Gonzalez-Gil *et al.* 2001; Saiki *et al.* 2002; Liu *et al.* 2002; Díaz *et al.* 2006). FISH combined with confocal laser scanning microscopy revealed the spatial distribution of bacteria and archaea (Fig. 2.3). When hybridized with

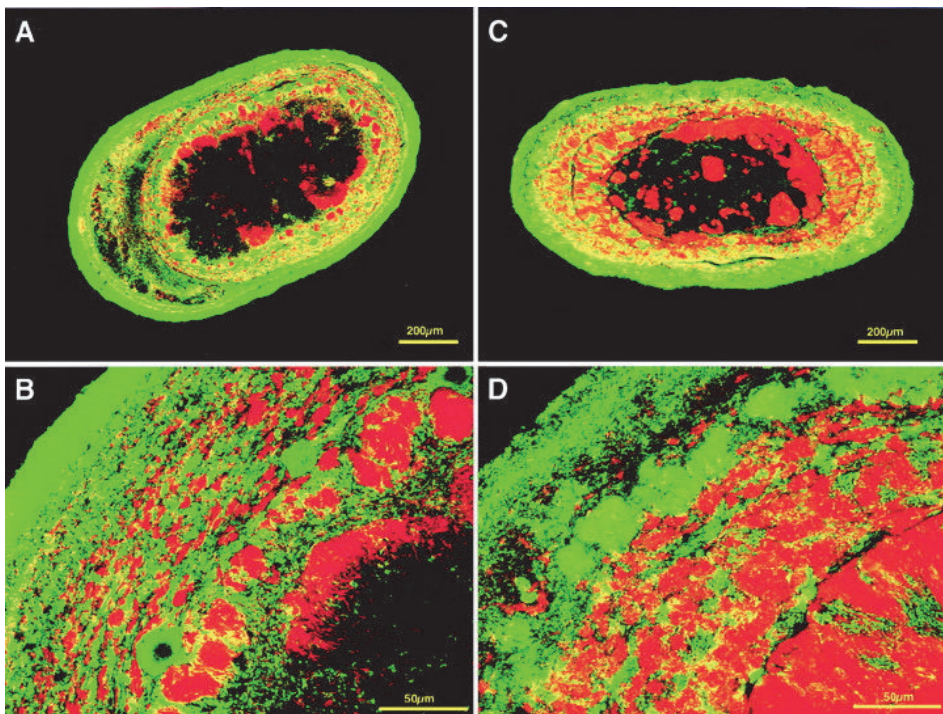


Fig. 2.3. In situ hybridization of sections from mesophilic and thermophilic granules viewed by CLSM. The sections were simultaneously hybridized with Cy-5-labeled bacterial-domain probe (EUB338) (green) and rhodamine-labeled archaeal-domain probe (ARC915) (red). Mesophilic (A and B) and thermophilic (C and D) sludge granules at universal and archaeal universal probes, the granular biofilm was found to be a layered or uneven structure. The outermost layer was dominated by bacterial cells, whereas the low magnification (A and C) and at higher magnification (B and D) are shown. The figure is taken from Sekiguchi *et al.* (1999).

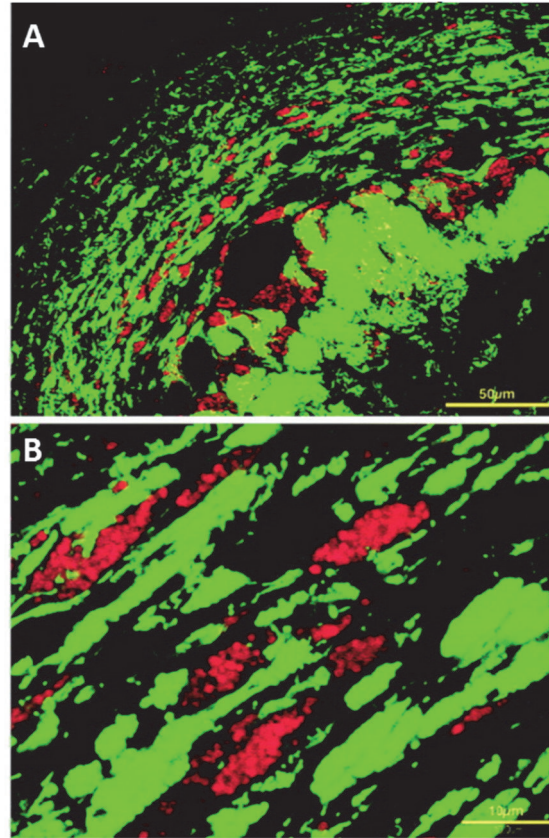


Fig. 2.4. In situ hybridization of sections from a mesophilic granule viewed by CLSM. (A) Mesophilic granule section hybridized with Cy-5-labeled archaeal probe (ARC915) (green) and rhodamine-labeled SYB701 probe specific for *Syntrophobacter* (red). (B) Higher magnification of mesophilic granule section of panel (A). The figure is taken from Sekiguchi *et al.* (1999).

bacteria, the inner layer was occupied mainly by archaeal cells. Since organic materials in the wastewater are present outside a granule, anaerobes in the outermost layer of granules ferment them to produce lower molecule metabolites such as C₄, C₃, C₂fatty acids, alcohols and H₂. The organisms present in the middle (or inner) layer contain syntrophs that oxidize these intermediates to form acetate and H₂. As the H₂ partial pressure must be kept sufficiently low for syntrophic degradation to proceed, H₂-consuming methanogens should be present close by. Indeed, they are spatially associated with syntrophs in their immediate proximity forming a beautifully arranged architecture (Fig. 2.4).

For an in-depth understanding of such a close association between H₂-producing fermentative bacteria and H₂-consuming methanogens — namely, interspecies H₂ transfer between organisms — the possible physical distance (proximity) between the two have been estimated. In the earliest study by Boone *et al.* (1989), diffusion rate and mass transfer of H₂ was theoretically considered, assuming that two organisms are evenly dispersed. As shown in the confocal laser scanning microscopy, however, it was evident that syntrophs are often tightly bound with methanogens to make methanogenic reactions

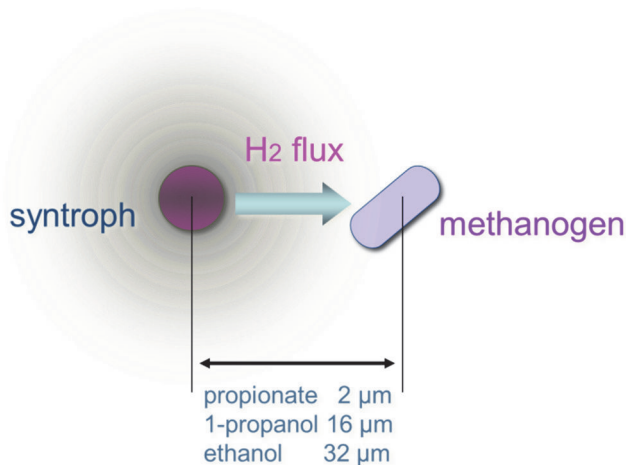
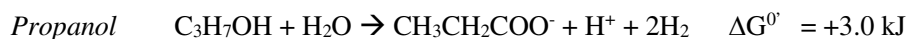
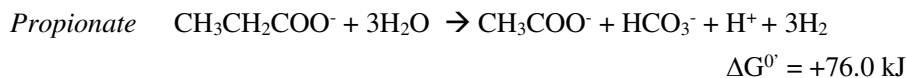


Fig. 2.5. Proximity needed for making interspecies H_2 transfer energetically favorable. Image is created based on Ishii *et al.* (2005).

energetically favorable. Later, Ishii *et al.* (2005, 2006) cultivated a syntroph with propionate, ethanol and propanol in the presence of methanogen, and calculated the necessary proximities between the organisms based upon their findings that the less energetically favorable the substrate was for the H_2 -producing syntrophs, the closer they formed relationships with the H_2 -consuming methanogens, resulting in tight coaggregation.



Indeed, when a propionate-oxidizing syntroph (*Pelotomaculum thermopropionicum*) is co-cultivated with a H_2 -consuming methanogen (*Methanothermobacter thermautotrophicus*) with ethanol, propanol, or propionate (note that *P. thermopropionicum* was isolated as propionate-oxidizing bacterium, but it was also found that other substrates such as ethanol can be oxidized in the presence of methanogen), cells were tightly coaggregated in propionate culture, whereas with ethanol and propanol, co-cultures were greatly dispersed. They estimated the allowable distance between the syntroph and methanogen based on Fick's diffusion law, and it turned out that the oxidation of and subsequent methanogenesis from propionate, ethanol and propanol require the cell-cell closeness of 2, 16 and 32 μm , respectively (Fig. 2.5). This estimation is notable as the FISH image of a UASB granule, in which propionate was fed exactly, shows the juxtaposition of two functionally identical microbes consistent with the calculated distance. In addition, during co-culturing of *P. thermopropionicum* and *M. thermautotrophicus*, monitoring growth in the laboratory by shaking the culture bottle to stir up settled cells altered the existing fine-tuned physical distance between the two cells. Consequently, without the required cell proximity necessary for propionate degradation, there were negative, or even inhibitory effects on subsequent cell growth (Imachi *et al.* 2000 and personal communication). Only when aggregated cells remained at the bottom

of the culture bottles, propionate degradation continued to occur. These findings strongly indicate that propionate is among the most difficult to be degraded, requiring syntrophs and methanogens to work very closely together.

5. Entity of Electron Transfer between Organisms

In a broad sense, interspecies H_2 transfer is also referred to as “interspecies electron transfer”, as H_2 is an electron carrier. However, there has long been controversy over the entity of interspecies H_2 transfer. Can H_2 transfer actually be formate transfer, electron (e^-) transfer, or some other electron-carrier transfer? Even in the early era of syntroph study, formate was thought to be an alternative carrier (Thiele and Zeikus 1988; Boone *et al.* 1989). This is because a number of H_2 -consuming organisms also utilize formate, indicating that they can receive formate instead of H_2 if syntrophs oxidize substrates to produce formate from H_2 or other reducing equivalents (such as ferredoxin). Indeed, the exergonic nature of this reaction indicates that this may readily take place:



Thiele and Zeikus (1988) operated a mesophilic digester sludge fed with ethanol or lactate. They detected a significant amount of formate in the sludge and demonstrated that methanogenesis from formate accounts for >90%, with the remaining methanogenesis occurring from H_2/CO_2 . The plausible reason why formate is an important electron shuttle in interspecies electron transfer was clarified by Boone *et al.* (1989).

They calculated the potential H_2 and formate diffusion between two organisms, *Syntrophomonas wolfei* and *Methanobacterium formicicum*, and found that at natural H_2 concentrations (some 10 Pa), H_2 could not diffuse rapidly enough to disperse methanogenic cells to account for the rate of methanogenesis, but formate could. Also, both *S. wolfei* and *M. formicicum* contained formate dehydrogenase that catalyzes formate formation from H_2/CO_2 .

Since then, several studies have reported the possible involvement of formate as an interspecies electron carrier. *Syntrophobacter fumaroxidans* was found to grow well on propionate in co-culture with methanogens that use hydrogen and formate, but no measurable growth was observed with methanogens that use only hydrogen (Dong *et al.* 1994). Hattori *et al.* (2001) showed that the acetate-oxidizing H_2 -producing bacterium *Thermacetogenium phaeum* co-cultured with *Methanothermobacter thermautotrophicus*, that has a formate dehydrogenase, grew and produced methane much faster than *T. phaeum* co-cultured with another *M. thermautotrophicus* strain that is deficient in formate dehydrogenase, suggesting that extracellular formate contributes to interspecies electron transfer to a great extent.

Recently, Ishii *et al.* (2005) and Gorby *et al.* (2006) reported that the flagellum-like appendages of *Pelotomaculum thermopropionicum* cells connecting with *Methanothermobacter thermautotrophicus* cells were electrically conductive nanowires based on scanning tunneling microscopy and tunneling spectroscopy, indicating that electron transfer is occurring through the wire (Fig. 2.6). Shimoyama *et al.* (2009).

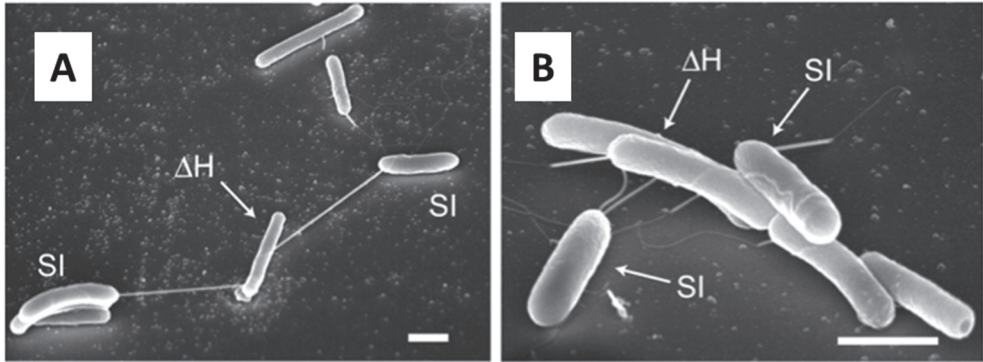


Fig. 2.6. Representative SEM images of a monoculture of *Pelotomaculum thermopropionicum* strain SI co-cultured with *Methanothermobacter thermautotrophicus* strain ΔH cells grown on propionate. A and B are different magnifications. Bars, 1 μm . The picture is taken from Ishii *et al.* (2005).

later found that FlhD (flagellin cap proteins) of *P. thermopropionicum* adhered onto *M. thermautotrophicus* cells, and in two hours, it altered the expression of over 50 genes, including upregulation of methanogenesis, ATP synthesis and hydrogenase genes. This study changed our view to syntrophy: bacterial flagellum maintains syntrophy not only to ensure proximity between specific partners, but also to synchronize their metabolism. The liaison between *P. thermopropionicum* and *M. thermautotrophicus* represents a protein-mediated communication system that has specifically evolved for interspecies interactions. Studies on syntrophs are now progressing to nano-scale physiology and electrochemistry to understand interspecies electron transfer. Kato *et al.* (2012) recently reported that electrons are transferred from one organism to another via electron conductive materials. They also found that (Yamada *et al.* 2014) supplementation of magnetite accelerated methanogenesis from acetate and propionate under thermophilic conditions, while supplementation of ferrihydrite also accelerated methanogenesis from propionate. Microbial community analysis revealed that supplementation of magnetite drastically changed bacterial populations in the methanogenic acetate-degrading cultures. Through these studies, a new term — “electric syntrophy” — has been created, demonstrating that novel interspecies electron transfer continues to be discovered.

6. Syntrophy Based on Genomic and Metagenomic Analyses

The advent of the high-throughput sequencing technologies and genome informatics is now unveiling the structures of single genomes and community genomes. Two different approaches to understanding syntrophy are currently expanding. One is based on genome analyses of pure culture or defined co-culture. This approach is straightforward and makes it possible to obtain solid and convincing data covering taxonomy, physiology, biochemistry and genomics (Sieber *et al.* 2012). The other approach is based on metagenomic analyses of methanogenic ecosystems. Once DNAs or RNAs are obtained from methanogenic ecosystems, one could investigate the genome structures of extant organisms (communities) regardless of their cultivability. Organisms that elude cultivation cannot elude genome sequencing unless they were numerically far less dominant within the guild.

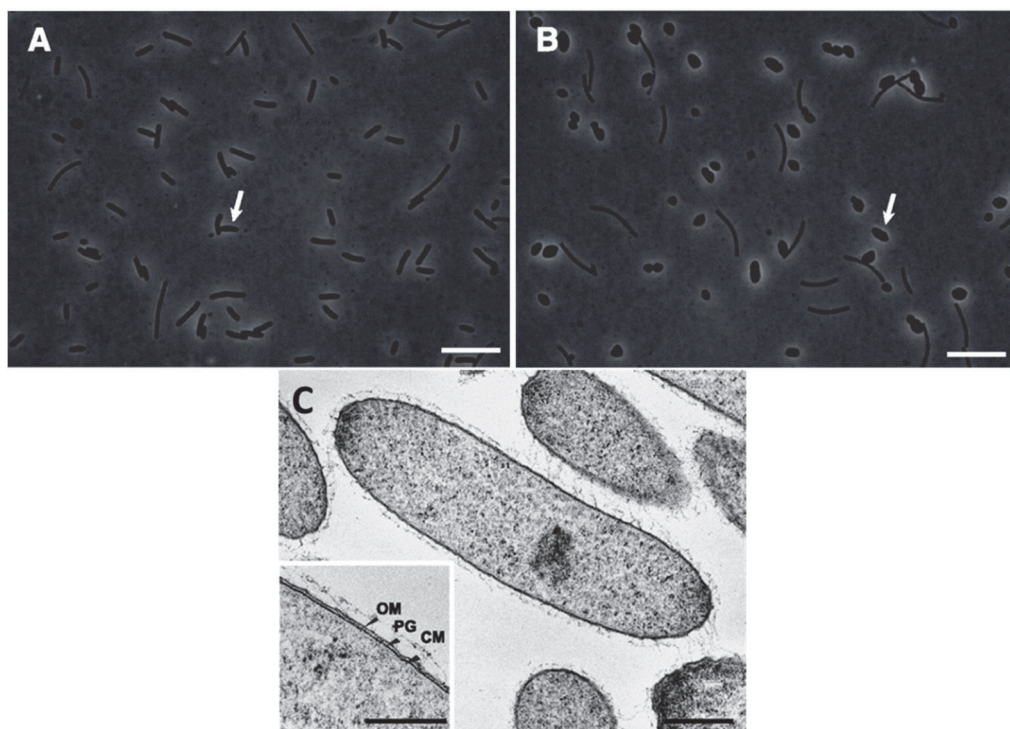


Fig. 2.7. Phase-contrast micrographs of *Syntrophorhabdus aromaticivorans* strain UI (indicated by white arrows) in co-culture with *Methanospirillum hungatei* grown on isophthalate (A) and grown on phenol (B). Bars, 10 μm . (C) Thin-section electron micrographs of strain UI in coculture with *M. hungatei* grown on 4-hydroxybenzoate, illustrating typical gram-negative cell wall structure (OM, outer membrane; PG, peptidoglycan; CM, cytoplasmic membrane). The bar for the large panel represents 0.5 μm . The inset of the panel shows a magnified view of the cell wall structure (bar 0.25 μm). The pictures are taken from Qiu *et al.* (2008).

One of the earliest studies on single genome-based study on syntrophy was to address whether methanogens respond to syntrophic association. All research done on the genetics and biochemistry of methanogens until a decade ago were based on pure cultures grown under high H_2 partial pressures, such as 10^5 Pa, to yield sufficient amounts of cells for further study. However, in syntrophic association with H_2 -producing bacterium, the H_2 partial pressures are kept three to four orders of magnitude lower than laboratory culture conditions. Methanogens constantly receiving some 10 Pa should be under significantly different physiological conditions from those in pure culture under plenty of H_2 . Luo *et al.* (2002) and Enoki *et al.* (2011) took full advantage of genomic information of *Methanothermobacter thermoautotrophicus* and investigated what genes are up- or downregulated when a methanogen is associated with a syntroph by comparing pure cultures under a high H_2 partial pressure. They clearly showed that genes responsible for several steps of methanogenesis, carbon fixation, amino acid syntheses and DNA/RNA metabolism are downregulated, whereas methanogenesis-driven energy generation appeared to be maintained by shifting the pathway to the alternative methylcoenzyme M reductase isozyme I and cofactor F_{420} -dependent process. These results clearly suggest

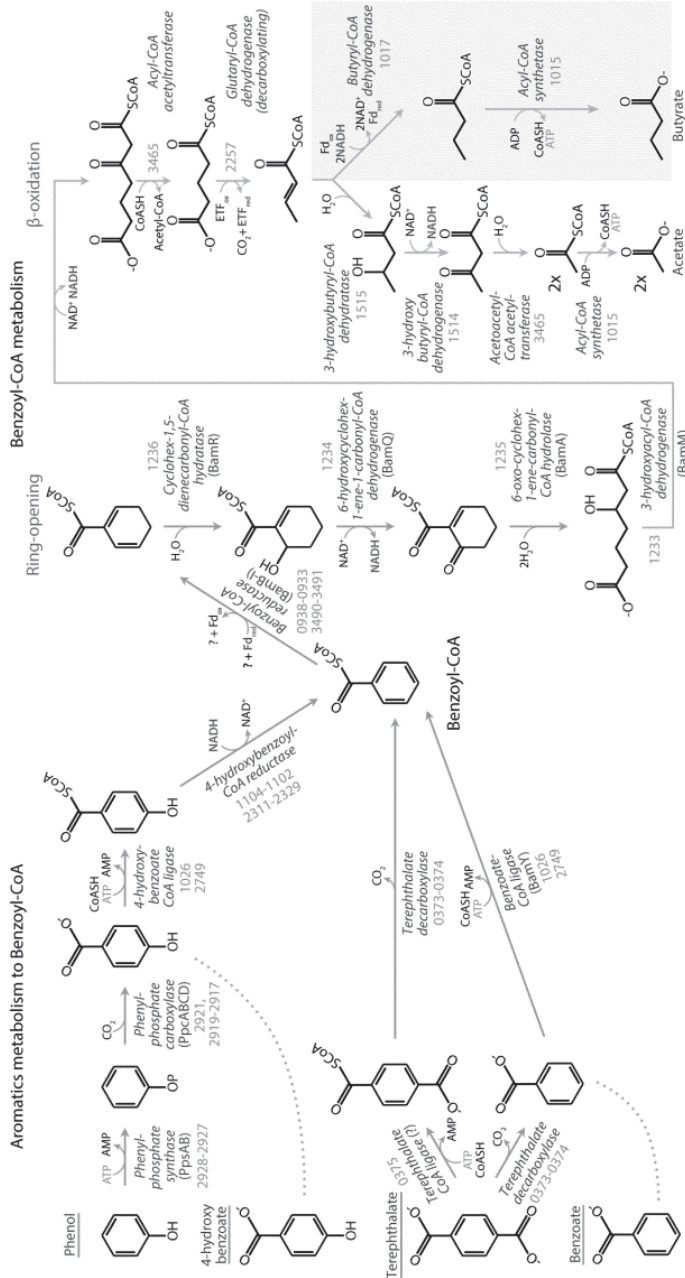


Fig. 2.8. Aromatic compound metabolism to benzoyl-CoA and following benzoyl-CoA degradation pathway in *Syntrophorhabdus aromaticivorans*. Phenol, 4-hydroxybenzoate, TA, and benzoate degradation to benzoyl-CoA is shown on the left. TA degradation has two potential pathways with terephthalyl-CoA or benzoate as an intermediate. Benzoyl-CoA degradation to acetate (white background) and possibly butyrate (gray background) are shown on the right side. Benzoyl-CoA degradation is split into reductive ring de-aromatization/opening and β -oxidation. Each reaction is labelled with the abbreviated strain UI locus tag (e.g. 'SynarDRAFT_0001' as '0001') and protein name (italicized) of the gene encoding the function. The figure is taken from Nobu *et al.* (2014).

that the physiology of methanogens when associating with syntrophic partners is distinct from free-living methanogens.

One of the landmark genomics studies in cultured syntrophs is the genome analysis of *Syntrophorhabdus aromaticivorans* (Nobu *et al.* 2014a). The organism is the first and only cultured organism capable of degrading phenol and its derivatives (*p*-cresol, 4-hydroxybenzoate, isophthalate and benzoate) in syntrophic association with an H₂-consuming methanogen under strictly anaerobic conditions (Qiu *et al.* 2008) (Fig. 2.7). The study identified the first syntrophic phenol-degrading phenylphosphate synthase (PpsAB) and phenylphosphate carboxylase (PpcABCD) and syntrophic terephthalate-degrading decarboxylase complexes. The strain UI genome also encodes benzoate degradation through the hydration of the dienoyl-coenzyme A intermediate in which reduced ferredoxin-oxidizing electron bifurcation is involved. As shown in Fig. 2.8, an entire picture of aromatic compounds degradation is proposed.

Metagenomics, including functional genes using high-throughput sequencing, is now extensively underway (Lykidis *et al.* 2011; Nelson *et al.* 2011; Sundberg *et al.* 2013; Werner *et al.* 2011; Narihiro *et al.* 2014; Nobu *et al.* 2014b). This striking approach is now uncovering a wider variety of organisms and functions within AD than ever thought. Likidis *et al.* (2011) reported the community genomics combined with FISH of a methanogenic reactor treating terephthalate-containing wastewater at moderately high temperatures (46–50 °C). They identified 22 phyla with *Firmicutes*, *Thermotogae*, *Spirochaetes*, *Synergistetes*, OP5 and *Chloroflexi* being the dominant ones. They also identified genes belonging to dominant *Pelotomaculum* species presumably involved in terephthalate degradation through decarboxylation, dearomatization, and modified β -oxidation to produce H₂/CO₂ and acetate. Interestingly, by using FISH analysis, they observed not only close physical proximity among methanogens and *Pelotomaculum*, but also the presence of other microbes such as OP5, WWE1, *Thermotogae* and *Syntrophus* associating with syntrophs and methanogens, suggesting that additional secondary syntrophic interactions can be predicted (Fig. 2.7). All of these results are speculative, but massive sequencing can uncover intriguing syntrophic network that would otherwise not be seen by conventional sequencing or a culture-dependent approach.

7. Concluding Remarks

Over decades, microbiologists have been interested in syntrophy because it plays a key role in anaerobic ecosystems. Syntrophy is a common phenomenon and lifestyle, but it has been very challenging to obtain an in-depth understanding because of difficulties in cultivation. However, tremendous efforts have been made to isolate syntrophic microorganisms and analyze their physiology, biochemistry and energetics sustaining syntrophy. Syntrophy is characterized by sharing energy between at least two distinct organisms at the thermodynamic limits of life in which interspecies electron transfer is involved. With the advent of massive sequencing technology together with the efforts to cultivate syntrophs, we will be able to see much more variety of syntrophic microorganisms and perhaps alternative modes of interspecies material transfer. Methane

fermentation technology will continue to evolve by gaining an in-depth understanding of syntrophy.

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Chapter 3

Microbial Community Involved in Anaerobic Purified Terephthalic Acid Treatment Process

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Purified terephthalic acid is one of the most important petrochemical products for our modern life. From an environmental viewpoint, wastewater from the purified terephthalic acid production industry should be treated using effective technologies that guarantee the protection of human health and environmental safety. To do so, anaerobic biological wastewater treatment process is successfully applied for the treatment of purified terephthalic acid wastewater in the world. In the process, methanogenic microbial consortia are key players in the degradation of purified terephthalic acid-related compounds, such as terephthalic acid, *p*-toluic acid, and benzoate. To date, several studies have been conducted to understand microbial community structures in anaerobic purified terephthalic acid wastewater treating processes and to elucidate their physiology and ecological functions. This chapter will describe the current knowledge of the microbial community structures involved in the degradation of purified terephthalic acid-related compounds in anaerobic wastewater treatment processes.

1. Introduction

The petrochemical industry produces thousands of materials essential for our modern life. The market size of the global petrochemical industry is expected to be \$609.3 billion in 2012 (Visiongain 2013). However, despite the social and economic benefits provided by the petrochemical industry, a large amount of wastewater is inevitably produced from petroleum refinery and petrochemical manufacturing processes. In typical petroleum refinery processes, 3,600 m³·day⁻¹ of crude oil are refined along with the production of 28,000 m³·day⁻¹ of produced water (Chen 2008; Chen *et al.* 2004; Dórea *et al.* 2007). In addition, the wastewater discharged from the petrochemical manufacturing processes contains a high proportion of toxic compounds, such as polycyclic aromatic hydrocarbons (Dórea *et al.* 2007; Sponza and Gok 2011), volatile organic compounds (Dórea *et al.*

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2007; Ghasemian *et al.* 2012), chlorinated solvents (Tobiszewski *et al.* 2012), metals (Dórea *et al.* 2007; Malakahmad *et al.* 2011), naphthenic acid (Wang *et al.* 2013), and aromatic carboxylic acid compounds (Verma *et al.* 2010; 2012). Such harmful wastewater should be treated using effective technologies that guarantee protecting human health and environmental safety.

Purified terephthalic acid (PTA) is one of the most important petrochemical products for the synthesis of polyethylene terephthalate, which has many valuable applications in our lives, such as in textile fibers, films, and food storage items (Razo-Flores *et al.* 2006). In 2011, global PTA production was estimated to be 40.6×10^6 tons·year⁻¹, and it is expected to be 58.3×10^6 tons·year⁻¹ in 2017, with an annual increasing rate at 6.2% (METI 2013). Production of PTA involves a series of chemical processes with *p*-xylene as the precursor (Roffia *et al.* 1984; Tomás *et al.* 2013). This consequentially produces wastewater containing high concentrations of organic by-products, the majority of which are composed of terephthalate (TA), *para*-toluate (*p*-Tol), benzoate, acetate, and 4-carboxybenzaldehyde (Fig. 3.1) (Kleerebezem *et al.* 1999b; Razo-Flores *et al.* 2006; Roffia *et al.* 1984; Tomás *et al.* 2013). Given that the production of one ton of PTA generates 2.5–10 m³ of wastewater (Kleerebezem *et al.* 2005; Razo-Flores *et al.* 2006), approximately 100–400 × 10⁶ m³·year⁻¹ of wastewater is generated and discharged from global PTA production. Thus, to complement the high global demand for PTA synthesis, it is necessary to effectively treat the by-products and residues generated from PTA manufacturing processes.

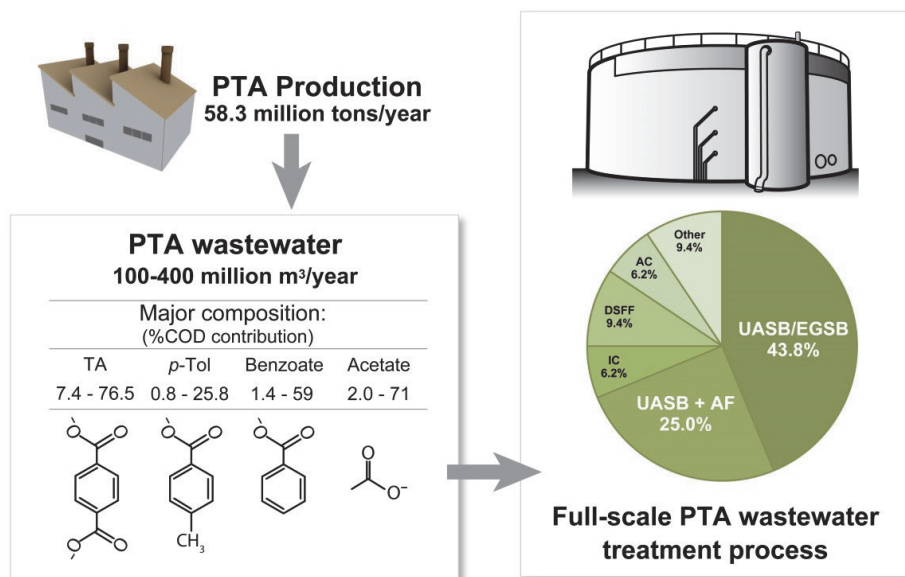


Fig. 3.1. Annual purified terephthalic acid production and discharged wastewater. The average composition of PTA wastewater is shown in the left top box. The right top box indicates the percentages of reactor type of full-scale anaerobic PTA wastewater treatment processes operated worldwide (van Lier *et al.* 2008). Abbreviations: UASB (up-flow anaerobic sludge blanket); EGSB (expanded granular sludge blanket); AF (anaerobic filter); IC (internal circulation); DSFF (down-flow stationary fixed film); AC (anaerobic contact).

Aerobic biological processes were initially applied to remediate PTA wastewater (Lau 1977; Noyola *et al.* 2000) due to their efficient chemical oxygen demand (COD) removal. However, known potential disadvantages are long hydraulic retention time, high aeration requirement, large site area requirement, excess sludge production, and poor biomass settling property due to 'sludge bulking' or overgrowth of filamentous bacteria (Noyola *et al.* 2000; Razo-Flores *et al.* 2006). To overcome these obstacles, BP-Amoco has developed anaerobic treatment systems under mesophilic conditions (35 °C) and achieved over 80–85% reduction in COD in the late 1980s (Razo-Flores *et al.* 2006). In further technological development, several types of anaerobic systems have been applied to treat PTA wastewaters, including anaerobic contact, down-flow stationary fixed film, internal circulation, anaerobic filter, up-flow anaerobic sludge blanket (UASB), and expanded granular sludge blanket (EGSB) (Feng *et al.* 2012; Guyot *et al.* 1990; Joung *et al.* 2009; Kim *et al.* 2012; Kleerebezem and Macarie 2003; Macarie 2000; Pereboom *et al.* 2012; Pophali *et al.* 2007; Stergar *et al.* 2003; Veolia Water Solutions & Technologies 2012; Zhu *et al.* 2010). In particular, granular sludge bed-type anaerobic systems, including UASB and EGSB, have been widely used (Fig. 3.1). These are mainstream systems for anaerobic biological treatment due to their capability for retaining a high concentration of granular sludge with a high wastewater loading rate ($>25 \text{ kg COD} \cdot \text{m}^{-3} \cdot \text{day}^{-1}$). For example, these systems are used for treating various types of wastewater discharged from agro-food, pulp/papermill, and petrochemical industries (Kleerebezem and Macarie 2003; Lettinga 1995; van Lier 1996; van Lier *et al.* 1996).

In such anaerobic processes, two major microbial guilds (i.e., syntrophs and methanogens) are consistently observed to support the conversion of wastewater organic compounds to biogas. In particular, these organisms are central to the treatment of PTA-related compounds, in which syntrophic substrate-oxidizing bacteria (syntrophs) degrade aromatic compounds to a mixture of acetate, H_2 , and formate, and methanogenic archaea (methanogens) further convert these compounds to CH_4 and CO_2 , as revealed by a combination of confocal scanning laser microscopy and fluorescence *in situ* hybridization (CSLM-FISH) targeting the 16S rRNA of syntrophs and methanogens (Chen 2008). These two guilds interact syntrophically because the initial oxidation of aromatic compounds (e.g., TA, *p*-Tol, and benzoate) is energetically unfavorable ($\Delta G^0 > 0$) (Figs. 3.2a and 2b) and requires coupled methanogenesis reactions (Fig. 3.2c and/or 2d) to proceed (McInerney *et al.* 2007; Qiu *et al.* 2008; Qiu *et al.* 2006; Schink and Stams 2013).

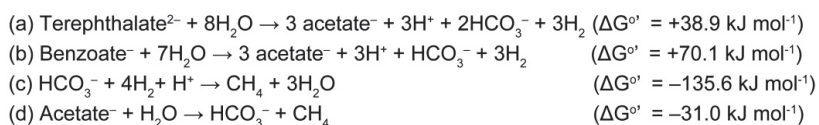


Fig. 3.2. Reactions involved in syntrophic degradation of aromatic compounds (McInerney *et al.* 2007; Qiu *et al.* 2006; Qiu *et al.* 2008). (a) terephthalate degradation; (b) benzoate degradation; (c) hydrogenotrophic methanogenesis; (d) acetoclastic methanogenesis.

From engineering and microbiological perspectives, it is important to identify the key members contributing to each microbial guild and further understand how they interact and metabolize aromatic compounds in PTA wastewater. So far, a fairly limited number of the microbial community analysis for anaerobic reactors treating PTA-related compounds, such as TA (Chen 2008; Chen *et al.* 2004; Li *et al.* 2014; Lykidis *et al.* 2011; Perkins *et al.* 2011; Wu *et al.* 2001b), *p*-Tol (Wu *et al.* 2001a), and benzoate (Narihiro *et al.* 2015; Wu *et al.* 2001b) has been investigated using both 16S rRNA gene-based molecular tools (e.g., Sanger-based clone libraries, pyrosequencing, and Illumina sequencing) and microscopic analyses (e.g., transmission electron microscope; TEM, scanning electron microscope; SEM, and CSLM-FISH). Although information regarding the microbial consortia responsible for PTA wastewater remediation is still limited, these pioneering studies provide essential insights into anaerobic microbial biodegradation of PTA-related compounds. This chapter will describe the current knowledge of microbial constituents in anaerobic bioprocesses treating PTA-related wastewater.

2. Syntrophic Aromatic Compound-Degrading Organisms

Thus far, only a handful of organisms capable of aromatic compound metabolism under methanogenic conditions have been described. Previous studies on mesophilic reactors degrading PTA-related compounds successfully isolated unique organisms capable of syntrophically degrading phthalate isomers (i.e., phthalate, isophthalate, and TA) from two genera: *Syntrophorhabdus* (i.e., *S. aromaticivorans* strain UI) and *Pelotomaculum* (*P. isophthalicum* strain II and *P. terephthalicum* strain JT) (Qiu *et al.* 2008; Qiu *et al.* 2004). Physiological and genomic studies reveal that the *S. aromaticivorans* strain UI can utilize *p*-cresol, 4-hydroxybenzoate, isophthalate, benzoate, and phenol in syntrophic association with methanogens (Nobu *et al.* 2014a; Nobu *et al.* 2014b; Qiu *et al.* 2008). As for the genus *Pelotomaculum*, Strain JT can degrade isophthalate, TA, and several other aromatic compounds (e.g., benzoate, hydroquinone, 2-hydroxybenzoate, 3-hydroxybenzoate, 2,5-dihydroxybenzoate, and 3-phenyl-propionate) in co-culture with the hydrogenotrophic methanogen, *Methanospirillum hungatei* (Qiu *et al.* 2006). Strain II could utilize all three phthalate isomers, benzoate, and 3-hydroxybenzoate in co-culture with *M. hungatei* (Qiu *et al.* 2006). Although incapable of degrading phthalate isomers, members of the genus *Syntrophus* are also known to specifically metabolize benzoate syntrophically (Jackson *et al.* 1999; Mountfort *et al.* 1984; Schocke and Schink 1997). As pointed out above (Fig. 3.2), these syntrophs degrade TA and/or benzoate to acetate, H₂, and CO₂ and partner acetate-degrading (acetoclastic) and H₂-oxidizing (hydrogenotrophic) methanogens further convert these compounds to CH₄ and CO₂. Thus, cooperation between the three aromatic compound-degrading syntrophic bacteria and methanogens is thought to play a central role in PTA wastewater treatment.

2.1. Distribution of known syntrophs and methanogens

PCR-based molecular techniques targeting the 16S rRNA gene have been extensively used for microbial community analysis of microbial consortia-degrading PTA-related compounds. Figure 3.3 summarizes the relative abundances of known methanogens

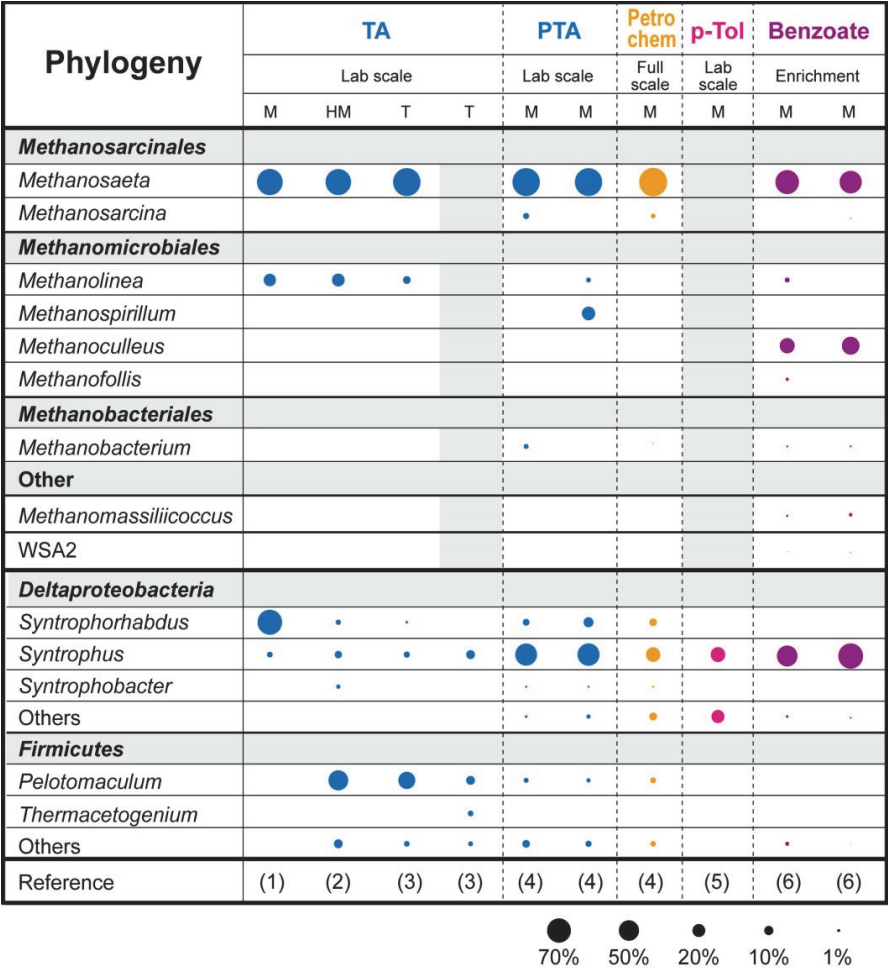


Fig. 3.3. The relative abundances of microorganisms related to known methanogens and syntrophs in previously reported microbial community analyses of anaerobic bioprocesses treating PTA-related compounds. Abbreviations: TA, terephthalic acid; PTA, purified terephthalic acid; petrochem, petrochemical industry wastewater; p-Tol, *p*-toluic acid; M, mesophilic (~35 °C); HM, hyper mesophilic (46–48 °C); T, thermophilic (~50 °C). References: 1, Wu *et al.* 2001b; 2, Chen *et al.* 2004; 3, Chen *et al.* 2004; 4, Perkins *et al.* 2011; 5, Wu *et al.* 2001a; 6, Narihiro *et al.* 2015.

and syntrophs in previously reported microbial community analyses of anaerobic processes degrading PTA-related compounds, including full-scale reactors (Perkins *et al.* 2011), lab-scale reactors (Chen 2008; Chen *et al.* 2004; Perkins *et al.* 2011; Wu *et al.* 2001a, b), and batch enrichment cultures (Narihiro *et al.* 2015). For methanogenic archaea, *Methanosaeta* populations were detected as the major constituent with relatively high abundances in all reactors (>60% of total archaeal populations). Given their specific acetoclastic capability, they are thought to mediate TA and benzoate degradation by syntrophically metabolizing the by-product acetate. Furthermore, the genus *Methanosaeta* includes both mesophilic (*M. concilii* and *M. harundinacea*) and thermophilic (*M. thermophila*) members, supporting their involvement in reactors

treating PTA-related compounds at these temperature ranges (Liu and Whitman 2008). The hydrogenotrophic methanogens predominant in the communities were observed to be substrate-dependent. *Methanolinea* were abundant (2.6–19.5%) in the PTA/TA-treating communities (Chen 2008; Chen *et al.* 2004; Perkins *et al.* 2011; Wu *et al.* 2001b), whereas *Methanoculleus* (27.3–38.0%) were found in the benzoate-fed batch enrichment cultures (Narihiro *et al.* 2015), respectively. In addition, *Methanospirillum*, *Methanobacterium*, and *Methanomassiliicoccus* were detected as minor hydrogenotrophic populations in several processes. Although another archaeal clade, WSA2 (also known as ArcI), thought to contribute to methanogenesis was also observed, its metabolic capacity remains unclear (Chouari *et al.* 2005).

The dominant syntrophic aromatic compound-degrading bacteria in the communities depend on the provided substrate and reactor temperature (Fig. 3.3). In TA-treating processes, *Syntrophorhabdus*-related populations predominated in reactors operated under mesophilic conditions (74.5% of total bacterial population) (Wu *et al.* 2001b), while those related to *Pelotomaculum* were abundant (9.2–47.9%) in higher temperature processes (hypermesophilic and thermophilic) (Chen 2008; Chen *et al.* 2004). In addition to these syntrophs, members of the benzoate-degrading genus *Syntrophus* were also identified in mesophilic communities treating PTA and other petrochemical process wastewaters and also specifically degrading *p*-Tol and benzoate (25.0–75.6%) (Perkins *et al.* 2011; Narihiro *et al.* 2015; Wu *et al.* 2001a).

In addition to the aforementioned aromatics degraders, a few populations of *Syntrophobacter* (0.4–2.1%) and *Thermacetogenium* (3.7%) were observed in four mesophilic reactors and one thermophilic reactor, respectively. According to their physiological traits, they may play a role in syntrophic propionate and acetate oxidation in the processes (Boone and Bryant 1980; Chen *et al.* 2005; Harmsen *et al.* 1998; Hattori *et al.* 2005; Hattori *et al.* 2000; Oehler *et al.* 2012; Wallrabenstein *et al.* 1995).

3. Microscopic Observations for PTA-Degrading Microbial Consortia

As a complementary approach to 16S rRNA-based community analyses, microscopic approaches have been used to elucidate the morphology and physical association of organisms in methanogenic consortia-degrading PTA wastewater, which is especially important for syntrophy. SEM and TEM observations of mesophilic UASB reactor granules showed three morphotypes (Wu *et al.* 2001b) (Fig. 3.4): chaining oblong cylinders (arrow 1), single or paired oval rods (arrow 2), and short rods (arrow 3), respectively resembling an acetoclastic methanogen genus *Methanosaeta* (Whitman *et al.* 2006), *Syntrophus* (Mountfort *et al.* 1984), and a hydrogenotrophic methanogen family *Methanobacteriaceae* (Fig. 3.4b). The *Syntrophus*- and *Methanobacteriaceae*-like morphotypes are often physically associated, suggesting that they may form syntrophic interactions. Using CLSM-FISH, the cylinder and short rod morphotypes were also found to be in close association and confirmed as *Methanosaeta* (MX825, green, Fig. 3.5b) and *Methanobacteriaceae* (MB1174, red, Fig. 3.5c). On the other hand, the *Syntrophus*-

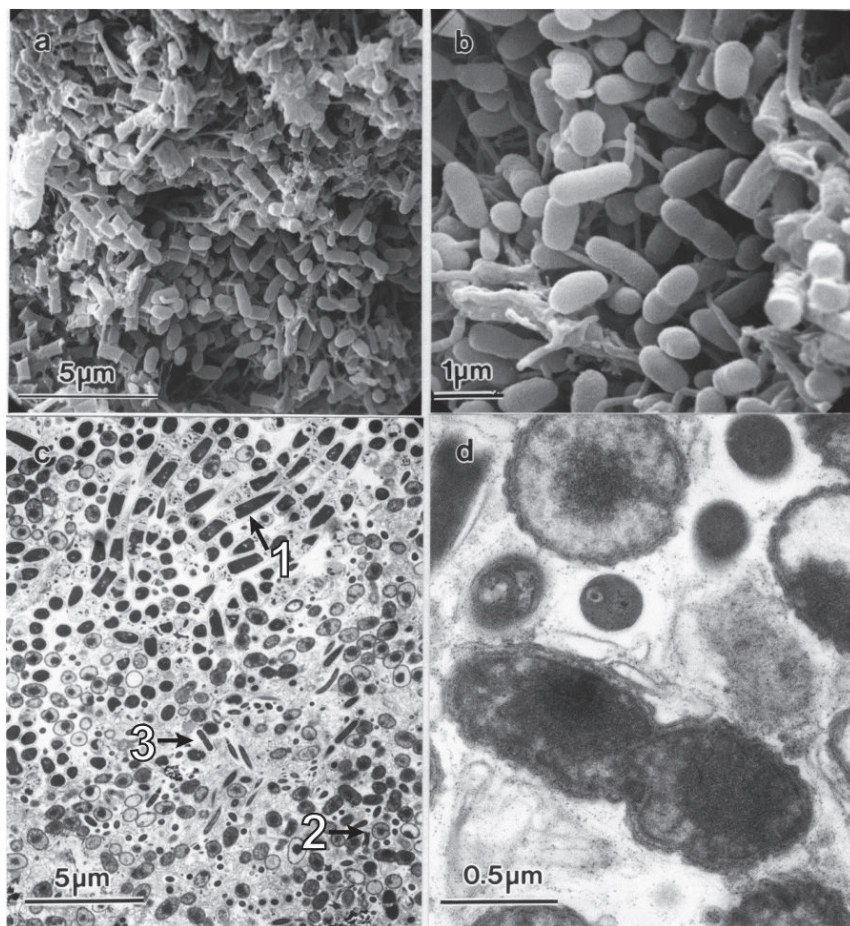


Fig. 3.4. Microphotographs of various predominant microorganisms observed in mesophilic terephthalate-degrading granules (Wu *et al.* 2001b). Scanning electron microscope (SEM) at (a) low magnification and (b) high magnification. Transmission electron microscope (TEM) at (c) low magnification and (d) high magnification.

like cells were found to be two *Syntrophorhabdus*-related populations using the probes delta-TA1 (green, overlapping with red EUB338 probe appearing yellow in Fig. 3.5d) and more specific delta-TA2 (red, overlapping with delta-TA1 appearing yellow in Fig. 3.5e). Based on these microscopic approaches, two *Syntrophorhabdus* populations predominated granules in physical association with *Methanosaeta* and *Methanobacteriaceae*, perhaps indicative of syntrophic interactions between these clades likely for TA degradation (Wu *et al.* 2001b).

In a thermophilic (55 °C) lab-scale hybrid reactor treating TA, SEM analysis revealed oblong cylinders (chaining: arrow 1, and isolated: arrow 2), oval rods (arrow 3), small rods (arrow 4), oblong rods (arrow 5), and thin filaments (arrow 6) as major morphotypes (Fig. 3.6) (Chen *et al.* 2004). Among these cells, the oblong cylinders and oval rods (arrow 3) predominating in this reactor correspondingly resembled *Methanosaeta* (Whitman *et al.* 2006) and *Pelotomaculum* (e.g., *P. terephthalicum* and *P. isophthalicum*)

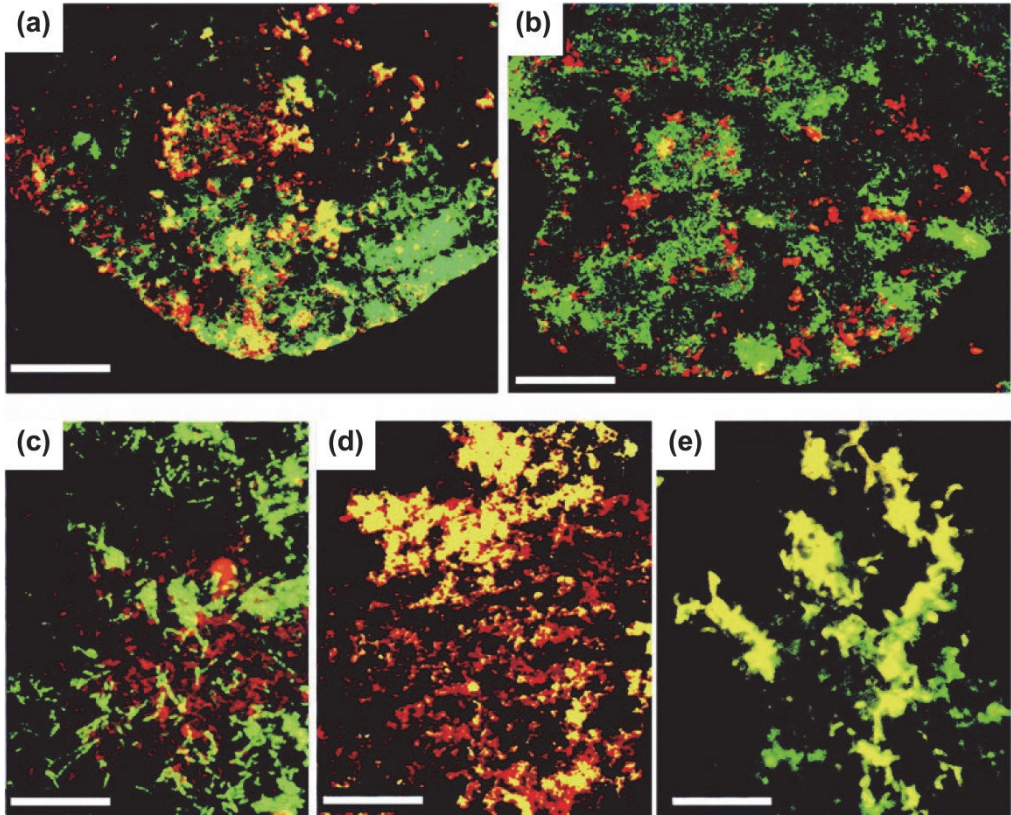


Fig. 3.5. FISH of granule sections for the mesophilic UASB reactor-degrading terephthalate (Wu *et al.* 2001b). (a) Topographical view of a granule section that was simultaneously hybridized with a bacterial-domain probe (EUB338) labeled with Cy3 (red) and an archaeal-domain probe (ARC915) labeled with FITC (green) (bar, 100 μ m). (b) a granule section with the MX825 probe (Cy5, green) for *Methanosaeta* and the MG1200 probe (Cy3, red) for *Methanomicrobiales* (bar, 100 μ m). (c) a granule section with the MX825 probe (Cy5, green) for *Methanosaetaceae* and the MB1174 probe (Cy3, red) for *Methanobacteriaceae* (bar, 20 μ m). (d) a granule section with the EUB338 probe (Cy3, red) and the delta-TA1 probe (Cy5, green), which targeted the *Syntrophorhabdus* group TA1 (bar, 20 μ m). (e) The diversity of the *Syntrophorhabdus* group as revealed by sequential FISH with the delta-TA1 probe (Cy5, green) and the delta-TA2 probe (Cy3, red) targeting the different *Syntrophorhabdus* groups (bar, 20 μ m).

(Qiu *et al.* 2006). FISH not only confirmed that the microbial community is dominated by *Archaea* (ARC915, red) and *Bacteria* (EUB338, green) (Fig. 3.7c), but also verified that the observed morphotypes were *Methanosaeta* (MX825, red) and *Pelotomaculum* (DFMI227a, green) (Fig. 3.7d). Likewise, using the same probes, *Pelotomaculum* and *Methanosaeta* were found to be abundant in a hyper-mesophilic (46–50 °C) reactor treating TA (Figs. 3.7a and 7b). Thus, instead of *Syntrophorhabdus*, *Pelotomaculum* clearly dominated TA degradation at higher temperatures through syntrophic association with *Methanosaeta*.

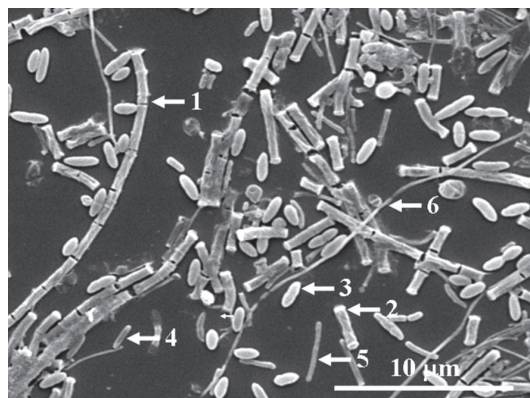


Fig. 3.6. Scanning electron microscope (SEM) microphotograph of various predominant microorganisms observed in a thermophilic terephthalate-degrading granules (Chen *et al.* 2004). Arrows indicate the six dominant morphotypes (see text for details).

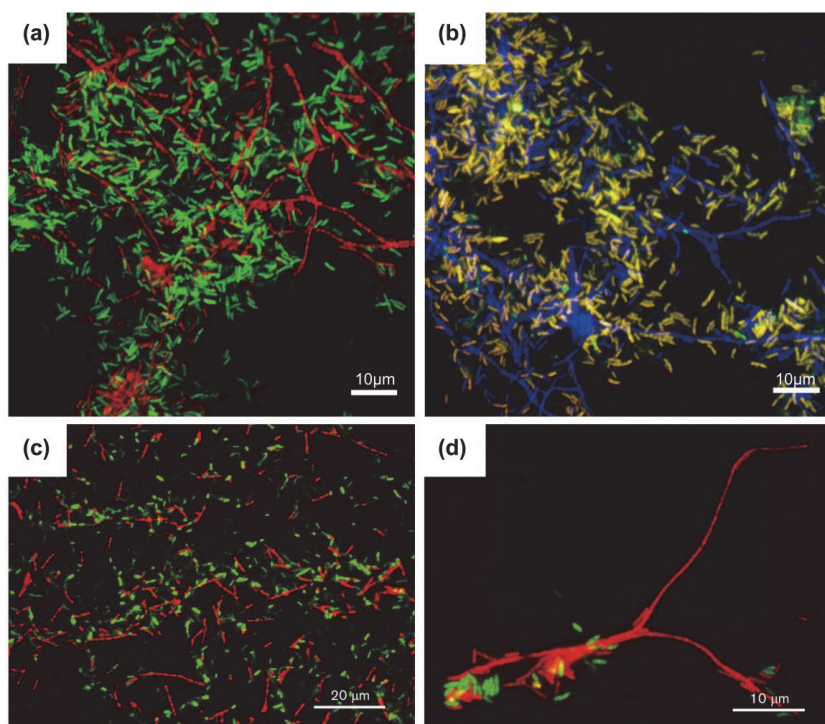


Fig. 3.7. FISH of biofilms for the hyper-mesophilic (a and b) and thermophilic (c and d) reactors degrading terephthalate (Chen 2008; Chen *et al.* 2004). (a and c) Hybridized with a bacterial-domain probe (EUB338, green) and an archaeal-domain probe (ARC915, red). (b) Hybridized with the EUB338 probe (green), the Ih821 probe for *Desulfotomaculum* group Ih (including *Pelotomaculum* spp.) (red), and the MX825 probe for the *Methanosaetaceae* (blue). (d) Hybridized with the DFMI227a probe for *Desulfotomaculum*-*Pelotomaculum* group (green) and MX825 probe (red).

To investigate the microorganisms involved in *p*-Tol acid degradation, SEM and TEM approaches were used to investigate a microbial community derived from a PTA wastewater treating reactor specifically fed with *p*-Tol (Wu *et al.* 2001a). In the original inoculum, long rods with a distinct collar structure (arrow A) and short rods (arrow B) were abundant (Fig. 3.8a). The short rods resembled the morphology of hydrogenotrophic methanogens, including *Methanobrevibacter* and *Methanobacterium* (Whitman *et al.* 2006). In the *p*-Tol degrading community, *Methanosaeta*-like cylinders were frequently observed through SEM, as with the mesophilic and thermophilic TA-treating reactors (Fig. 3.8b). Additional TEM analysis allowed for clear visualization of the long rods with collars (Fig. 3.8c), which have been reported to arise during cell division at the internal cell membrane in *Desulfomonile tiedjei* (Fig. 3.8d) (Deweerd *et al.* 1990; Mohn *et al.* 1990). However, there is currently no direct evidence that *Desulfomonile*-related organisms contribute to the syntrophic degradation of *p*-Tol.

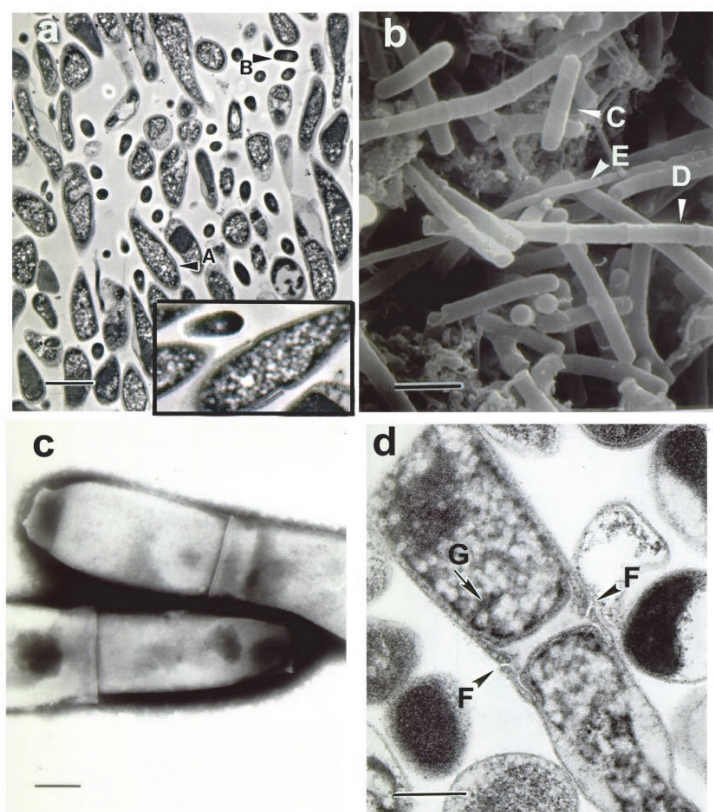


Fig. 3.8. Microphotographs of various predominant microorganisms observed in the inoculum sludge taken from PTA wastewater treatment processes (a) and a mesophilic *p*-Tol-degrading community (b, c, and d) (Wu *et al.* 2001a). (a) TEM of the inoculated sludge. Bar = 1.0 μ m. (b) SEM of the *p*-Tol-degrading microbial community. Bar = 1.5 μ m. (c) TEM of the phosphotungstic acid-stained rod cells that predominated in the *p*-Tol-degrading community. Bar = 0.4 μ m. (d) TEM of the predominated rod cells in the *p*-Tol-degrading community. Bar = 0.4 μ m.

In short, microscopic observations using TEM, SEM, and CSLM-FISH approaches provide insights into the morphology and organization of the predominant microbial populations in methanogenic consortia degrading PTA-related compounds. Specifically, the juxtaposition of syntrophs and methanogens is critical evidence for syntrophic degradation of PTA-related compounds. Furthermore, these findings are in good accordance with the results of microbial community structures revealed by PCR-based molecular techniques presented in the previous section.

4. Other Microbial Populations

Besides known methanogens and syntrophs, significant amounts of functionally unknown bacterial phyla were found in all processes (Fig. 3.9). In particular, *Bacteroidetes*, *Chloroflexi*, and *Synergistetes* populations were detected in almost all of the communities. *Bacteroidetes* and *Chloroflexi* populations were reported to contribute to fermentative degradation of macromolecular organic compounds, such as sugars, proteins, and lipids, in anaerobic wastewater treatment processes (Ariesyady *et al.* 2007; Ito *et al.* 2012; Kampmann *et al.* 2012). *Synergistetes* populations were frequently found in various types of anaerobic wastewater treatment processes (Godon *et al.* 2005; Narihiro *et al.* 2009), and some of the *Synergistetes* members may contribute to acetate oxidation (Ito *et al.* 2011). In thermophilic TA-degrading processes, *Caldiserica*-related organisms were frequently observed (10.2–16.5%) (Chen *et al.* 2004). Besides, *Spirochaetes*-related organisms predominated in benzoate-fed enrichment cultures; however, their ecological functions in the cultures are still unclear (Narihiro *et al.* 2015).

Moreover, a significant amount of bacterial populations were associated with candidate phyla with no cultured representatives. For example, the candidate phylum AC1 predominated in the communities treating TA (12.3–26.9%) (Chen *et al.* 2004; Wu *et al.* 2001b), *p*-Tol (7.2%) (Wu *et al.* 2001a), and complex petrochemical wastewater (22.9%) (Perkins *et al.* 2011). The members of ‘*Ca. Aminicenantes*’ (formerly known as candidate phylum OP8) were found in thermophilic TA-treating reactors (Chen *et al.* 2004). In hyper-mesophilic TA-degrading reactors, ‘*Ca. Atribacteria*’ (OP9), ‘*Ca. Cloacimonetes*’ (WWE1), and ‘*Ca. Hydrogenedentes*’ (NKB19) were detected (Lykidis *et al.* 2011). ‘*Ca. Atribacteria*’-related populations were also found in *p*-Tol-degrading processes along with significant populations of candidate phylum SC4 (Wu *et al.* 2001a). ‘*Ca. Cloacimonetes*’-related organisms predominated in benzoate-degrading cultures (Narihiro *et al.* 2015). Metagenomic analysis of ‘*Ca. Cloacimonas acidaminovorans*’ suggests that they may contribute to propionate-degradation in anaerobic digestion (Pelletier *et al.* 2008). Due to the lack of cultured isolates, their *in situ* physiological traits still remain unknown. We speculate that these functionally unknown populations play an important role in the degradation of PTA-related waste compounds. Further studies on these functionally unknown populations are necessary and can be achieved by using state-of-the-art ecogenomic approaches, including metagenomics, metatranscriptomics, and single-cell genomics (Lykidis *et al.* 2011; Rinke *et al.* 2013).



Fig. 3.9. The relative abundances of functionally unknown bacteria in previously reported microbial community analyses of anaerobic bioprocesses treating PTA-related compounds. Abbreviations: TA, terephthalic acid; PTA, purified terephthalic acid; petrochem, petrochemical industry wastewater; p-Tol, *p*-toluic acid; M, mesophilic (~35 °C); HM, hyper mesophilic (46–48 °C); T, thermophilic (~50 °C). References: 1, Wu *et al.* 2001b; 2, Chen *et al.* 2008; 3, Chen *et al.* 2004; 4, Perkins *et al.* 2011; 5, Wu *et al.* 2001a; 6, Narihiro *et al.* 2015.

5. Conclusions

5.1. Linking the microbial populations with the process operation

Overall, the major microbial constituents in methanogenic consortia-degrading PTA-related chemicals (i.e., TA, *p*-Tol, and benzoate) have been revealed by polyphasic approaches using microscopic and molecular techniques. The major populations of syntrophs were strongly associated with process temperature. In mesophilic processes, members of *Syntrophorhabdus* and *Syntrophus* likely took part in the degradation of TA and/or benzoate. *Pelotomaculum*-related organisms predominated in the reactors operated under hyper-mesophilic and thermophilic conditions. In contrast, regardless of operational temperature, acetoclastic methanogens assigned to the genus *Methanosaeta* are the dominant methanogens in PTA-degrading microbial consortia. They may utilize the acetate produced by the degradation of PTA-related compounds. The dominant hydrogenotrophic methanogens were found to be substrate-dependent. For example, *Methanolinea* and *Methanoculleus* populations predominated in TA- and benzoate-degrading consortia, respectively. Besides the syntrophs and methanogens, phylogenetically diverse bacterial populations were observed in all types of processes. Albeit their ecological traits are still unclear, they may contribute to process stability and efficiency.

5.2. Potential inhibitory mechanism of TA degradation

Although anaerobic processes have been demonstrated as a viable technology to treat PTA wastewater, these processes still face some critical challenges during operation. One major challenge is a decrease in total microbial inventory or biomass, and extensive loss of biomass can lead to poor COD removal and eventually, process failure. While the exact cause still remains unknown, high volumetric loading rates and inhibition of TA degradation by high concentrations of acetate and benzoate have been suggested as two causes, which are likely inter-related (Cheng *et al.* 1997; Joung *et al.* 2009; Kleerebezem *et al.* 1999a; Kleerebezem *et al.* 1997; Kleerebezem *et al.* 1999d). High volumetric loading rates are due to a surge in influent flow rate. An abrupt increase in influent flow rate can lead to an increase in upward flow velocity, causing smaller granules to be washed out from the reactor. In the PTA wastewater, TA, benzoate, and acetate concentrations can also fluctuate (Fig. 3.1). Under these conditions, inhibition of TA degradation by high concentrations of benzoate and acetate was speculated (Kleerebezem *et al.* 1999c, d). Kleerebezem and co-authors (1999c, d) observed inhibition of TA degradation with a lag time (>40 days) when fresh biomass from a TA-grown enrichment culture was used under batch tests, where 12 mM benzoate or 50 mM acetate was added concurrently with 14 mM TA. Still, the culture could degrade benzoate or acetate immediately, but the inhibition of TA degradation was observed. They postulated that the enzyme responsible for TA degradation through decarboxylation via benzoate was inhibited irreversibly under high benzoate concentrations and suggested that TA-degrading syntrophs may preferentially degrade benzoate over TA when both are present. They also suggested that the inhibition of TA degradation by high acetate concentration

was likely due to a non-competitive inhibition on methanogens. Inhibition of TA degradation was also observed after subjecting samples taken from a TA-grown enrichment culture under short-term starvation. However, the ecological and biochemical mechanisms behind this inhibition remains unclear. Recent developments in environmental genomics and advances in our understanding on syntrophy may open new windows to investigation of this phenomenon.

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Chapter 4

State-of-the-Art Anaerobic Ammonium Oxidation (Anammox) Technology

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The nitrogen (N) cycle, one of the most important biospheres on Earth, consists of complex interactions among various nitrogen compounds. Anaerobic ammonium oxidation (Anammox) is a N-transformation pathway that was discovered in the 1990s. The metabolism of Anammox bacteria relies on the oxidation of ammonium for energy using nitrite as the electron acceptor to form nitrogen gas. Due to its chemolithoautotrophic nature, lack of requirement for aeration and organic carbon (C), as well as the high efficiency of N-compounds' conversion to nitrogen gas, Anammox holds an attractive potential for N-removal from wastewaters, especially for those with a low "carbon/nitrogen (C/N)" ratio. This chapter presents the up-to-date information of Anammox bacteria genera and their versatile metabolic pathways found by a number of advanced molecular techniques, and also gives a brief summary on their potential nitrogen sources of cell synthesis through microbial thermodynamic calculations. Considering its applicability for nitrogen removal from wastewaters (both for high-strength and dilute strength), the effect of solid retention time on the nitrogen removal efficiency and inhibitors on Anammox process are discussed. Moreover, its possible involvement in syntrophy for sulfate reduction and ammonium oxidation triggers scientists to wonder if it plays a significant role in the global nitrogen-sulfur cycle.

1. The Nitrogen Cycle

Nitrogen forms the majority of the gas in Earth's atmosphere (78%), and thus leads to nitrogen transformation among its various chemical forms, carried out by biological and physical reactions. The biological nitrogen transformation pathways discovered by the end of the 19th century, consisted of assimilation, ammonification, nitrification, denitrification, and nitrogen fixation (Ahn 2006; Paredes *et al.* 2007; You *et al.* 2009).

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An overlooked pathway of anaerobic ammonium oxidation (Anammox) was predicted by Broda (1977). This theoretical prediction stated that the existence of Anammox should be carried out by lithotrophic organisms by driving ammonium as the electron donor and nitrite or nitrate as the electron acceptor(s). Its existence was confirmed in a denitrifying bioreactor treating the Gist-Brocades fermentation effluents in the 1990s, due to the observation of an ammonium concentration decrease alongside an increase in dinitrogen gas concentration (Mulder *et al.* 1995; Kuenen and Jetten 2001). Subsequently, the mechanism of this concealed pathway has been revealed and the results showed its unconventional metabolism and attractive applications in nitrogen removal for wastewaters (Van de Graaf *et al.* 1996; Jetten *et al.* 1997; Strous *et al.* 1998). This knowledge completes the current understanding of the global nitrogen cycle.

1.1. *Nitrogen transformations*

The nitrogen cycle (see Fig. 4.1) involves the following processes:

- (1) Ammonification: Organic nitrogen, normally from cell lysis of deceased plants and animals, is converted to ammonium (NH_4^+) by microorganisms.
- (2) Assimilation: Ammonium is utilized biologically and converted to components of organisms (organic nitrogen).
- (3) Nitrification: Low-valence nitrogen is converted to high-valence nitrogen by ammonia-oxidizing bacteria and nitrite-oxidizing bacteria when electron acceptors are provided. Electron acceptors could be oxygen, or other oxidized compounds, e.g., sulfate, ferric (Fe^{3+}).
- (4) Assimilative reduction: Nitrate (NO_3^-) or nitrite (NO_2^-) is reduced to ammonium for cell synthesis by organisms.
- (5) Denitrification: High-valence nitrogen is converted to low-valence nitrogen by microorganisms. Nitrate (NO_3^-) is reduced to nitrogen gas through an array of intermediate products (NO_2^- , NO , N_2O) combined with electron donors (e.g., organic matters, sulfide and *etc.*).
- (6) Nitrogen fixation: The atmospheric nitrogen is fixated to ammonium by symbiotic bacteria (mainly symbiosis with leguminous plants) or some non-symbiotic bacteria.
- (7) Anammox: Autotrophic bacteria utilize ammonium as the electron donor and nitrite as the electron acceptor in a ratio of 1:1.3 during the growth phase, converting them into nitrogen gas and nitrate under anaerobic conditions in the absence of organic carbons.

1.2. *Conventional nitrogen removal*

Due to the excess nitrogen discharge from waste streams directly into water bodies without advanced treatment, receiving waters are suffering from eutrophication and hypoxia. Nutrient pollution control strategies pursued by environmental authorities worldwide is calling for enforcing numerous nitrogen discharge criteria. Conventional nitrogen removal integrates nitrification (autotrophic conversion of ammonium into

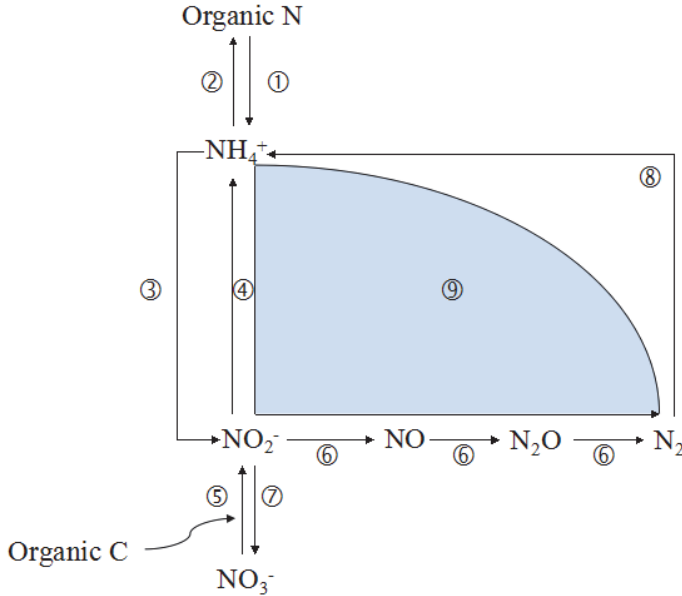


Fig. 4.1. Schematic of microbial nitrogen cycle in activated sludge floc. ① Ammonification; ② Assimilation; ③ Nitrification; ④ Assimilation reduction; ⑤ Denitrification; ⑥ Denitrification; ⑦ Nitrification; ⑧ Nitrogen fixation; ⑨ Anammox.

nitrite and further to nitrate) and denitrification (heterotrophic transfer of nitrate into nitrogen gas). Those conventional nitrogen removal processes require a large amount of oxygen, produce a substantial amount of sludge, and generate a considerable volume of N₂O (a greenhouse gas with a potential roughly 300 times higher than CO₂) (Peng and Zhu 2006; Sun *et al.* 2010). Anammox offers a sustainable alternative for the same purpose. Its merits are: (1) a 60 % energy reduction for aeration (van Dongen *et al.* 2001; Paredes *et al.* 2007; Siegrist *et al.* 2008); (2) no organic donor needed for denitrification, which could otherwise be converted to energy in the form of methane (McCarty and Bae 2011); (3) a 90% cost reduction for sludge management (Mulder 2003; De Clippeleir *et al.* 2011); and (4) less production of nitrous oxide (N₂O) (ICCP 2013; Kampschreur *et al.* 2009a). Fux and Siegrist (2004) estimated that, collectively, a 30–40% cost reduction, as well as a reduction in the environmental costs of greenhouse gas emissions, could be achieved by Anammox.

2. Anaerobic Ammonium Oxidation (Anammox)

2.1. *Anammox* genera

Anammox bacteria are chemolithoautotrophic organisms that use bicarbonate as the sole carbon source for the biosynthesis of cell material, and derive their energy from the conversion of ammonium and nitrite into dinitrogen gas (van Niftrik *et al.* 2004). Anammox bacteria are not only found in bioreactors, but have also been observed in natural environments, such as coastal sediments, lakes, and marine sub-oxic zones

(Kuene and Jetten 2001; Schmid *et al.* 2007). As members of the Planctomycetales order from the bacterial domain, they are considered to be an ecologically and environmentally important group of microorganisms (Jetten *et al.* 2009).

Table 4.1 summarizes Anammox species that have been discovered. Five known Anammox genera have been described, including the genus of *Ca. Brocadia anammoxidans*, which was the first genus discovered in the denitrifying bioreactor mentioned previously (Mulder *et al.* 1995). Three other characterized species within the *Ca. Brocadia* genus are *Ca. Brocadia fulgida* (Kartal *et al.* 2008), *Ca. Brocadia sinica* (Oshiki *et al.* 2011), and *Ca. Brocadia caroliniensis* (Rothrock *et al.* 2011). *Ca. Brocadia fulgida* was an autofluorescent bacterium; *Ca. Brocadia sinica* can sustain high ammonium and nitrite load treating concentrated wastewaters; *Ca. Brocadia caroliniensis* was identified from a bioreactor treating animal waste sludge. All of these were enriched in Anammox bioreactors.

The only species reported within the *Candidatus Kuenenia* genus is *Ca. Kuenenia stuttgartiensis* (Schmid *et al.* 2000). The *Ca. Scalindua* genus consists of nine proposed species, six of which were discovered in marine environments (Kuypers *et al.* 2003; Woebken *et al.* 2008; Hong *et al.* 2011; Fuchsman *et al.* 2012; Dang *et al.* 2013; van de Vossenberg *et al.* 2013). *Ca. Scalindua sorokinii* was the first Anammox species found in a natural environment (Black Sea), while *Ca. Scalindua richardsii* was also discovered from the Black Sea (Kuypers *et al.* 2003; Fuchsman *et al.* 2012). Although these two species originated from the Black Sea, they also dominated in various natural environments. A cluster associated with *Ca. Scalindua sorokinii* was detected in the lower sub-oxic zone where the ammonium concentration was high, but nitrite concentration was low, whereas another cluster associated with *Ca. Scalindua richardsii* was found in the upper sub-oxic zone with a low ammonium concentration, but a high nitrite concentration (Fuchsman *et al.* 2012). *Ca. Scalindua brodae* and *Ca. Scalindua wagneri* were both identified in wastewater treatment plants (WWTPs) (Li *et al.* 2010). *Ca. Scalindua arabica* originated from the Arabian Sea and the Peruvian oxygen minimum zone (Woebken *et al.* 2008). *Ca. Scalindua pacifica* (Dang *et al.* 2013) and *Ca. Scalindua profunda* with pili-like structures (van de Vossenberg *et al.* 2013) were retrieved from the Bohai Sea and marine sediment of a Swedish fjord, respectively. Two additional species' names were tentatively proposed from molecular surveys: *Ca. Scalindua sinooilfield* from a high-temperature petroleum reservoir (Li *et al.* 2010) and *Ca. Scalindua zhenghei* from deep-sea subsurface sediments (Hong *et al.* 2011). The only known species affiliated with the *Ca. Anammoxoglobus* genus was *Ca. Anammoxoglobus propionicus*, enriched from an Anammox reactor (Kartal *et al.* 2007a). *Ca. Jettenia asiatica* was retrieved from a granular sludge Anammox reactor (Quan *et al.* 2008). These studies have demonstrated Anammox's ubiquity in diverse natural habitats.

2.2. Metabolism of Anammox bacteria

Anammox's metabolism shown in Fig. 4.2 possesses a unique pathway (Strous *et al.* 2002). In Anammox catabolism, ammonium is oxidized using nitrite as the electron

Table 4.1. Anammox bacterial species.

Anammox bacterial species	References
<i>Ca. Brocadia anammoxidans</i>	Mulder <i>et al.</i> 1995
<i>Ca. Brocadia fulgida</i>	Kartal <i>et al.</i> 2008
<i>Ca. Brocadia sinica</i>	Oshiki <i>et al.</i> 2011
<i>Ca. Brocadia caroliniensis</i>	Rothrock <i>et al.</i> 2011
<i>Ca. Kuenenia stuttgartiensis</i>	Schmid <i>et al.</i> 2000
<i>Ca. Scalindua sorokinii</i>	Kuypers <i>et al.</i> 2003
<i>Ca. Scalindua richardsii</i>	Fuchsman <i>et al.</i> 2012
<i>Ca. Scalindua brodae</i>	Schmid <i>et al.</i> 2003
<i>Ca. Scalindua wagneri</i>	Schmid <i>et al.</i> 2003
<i>Ca. Scalindua arabica</i>	Woebken <i>et al.</i> 2008
<i>Ca. Scalindua pacifica</i>	Dang <i>et al.</i> 2013
<i>Ca. Scalindua profunda</i>	van de Vossenberg <i>et al.</i> 2013
<i>Ca. Scalindua sinooilfield</i>	Li <i>et al.</i> 2010
<i>Ca. Scalindua zhengheii</i>	Hong <i>et al.</i> 2011
<i>Ca. Anammoxoglobus propionicus</i>	Kartal <i>et al.</i> 2007a
<i>Ca. Jettenia asiatica</i>	Quan <i>et al.</i> 2008

acceptor for the creation of a proton motive force (PMF) over the anammoxosomal membrane. With the uptake of one (+0.38 V) plus three (+0.34 V) low-energy electrons, nitrite is reduced to nitric oxide, which then reacts with ammonium to produce hydrazine. The conversion of hydrazine to nitrogen yields four high-energy electrons (-0.75 V), which subsequently generates a positive PMF. Following this, the ATPase is energized by the PMF, and further ATP is yielded in the riboplasm, as demonstrated in Fig. 4.2a. To initiate high-energy electrons for carbon dioxide reduction in cell synthesis, the central catabolism is combined with nitrite reduction to nitrate through the PMF-driven reverse electron transport (RET), during which hydrazine donates high-energy electrons to ferredoxin (but not recycled). Figure 4.2b elucidates that nitrite oxidation to nitrate yields four low-energy electrons, which must be energized by the PMF, to subsidize back into the Anammox reaction for the compensation of the four high-energy electrons flowing to ferredoxin (Kuenen 2008). Anammox's distinctive metabolism would be beneficial to the understanding of biochemistry and medicinal applications.

2.3. Versatility of Anammox

Although Anammox bacteria are recognized as chemolithoautotrophs, recent studies have suggested their versatility of utilizing a wide range of electron donors/acceptors for their catabolism, including propionate, acetate, formate, ferric ions, manganese oxides, ammonium, and nitrite/nitrate, as summarized in Fig. 4.3 (Strous *et al.* 2002; Strous *et al.* 2006; Kartal *et al.* 2007b). The interesting findings at present are the ability of using

propionate and acetate with the presence of ammonium and nitrate by *Candidatus Anammoxoglobus propionicus* and *Candidatus Brocadia fulgida propionicus* (Kartal et al. 2007a). These two species were capable of reducing nitrite and nitrate into nitrogen while oxidizing organic carbon into carbon dioxide for cell synthesis. The information advises that Anammox may have potential for concurrent organic and nitrogen removal.

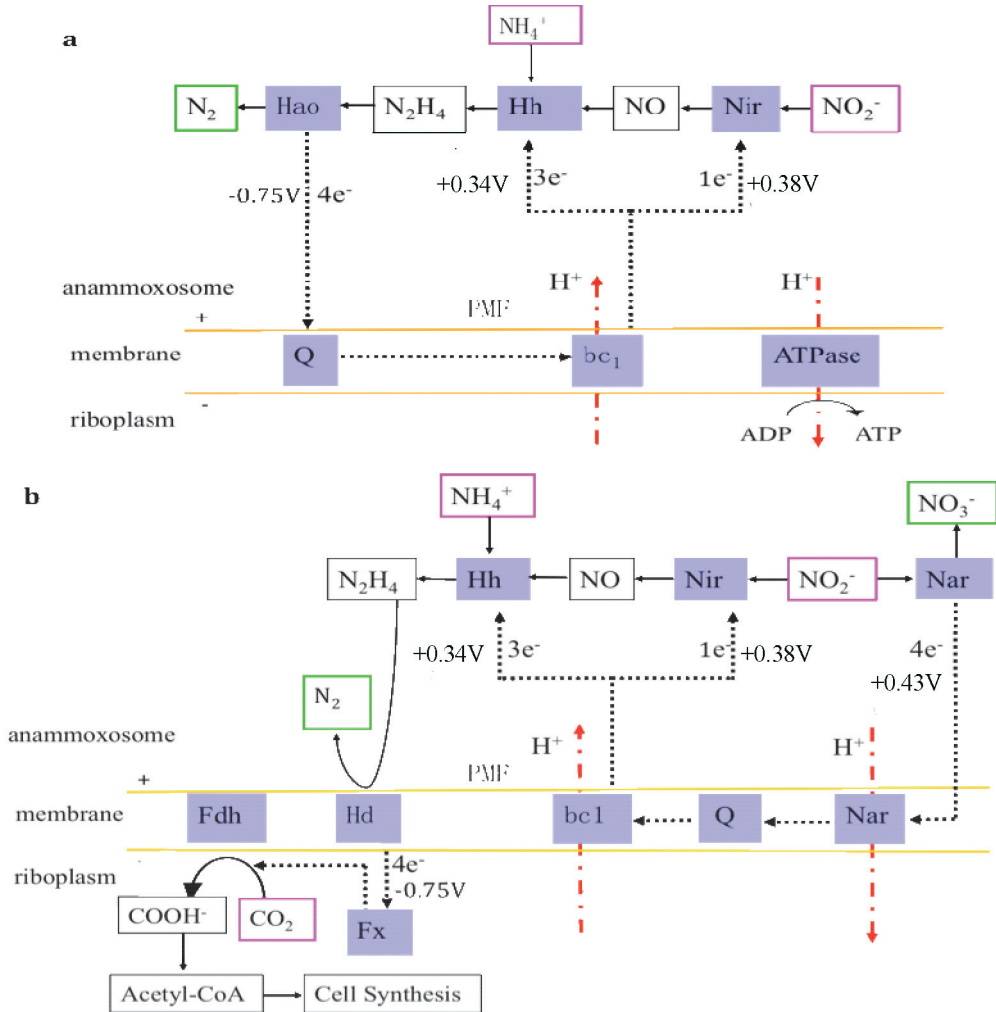


Fig. 4.2. Metabolic pathways and reversed electron transport (RET) in Anammox bacteria. a) Anammox central catabolism; b) PMF-driven RET combines central catabolism with nitrate reductase to generate high potential electrons for the acetyl-CoA pathway. Hao, hydrazine oxidoreductase; Hd, hydrazine dehydrogenase; Hh, Hydrazine hydrolase; Nir, nitrite oxidoreductase; Fx, ferredoxin; Fdh, formate dehydrogenase; bc₁, cytochrome bc₁ oxidoreductase; Q, ubiquinone; Nar, nitrate reductase. Figures illustrated based on Kuenen (2008), Strous et al. (2006), and Jetten et al. (2009) from present genomic data, experimental evidence, and thermodynamic calculations.

2.4. Syntrophy involving Anammox

Some researchers have found that Anammox may participate, in syntrophy, in the coupling of the electron donor of sulfate to ammonium oxidation, which is expected to exert a significant impact on the further understanding of sulfur and nitrogen cycles and their interactions on Earth. The annual N and S fluxes between the ocean and the atmosphere have been estimated to be 140 and $15\text{--}33 \times 10^{12}$ g, respectively (Gruber and Galloway 2008; Malin 2006). As the oceans have been considered to be a sink of ammonium and sulfate, it has been hypothesized that a significant contribution may come from the biological interaction among the N and S cycles in oceans (Cai *et al.* 2010; Schrum *et al.* 2009). It is speculated that ammonium acts as the electron donor to reduce sulfate to elemental sulfur and generates nitrogen through a process termed “Sulfate Reduction and Ammonium Oxidation (SRAO)”.

Fdz-Polanco *et al.* (2001; 2001a; 2001b) have found simultaneous SRAO in a granular anaerobic fluidized-bed reactor treating vinasse from an ethanol distillery of sugar beet molasses. They proposed that this biochemical reaction could be obtained by combining reactions involved in nitrite formation and Anammox reactions. This phenomenon has also been observed under organic conditions by other researchers in organic conditions (Reyes-Avila *et al.* 2004; Zhao *et al.* 2006). Zhang *et al.* (2009) reported that ammonium could be oxidized anaerobically with sulfate as the electron acceptor in the presence of digested sludge under inorganic conditions. The condition of high substrate concentrations and low oxidation–reduction potential (ORP) might be favorable for such a syntrophic relationship, even with its low negative Gibbs free energies gained. Liu *et al.* (2008) and Yang *et al.* (2009) have detected simultaneous ammonium and sulfate removal in an Anammox reactor, which consisted of ammonium oxidation to nitrite and sulfate deoxidization to sulfur. A new species, “*Anammoxoglobus sulfate*”, was considered to perform the critical role for SRAO reactions (Liu *et al.* 2008).

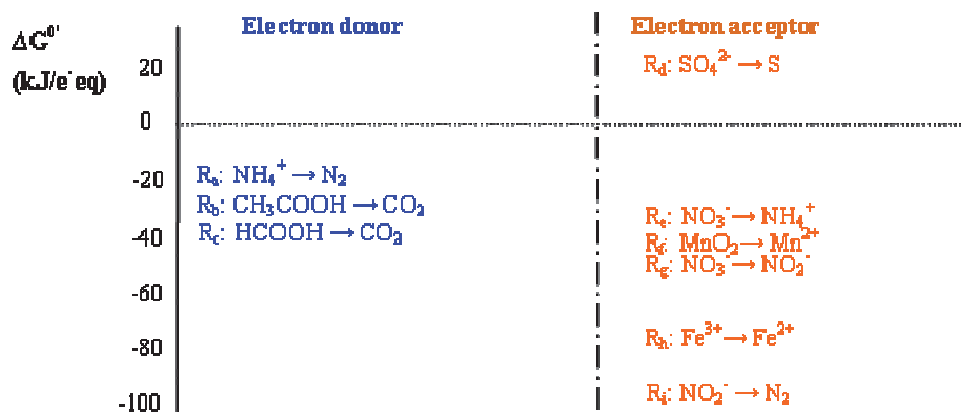


Fig. 4.3. Half-reactions and their energy yields involved in versatile Anammox processes based on Gibbs standard free energy at pH 7, modified from (Gao and Tao 2011). The thermodynamic data obtained from Rittmann and McCarty (2001).

On the other hand, the existence of SRAO in sub-seafloor has already been demonstrated through thermodynamic calculations and chemical profile analysis of sedimentary pore water (Schrump *et al.* 2009). Most recently, a successful stable co-culture of Anammox bacteria and *Sedimenticola* sp. in a laboratory-scale system validated its achievability with the media containing sulfide, nitrate, ammonium, and CO₂ (Russ *et al.* 2014). Although much research has been carried out, the functional microorganisms and mechanism of SRAO processes, whether in laboratory-scale microbial systems or natural environments, have not yet been fully identified.

2.5. N source for Anammox bacteria

Microorganisms prefer ammonium as their nitrogen source since it holds the same valence as the nitrogen in protein. Most of them can also utilize nitrite, nitrate, or even nitrogen gas for nitrogen requirements during anabolism (Rittmann and McCarty 2001). It is confirmed that Anammox bacteria use ammonium as the electron donor and nitrite as the electron acceptor. Still, based on the microbial thermodynamic modeling (microbial energetics), it is proposed that Anammox bacteria may use nitrite, instead of the more general ammonium, as a nitrogen source for cell synthesis, based on microbial thermodynamic modeling (microbial energetics). The stoichiometry of Anammox reactions with the assumption of different nitrogen sources could be derived from the thermodynamic electron equivalents model (TEEM) by McCarty (1975), as shown in Table 4.2 (detailed calculations not shown here). By comparing the coefficients of involved nitrogen compounds (ammonium, nitrite, nitrate, and nitrogen gas) in Anammox metabolism between the model-derived equation and the experimentally-observed value provided by Strous *et al.* (1998), it is obvious that there is a higher agreement between the values when nitrite is assumed as nitrogen source. Further evidence supporting this hypothesis came from numerous studies, which found that the pH of Anammox systems would increase during cultivation (Strous *et al.* 1998; van de Graaf 1996). Anammox bacteria utilize carbon dioxide as the carbon source and protons would be produced during the conversion. The protons could be consumed and result in pH increase if nitrite is used as the nitrogen source ($4\text{NO}_2^- + \text{H}_2\text{O} + \text{H}^+ = \text{NH}_3 + 3\text{NO}_3^-$). Conversely, the pH would be lower if ammonium is utilized as the nitrogen source instead ($\text{NH}_4^+ + \text{OH}^- = \text{NH}_3 + \text{H}_2\text{O}$). Additionally, nitrite reductase was detected in Anammox bacteria, which makes the conversion of nitrite to ammonia possible (Strous *et al.* 2006). Accordingly, it can be deduced that nitrite not only served as the electron acceptor and reductant for carbon fixation in Anammox metabolism, but also served as the nitrogen source.

Table 4.2. Stoichiometry of modeling results with NH₄⁺/NO₂⁻ as N-source.

N source	NH ₄ ⁺	NO ₂ ⁻	NO ₃ ⁻	N ₂	References
NH ₄ ⁺ -N	1	1.2	0.21	0.99	Strous <i>et al.</i> (1998)
NO ₂ ⁻ -N	1	1.27	0.26	1.00	
Experimental	1	1.32	0.26	1.02	

2.6. Long solid retention time for less nitrate formation

According to the stoichiometry of Anammox reactions, a large percentage of nitrate, over 10% of the total nitrogen, would be generated due to the use of nitrite as the reductant for cell synthesis (Strous *et al.* 1998). Undoubtedly, it would not allow the nitrogen removal efficiency to reach more than 90%, which is usually undesirable in Anammox applications for wastewater treatment. However, a recent study exhibited that there may be exceptions with a conversion rate of only 2–4% to nitrate (Kwak *et al.* 2012). To decipher the mystery for such a low nitrate formation, Lee *et al.* (2013a) developed combined stoichiometric equations based on TEEM for overall Anammox metabolism. This supported that only a small fraction of ammonium would be converted into nitrate, in the range of 2% at a relatively long solid retention time (SRT), during a low growth rate. Hence, less amount of reductant, nitrite in the case of Anammox process, would be required for cell synthesis, which results in less formation of nitrate. This observation of a low SRT operation would enhance the nitrogen removal efficiency to over 90%, while generating less sludge.

2.7. Inhibition of Anammox

Anammox has been widely studied worldwide due to its economic advantages and high nitrogen removal capacity compared to conventional nitrification–denitrification (Van Hulle *et al.* 2010). During the last two decades, the Anammox process has been developed and applied to treat wastewater with high ammonium concentration content from the laboratory set-ups to full-scale plants (Hwang *et al.* 2005; Kimura *et al.* 2013; Shalini and Joseph 2012). However, it is restricted by the low growth rate and numerous inhibition factors, e.g., substrate, organic matters, etc. (Jin *et al.* 2012). Similarly, various wastewater types and their impurities contributing to various restrictions in the application of Anammox have been investigated. Here, the inhibiting factors are summarized as follows.

2.7.1. Substrates

Although ammonium and nitrite are the substrates for Anammox reactions, substrate inhibition should not be ignored. Results displayed that a high ammonia concentration can suppress Anammox bacteria (Dapena-Mora *et al.* 2007; Puyol *et al.* 2014a). However, molecular ammonia (NH_3), caused by high ammonia and nitrite concentrations at high pH, is considered as the key inhibitor rather than ammonium itself due to a relationship between ammonium ions and NH_3 (Fernandez *et al.* 2012; Aktan *et al.* 2012). The molecular NH_3 diffusing into the Anammox cell, when there is a high pH gradient between extracellular and intracellular membranes, would cause cell lysis (Kadam and Boone 1996). Researchers testified that the threshold molecular ammonia concentration that greatly inhibits Anammox activity is $150 \text{ mg}\cdot\text{l}^{-1}$, but even when the ammonia and nitrite concentrations are up to $1,500 \text{ mg}\cdot\text{l}^{-1}$ and $500 \text{ mg}\cdot\text{l}^{-1}$, respectively, no serious interference to its bacterial activity was found (Aktan *et al.* 2012). Nevertheless, a firm conclusion of the inhibition threshold value could not be determined, due to the diverse features of various Anammox species (Oshiki *et al.* 2011). Similar to ammonium,

biotoxicity of Anammox cells would occur if nitrite reaches up to a threshold concentration (Isaka *et al.* 2007; Bettazzi *et al.* 2010). However, the threshold values reported varied from 5–20 mM. Results revealed that ionized nitrite is the main inhibitor of Anammox reactions at $\text{pH} > 7.1$ when nitrite exists (Puyol *et al.* 2014b). However, molecular nitrous acid (HNO_2), influenced by pH, is another factor impeding Anammox cells (Fernandez *et al.* 2012). Thus, pH value is the primary factor influencing Anammox significantly. pH beyond neutral conditions is unbeneficial to Anammox bacteria owing to the increase of either HNO_2 or NH_3 (Mosquera-Corral *et al.* 2005). Normally, a feed ratio of ammonium to nitrite close to 1 or slightly lower would be advantageous to Anammox reactions.

2.7.2. Organic matters

Due to the chemoautotrophic nature of Anammox bacteria, their slow growth rate is notorious. Additionally, organic carbon components in wastewaters would boost the growth of heterotrophic bacteria (denitrifying bacteria) and out-compete Anammox (Chamchoi *et al.* 2008). Even though the doubling time of heterotrophic bacteria reported are much greater than that of autotrophic bacteria, Anammox bacteria could still out-compete heterotrophic denitrifying bacteria by applying their organotrophic pathway, if the influent C/N could be lower than 2. (Strous *et al.* 2002; Strous *et al.* 2006; Kartal *et al.* 2007b; Guven *et al.* 2005). Nonetheless, some toxic organic matters (e.g., methanol (Isaka *et al.* 2008), toluene (Martinez Hernandez *et al.* 2013) and antibiotic (Fernandez *et al.* 2009)) can severely deter Anammox bacteria. Isaka *et al.* (2008) exhibited that methanol is not the direct inhibitor, while formaldehyde converted by methanol is the key element due to its toxicity towards enzyme and protein.

2.7.3. Other factors

Other factors which influence Anammox processes negatively include salinity, heavy metals, phosphate and sulfide. High salinity will cause microbial death due to the rise in cellular osmotic pressure. Heavy metals are toxic to organisms through their bioaccumulation in cells. The existence of sulfate and organics could facilitate sulfate to act as the electron acceptor for hydrogen sulfide formation, which will be toxic to Anammox cells. Specifically, the half maximal inhibitory concentration (IC_{50}) of sulfide-S for Anammox bacteria is $264 \text{ mg}\cdot\text{l}^{-1}$ at a nitrogen concentration of $200 \text{ mg}\cdot\text{l}^{-1}$ (Jin *et al.* 2013). Moreover, the optimum temperature for Anammox bacteria is from 30°C to 40°C (Dosta *et al.* 2008). Higher or lower temperatures would reduce the Anammox activity. More studies are needed to understand how to enhance the Anammox bacterial activity through minimizing the effect of inhibitors. Several research aspects are suggested: (1) the inhibitor species; (2) the mechanisms of the inhibitors on Anammox bacteria; (3) the acclimation of Anammox species to tolerate different conditions.

2.8. N₂O emission

Nitrous oxide, which depletes stratospheric ozone and hastens climate change, is produced during conventional nitrification–denitrification (IPCC 2013; Kramlich and Linak 1994). In this regard, controlling its production and emissions from WWTPs garnered significant attention (Kampschreur *et al.* 2009b). The mechanism of N₂O formation through the conventional nitrification–denitrification lies in reducing nitrite- (nitrifier denitrification) and intermediate-formation during the oxidation of hydroxylamine (MH₂OH) to NO₂⁻ (Wunderlin *et al.* 2013; Scherson *et al.* 2014). Through this, N₂O emission can alleviate up to 14.6% of the nitrogen load (Kampschreur *et al.* 2009b). Conversely, due to the lack of heterotrophic denitrifiers in partial nitrification/Anammox systems, N₂O emission through these systems could be negligible (Desloover *et al.* 2012; Scaglione *et al.* 2015). Kampschreur *et al.* (2009a) reported the N₂O emission from a single-stage nitrification–Anammox process was 1.2% of the nitrogen load. Nonetheless, Scaglione *et al.* (2014) described that approximately 3–15% of the nitrogen load was removed as N₂O in the partial nitrification reactor, indicating that a combined system integrated with Anammox would be a better alternative to reduce N₂O formation. In another words, N₂O emission can be restricted through the immediate consumption of nitrite owing to higher *in situ* Anammox activity (Desloover *et al.* 2012). Yet, N₂O has also been recognized as a rocket fuel, and thus prompts a concept of N₂O generation for energy production from wastewaters, as pursued by Scherson *et al.* (2014). They successfully generated and collected N₂O from high ammonium content wastewaters, and used it for co-combustion with methane, achieving about 30% more energy yield in comparison with the combustion with O₂ and CH₄.

3. Analysis Methodologies

3.1. Specific Anammox activity (SAA)

In order to assess the applicability of Anammox processes at industrial scales, it is necessary to estimate the maximum specific Anammox activity (SAA) towards different compounds present in industrial effluents. To determinate the conditions of the maximum SAA, nitrogen gas production in batch tests are commonly used as the same concept of anaerobic digestion. Once these conditions were determined, the effects of different compounds present in wastewaters (NH₄⁺, NO₂⁻, NO₃⁻, Na⁺, Cl⁻, PO₄³⁻, SO₄²⁻, S²⁻, acetate, flocculants, allylthiourea, chloramphenicol) on the maximum SAA could be discovered. The total amount of N₂ gas produced was obtained from the overpressure measured in the headspace of each vial at the end of the assay based on the ideal gas law. The amount of nitrogen removed from the liquid phase was also calculated by measuring the ammonium, nitrite, and nitrate concentrations at the beginning and the end of the experiment and taking into account the volume of the liquid phase (Dalsgaard and Thamdrup 2002; Dapena-Mora *et al.* 2007).

3.2. Anammox activity–isotope pairing technique

The isotope pairing technique (IPT) is a well-established ^{15}N approach for estimating denitrification of bottom-water and of NO_x^- produced via sedimentary nitrification. During the past decade, this technique has been used in numerous studies. The IPT aims to quantify the genuine production rate of N_2 gas, i.e., ^{14}N - N production as it would occur without the addition of $^{15}\text{NO}_x^-$. This corresponds to the production rate of $^{28}\text{N}_2$ ($^{14}\text{N}^{14}\text{N}$) times two plus the production rate of $^{29}\text{N}_2$ ($^{14}\text{N}^{15}\text{N}$) after addition of $^{15}\text{NO}_x^-$ to the system. This production (p^{14}) is estimated from the production rates of $^{29}\text{N}_2$ ($p^{29}\text{N}_2$) and $^{30}\text{N}_2$ ($p^{30}\text{N}_2$) using the following expression of Nielsen (1992).

$$p^{14} = \frac{p^{29}N_2}{2 \cdot p^{30}N_2} \cdot (2 \cdot p^{30}N_2 + p^{29}N_2) \quad (4.1)$$

The supposition that the genuine production rate of N_2 equals $2 \cdot ^{28}\text{N}_2 + ^{29}\text{N}_2$ production and this production can be calculated from Equation 4.1 according to a number of assumptions (Risgaard-Petersen *et al.* 2003; Sato *et al.* 2012)

3.3. Next-generation sequencing (NGS)

The recent emergence of novel (next-generation) sequencing technologies, resulting in higher sequence output and dramatic drop in the price, defines a new era in metagenomics. NGS is a powerful technique to obtain genomic information of marine Anammox bacteria in oxygen minimum zone (OMZ) ecosystems. The coverage by solid and Ion Torrent was high enough to analyze the important *hzsA* and *hdh* core in Anammox genes. Together with 16S rRNA gene phylogenetic analysis, it was shown that the diversity of the marine Anammox in the Arabian Sea was lower than previously determined by polymerase chain reaction (PCR) methods (Villanueva *et al.* 2014).

3.4. Fluorescence in situ hybridization (FISH)

FISH uses fluorescent probes that can bind to only those parts of the chromosome with which they show a high degree of sequence complementarity. Successively, fluorescence microscopy is used to identify the location of the fluorescent probe bounded to the chromosomes, so that the structure of the Anammox biofilm can be observed. FISH is often used for finding specific features in DNA for use in genetic counseling, medicine, and species identification (Schmid *et al.* 2005).

4. Anammox Applications

4.1. Concentrated wastewaters

Even though the Anammox process was discovered in the full-scale reactor and has been successfully studied in the laboratory, the bottleneck for its full-scale applications includes the slow growth nature of Anammox bacteria, substrate toxicity, accumulation

of nitrite, foaming, scaling and sludge retention/settling/solids separation, and other operational problems. Generally, the processes of granule or biofilm are encouraged (Fernandez *et al.* 2008; Figueroa *et al.* 2012). Besides, organic matters contained in wastewaters may lead heterotrophic bacteria to out-compete with Anammox bacteria as aforementioned. Furthermore, coupled to the application of Anammox systems, sustainable partial nitrification is required in order to provide the electron acceptor for Anammox to complete ammonium oxidation into nitrogen gas. This niche could be accomplished by a process termed “Single reactor system for High activity Ammonium Removal Over Nitrite (Sharon)”. It uses high temperature (35 °C) and low SRT to enrich the ammonium-oxidizers and inhibit the nitrite-oxidizers. This process is suitable to treat wastewaters in the meso-temperature range and with high ammonium content such as industrial reject waters (van Dongen *et al.* 2001). This lays the primarily groundwork for scale-up implementation of Anammox.

The first full-scale Anammox reaction (70 m³) was built in Rotterdam, Netherlands (van der Star *et al.* 2007). However, the start-up time is up to 3.5 years due to a lack of Anammox sludge and its low growth trait. To worsen the situation, several issues occurred during the start-up period: (1) incidental nitrite toxicity; (2) biomass washout; (3) toxicity to Anammox biomass caused by excess methanol; and (4) unexpected operational problems (van der Star *et al.* 2007). Nevertheless, this study still managed to treat a flow of 750 kg·d⁻¹, which was 50% higher than the design load. Joss *et al.* (2009) reported that a full-scale partial nitritation and Anammox system, treating high ammonium wastewater, was accomplished with over 90% nitrogen conversion at an ammonium oxidation rate of 0.5 kg·Nm⁻³·d⁻¹. Beside the first discovery of Anammox in the Netherlands, a full-scale landfill leachate plant operation in Keelung, Taiwan found that 80% and 27% for total nitrogen and chemical oxygen demand (COD) removals, respectively, were associated with Anammox (see Fig. 4.4). This unexpected process, termed as simultaneous partial nitrification, anaerobic ammonium oxidation and denitrification (SNAD), resulted from a low dissolved oxygen (DO) level of 0.5 mg·l⁻¹ caused by the under-estimated aeration supply (Wang *et al.* 2010). Additionally, this patented process has proven to be reproducible in another landfill leachate plant in Bali, Taiwan, with treatment capacity of 800 cubic meter per day (CMD) and a nitrogen loading rate of 0.6 kg·Nm⁻³·d⁻¹ by inoculating the SNAD seed. This process also showed the practicality of treating opto-electronic wastewaters (Daverey *et al.* 2012, 2013). SNAD alleviates the Anammox horizon by integration of denitrification for COD removal in single-stage systems.

Over the past decade, many full-scale partial nitrification–Anammox facilities were constructed. By 2014, more than 100 facilities were in operation. Among them, over 50% of plants are sequencing batch reactor (SBR), 88% of plants are single-stage systems, and 75% of plants are utilized to treat sidestream of municipal wastewaters (Lackner *et al.* 2014). Most of them focus on treating high ammonium concentration wastewaters,



Fig. 4.4. The full scale Anammox reactor in Taiwan treating landfill leachate (a) Aeration tank; (b) suspended Anammox biomass; (c) attached Anammox biomass (Wang *et al.* 2010).

Table 4.3. Scale-up Anammox systems (Adapted from Ni and Zhang 2013; Lackner *et al.* 2014).

Location	Influent	Reactor volume·m ⁻³	Designed load·kgNd ⁻¹	Operational year
Rotterdam, NL	Reject water	72	750	2002
Lichtenvoorde, NL	Tannery	100	150	2004
Olburgen, NL	Potato processing	600	700	2006
Mie prefecture, JP	Semiconductor	50	220	2006
Stockholm, SE	Reject water	1,400	600	2007
Niederglatt, Switzerland	Reject water	180	60	2008
Tongliao, China	Monosodium glutamate (MSG)	6,600	1,100	2009
Yichang, China	Yeast production	500	1,000	2009
NL	Reject water	425	600	2010
Tai'an, China	Corn starch and MSG	4,300	6,090	2011
Poland	Distillery	900	1,460	2011
Wuxi, China	Sweetener	1,600	2,180	2011
Shaoxing, China	Distillery	560	900	2011
Pfannenstiel, CH	Reject water	320	75	2012
Breda, NL	Reject water	1,000	990	2013
Grindsted, DK	Reject water	140	100	2013

especially reject waters. Lackner *et al.* (2014) summarized current full-scale Anammox systems. Table 4.3 provides a summary of the operational information for 14 of such systems (Adapted from Ni and Zhang 2013; Lackner *et al.* 2014). These applications of full-scale Anammox processes have shown their commercial feasibility.

4.2. Dilute wastewaters (mainstream)

Although previous studies of Anammox-related systems have mostly emphasized the treatment of high ammonium content wastewaters, the potential for extending process application to dilute wastewaters, particularly for sewage, has received increasing attention recently (Abbassi *et al.* 2014; Kartal *et al.* 2010). Anammox bacteria has been found in various environments under a wide temperature range, providing the practicability with dilute wastewaters (Rysgaard and Glud 2004; Byrne *et al.* 2009; Hu *et al.* 2011; van de Vossenberg *et al.* 2008). Yet, the challenges of ambient temperature and low ammonium concentration within dilute wastewaters are the limiting factors for nitrification (Dosta *et al.* 2008; Vazquez-Padin *et al.* 2011). Furthermore, dilute influents make it challenging to maintain a sustainable Anammox biomass population in this process. Plus, hydraulic retention times (HRTs) are required to meet with the conventional nitrification–denitrification. Moreover, heterotrophic bacteria competition should be a concern with organic carbon matters exiting in dilute wastewaters.

To this end, completely autotrophic nitrogen removal over nitrite (CANON), SHARON-Anammox, denitrifying ammonium oxidation (DEAMOX) have been studied with dilute wastewaters or sewage (Nozhevnikova *et al.* 2012; Schmidt *et al.* 2003). These studies showed the importance of DO control for nitrite formation so that complete nitrogen removal could be further carried out by Anammox. If too little DO is delivered, incomplete ammonium oxidation would occur. However, if too much DO is supplied, nitrifiers may out-compete the Anammox organisms, resulting in excess oxidization of ammonia to nitrate (Li *et al.* 2011). In both cases, total nitrogen removal efficiency would be hindered. Lee *et al.* (2013b) reported that a robotic DO supply strategy in a membrane biofilm reactor (MBfR) at 20 °C could treat synthetic diluted wastewater without organic matters. This system accumulated about 88% to 94% nitrite when feeding at a stoichiometric mole ratio of 1.5 mol O₂/ammonium fed. This research sets the foundation for sustainable nitrification by providing a benchmark oxygen control for diluted wastewaters. Based on this finding, they applied the same oxygen benchmark strategy in a single-stage nitrogen removal biofilter (NRBF). Through close control of the oxygen to ammonia ratio of 0.75 mol O₂/ammonium fed, higher than 90% nitrogen can be removed from dilute wastewaters at ambient temperature and with HRTs as short as 1-hour HRT (Kwak *et al.* 2012). These studies indicate that the DO control strategy for autotrophic removal of nitrogen from dilute wastewaters is a promising strategy.

Hendrickx *et al.* (2012) treated synthetic wastewater of 69 mg-N·l⁻¹ (NH₄⁺+NO₂⁻) at a loading rate of 0.31 g-N·(l·d)⁻¹ in a 4.5-l gas-lift reactor. In this study, a net Anammox bacteria growth rate of 0.040 d⁻¹ at 20 °C and less than 0.2 mg-N·l⁻¹ (NH₄⁺+NO₂⁻) of effluent concentrations were reported. Anammox activity was maintained at a COD/N ratio up to 0.5 and ambient temperatures (18±3 °C) in a laboratory-scale anoxic/aerobic reactor, which meant that Anammox bacteria successfully competed with heterotrophic bacteria (Winkler *et al.* 2012). Anammox granules can be formed in the upflow anaerobic sludge bed (UASB) reactors at 30 °C, and even when the temperature decreased to 16 °C, the sludge granules can still be maintained, which contributed to a high nitrogen removal

rate (NRR) treating low-strength wastewater (Ma *et al.* 2013). Anammox bacteria occurred in an oxygen-limited autotrophic nitrification denitrification (OLAND) process and DO could greatly affect the Anammox community when treating low-strength nitrogen wastewater (ammonium concentrations from 66 to 29 mg-N·l⁻¹) (De Clippeleir *et al.* 2011). Ma *et al.* (2011) stated that a 88.38% removal efficiency for total nitrogen was obtained in one nitrification reactor and one Anammox reactor for treating sewage containing 44.4 mg·l⁻¹ ammonium and 44.4 mg·l⁻¹ soluble COD at HRT of 4.6 h and 27–30 °C. However, the temperature of wastewater is a critical problem for Anammox application for mainstream treatment. Even though the optimum temperature for Anammox is described from 30–40 °C (Dosta *et al.* 2008), Gilbert *et al.* (2014) found that Anammox activity for low-strength wastewater is not severe in a biofilm reactor, using 10-mm carriers, when temperature is dropped from 20 °C to 13 °C. Furthermore, Huang *et al.* (2013) made an important discovery that Anammox bacteria can change the feeding intensity with the oxygen supply control when partial nitrification and Anammox are carried out in a single-stage reactor for diluted wastewater treatment. We believe that these results will help stimulate the greater application of this energy-efficient nitrogen removal process for concentrated as well as dilute wastewaters.

5. Summary

Anammox has received an increased interest due to its sustainable characteristics. This chapter provides detailed information on Anammox technologies, including its relation to the nitrogen cycle, Anammox species, molecular analysis techniques, scale-up applications, and commercialization potential. With growing populations and climate change along with diminishing energy, energy-efficient Anammox systems offer a promising nitrogen removal alternative to meet lower energy consumption, with no requirement of an organic donor, N₂O emission reduction, and lower sludge handling costs. Furthermore, it is conceived that Anammox's involvement in syntrophy for SRAO may play a significant role in global nitrogen–sulfur cycle.

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Chapter 5

Application of Metagenomics in Environmental Anaerobic Technology

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Anaerobic technology has been applied for the treatment of solid wastes and wastewater since the 1880s. This chapter reviews the most recent advance in molecular microbial characterization techniques, i.e., metagenomics, as well as its applications in anaerobic technology, from the exploration of the intriguing science of anaerobes to industrial engineering applications. With the rapidly decreasing cost of high-throughput sequencing technologies and timely updating of state-of-the-art bioinformatics analysis tools, metagenomics has revolutionized the study of microbiology and demonstrated the great potential of the development of novel microbial resources (e.g., industrial biomolecules and enzymes and biodegradation genes), as it discloses unprecedented genetic information of uncultivated microorganisms at an amazingly fast rate. This chapter gives a detailed introduction to the technical procedures of metagenomics, from preliminary molecular experiments to high-throughput sequencing, as well as its application in environmental anaerobic technology.

1. Introduction

Anaerobic technology has been a proven biological method applied to organic waste and wastewater treatment for over 130 years, demonstrating its intrinsic advantages of energy-saving, reduced sludge yield, and, more attractively, the production of bioenergy (Lettinga *et al.* 1980; McCarty 1982). The past three decades have witnessed great inventions with optimized reactor design, configurations, and operation (Lettinga *et al.* 1980; Habets *et al.* 1997; Kato *et al.* 1994; Barber and Stuckey 1999) that largely promote the widespread industrial applications of anaerobic technology for biofuel production and/or pollution control around the world (Fang 2010). Better still, advances in molecular techniques in the 1990s further facilitated the development of anaerobic technology, as thereafter, the microorganisms and enzymes responsible for bioenergy production (Rittmann *et al.* 2008; Narihiro and Sekiguchi 2007) and/or pollution control (Zhang *et al.* 2005; Sanz and Köchling 2007) from waste and wastewater have been continuously identified.

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The application of molecular techniques, such as PCR cloning, fluorescent *in situ* hybridization (FISH), denaturing gradient gel electrophoresis (DGGE), and terminal restriction fragment length polymorphism, in various anaerobic digesters discloses the identities of contributing anaerobes that have been previously mysterious, and helps us to realize that they rarely work alone, but typically establish tightly coupled syntrophic interactions to achieve biodegradation and biogas production (Sieber *et al.* 2012). For instance, the minimum communities for anaerobic conversion of phenol to methane require an obligate syntrophic association between *Syntrophorhabdus aromaticivoran* UI and a hydrogenotrophic methanogen (Qiu *et al.* 2008). While most of these powerful molecular techniques are designed to characterize microbes based on currently available sequence information of microorganisms, they are limited in interpreting the microbial interactions and function, especially when considering the high microbial diversity in anaerobic digesters, as well as the unculturability of the vast majority of microbes.

With the rapidly reducing cost of revolutionary next-generation sequencing (NGS) and the fast development of state-of-the-art bioinformatical analyses, metagenomic approaches, including 16S rRNA gene sequencing, metagenome, metatranscriptome, and metaproteome, have been widely applied to improve our understanding of microbial biodiversity and functions in various natural ecosystems (Fig. 5.1), such as soil (Costello *et al.* 2009; Benndorf *et al.* 2007), ocean (Pernthaler *et al.* 2008; Frias-Lopez *et al.* 2008), sediment (Benndorf *et al.* 2007; Jorgensen *et al.* 2012), typical human or animal ecosystems, such as human gut (Qin *et al.* 2010; Verberkmoes *et al.* 2008) and cow rumen (Hess *et al.* 2011), and engineered biological waste/wastewater treatment systems, including activated sludge (Zhang *et al.* 2012; Yu and Zhang 2012), drinking water or wastewater biofilms (Rademacher *et al.* 2012; Shi *et al.* 2013), anaerobic digesters (Vanwonterghem *et al.* 2014; Ju and Zhang 2014), etc. On the one hand, 16S rRNA gene high-throughput sequencing (HTS), which sequences different hypervariable regions of 16S rRNA gene, brings comprehensive insights into whole microbial communities

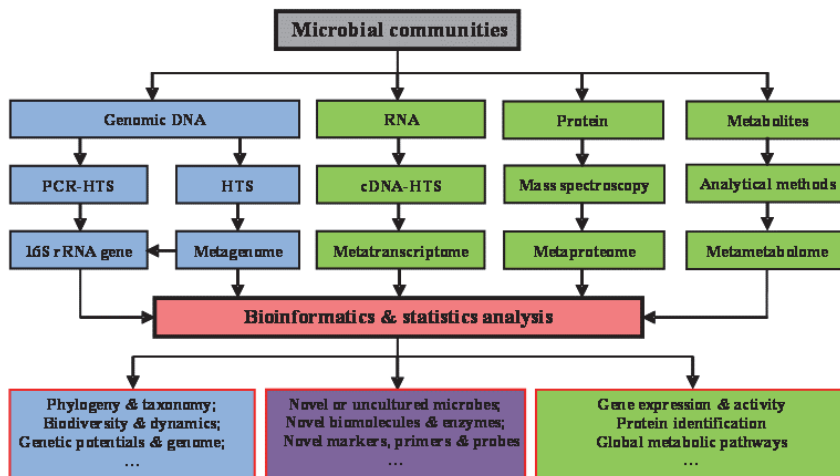


Fig. 5.1. Diagram of branching structures of HTS-based metagenomics studies.

in terms of taxonomy and diversity (Ye and Zhang 2013), especially for those rare but functionally important species. Furthermore, shot-gun metagenome and metatranscriptome, which directly sequence metagenomic DNAs (Yang *et al.* 2014b) or RNAs (Xia *et al.* 2014), result in much greater coverage and far more informative sequence data than those that could be achieved by any technologies before. Moreover, in recent years, diverse high-performance computing tools have sprung up like mushrooms to cope with the explosive expansion of sequencing data from next and third-generation HTS, and have helped dig into the huge informative sequences (Meyer *et al.* 2008; Yu and Zhang 2013; Caporaso *et al.* 2010). Analyses of HTS data disclose enormous microbial diversity and plentiful novel gene resources, unravel their temporal and spatial distributions mediated by environment gradients, and improve our understandings of their genetic novelties, and biological relevance and significance. This collective knowledge is essential to fully exert the potential of anaerobic technology in industrial applications.

This chapter reviews the application of metagenomics and the state-of-the-art bioinformatics tools in the analysis of microbial communities in anaerobic reactors. First, different sequencing strategies (e.g., 16S rRNA gene, DNA, messenger RNA) and prevalent NGS and third-generation sequencing (TGS) platforms are introduced in the context of applying them to various research aims. Second, the molecular operations, including extraction of nucleic acid, selection of biomarkers and PCR amplification, and library preparation before sequencing, are discussed in detail with an emphasis on special difficulties/concerns in handling anaerobic sludge samples. Moreover, the applications of metagenomics in anaerobic technology are introduced in detail, focusing on identifying new species, novel genes, as well as exploring relationships between microbial structure and environment or functioning in the anaerobic digestion processes operated for the production of methane and hydrogen. In addition, the application of metagenomics in monitoring genetic biohazards, such as antibiotic resistance genes (ARGs) and pathogens in anaerobic digestion processes is briefly introduced to sound an alarm to the sanitary safety of future disposal and management of anaerobic digestion sludge.

2. Sequencing Strategies and Platforms

In the studies of metagenomics, different sequencing strategies that target diverse genetic materials, including 16S rRNA gene, genomic DNA, messenger RNA, and protein, are adopted to answer questions regarding the structural and functional diversity of microbial communities. To fulfill these challenging tasks, various HTS-based technically mature NGS platforms (Fig. 5.2, I–III), such as MiSeq and HiSeq platforms of Illumina, GS Junior and FLX systems of Roche, PGM of Ion Torrent, and SOLiD System of Life Technologies, are developed, commercialized, and continuously upgraded to yield sequence data that differ in throughput, read length, price per base, and sequencing quality. Among them, Illumina HiSeq has been the market leader in recent years, mainly due to the largest throughput and the highest base quality at the lowest per base price.

Meanwhile, tag-based methods, such as SafeSeq, CircleSeq, and Duplex Sequencing, are applied to largely increase accuracy in NGS by magnitudes and reduce the error rate down to 10^{-5} – 10^{-8} (Fox *et al.* 2014). Noteworthy, TGS platforms (i.e., single molecule



Fig. 5.2. Next-generation (I–III) and third generation (IV–VI) sequencing platforms (photos were downloaded from websites of respective providers).

real time sequencing) (Schadt *et al.* 2010), such as PacBio RS of Pacific Biosciences and MinION™ of Oxford Nanopore (Fig. 5.2, IV–VI), are under improvement or development. Once the single molecule real time sequencing technique becomes mature and more robust, these TGS platforms will have a promising application in single-cell sequencing, leading to better understanding the function of an individual cell in its surrounding microenvironment (Eberwine *et al.* 2014). Readers may refer to the works of Shendure and Ji (2008) and Metzker (2009) and of Schadt *et al.* (2010) for introduction to the sequencing principles of NGS and TGS platforms, respectively.

2.1. 16S rRNA gene high-throughput sequencing

16S rRNA gene amplicons HTS has now been extensively used to fingerprint the diversity of prokaryotic communities. The Roche's 454 pyrosequencing and Illumina's HTS sequencing have currently been the world's most popular NGS methods for 16S rRNA gene amplicons. The earliest entry into the market of the pioneering pyrosequencing (in 2005) and its relative longer read length compared with latter competitors have made it as the most attractive choice for 16S-based environmental survey at the early stage of the NGS era. The latest Roche technologies (December 2014), i.e., GS junior and GS FLX systems, can generate up to a million of reads in a run, with adequate lengths (450–1,000 bases) to approximately cover at least two multiple hypervariable regions or even over half of the length of the 16S rRNA gene. Up to now, 16S rRNA gene pyrosequencing has been widely applied to characterize microbial composition and dynamics in various environmental habitats (e.g., soil, water, air, human, and wastes and wastewater treatment facilities) and across multiple research disciplines. However, MiSeq and HiSeq platforms of the late-comer, Illumina, are rapidly taking over the sequencing market from Roche by using sequencing-by-synthesis to generate hundreds of millions to billions of higher quality reads per flow cell at far lower prices. Moreover, the read length could reach up to over 500 bps by overlapping the paired-end reads (e.g., of 300 bps) and is promised to be further extended in the near future with the rapid development of Illumina technologies.

To sequence large numbers of samples in parallel, short nucleotide barcodes (usually between 6–12 nucleotides) are developed, evaluated, and applied to multiplex samples on different NGS platforms (Mir *et al.* 2013; Parameswaran *et al.* 2007; Bartram *et al.* 2011; Smith *et al.* 2010; Hamady *et al.* 2008). For pyrosequencing, lists of 1,544 error-correcting forward barcodes and 48 forward–reverse barcode pairs (10-base) were provided in publications of Hamady *et al.* (2008) and Parameswaran *et al.* (2007), respectively. For Illumina paired-end sequencing, lists of 96 barcodes (6-base) designed for Illumina paired-end sequencing are available at Bartram *et al.* (2011). Recently, a dual-index sequencing strategy and a coupled curation pipeline have been developed for analyzing amplicon sequence data on Illumina’s MiSeq. It is demonstrated that large numbers of samples could be multiplexed in a total of 1,536 combinations from 32 i5-forward primers (8-base) and 48 i7-reverse primers (8-base), and sequenced in parallel with shot-gun metagenomes, yielding data with considerably higher sequencing coverage than the 454 pyrosequencing platform, while at least keeping equal quality (Kozich *et al.* 2013). In this way, it is feasible and reasonable to multiplex up to hundreds or a thousand of anaerobic sludge samples with rigorous experimental design in a single Illumina lane, which includes enough biological and technical replicates to allow for strict statistical analysis and to cover enough environmental gradients (temporally and/or spatially) to resolve their effects on the microbial community structure.

2.2. Metagenome and metatranscriptome

The wide application of metagenome and metatranscriptome necessitates considerations of different sequencing platforms as to the cost, data throughput, sequencing accuracy, read length, sequencing time, as well as potential limitations and biases as manifested in metagenomics studies.

A recent study compared the performance of three benchtop HTS platforms — i.e., Roche 454 GS Junior, Illumina MiSeq, and Ion Torrent PGM — in sequencing an isolate of *Escherichia coli* O104:H4 (Loman *et al.* 2012). While each platform could produce enough sequences required for assembling a draft genome in days (e.g., needs from 3 hours to 12.6 days to generate 1 Gb of sequence data), their performance in price, quality, and speed differed greatly. The MiSeq had the highest data throughput (1.6 Gb·run⁻¹, 60 Mb·h⁻¹) at the lowest unit price (\$0.5·Mb⁻¹) and error rates, whereas the 454 GS Junior had the lowest data throughput (70 Mb·run⁻¹, 9 Mb·h⁻¹) at the highest unit price (\$31·Mb⁻¹), but produced the longest sequences (up to 600 bases) and most contiguous assemblies. Besides, the 454 GS Junior and Ion Torrent PGM both generated homopolymer-associated indel errors (an error rate of 0.38% and 1.5%, respectively), compared with the Miseq. In another comparative study, Ion Torrent’s PGM, Pacific Biosciences’ RS, and Illumina’s MiSeq and Hiseq sequencers were adopted to sequence four bacterial genomes with quite representative GC contents ranging from 19.3% to 67.7% (Quail *et al.* 2012). While all sequencers retrieved near perfect coverage for the three genomes with relatively higher GC content, the PGM displayed a profound bias (i.e., no coverage of about 30% of the genome) in sequencing the extremely low GC (19.3%), high AT genome of *Plasmodium falciparum*. Moreover, slightly more single nucleotide polymorphisms (SNPs, i.e., variants) could be called (82%) from the PGM

data than for Illumina data (68–76%), but at the cost of higher false positives of SNPs and raw error rate (1.8%) than the latter (0.4%). In addition, the high error rate (13%) of long (~1,500 bases) PacBio reads hindered its detection of SNPs, although these long reads are useful for scaffolding *de novo* assemblies.

Overall, while the Illumina HiSeq platform leads today's sequencing market due to its surprisingly high-throughput per unit price, other platforms may be chosen out of tradeoffs between data cost effectiveness and other factors to consider, such as read length, equipment price or simplicity, sequencing speed, and efficiency in scaffolding. For instance, the high-quality short metagenomic reads generated by Illumina platforms, via co-assembly, could be used to connect and rectify the low-quality long reads obtained by the PacBio platform from the same sample.

3. Molecular Experiments and Sequencing

3.1. *Extraction of nucleic acids*

The quality and representation of RNA or DNA extracted from biological samples in the anaerobic reactors is another key factor affecting the reliability of obtained sequence data. The amount of starting samples for nucleic acid extraction should be quantified in terms of cell number, mass of sludge (or sediment/soil), or volume of slurry. Such information is crucial for absolute quantification of specific microorganisms, such as methanogens. RNA or DNA replicates must be taken to evaluate the reproducibility of biological samples from the same reactor, thus enabling an effective comparison across reactors. Technical replicates in DNA/RNA extraction are also an issue to consider in the experiment design. For details on the extraction of nucleic acids, readers may refer to the book chapter, "Application of Molecular Methods for Anaerobic Technology" (Fang 2010).

No matter what method has been used, the extraction protocols should be kept the same throughout a study. The use of commercial kits may increase the reproducibility of extracted nucleic acid, allowing for a more equal comparison between independent studies. The FastDNA® Spin Kit for Soil is a popular commercial kit for DNA extraction from aerobic activated sludge (Zhang *et al.* 2012; Guo and Zhang 2013) and anaerobic sludge (Martín *et al.* 2006). For RNA isolation, TRIzol is commonly applied (Jahn *et al.* 2008). Readers may refer to the manufacturer's instructions for the detailed procedures.

3.2. *Selection of biomarker*

The 16S rRNA gene is the most commonly used phylogenetic biomarker for fingerprinting of microbial communities because of its characteristics, such as essential and unchanged function, high conservation and abundance (i.e., ubiquitous in almost all prokaryotes, and usually in numerous copies), various regions at different conservation levels, relatively long length (~1,500 nucleotides), and widely believed unlikelihood of horizontal gene transfer. Other rRNA genes, including the 5S rRNA gene and 23S rRNA gene, have also been used. However, the 5S rRNA gene is too short (i.e., ~120 nucleotides) to give solid identification. The length of the 23S rRNA gene is much longer

(~2,900 nucleotides), but its application has been largely restrained by the lack of reference databases and the increased cost for extended length.

The 16S rRNA gene is composed of conserved and variable regions based on their genetic stability. According to differences in sequences in these regions, organisms can be distinguished and classified into different taxonomy ranks from domain down to the genus/species levels. However, while the phylogenetic properties of the 16S rRNA gene and the large amount of reference sequences publicly available favor its wide application, the disadvantages and pitfalls of using the 16S rRNA gene as a molecular marker should not be overlooked. For instance, intragenomic heterogeneity in multiple 16S rRNA genes may lead to the identification of multiple ribotypes for a single organism (Case *et al.* 2007). Moreover, even ignoring intragenomic heterogeneity, sequence clustering at 99% identity may result in diverse ribotype microdiversity (Acinas *et al.* 2004). For instance, even a single ribotype of 16S rRNA gene (100% identity) of a natural *Vibrio splendidus* population incorporates significant genotypic variations (Thompson *et al.* 2005). Overall, the presence of intragenomic heterogeneity and ribotype microdiversity of the 16S rRNA gene reveals that it may lack resolution at the species levels for some microbial groups. Another limitation lies in the difficulty in using 16S rRNA gene for microbial quantification in terms of the cell number, because of the great variability in the copy number of 16S rRNA genes (e.g., from one copy in *Nitrosomonas sp.* AL2012 to 15 copies in *Clostridium paradoxum* DSM 7308) and little information about them in different bacteria (Zhang *et al.* 2009a). In addition, some researchers presented evidences for horizontal gene transfer of the 16S rRNA gene (Kitahara and Miyazaki 2013; Schouls *et al.* 2003; Badger *et al.* 2005), which may shadow the integrity of the 16S rRNA gene as “gold standard” for microbial fingerprinting.

To make up for those inadequacies of the 16S rRNA gene, database resources, such as Ribosomal RNA Operon Copy Number Database (*rrndb*), can be used to transform 16S rRNA gene sequence-based quantification into cell-based estimation by normalizing the sequence abundance of each taxon against its 16S copy number (Zhang *et al.* 2009a). Other molecular marker genes, such as the single-copy *rpoB*, 23S rRNA gene, *pmoA* (for methanotrophic bacteria), *mcrA* (for methanogens), formyltetrahydrofolate synthetase (for homo-acetogens), can also be used for microbial fingerprinting. The 23S rRNA gene (about 67,064 sequences in August 2014, 4.5% of 16S rRNA sequences in SILVA databases) may get more popular as it is much longer than the 16S rRNA gene and may resolve very closely related species. Moreover, other marker genes, such as *rpoB*, rRNA internal transcribed spacer, and functional genes, have also been used as auxiliary microbial fingerprinting methods, especially in the case of profiling bacteria or archaea with identical or near identical 16S rRNA genes but divergent genomes and ecophysologies (Jaspers and Overmann 2004).

3.3. Polymerase chain reaction (PCR)

For many HTS-based metagenomics methods introduced in this chapter, PCR amplification is an initial step following nucleic acid extraction to amplify target fragments of DNA or RNA, respectively. The PCR cycle proceeds in three steps; namely denaturation, annealing, and primer extension.

The crucial prerequisite of a PCR reaction is the selection of primer sets that can effectively target genes of interest with high coverage, enough specificity, and less bias (e.g., preferential amplification). These primers may be either self-designed based on the alignments of relevant DNA sequences, or directly adopted from literatures. Currently, popular primer/probe design tools include online designing tools, e.g., Primer3Plus (Untergasser *et al.* 2007), NCBI's Primer-BLAST (Ye *et al.* 2012), and locally installed software, e.g., Primer3 (Untergasser *et al.* 2012), ProSig (<http://public.lanl.gov/jgans/>), ARB (Ludwig *et al.* 2004), and Primer Prospector (Walters *et al.* 2011). The coverage of designed primers can be evaluated *in silico* using the online ProbeMatch at the Ribosomal Database Project (RDP) website (Cole *et al.* 2005). Ideally, designed primers must be both specific (to the group in question) and complete (being complementary to sequences in all taxa within that group). In addition, the rich resources of reference sequences in the databases, such as GenBank, RDP, and ARB, are available to support the design of PCR primers that target the whole microbial community or specific microbial groups.

Traditionally, *Bacteria*- and *Archaea*-specific primer sets, i.e. EUB8F/UNIV1492R for *Bacteria* (Lane 1991) and A109F/A934B for *Archaea* (Grosskopf *et al.* 1998; Stahl and Amann 1991), were widely used for the investigation of microbial diversity of anaerobic communities. For HTS-based microbial fingerprinting, many primer sets have been designed to target different hypervariable regions of 16S rRNA genes and to yield fragments of varied length and coverage for bacteria and/or archaea that can be fully covered and sequenced on currently prevalent NGS platforms (Table 5.1). Among them, 515F/806R is optimized (ArBa515F/ArBa806R) as nearly universal to both *Archaea* and *Bacteria* (Walters *et al.* 2011) and has succeeded in amplifying numerous environmental samples (Barberán *et al.* 2011; Fierer *et al.* 2008; Caporaso *et al.* 2011). This primer set, which targets approximately 290 bp of the V4 hypervariable region, can be fully covered by the Illumina Miseq (e.g., PE200×2); thus, it is quite suitable for monitoring bacterial and archaeal populations in anaerobic digesters. However, 16S rRNA gene fragments amplified by 515F/806R may be too short to assign some microbial groups to lower taxonomic ranks, such as species level. Noteworthy, 341F/806R (U-V3V4) can simultaneously match >99% bacterial and archaeal sequences in the RDP database (Table 5.1). The length of yielded fragments (~465 bp) can provide higher taxonomic resolution and be fully covered and sequenced on the GS junior and GS FLX systems of 454 Life Sciences. By contrast, 27F/518R merely matches a small proportion (21.6%) of bacterial sequences, although it has been used for bacterial fingerprinting in many studies. Other universal primer sets, such as 926F/1392R, 787F/1391R, and ArBa515F/909R, are also applied to obtain relatively long 16S fragments (up to 600 nucleotides), but at the expense of reduced coverage. In particular, for the amplification of bacterial DNAs in activated sludge, biased diversity metrics of seven primer sets targeting V1 to V9 hypervariable regions of the 16S rRNA gene had been evaluated (Cai *et al.* 2013), and V3/V4 regions were suggested due to greater diversity coverage. However, V1 and V2 regions were recommended to identify functional bacterial groups (e.g., nutrient-removing bacteria), as they provide more consistent taxonomic assignment for a relatively wide range of genera than other considered regions (Guo *et al.* 2013).

Biases introduced into PCR amplification steps are inevitable, no matter what kind of gene is targeted. Thus, full awareness of what/how/when biases are generated is beneficial to minimizing the adverse effects to downstream analysis. In general, a low concentration of DNA template and a high number of PCR cycle are known to raise PCR bias. To minimize such bias, it is preferential to start with uniform and relatively high concentrations of DNA template for all samples, to minimize the number of PCR cycles,

Table 5.1 Oligonucleotide primers to amplify various hypervariable regions of the 16S rRNA gene. The primer coverage was evaluated by RDP ProbeMatch on Jan 20, 2015. Only good-quality 16S rRNA gene sequences $\geq 1,200$ bp in length including 1,408,156 bacterial and 25,760 archaeal sequences are used as reference for probe match, allowing two or zero (percentages in brackets) mismatches. Abbreviations: U, Universal; B, Bacteria; A, Archaea; Ref, References.

Name and sequences of primer set	Regions	Length (bp)	Coverage (%) by ProbeMatch		Ref
			B	A	
ArBa515F: 5'-(TA)GTGCCAGCMGCCGCGGTAA-3' ArBa806R: 5'-(AC)GGACTACVSGGGTATCTAAT-3'	U-V4	~290	99.1 (5.6)	98.7 (53.0)	(1)
ArBa515F: 5'-(TA)GTGCCAGCMGCCGCGGTAA-3' ArBa909R: 5'-TTTCAGYCTTGCGRCCGTAC-3'	U-V4V5	~390	83.9 (7.0)	69.0 (36.2)	(2)
926F: 5'-AAACTYAAAKGAATTGRCGG-3' 1392R: 5'-ACGGGCGGTGTGTRC-3'	U-V6V8	~460	56.5 (47.8)	58.6 (46.8)	(3)
341F: 5'-CCTAYGGRBGCASCAG-3' 806R: 5'-GGACTACNNGGGTATCTAAT-3'	U-V3V4	~465	99.4 (84.3)	99.2 (87.2)	(4)
787F: 5'-ATTAGAWACCCBGGTAGTC-3' 1391R: 5'-GACGGGCRGTGWGTRCA-3'	U-V5V8	~600	56.0 (47.7)	56.9 (47.0)	(5)
ArBa515F: 5'-GTGCCAGCMGCCGCGGTAA-3' Ba806R: 5'-GGACTACHVGGGTWCTAAT-3'	B-V4	~290	99.4 (87.2)	98.7 (53.6)	(6)
338F: 5'-ACTCCTACGGAGGCAGCAG-3' 802R: 5'-TACNVGGGTATCTAATCC-3'	B-V3V4	~465	98.5 (84.3)	0.0 (0.0)	(7)
27F: 5'-GAGTTTGATCMTGGCTCAG-3' 518R: 5'-WTTACCGCGGCTGCTGG-3'	B-V1V3	~490	21.6 (16.7)	0.0 (0.0)	(8)
ArBa515F: 5'-GTGCCAGCMGCCGCGGTAA-3' Ar806R: 5'-GGACTACNSGGGTMTCTAAT-3'	A-V4	~290	99.1 (6.6)	0.0 (53.8)	(9)
A571F: 5'-GCYTAAAGSRICCGTAGC-3' UA1204R: 5'-TTMGGGGCATRCIKACCT-3'	A-V4V7	~630	0.2 (0.0)	96.1 (54.2)	(10)
340F: 5'-CCCTAYGGGGYGCASCAG-3' 1000R: 5'-GGCCATGCACYWCYTCTC-3'	A-V3V6	~660	0.1 (0.0)	97.7 (84.5)	(11)

(1) (Barberán *et al.* 2011; Fierer *et al.* 2008); (2) (Wang and Qian 2009; Ye *et al.* 2013); (3) (Vanwonterghem *et al.* 2014; Baker *et al.* 2003); (4) (Yu *et al.* 2005; Sundberg *et al.* 2013); (5) (Tiao *et al.* 2012; Tyson *et al.* 2004); (6) (Kozich *et al.* 2013); (7) (Claesson *et al.* 2010; Ju *et al.* 2013); (8) (Ibarbalz *et al.* 2013; Ha *et al.* 2012); (9) (Porat *et al.* 2010); (10) (Baker *et al.* 2003); (11) (Gantner *et al.* 2011).

and to perform pooled multiple (e.g., triplicate) PCRs for each sample. It has also been known that low annealing temperatures allow for more mismatches and increases the diversity of PCR products, but it might also generate undesirable PCR products (so called “non-specific amplification”). Moreover, PCR artifacts, such as chimeras, are often formed owing to a number of mechanisms, such as partially reverse transcribed DNA, premature PCR products acting as primers in a subsequent PCR cycle, or the presence of partial fragments of rDNA in DNA extracts (Amann *et al.* 1995). Among them, the majority of chimeras are believed to be caused by incomplete template extension. It has also been noted that chimeras are rare with shot-gun sequencing (e.g., DNA-based metagenome HTS), but are quite common in amplicons-based sequencing, where closely related sequences are amplified (e.g., 16S rRNA gene amplicons HTS). In addition, although multiplex PCR can be conducted to simultaneously amplify two or more target sequences with great efficiencies (saving time and efforts), the presence of multiple primer pairs may give rise to the formation of primer dimers, preferential amplification of undesirable products, and poor sensitivity (Markoulatos *et al.* 2002). Limitations also lie in differential optimal annealing temperatures required by individual primers when they are combined in a single-tube multiplex PCR reaction.

3.4. Library construction and sequencing

Library construction typically consists of several steps to prepare the target nucleic acid, DNA, or RNA into a form that is compatible with the sequencing platforms to be used, including fragmentation of target sequences into a desired length, conversion of sequence fragments into double-stranded DNAs, attachment of sequencing adaptors to the ends of target fragments, and quantification of yielded library products for final sequencing (Head *et al.* 2013). While various strategies have been developed for library preparation on different NGS platforms, they share similar principles, considerations, or challenges, so as to construct high-quality libraries with minimized biases.

The major concerns in library construction are to maximize complexity and minimize biases (e.g., preferential amplification, batch effects, adaptor dimers) introduced by PCR or other amplification-based cloning, especially for those libraries with limited DNA/RNA from a small number of cells. High library complexity, as usually manifested by low duplicate read rates in the sequencing data, is believed to reflect high fidelity of the original complexity of nucleic acid samples, thus suggesting less bias introduced into an experimental design; whereas extensive PCR amplification gives rise to preferential amplification of different sequences, as typically observed nowadays for single-cell DNA or RNA sequencing, leading to the generation of biased resulting data (Head *et al.* 2013). The most direct way to increase library complexity should be less amplification and more starting genetic materials. For RNA sequencing, it has also been demonstrated that real biological duplicate reads can be differentiated from duplicate PCR-derived artifacts by a commercial kit of digital sequencing that incorporates multiple combinations of indexed adaptors (Shiroguchi *et al.* 2012). Readers may refer to Head *et al.* (2013) and Kucuktas *et al.* (2010) for more details on the library construction for NGS applications.

Another issue in library construction is the determination of insert size of the library, which may depend on both the practical sequencing application and the limitations of different NGS platforms. For example, the 2×250 paired-end reads can be run on a sequencing library of 16S rRNA gene amplicons with different lengths to match multiple adjacent or segregated hypervariable regions, and with or without overlapping paired reads. Readers may refer to the manufacturers' guidelines for technically feasible library inset size and detailed sequencing procedures on various NGS instruments.

4. Applications of Metagenomics in Anaerobic Technology Studies

The application of metagenomics helps to elucidate microbial community structure and function in anaerobic digesters or bioreactors, especially for revealing uncultured microorganisms or novel genes and enzymes. The first application of HTS-based metagenomics in anaerobic technology studies dates back to 2005 and involves the identification of a novel alcohol/aldehyde dehydrogenase from metagenomic DNA libraries of an anaerobic digester (Norfolk, UK) by 454 pyrosequencing, followed by the successful validation of its enzyme activity in ethanol degradation (Wexler *et al.* 2005). Later, the rapidly decreasing sequencing cost and the prevalence of NGS technologies other than 454 pyrosequencing allowed for more extensive applications of HTS-based metagenomics approaches, including 16S rRNA gene amplicons sequencing and metagenome for novel microbial insights into the anaerobic digestion processes operated for bioenergy production (e.g., methane, biohydrogen, bioethanol, fatty acids) and/or pollution control (e.g., refractory compounds, bacterial pathogens, antibiotics resistance genes). A few pioneering studies even applied metatranscriptome (Xia *et al.* 2014; Zakrzewski *et al.* 2012) or metaproteome (Lü *et al.* 2013; Kohrs *et al.* 2013; Hanreich *et al.* 2013; Hanreich *et al.* 2012; Abram *et al.* 2011; Heyer *et al.* 2013; Wu *et al.* 2013) to gain some preliminary insights into the gene expression and active enzymes in anaerobic digestion processes.

4.1. Microbial community structure

HTS-based metagenomic approaches have provided deeper and more thorough understandings of the structure of microbial communities involved in bioenergy (e.g., methane, hydrogen) production from large varieties of organic wastes (e.g., sewage sludge, manure, food residues, cellulose, vinasses, agricultural wastes) or high-strength wastewater (e.g., municipal, brewery, leachate, molasses, food and distillery wastewater) in anaerobic digesters of various designs, including Upflow Anaerobic Sludge Bed (UASB) (Kubota *et al.* 2014; Liu *et al.* 2012; Kim *et al.* 2012), Expanded Granule Sludge Bed (Werner *et al.* 2011), Anaerobic Dynamics Membrane Bioreactor (AnDMBR) (Yu *et al.* 2012; Ma *et al.* 2013; Smith *et al.* 2013; Xie *et al.* 2014; Yu *et al.* 2014), Continuously Stirring Tank Reactor (CSTR) (Ju and Zhang 2014; Lee *et al.* 2014a; Smith *et al.* 2014; Lee *et al.* 2014b; Wang *et al.* 2013; Xia *et al.* 2013), Plug-flow Anaerobic Digester (St-Pierre and Wright 2014; 2013), etc.

4.1.1. *Methanogenic bioreactors*

4.1.1.1. Microbial diversity and composition

HTS-based metagenomic approaches support that while the microbial community is mainly composed of *Bacteria* and *Archaea*, *Bacteria* generally dominate over *Archaea* in terms of both sequence abundance and diversity in full-scale methanogenic bioreactors. Sundberg *et al.* (2013) investigated microbial richness in 21 full-scale mesophilic (35–40 °C) or thermophilic (51–55 °C) biogas digesters treating sewage sludge or co-digested wastes from slaughter houses, restaurants, households, etc. Analysis of over 160,000 16S rRNA gene sequences of V3 and V4 regions from 454 pyrosequencing showed that bacterial communities for each digester were widely distributed into 5–10 phyla, 7–20 classes, and 14–65 genera, accounting for 80–100% (of 16S) of total sequences, whereas archaeal communities were merely composed of one phyla, 1–3 classes, and 1–7 genera, occupying no more than 20% of total sequences. The higher relative abundance and diversity of bacteria than archaea in methanogenic digesters is in agreement with the results of other recent HTS-based studies (Ju and Zhang 2014; Yang *et al.* 2014b; Wirth *et al.* 2012) and earlier studies using traditional molecular techniques (Godon *et al.* 1997; Regueiro *et al.* 2012; Cardinali-Rezende *et al.* 2009). Overall, the larger throughput and higher coverage of HTS-based approaches make them more robust and powerful in resolving and quantifying complex microbial diversity in anaerobic bioreactors, compared with molecular techniques such as clone library and DGGE, especially for identifying those rare and/or novel species (Zhang *et al.* 2009b; Kröber *et al.* 2009; Tuan *et al.* 2014).

Although it is generally agreed that *Bacteria* dominated over *Archaea* in methanogenic anaerobic digesters, the proportions between *Bacteria* and *Archaea* in methanogenic digesters measured previously using traditional molecular techniques or chemical composition analysis greatly disagree with one another. The archaeal populations were estimated as 4–8% of the total microbial community in laboratory-scale mesophilic (37 °C) biogas reactors fed with mixed cellulose and egg albumin by membrane phospholipids analysis (Sundh *et al.* 2003), 6% in a CSTR-fed with fodder beet silage by amplified rDNA restriction analysis (ARDRA), 6% in a thermophilic (50–55 °C) plug-flow reactor treating biowaste sludge (Goberna *et al.* 2009) and 14% in a mesophilic (35 °C) household waste digester (Cardinali-Rezende *et al.* 2009) both by PCR-cloning, 5% to 48% in a laboratory-scale thermophilic synthetic solid waste digester (Montero *et al.* 2008), and 0% to 40% in six full-scale anaerobic digesters (one UASB and five CSTR fed with various substrates) both by FISH (Regueiro *et al.* 2012), etc. Generally, 16S rRNA gene sequences from HTS-based amplicons dataset (PCR-based) or metagenome (non-PCR based) provide relatively stable and conserved estimations of archaeal populations, compared with cell-based semi-quantitation by FISH. 454 pyrosequencing of 16S rRNA gene amplicons showed that the proportions of *Archaea* in total 16S were 2–20% in six mesophilic (28–35 °C) and one thermophilic (51–53 °C) full-scale sewage sludge CSTR digesters, and 0–7% in ten mesophilic (37–40 °C) and four thermophilic (51–55 °C) full-scale CSTRs co-digesting various wastes (Sundberg *et al.* 2013). Analysis of 16S rRNA gene fragments in metagenomes found that the archaeal

proportions in total 16S were 3–5% in two saline and fresh sewage sludge CSTR digesters (Yang *et al.* 2014b), <4% in methanogenic enrichments fed with straw and hay (Hanreich *et al.* 2013), 12% in methanogenic biofilm of a two-phase thermophilic leached biogas system (Rademacher *et al.* 2012), 9–13% in two methanogenic phenol-degrading reactors at 20 °C and 37 °C (Ju and Zhang 2014), etc. Notably, the percentage of archaeal populations in sewage sludge full-scale digesters (2–20%, averaged 10%, n=7) was generally much higher than in full-scale reactors co-digesting various organic wastes (<4.4%, averaged 1.8%, n=14) (Sundberg *et al.* 2013).

4.1.1.2. Bacteria

The bacterial 16S rRNA gene sequences obtained from 21 full-scale anaerobic digesters by Sundberg *et al.* (2013) were dominated by *Firmicutes* (53%, mainly *Clostridia*) and *Bacteroidetes* (13%), followed by *Proteobacteria* and *Chloroflexi*. This result is well in agreement with observations by other researchers based on 16S rRNA HTS data of nine full-scale granulated sludge reactors treating brewery wastewater (Werner *et al.* 2011), six full-scale reactors treating sewage sludge (Lee *et al.* 2012), three mesophilic dairy manure biogas digesters (St-Pierre and Wright 2014; 2013), a mesophilic (41 °C) agricultural biogas reactor fed with maize silage (63%), green rye (35%) and chicken manure (2%) (Schlüter *et al.* 2008), and a thermophilic anaerobic swine manure digester (Tuan *et al.* 2014), where *Proteobacteria*, *Bacteroidetes*, *Firmicutes* (mainly *Clostridia*), and *Chloroflexi* were congruously tagged as dominant bacterial phyla. The dominance of these four phyla in anaerobic digesters, as revealed by metagenomics studies, is well in agreement with the previous studies by cloning and Sanger sequencing (Nelson *et al.* 2011; Riviere *et al.* 2009), although the rank orders of their abundance may be different. In addition, 16S rRNA gene HTS data also implicated the presence of less abundant (generally ~1% in average) but cosmopolitan (i.e., frequently detected) phyla in anaerobic digesters, typically including *Actinobacteria*, *Spirochetes*, and *Synergistetes* (Sundberg *et al.* 2013; Werner *et al.* 2011; St-Pierre and Wright 2014; Lee *et al.* 2012; Ye and Zhang 2013).

4.1.1.3. Archaea

The archaeal populations in methanogenic digesters are known to be predominated by methanogens (i.e., methane-producing *Archaea*) in the phylum *Euryarchaeota* (Sundberg *et al.* 2013). HTS-based 16S rRNA gene sequencing by different researchers reveals highly variable compositions of methanogens in different digesters, or within one single digester across temporal scales.

It has been found that methanogen composition is differentiated by the nature of substrates. The ten codigesters disposing various organic wastes examined by Sundberg *et al.* (2013) were dominated by hydrogenotrophic methagens, mainly *Methanoculleus* and *Methanobacterium*, whereas the seven sewage sludge digesters were dominated by acetoclastic methanogen *Methanosaeta*, which was totally absent from those co-digesters. St-Pierre and Wright (2013) examined methanogen populations in three (two plug-flow and one complete mix design) full-scale mesophilic anaerobic manure digesters

(Vermont, USA) treating dairy manure. There were 25–269 species-level OTUs for each sample (V1–V3 hypervariable regions). The two plug-flow anaerobic digesters were predominated (98.5–99.7%) by an acetoclastic methanogen, while the complete mix reactor was occupied by diverse acetoclastic and hydrogenotrophic methanogens affiliated to four distinct phylogenetic groups: namely, *Methanomicrobiales*, *Methanosarcinales*, *Methanoplasmatales*, and *Methanobacteriales*. Lee *et al.* (2014a) investigated the methanogens in four methanogenic digesters fed with food waste-recycling wastewater and found that the plug-flow thermophilic digester was dominated by *Methanoculleus* (97%), the thermophilic CSTR by either hydrogenotrophic *Methanoculleus* (95%) or *Methanothermobacter* (98%, when pH increase), whereas the UASB mesophilic digester by acetoclastic *Methanosaeta* (87.2%), and mesophilic CSTR by hydrogenotrophic *Methanoculleus* (75%) or acetoclastic *Methanosaeta* (63%, with low acetate concentrations of 0.1 g·l⁻¹).

4.1.1.4. Environmental and operational impacts on community structure

Despite a similar microbial composition at the domain and phylum levels among different anaerobic digesters, the microbial diversity and composition, however, are far more differentiated at lower taxonomic ranks, e.g., from class to species levels, which may be governed by multiple factors, including nature of substrates (e.g., codigestion), digester design, operational parameters (e.g., temperature, pH), etc.

The sources (or nature) of substrates (sewage sludge vs. codigested organic wastes) and temperature (mesophilic vs. thermophilic) are found as deterministic factors shaping the microbial richness and composition in 21 full-scale anaerobic digesters studied by Sundberg *et al.* (2013). Richness analysis of species-level OTUs (clustered at 3% dissimilarity; 1,760 random sequences for each digester) showed that the sewage sludge digesters had higher microbial richness (OTU: 329±46, Chao1: 707±145; n=7), compared with mesophilic (OTU: 296±58, Chao1: 536±123; n=10) or thermophilic (OTU: 248±45, Chao1: 437±101; n=4) organic wastes codigesters. Principal Component Analysis of the microbial composition indicated that the digesters were clearly separated by substrate type and operation temperature. At class and order levels, heterotrophic classes, such as *Clostridia* and *Sphingobacteria* (phylum *Bacteroidetes*), and two methanogenic orders, including *Methanobacteriales* and *Methanomicrobiales*, were more represented in organic wastes co-digesters, while another two classes, including *Deltaproteobacteria* and *Bacteroidetes* (phylum *Bacteroidetes*), and one methanogenic order, *Methanosarcinales* were more abundant in sewage sludge digesters. Noteworthy, class *Bacteroidetes* were nearly absent from those thermophilic organic wastes digesters, whereas phylum *Thermotoga* were almost only abundant (0.1–48%, averaged 20%) in thermophilic digesters.

The effects of substrate type and temperature on microbial diversity and composition in anaerobic digestion process have also been documented by other researchers using either HTS-based or traditional molecular techniques. Regueiro *et al.* (2012) investigated microbial structure in seven anaerobic digesters treating various substrates using DGGE and cluster analysis, and showed that reactors treating similar substrates (e.g., dairy and dairy-fish wastes; sugar and yeast) were grouped together. Lee *et al.* (2012) compared

seven full-scale anaerobic digesters (six fed with sewage sludge and one with night soil) using 16S rRNA gene 454 pyrosequencing (V1, V2, and V3 regions) and Non-metric Multidimensional Scaling Analysis, and demonstrated that the bacterial structure was mainly influenced by temperature. For example, more sequences were affiliated with *Firmicutes* in the thermophilic ($24.3 \pm 10.5\%$) than mesophilic ($12.6 \pm 5.0\%$) digesters ($P < 0.01$, ANOVA), whereas more sequences were assigned to *Bacteroidetes* ($22.0 \pm 9.2\%$) in the mesophilic than thermophilic digesters ($17.0 \pm 9.2\%$) ($P < 0.05$, ANOVA). Moreover, the variability of bacterial community was larger across digesters than within one digester in time scales (in six months), as had also been observed in another study (Werner *et al.* 2011). Carballa *et al.* (2011) used 16S rRNA gene-based DGGE and terminal restriction fragment length polymorphism and clustering analysis to compare bacterial and archaeal communities in two mesophilic (34 ± 2 °C) and two thermophilic (53 ± 2 °C) anaerobic digesters treating sewage diluted kitchen wastes and demonstrated a clear segregation of them by temperature.

Apart from substrate type and operational temperature, other factors such as reactor design and configuration have also been reported to affect microbial composition in methanogenic digesters. Leclerc *et al.* (2004) compared the archaeal structure in 44 anaerobic digesters with various configurations and digesting substrates using conformation polymorphism analysis and PCR cloning, and it was concluded that the applied reactor design (e.g., UASB, CSTR, and fixed-bed) partially determined the distribution of different *Archaea*, with no clear relationship observed between the archaeal communities and digesting substrate types. For example, *Methanosaeta* sp. appeared to favor the presence in UASB reactors and was located in the inner part of UASB granules (Sekiguchi *et al.* 1999). In contrast, *Methanosarcina frigus* tended to be more significant in fixed-bed and CSTR digesters (but relatively low levels in UASB). However, Werner *et al.* (2011) detected no clear grouping patterns of bacterial communities by reactor configurations, i.e., UASB, Expanded Granule Sludge Bed, and Internal Circulation Reactor, based on >400,000 16S rRNA gene pyrotags for 112 samples collected monthly from full-scale bioreactor facilities treating brewery wastewater. Instead, they highlighted the importance of maintaining high bacterial evenness in full-scale systems operated for four years (rather than weeks), as methanogenic activity of the digesters were observed as positively correlated with their community evenness. Lee *et al.* (2013) found no apparent difference in the bacterial community structure between single-stage (four reactors) and two-stage (three reactors) digesters, based on 16S rRNA gene pyrosequencing.

In addition, the impacts of pre-treatment methods on the microbial composition and functional performance in methanogenic digesters have also been evaluated. Zhang *et al.* (2009b) used focused-pulse sludge pre-treatment to increase the methane production rate (by 15–30%). Analysis of 36,797 pyrotags of the V6 region of bacterial 16S rRNA genes indicated that the pre-treatment resulted in a markedly increased bacterial diversity by ~250 folds (over 2,000 phylotypes), higher abundances of *Ruminococcus* (cellulose fermentation), *Chloroflexi* (biomass-derived organic carbon scavenger), and *Treponema* (homo-acetogenesis), as well as a transition of methanogens from H_2 -oxidizing *Methanoculleus* to acetate-cleaving *Methanosaeta*. Lee *et al.* (2013) discovered that the

abundance and diversity of *Spirochaetes* in anaerobic digesters were mainly affected by alkalinity and temperature, based on 16S rRNA pyrosequencing, qPCR and partial correlation analysis.

4.1.2. Biohydrogen bioreactors

The microbial community in hydrogen-producing acidogenic sludge is generally regarded as relatively simple, compared with those contributing to methane fermentation, where multiple biological processes are involved and typically fulfilled by difference microbial groups, such as hydrolyzers, acidogens, acetogens, and methanogens (Fang *et al.* 2002; Fang and Liu 2002; Chojnacka *et al.* 2011). The major contributing microorganisms involved in hydrogen production in anaerobic digesters, as revealed by HTS-based 16S rRNA gene sequencing, are limited to relatively narrow taxonomic divisions, including *Thermoanaerobacter* (49%), *Clostridium* (30%), and *Acetivibrio* (21%) in microcrystalline cellulose-degrading thermophilic (50–60 °C) enrichments (Carver *et al.* 2012), *Ruminococcaceae* (52–74%), *Clostridiaceae* (13–38%) and *Prevotellaceae* (6–9%) in two glucose-degrading UASB reactors (Liu *et al.* 2012), *Clostridiaceae* and *Leuconostocaceae* in stone biofilm of a packed bed reactor fed with molasses (Chojnacka *et al.* 2011), *Clostridium* (71–84%) and *Sporolactobacillus* (14–28%) in ambient batch reactors co-digesting sucrose and cattle manure (Perera and Nirmalakhandan 2010), *Thermoanaerobacter* (58–93%) and *Clostridium* (2–20%) in 35 and 55 °C batch reactors fed with pretreated sludge (Zheng *et al.* 2014). Therefore, members of phylum *Firmicutes* are typically the most predominant players in both mesophilic (mainly order *Clostridiales*) and thermophilic (mainly order *Thermoanaerobacteriales*) hydrogen-producing anaerobic reactors, as has also been widely documented by previous studies on biohydrogen production using anaerobic technologies and molecular techniques (Fang *et al.* 2003; Kim *et al.* 2004; Li and Fang 2007; Kim *et al.* 2006).

Many operational attempts have been made to increase hydrogen production, and enrichment or retention of hydrogen-producing bacteria in anaerobic digestion processes by physical (e.g., heat treatment), chemical (pH adjustment by acid or alkaline addition), or biological treatment of the feed and/or seed sludge, co-digestion (e.g., manure, food wastes), addition of microbial carriers (e.g., activated carbon, carbon nanotubes), etc. Besides the traditionally used physical or chemical pre-treatment methods, a novel biological pre-treatment method, termed as “thermophilic bacteria sludge pre-treatment”, has been recently proposed by using *Geobacillus stearothermophilus* to promote hydrogen production from wasted sewage sludge by selectively enriching hydrogen-producing bacteria (Zheng *et al.* 2014). 454 pyrosequencing analysis of bacterial 16S rRNA genes showed that bacterial richness and evenness were sharply reduced and a high proportion of hydrogen-producing *Thermoanaerobacterium* (58.61–93.75%) colonized the two digesters fed with pre-treated sludge, especially under thermophilic conditions (93.75%).

The effect of the addition of microbial carriers and co-substrates on the hydrogen-producing microbial community has also been investigated. Liu *et al.* (2012) evaluated the effects of the addition of carbon nanotubes and activated carbon particles on the biohydrogen production and microbial community structure in two glucose-fed UASB

reactors (operated at room temperature). Pyrosequencing of 16S rRNA gene amplicons of V1, V2, and V3 regions showed that both UASB reactors were predominated by *Clostridia*, revealing the selective enrichment of hydrogen-producing bacteria induced by the addition of carbon-based carriers to UASB reactor. The carbon nanotubes provided better surface adhesion for retention of a higher proportion of cellulose-degrading and acetate- and ethanol-producing *Ruminococcaceae* (74%) over butyrate-producing *Clostridiaceae* (13%) in UASB, compared with activated carbon (52% vs. 38%), resulting in a quicker startup (seven days vs. 21 days to form visible granules) and better performance of hydrogen production (2.45 vs. 1.42 mol·mol⁻¹ glucose of maximal hydrogen yield). Perera and Nirmalakhandan (2010) enhanced fermentative hydrogen production (by ~10%) from sucrose in ambient batch reactors by supplementing with co-digesting cattle manure, which provided hydrogen-producing seed, nutrients, and buffering capacity. 454 pyrosequencing suggested the dominance of *Clostridium pasteurianum* (71–84%), *Sporolactobacillus laevolacticus* (14–28%) and other members of *Firmicutes* (2%).

4.1.3. Novel microorganisms

The most prominent advantage of metagenomic approaches over traditional molecular techniques lies in its power in revealing uncharacterized or novel microorganisms. Metagenomics studies in the past three years indicate that anaerobic bioreactors shelter tremendous yet-to-be-cultured or novel microorganisms (Ju and Zhang 2014; Sundberg *et al.* 2013; Lee *et al.* 2012; Jang *et al.* 2014). One striking example is the recently successful characterization of a novel archaeal lineage, ANME-2d, and the identification of its roles in nitrate-driven anaerobic methane oxidation in a lab-scale anaerobic reactor using multiple HTS-based omics technologies (Haroon *et al.* 2013).

16S rRNA gene amplicons or metagenome-based studies unravel extremely high diversity of novel microorganisms in anaerobic digestion processes. Lee *et al.* (2012) discovered that unclassified phyla had surprisingly high and changeable proportions with time (from 5.2–71.8%) in 40 digester sludge samples collected monthly from seven full-scale anaerobic digesters over six months. Phylogenetic analysis indicated that novel OTUs of unclassified phyla were mostly affiliated with *Spirochaetes* and *Firmicutes*. The relative proportion of *Spirochaetes* in total 16S rRNA gene sequences for each sample varied from 1.3% to 30.0% in different anaerobic digesters (Lee *et al.* 2013). Known members within this phyla have been reported to play a role in degrading cellulose or other polysaccharides (Leschine *et al.* 2006) (e.g., genera *Treponema* and *Spirochaeta*), or H₂/CO₂-reductive homo-acetogenesis (e.g., *Treponema primitia*) (Graber and Breznak 2005). Lee *et al.* (2013) proposed that *Spirochaetes* might fulfill syntrophic acetate oxidation in the tested anaerobic reactors where hydrogen partial pressure was kept low (<20 Pa). Ju and Zhang (2014) discovered the dominance of uncultured W22 (belonging to candidate division WWE1, 25%), *Sulfurovum*-like uncultured epsilon-proteobacteria (35%), and other uncultured bacteria (e.g., phylum OP8, *Chloroflexi* T78 clone) in the 16S rRNA gene pyrotags retrieved from two phenol-degrading metagenomic reactors. Pelletier and its co-authors suggested that WWE1 were probably syntrophic amino acid

metabolizers because of the presence of genes that are typically involved in amino acid fermentation in the reconstructed genome of its member *Candidatus Cloacamonas* str. Evry (Pelletier *et al.* 2008). A recent study based on 16S rRNA gene-based stable isotope probing, FISH, and secondary ion mass spectrometry-*in situ* hybridization revealed that WWE1 might play a role in either extracellular cellulose hydrolysis processes and/or in the uptake of fermentation products (Limam *et al.* 2014).

Other studies also present evidence of a high proportion of unclassified bacteria in anaerobic digesters, although their roles in the digester are unknown. St-Pierre and Wright (2014) documented that the majority of bacterial OTUs (5% dissimilarity, 20,366 non-chimeric 16S sequences) in three full-scale anaerobic dairy manure digesters could not be assigned, revealing the presence of novel bacterial taxonomic groups that have yet to be described. Sundberg *et al.* (2013) found that bacterial OTUs (3% dissimilarity) clustered from the unclassified *Bacteria* sequences from different digesters were not closely related, accounting for an average of 33% (12–48%) and 10% (2–41%) of the bacterial sequences in full-scale anaerobic digesters fed with sewage sludge (n=7) and various organic wastes (n=14), respectively. Guermazi *et al.* (2008) used a metagenomic approach to discover and characterize a new bacterial candidate division WWE3, which could not have been detected using traditional 16S rDNA PCR primers or FISH probes because of their unusual 16S rRNA gene. Based on the HTS data, new PCR primers and FISH probes were designed for the quantification and visualization of these novel bacteria in various environments. Overall, future studies are needed to identify the roles of novel bacteria, probably via HTS of genomic DNA and/or messenger RNA rather than merely the 16S rRNA gene.

As opposed to the widespread occurrence of novel or yet-to-be-cultured bacteria, novel archaea (e.g., methanogens) are rarely reported in anaerobic digesters by studies using 16S rRNA as the marker gene. Only one single study on methanogens employed 454 pyrosequencing to deeply sequence the methanogen-specific *mcrA* gene in an algal-fed anaerobic digester, and discovered the presence of numerous novel methanogens (Ellis *et al.* 2012). More studies are needed to confirm the presence of these novel methanogens in anaerobic digesters by amplifying and sequencing both the 16S rRNA gene and *mcrA* with more environmental samples.

4.1.4. *Syntrophs*

Syntrophy, a tightly coupled mutualistic interaction between two microorganisms that favorably exchange intermediates (Sieber *et al.* 2012), is ubiquitous and essential for efficient waste decomposition and biofuel production in anaerobic digestion processes. In anaerobic reactors, the efficient biodegradation of many organic compounds (e.g. aromatics, fatty acids) heavily relies on the syntrophic associations between two groups of microorganisms (e.g., close physical association between syntrophic bacteria and hydrogenotrophic methanogens) to prevent the accumulation of inhibitory metabolic intermediates (e.g., fatty acids and H₂), which render the biodegradation process thermodynamically unfavorable (Schink and Stams 2006). Typical syntrophs involved in fatty acids degradation in previously characterized anaerobic reactors (such as UASB) by

molecular techniques include *Syntrophobacter*, *Syntrophus*, and *Smithella* in the order *Syntrophobacterales*, and *Pelotomaculum*, *Syntrophospora*, *Syntrophomona*, and *Sporotomaculum* in the order *Clostridiales* (Zhang *et al.* 2005; Tartakovsky *et al.* 2001; Sekiguchi *et al.* 1998; Imachi *et al.* 2002; Liu *et al.* 1999; Qiu *et al.* 2003). Some *Deltaproteobacteria* species in sulfate-reducing *Desulfovibrio* (e.g., lactate and ethanol), iron-reducing *Geobacter* (e.g., acetate and ethanol) and *Pelobacter* (e.g., alcohols) were capable of syntrophic metabolism of various organic substrates (McInerney *et al.* 2008).

Recent application of metagenomics has brought deep insights into the syntrophic interactions in anaerobic reactors. A pioneering work by Haroon *et al.* (2013) using metagenome and metatranscriptome in combination with other techniques provides first genomic and metabolic evidences into the role of novel ANME-2d in nitrate-driven anaerobic methen oxidation (AOM) in a laboratory-scale anaerobic bioreactor, where nitrite generated by ANME-2d is discovered to be reduced to dinitrogen gas in syntrophy with an anaerobic ammonium-oxidizing bacterium. The syntrophic relationships established for AOM had also been well supported by previously observed close physical association between anaerobic methanotrophic (ANME) archaea, ANME-2 and ANME-3, and sulfate-reducing bacteria in biosolids (Boetius *et al.* 2000; Orphan *et al.* 2002).

The prevalence of syntrophs has also been widely detected in methanogenic reactors degrading aromatic compounds. From an anaerobic terephthalate-degrading enrichment metagenome, Lykidis *et al.* (2010) detected *Pelotomaculum*-related populations that putatively syntrophically degraded terephthalate into acetate, CO₂, and H₂, and other syntrophs, including *Syntrophus* and WWE1, that presumably metabolized benzoate and amino acids, respectively. By 16S rRNA gene pyrosequencing of two phenol-degrading methanogenic reactors, Ju and Zhang (2014) discovered that the diversity and abundance of syntrophic bacteria that contribute to syntrophic degradation of phenol and its metabolic products were different under mesophilic (37 °C) and ambient (20 °C) conditions. Unlike the prevalence of syntrophic bacteria capable of phenol (*Syntrophorhabdus aromaticivorans*) and benzoate degradation (*Syntrophus*) in both reactors, syntrophic genera, including W22 (in WWE1), *Moorella* (family *Thermoanaerobacteraceae*), and *Synergistes*, were more abundant at 37 °C, whereas *Sulfurovum*-like uncultured epsilon-proteobacteria, *Pelotomaculum* and *Desulfovibrio* (*mexicanus*), *Chlorobia* are more prevalent at 20 °C, suggesting a temperature-induced differentiation in membership of syntrophs. Notably, the structure of 20 °C phenol-degrading consortia presented by Ju and Zhang (2014) highly resembled those phylotypes involved in anaerobic benzene-degradation revealed by three previous studies using specific stable isotope and 16S rRNA gene cloning (Kleinstaub *et al.* 2008; Herrmann *et al.* 2010). Based on these 16S rRNA gene and metabolic evidences, the authors proposed that species of *Pelotomaculum*, *Sulfurovum*-like *Epsilonproteobacteria*, and sulfate-reducing *Desulfovibrio* were presumably responsible for syntrophic metabolism of aromatic compounds or their byproducts under sulfate-reducing conditions. However, genomic and transcriptomic evidences (other than merely the 16S rRNA gene) coupled to bioimaging techniques (e.g., microautoradiography-FISH,) and stable isotope probing are required to validate and elucidate the underlying philosophy of syntrophy among them.

In addition, metagenomics-based surveys have also detected less abundant but cosmopolitan bacterial groups in anaerobic reactors, which were previously known to harbor syntrophic species, such as phylum *Synergistetes*. The relative abundance of *Synergistetes* in total bacterial community in anaerobic digesters estimated by HTS-based 16S rRNA datasets was typically around 1.0% (St-Pierre and Wright 2014; Lee *et al.* 2012; Schlüter *et al.* 2008; Martínez *et al.* 2014; Li *et al.* 2014; Qiao *et al.* 2013), but *Synergistetes* could achieve high abundance (up to 6.7% and 19%) in anaerobic reactors treating vinasses from ethanol distilleries (Martínez *et al.* 2014) and linear alkylbenzene sulfonate-containing wastewater (Carosia *et al.* 2014), respectively. So far, this phylum has been known to be mainly comprised of syntrophic metabolizers of amino acids, including *Aminobacterium colombiense*, *Thermoanaerovibrio acidaminovorans*, and *Aminomonas paucivorans* (Sieber *et al.* 2012). Among them, *A. colombiense* can also metabolize peptides and organic acids, and *T. acidaminovorans* can degrade organic acids and sugars. However, the role of *Synergistetes* in anaerobic digestion processes remains invalidated (e.g., amino acids degradation) or unidentified.

4.2. Microbial community function

The direct shot-gun sequencing of genomic DNA (metagenome) or mRNA/cDNA (metatranscriptome) provides a fundamental overview of gene diversity and abundance, expressed activities, and contributing microorganisms involved in the global metabolic pathways (including hydrolysis, acidogenesis, acetogenesis, and methanogenesis), as well as novel sights into the novel microorganisms, enzyme-coding genes, biodegradation pathways, and microbial interactions in the anaerobic digestion process.

4.2.1. Metabolic pathway in anaerobic digestion

Metagenomic sequencing of genomic DNA provides insights into the potential metabolic pathways and contributing microorganisms involved in carbohydrate degradation and methanogenesis in several anaerobic digesters. Krause *et al.* (2008) investigated the metagenome of a biogas reactor fed with agricultural crops (98%) and small proportions of chicken manure (2%) by Gene Ontology and Pfam databases. The significant overrepresentation of *Firmicutes*-originated (mainly *Clostridia*) enzyme-coding genes involved in the degradation of xylans, oligosaccharides, disaccharides, and monosaccharides, and *Methanoculleus*-originated enzyme-coding genes coding a hydrogenotrophic methanogenesis pathway suggested that *Clostridia* were important for polysaccharides' and oligosaccharides' hydrolysis and *Methanoculleus*-like archaea were mainly responsible for methane production using CO₂ and H₂ (Schlüter *et al.* 2008; Krause *et al.* 2008). Further analysis of the same metagenome by Jaenicke *et al.* (2011) revealed that the enzymes responsible for polysaccharide degradation were mainly affiliated with *Firmicutes*, followed by *Bacteroidetes* and *Proteobacteria*, while *Clostridia* were mainly responsible for acetogenesis via the reductive CoA pathway (Wood–Ljungdahl pathway).

In other studies, Rademacher *et al.* (2012) compared the microbial enzymatic capacity of a hydrolytic/cellulolytic biofilm on the surface of substrates (rye silage and

winter barley) in the pre-hydrolysis reactor (55 °C) and an acidotrophic/methanogenic biofilm attached to the pickings of the subsequent anaerobic filter reactor (55 °C), respectively. Comparison of two biofilm metagenomes revealed that carbohydrate-degrading enzymes (mainly *Clostridia*) were highly enriched in the cellulolytic biofilm, whereas the methanogenic enzymes contributed to a hydrogenotrophic methanogenesis (mainly *Methanothermobacter*) pathway and were dominant in the methanogenic biofilm sample. However, the genes coding enzymes for an acetoclastic pathway were also identified, although they were not as abundant as that for a hydrogenotrophic pathway. The dominance of genes coding enzymes for cellulolytic hydrolysis by *Clostridia* and methanogenesis by hydrogenotrophic methanogens in anaerobic digestion processes, as described by Rademacher *et al.* (2012), was well in agreement with that documented by Krause *et al.* (2008). However, KEGG pathway annotation of four metagenomes of full-scale anaerobic sewage sludge digesters treating municipal sewage sludge by Yang *et al.* (2014b) supported that the raw reads involved in an acetotrophic pathway (mainly *Methanosarcinales*) were always more abundant than that for a hydrogenotrophic or methylotrophic pathway, and they suggested that the acetoclastic pathway was mainly responsible for methanogenesis. In addition, metagenomic analysis of two lab-scale UASBs fed with waste-activated sludge with and without pH 10 pre-treatment (for eight days) suggested the prevalence of gene-coding enzymes (mainly originated from *Methanosaeta* and *Methanothermobacter*) involved in both acetoclastic and hydrogenotrophic methanogenesis pathways (Wong *et al.* 2013).

Above all, it is wise to be aware that the aforementioned discrepancies in methanogenic pathways in anaerobic digestion processes reported by different researchers might be related to their differences in other experimental variables, such as substrate, reactor type, organic loading, operation time, etc., or even data-processing pipeline. It should also be kept in mind that all of these studies discuss metabolic potentials at the DNA level, which cannot represent RNA-level gene expressions or protein-level enzyme activities. The coupling of metagenome to metatranscriptome is an effective means to obtain a thorough understanding between “potential” and “active” metabolic pathways in anaerobic digestion processes. However, so far, little work has been done from this aspect. Only until quite recently, Xia *et al.* (2014) used metatranscriptomic and metagenomic approaches to re-characterize microbial cellulose anaerobic degradation and methanogenesis pathways in a lab-scale thermophilic cellulose-degrading reactor. They discovered that although genes involved in methanogenesis by acetoclastic order *Methanosarcinales* were less prevalent than hydrogenotrophic order *Methanobacteriales* (by 60%), the transcriptional activities of *Methanosarcinales* were remarkably higher (six times) than *Methanobacteriales*. More future studies in anaerobic digestion using coupled metatranscriptome and metagenome are required.

4.2.2. Novel genes for bioenergy and biodegradation

Functional metagenomics, typically including a high-throughput metagenomic library for functional screening for clones growing on target substrates (e.g., antibiotics, chitin, cellulose, etc.) coupled to HTS, has been widely applied to uncover a wide range of novel

biocatalytic enzymes in many habitats, such as soil, lake sediment, marine water, extreme environments (in temperature, pH, etc.) (Li *et al.* 2009). However, not much attention has been paid to its application in identifying novel enzymes for biofuels or biodegradation from anaerobic digesters.

Wexler *et al.* (2005) constructed a broad host-range metagenomic cosmid library (60–80 kb, 110,000 clones) from sludge of an anaerobic sludge digester, and screened for genes that confer ethanol utilization. They discovered an alcohol/aldehyde dehydrogenase (*adhE*), termed *adhE*_{Meta}, which most closely resembled the *adhE* of *Clostridium acetobutylicum*. Through multiple attempts *in vivo* and *in vitro* genetic manipulations, they successfully validated that cloned *adhE*_{Meta} enabled *Rhizobium leguminosarum* to grow on ethanol as the sole carbon and energy source.

Yan *et al.* (2013) utilized a metagenomic fosmid library from a biogas digester coupled to 454 pyrosequencing to screen (hemi-) cellulase genes. In total, 341, 246, and 386 positive clones were evidenced with endo- β -1,4-glucanase, β -glucosidase, and endo- β -1,4-xylanase activities, respectively. Among them, 21 unique glycosyl hydrolase (GH) genes showed 39–72% similarities to their nearest neighbors, suggesting the storage of diverse GHs in the biogas digester system as potential resources for future industrial application in lignocellulose hydrolysis. Apart from bioinformatics' prediction, nine GH genes were expressed and purified by Yan *et al.* (2013), and successfully validated of their activities in the four substrates. In agreement with the discovery by Yan *et al.* (2013), Xia *et al.* (2013) also discovered that 108 out of total 236 glycoside hydrolases in the metagenome of a thermophilic cellulose-degrading reactor showed less than 50% identity to known proteins in NCBI databases, revealing the presence of considerable novel thermo-stable cellulolytic genes awaiting further validation by future experiments.

4.2.3. Function prediction of uncultured microorganisms

The function of microorganisms responsible for anaerobic syntrophic degradation of aromatic compounds in a methanogenic hyper-mesophilic (46–50 °C) terephthalate-degrading hybrid reactor have been predicted by Lykidis *et al.* (2010) through metagenome assembly and binning. By applying metagenomes sequencing (totally 86 Mb) with three library inserts (~3, 8, and 40kb) and sequence composition-based binning using Phylopythia (with sample-specific training sets), genomic contigs (45 of them between 24 and 167 kb) assigned to *Pelotomaculum*, methanogens, *Syntrophaceae*, *Thermotogae*, WWE1 and OP5 were retrieved and annotated of their protein-coding genes. The authors proposed that *Pelotomaculum* species might presumably degrade terephthalate in syntrophy with other species via decarboxylation, dearomatization, and modified β -oxidation to hydrogen, CO₂, and acetate. Moreover, the OP5 genome was discovered to harbor genes for anaerobic autotrophic butyrate production, and the *Thermotogae*, *Syntrophus*, and WWE1 genomes carry genes to oxidize butyrate to CO₂, hydrogen, and acetate.

The application of metagenome and metatranscriptome also provides novel and intriguing insights into the microbial ecology in nitrogen and carbon cycling. Based on metatranscriptome and single-cell sequencing combined with ¹³C- and ¹⁵N-labelling and

bioreactor performance, Haroon *et al.* (2013) demonstrated that the novel archaeal ANME-2d (newly nominated by the authors as Candidatus “*Methanoperedens nitroreducens*”) could independently perform anaerobic methane oxidation via reverse methanogenesis, coupled to nitrate reduction to nitrite. Nitrite released by ANME-2d was then reduced to dinitrogen gas through a syntrophic interaction with an anaerobic ammonium oxidizing (anammox) bacterium (*Kuenenia*), outcompeting *Methylomirabilis oxyfera*, which was also able to couple AOM to nitrite reduction. In another study, Hu *et al.* (2012) investigated a granular sludge metagenome (37,432 contigs; average length of 571 nucleotides) of an Anammox reactor dominated by an Anammox bacterium Candidatus *Jettenia asiatica* (~50%), identifying 25 genes essential for Anammox. Intriguingly, three other microbial groups were also present in the different layers of the sludge granules, including (I) aerobic ammonia-oxidizing *Bacteria*, (II) methanogens, and (III) the denitrifying methanotroph Candidatus *Methylomirabilis oxyfera* (in phylum NC10). These microbial groups were presumably to work together with the Candidatus *Jettenia asiatica* to realize methane and nitrogen cycling within the reactor.

4.2.4. Fundamental novel insights into anaerobic digestion

The “open-ended” metagenomics approaches provide an informative overview of global structure, function, and metabolism in microbial communities, based on which novel hypotheses can be brought up and validated by “hypothesis-driven” studies. Recently, Rotaru *et al.* (2014) at the University of Massachusetts used metatranscriptome (i.e., global gene expression patterns) as a diagnostic tool to discover and elucidate a novel mechanism of interspecies electron transfer in triplicate UASBs that produce methane from brewery wastes. Metatranscriptomic analysis of methanogenic aggregates in the UASBs suggested that strictly acetoclastic *Methanosaeta* species, as the most dominant methanogen, displayed high transcription activities for these genes coding enzymes necessary for the reduction of carbon dioxide to methane, and simultaneously *Geobacter* species, as the most abundant bacteria, highly expressed gene activities involved in ethanol metabolism and structural protein for electrically conductive pili (PilA). The authors suggested that these two species exchanged electrons via direct interspecies electron transfer. This inference was later validated in co-cultures of *Geobacter metallireducens* and *Methanosaeta harundinace* using transcriptomic, isotope labeling, and genetic analysis, demonstrating that direct interspecies electron transfer, an alternative way in electron transfer for methanogenesis, dominated over interspecies H_2 /formate transfer to produce methane in UASBs fed with brewery wastes. The metatranscriptomic approach presented by the authors provides not only a tool to explore interspecies electron transfer in anaerobic digestion under more circumstances, but also a template as to how to make the best use of knowledge told by metagenomics to initiate a “hypothesis-driven” study.

In another metagenomics-based study, Xia *et al.* (2014) initially used coupled metatranscriptome and metagenome to gain novel insights into the thermophilic cellulose methanization process. By screening an unprecedented number of active genes (up to 40,000), the authors discovered for the first time the co-transcription of hemicellulases by

Clostridiales and *Spirochaetales* in the presence of cellulose stimulation alone, the comparable transcription of Sus-like polysaccharide utilization loci for polysaccharides breakdown by an unclassified order of *Bacteroidetes*, as well as the significant role of *Thermotogales* in synergistic beta-sugar consumption so as to make up for the inadequacies of cellulose degrader *Clostridiale* in metabolizing this substrate. Moreover, it had also been found that the less abundant acetate-utilizing *Methanosarcinales* (at the DNA level) were far more transcriptionally active than their hydrogenotrophic counterparts in methane production, which challenges the previous viewpoint that a minority of aceticlastic methanogens were associated with repressed metabolism.

4.3. Antibiotic resistance genes and pathogens

The presence of microbial genetic biohazards, such as ARGs, pathogens, and DNA viruses, poses great sanitary concern in further reclamation of anaerobically digested sludge (*e.g.*, land application). In recent years, HTS-based metagenomic methods have been widely applied to efficiently detect wide-spectrum profiles of ARGs, bacterial pathogens, or viral communities in water and wastewater treatment facilities, including drinking water disinfection systems (Shi *et al.* 2013; Gomez-Alvarez *et al.* 2012), activated sludge (Zhang *et al.* 2011; Yang *et al.* 2013; Ye and Zhang 2011; Szczepanowski *et al.* 2008; Parsley *et al.* 2010), sewage influent and effluent (Cai and Zhang 2013; Cai *et al.* 2013), and primary and secondary sewage sludge (Bibby and Peccia 2013; Tamaki *et al.* 2012). In contrast, little attention has been paid to the occurrence and fate of such microbial contaminants in anaerobic digestion processes.

Recently, Yang *et al.* (2014a) evaluated the fate of antibiotic resistance genes in a full-scale sewage treatment plant using a metagenomic approach. A total of 271 ARGs subtypes belonging to 18 ARGs types were identified from raw influent, activated sludge, anaerobic digester, and treated effluent, and the anaerobic digester was abundant with six ARGs types with resistance to tetracycline, macrolide-lincosamide-streptogramin, sulfonamide, multidrug, aminoglycoside, and acriflavine. It was estimated that while over 99.8% of wastewater-borne ARGs could be effectively removed by the sewage treatment process, the digestion process, however, could merely remove 20.7% of total ARGs in the sewage sludge, which sounds an alarm on the sanitary safety during the subsequent disposal of the digested sludge. In another recent study using metagenomic analyses, Resende *et al.* (2014) observed a marked reduction of ARGs with resistance to the macrolides (*ermB*), aminoglycosides (*aphA2*), and beta-lactams (*blaTEM-1*) after a 60-day digestion.

Bibby and Peccia (2013) applied shot-gun viral metagenomics sequencing and assembly to evaluate the infectious risks associated with direct land application of ten sewage sludge (without digestion) from five wastewater treatment plants in the U.S. and identified numerous different types of human (26 DNA and 17 RNA) viruses from the assembled sequences of sewage sludge, including a high abundance of three newly emerging viruses and *Enteroviruses* with relatively minor abundance and occurrence. The findings of this study necessitate cautious disposal of sewage sludge even after anaerobic digestion, although further studies are still needed to evaluate the effectiveness of

anaerobic digestion in minimizing these virus-brought infectious risks. Recently, Luo and Angelidaki (2014) used the combination of ethidium monoazide, PCR, and Ion Torrent sequencing to characterize “active” bacterial pathogens in a biogas plant. The sludge was pre-treated by ethidium monoazide so as to cleave DNAs from dead cells, thus excluding them from downstream PCR amplification. The results showed that anaerobic digestion reduced the richness and relative abundance of bacterial pathogens. However, the abundance and richness of bacterial pathogens (e.g., *Streptococcus bovis*) increased after 30 days’ post-digestion, indicating that attention must be paid to the management of post-digestion sludge.

5. Perspectives

The advent of the metagenomics era allows for the community-wide investigation of microbial structure, metabolism, and function in manually controlled biological engineering systems, such as anaerobic digesters. With the assistance of state-of-the-art bioinformatics tools, it is now easy to depict or compare the global microbial composition based on 16S rRNA gene HTS data, or predict the DNA-level functional potentials in anaerobic digestion processes from metagenomes. One major pitfall with current comparative metagenomics studies on anaerobic digestion lies in the fact that when the effect of one factor on microbial composition or functional categories is evaluated within one single study or across multiple studies (e.g., substrate types, temperature, reactor design), the inconsistencies in other influential factors, such as sludge retention time, organic loading rate, years of operation, feeding frequency, or even methodological differences (e.g., in selected PCR primer, read length, and data-processing pipeline), are regrettably ignored, which may lead to incomplete or biased annotation and contradicting conclusions drawn by different studies. Therefore, considerations should be taken into how to obtain enough comparable datasets via elaborate experimental design (e.g., single-factor experiment). Moreover, there has been an urgent need to properly translate the scientific knowledge obtained from metagenomics into engineering practices guiding design or operation of full-scale anaerobic bioreactors. To fulfill this goal, more emphasis should also be focused on investigating the core microorganisms by their expressed RNA active or functional proteins, rather than plainly describing whole microbial community diversity at the DNA-level with little reference to microbial interactions (e.g., syntrophy) and system functioning in bioenergy production and pollution control. Notably, metagenome *de novo* assembly and multidimensional coverage binning are powerful and increasingly popular ways to directly study uncultured or novel microorganisms, opening a broad gate to access to diverse novel microbial resources for application in genetic engineering towards enhanced biofuel production and pollution control. The genome annotation of reconstructed uncultured bacteria provides physiological and biochemical clues to help to isolate them, which eventually make the *in vitro* validation of their enzymatic activities feasible. Finally, metagenome is advised to work more in complement with metatranscriptome and metaproteome to resolve the differentiated RNA expression and enzyme activities, and with single-cell sequencing to obtain more genomes with higher quality probably at a much lower sequencing depth (thus less cost)

than at present. Above all, to decode the microbial behaviors in engineering biological systems is the key to manipulate them to fully exploit their economic and environmental benefits. Metagenomics is to fulfill this endeavor.

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Chapter 6

Transformations and Impacts of Ammonia and Hydrogen Sulfide in Anaerobic Reactors

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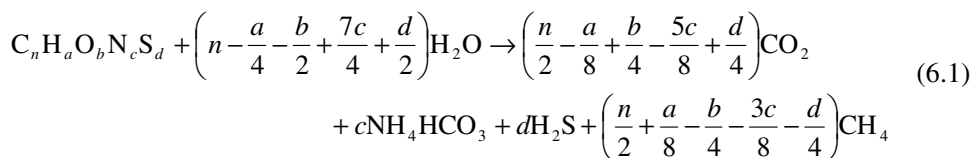
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In this chapter, the transformations and the response of ammonia and hydrogen sulfide in anaerobic systems are summarized and subdivided into several topics. Chemical quantitative analyses including fermentation stoichiometry, dissociation equilibrium, the gas–liquid equilibrium of NH₃ and H₂S and the pH buffer system in anaerobic processes are introduced. The positive and negative roles of ammonia in anaerobic digestion are discussed in detail based on recent research. The formation and harmful corrosion of hydrogen sulfide are elucidated, along with the technologies for sulfate-rich wastewater treatment and hydrogen sulfide removal from biogas.

1. Stoichiometry of Anaerobic Degradation Equation

Complete anaerobic degradation processes produce a biogas consisting of CH₄, CO₂, H₂S and NH₃. The detailed composition of a biogas is dependent on the substrate. Their quantities can be calculated by Eq. (6.1). Table 6.1 summarizes the typical biogas compositions produced from different wastes and wastewaters. Wastes with high content of nitrogen and sulfur could produce H₂S and NH₃ and their related soluble state compounds. H₂S and NH₃ may cause many problems for the utilization of biogas, and the soluble state of H₂S and NH₃ may also result in the inhibition of fermentation processes. Therefore, it is important to understand the behaviors of nitrogen and sulfur in anaerobic systems.

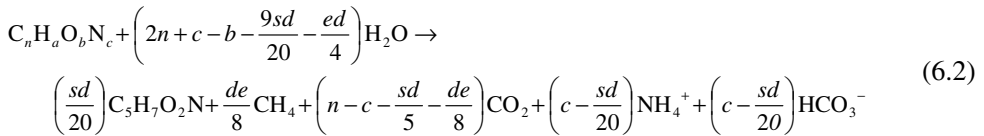


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Table 6.1. Biogas composition from different substrates.

Components	Household waste	Wastewater treatment plants sludge	Agricultural wastes	Waste of agri- food industry
CH ₄ %	50–60	60–75	60–75	68
CO ₂ %	38–34	33–19	33–19	26
N ₂ %	5–0	1–0	1–0	-
O ₂ %	1–0	< 0.5	< 0.5	-
H ₂ O%	6 (40 °C)	6 (40 °C)	6 (40 °C)	6 (40 °C)
Total%	100	100	100	100
H ₂ S mg·m ⁻³	100–900	1,000–4,000	3,000–10,000	400
NH ₃ mg·m ⁻³	-	-	50–100	-

McCarty (1964) proposed the following Eq. (6.2) to illustrate the elemental transformation and main products during anaerobic digestion. The synthesis of carbon, hydrogen, oxygen and nitrogen into biomass cells was taken into consideration in this equation.



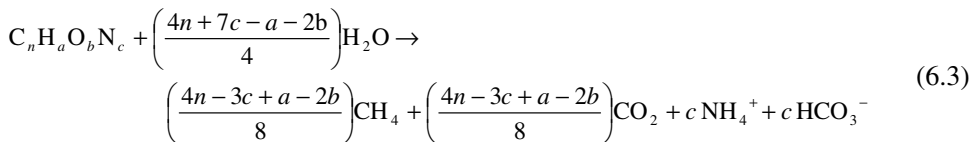
where:

$$d = 4n - a - 2b - 3c$$

s = the fraction of COD synthesized into biomass

e = the fraction of COD converted to methane

However, the fraction of COD synthesized to microbes is not clear and needs to be determined by experiment. This uncertainty greatly limits the application of Eq. (6.2). On the other hand, the growth yield of anaerobic microbes is very low, accounting for 5–15% of influent COD, as compared to aerobic microbes. Therefore, the cell growth term in Eq. (6.2) can be neglected in most cases. As a result, a simplified equation can be used to predict products of the anaerobic process, as shown in Eq. (6.3).



By using this simplified stoichiometric Eq. (6.3), the biogas and alkalinity produced from different kinds of wastes and organic compounds can be predicted according to the substrate formula, as listed in Table 6.2.

Table 6.2. Prediction of biogas yield from selected organic compounds.

Substrate	Biogas ($L \cdot kg^{-1} \cdot VS^{-1}$)	CH_4 (%)	CO_2 (%)	NH_3 Production (Yes/No)
Carbohydrate $C_n(H_2O)_n$	≈ 744	50	50	No
Protein $(C_8H_{12}O_3N)_n$	≈ 764	69	31	Yes
Lipid $C_nH_{2n}COOH$	$\approx 1,425$	70	30	No
Glucose $C_6H_{12}O_6$	744	50	50	No
Starch $(C_6H_{10}O_5)_n$	820	50	50	No
Cellulose $(C_6H_{10}O_5)_n$	820	50	50	No
Benzoic acid $C_7H_6O_2$	1,285	53.6	46.4	No
Phenol C_6H_6O	1,222	58.3	31.7	No
Formic acid CH_2O_2	608	25	75	No
Acetic acid $C_2H_4O_2$	748	50	50	No
Propionic acid $C_3H_6O_2$	908	58.4	41.6	No
Butyric acid $C_4H_8O_2$	1,018	62.5	37.5	No
Valeric acid $C_5H_{10}O_2$	1,098	65.0	35.0	No
Pamitate $C_{16}H_{32}O_2$	1,444	69.7	30.3	No
Oleic acid $C_{18}H_{34}O_2$	1,430	70.8	29.2	No
Methanol CH_4O	700	75	25	No
Ethanol C_2H_6O	971	75	25	No
Propanol C_3H_8O	1,211	75	25	No
Raw waste $C_{46}H_{73}O_{31}N$	473	53.3	46.7	Yes
Paper $C_{266}H_{434}O_{210}N$	848	60.9	39.1	Yes
Excess sludge $C_3H_7O_2N$	793	62.5	37.5	Yes
Primary sludge $C_{22}H_{39}O_{10}N$	986	61.9	38.1	Yes
Mixture sludge $C_{10}H_{19}O_3N$	1,003	69.4	30.6	Yes

2. Bicarbonate Alkalinity and Influence of Ammonia

2.1. Bicarbonate alkalinity

In an anaerobic system, around 40% of CO_2 in the biogas phase is balanced with the liquid phase. The dissolution of CO_2 into the liquid phase of a digester would seriously decrease the pH. Carbon acid and residue VFA are acid products in anaerobic digestion systems. Alkali products are needed to neutralize these acid products and then provide an essential pH buffer to maintain a suitable pH for anaerobic microorganisms. The alkalinity demand is calculated based on the following description.

Alkalinity is the parameter quantifying the capacity of a solution in dampening pH change. In a CO_2 – HCO_3^- – CO_3^{2-} aqueous system containing organic acids, alkalinity is often represented by the $mg \cdot L^{-1}$ of $CaCO_3$ in Eq. (6.4).

$$H^+ + \text{Alkalinity} = HCO_3^- + 2CO_3^{2-} + OH^- + RCOOH^- \quad (6.4)$$

As shown in the above equation, anions including HCO_3^- , CO_3^{2-} and RCOOH^- have the capacity to neutralize H^+ . As a result, all of those anions are sources of alkalinity. In an anaerobic system, the pH is often kept in the range of pH 7–8, at which concentrations of CO_3^{2-} and OH^- are substantially lower than HCO_3^- , as shown in Eq. (6.5).

$$\text{CO}_3^{2-} \ll \text{HCO}_3^-; \quad \text{OH}^- \ll \text{HCO}_3^- \quad (6.5)$$

In addition, in a properly operated anaerobic treatment system, there should be little VFA accumulation, and thus, the concentration of VFA is significantly lower than HCO_3^- , as shown in Eq. (6.6).

$$\text{RCOOH}^- \ll \text{HCO}_3^- \quad (6.6)$$

The alkalinity equation may be simplified to Eq. (6.7).

$$\text{H}^+ + \text{Alkalinity} = \text{HCO}_3^- \quad (6.7)$$

The relationship between pH, bicarbonate alkalinity and P_{CO_2} , i.e. partial pressure of CO_2 in the gas phase, can be described in Eq. (6.8).

$$\text{pH} = \text{pK}_{a,1} + \log \left(\frac{\text{Alkalinity}}{50,000} \div \frac{\text{P}_{\text{CO}_2}}{\text{K}_\text{H}} \right) \quad (6.8)$$

By using Eq. (6.8) for the relationships among the pH, CO_2 in biogas and alkalinity for mesophilic and thermophilic conditions may be calculated, as illustrated in Fig. 6.1. For the mesophilic condition, the minimum alkalinity needed to keep the pH at 7.0 with 40% CO_2 in biogas was around 2,500 $\text{mg} \cdot \text{L}^{-1}$.

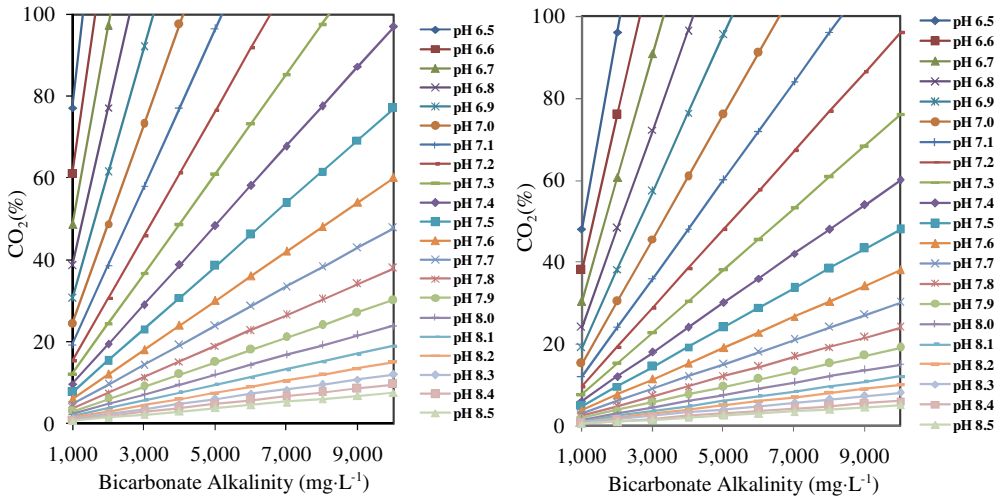
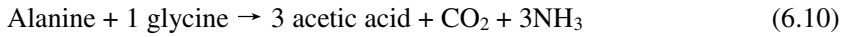
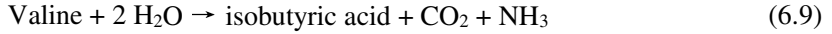


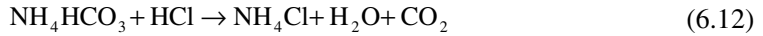
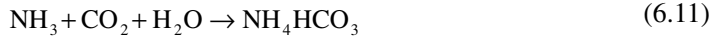
Fig. 6.1. The relationship among bicarbonate alkalinity, CO_2 and pH (Left: 35 °C; Right: 55 °C).

2.2. Ammonia production and its important role in pH buffering

During anaerobic digestion, NH_3 is produced from the breakdown of the components containing N. Proteins and amines are relatively easily degradable and total ammonia nitrogen (TAN) is therefore produced during the anaerobic degradation process. The degradation of amino acids takes place via the deamination/decarboxylation processor, known as the Stickland reaction, and is carried out by *Clostridium* that exists in abundance in animal manure (Sommer *et al.* 2013).



For specific wastes and wastewaters, the ammonia production can be calculated. As shown in Eq. (6.11), the NH_3 reacts with CO_2 to form ammonia bicarbonate, which is a weak acid–base solution. As calculated from Eqs. (6.11–14), one gram of ammonia (as N) is equivalent to 3.57 grams of bicarbonate alkalinity.



$$[\text{Alkalinity}] = \frac{\frac{1}{2}(\text{molecular weight of } \text{CaCO}_3)}{\text{molecular weight of N}} \cdot [\text{NH}_4^+ - \text{N}] = 3.57[\text{NH}_4^+ - \text{N}] \quad (6.14)$$

Some substrates, like cellulosic materials, have little nitrogen content. The high C/N ratio may cause a deficiency of bicarbonate alkalinity. Under such conditions, extra alkali compounds such as NH_4HCO_3 , NaHCO_3 , $\text{Ca}_2(\text{HCO}_3)_2$ or nitrogen-rich substrates should be supplemented into a digester. As a case study, Qiao *et al.* (2013) indicated that coffee grounds had a C/N ratio of 23.8 and nitrogen was not enough to produce NH_4^+ for providing alkalinity and pH control was the problem. For this case, a co-digestion treating coffee grounds with waste-activated sludge was suggested based on the element analysis as listed in Table 6.3. The ammonia concentration with 100% degradation can be predicted by Eq. (6.15) for coffee grounds and Eq. (6.16) for a mixture of coffee grounds and sludge based on dry matter. These results proved the effectiveness of the McCarty's Eq. (6.2). The sludge can produce eight times as much ammonia as that from coffee grounds as calculated in Table 6.4. As a result, the alkalinity from 100 grams substrate changed from 3.70 to 14.75 $\text{g}\cdot\text{L}^{-1}$, which are shown in Table 6.4. In Eq. (6.15), the biomass yield was not included. In fact, the ammonia concentration was very sensitive to the ratio of nitrogen transforming into biomass as $\text{C}_5\text{H}_7\text{O}_2\text{N}$ for low-nitrogen substrates. The mixture substrate fermentation stoichiometric reactions with biomass yields of 0.2 $\text{g-C/g-C}_{\text{substrate}}$ are listed in Eq. (6.16). Through a long term experiment, the ammonia concentration calculated in Eq. (6.16) consisted well with the analysis data as shown in Fig. 6.2. Using this methodology, whether a substrate is suitable for an anaerobic system could be pre-evaluated according to the alkalinity requirement.

Table 6.3. The elemental composition of coffee grounds and sludge.

<i>C</i>	<i>H</i>	<i>O</i>	<i>N</i>	<i>C/N</i>	<i>Molecular Weight</i> (<i>g/mol</i>)	<i>Nitrogen</i>
5	8.38	2.34	0.18	23.8	108	2.3%
5	9.40	5.8	0.74	5.8	173	6.0%

Table 6.4. Alkalinity with different amounts of sludge in the mixture substrate.

	<i>C/N</i>	<i>N/100g-TS</i>	<i>Alkalinity/100g-TS</i>
Coffee	23.8	1.04	3.70
Coffee (95%):Sludge(5%)	22.1	1.15	4.09
Coffee (90%):Sludge(10%)	20.5	1.36	4.85
Coffee (67%):Sludge(33%)	14.7	2.06	7.35
Coffee (50%):Sludge(50%)	11.7	2.61	9.29
Sludge	5.8	4.14	14.75

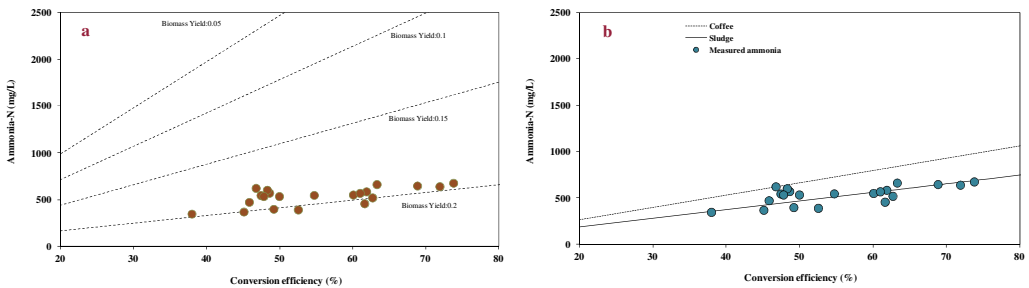
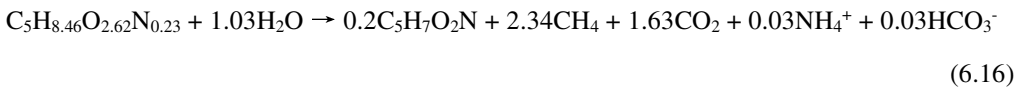


Fig. 6.2. Ammonia and bicarbonate alkalinity variation (Qiao *et al.* 2013).



2.3. Ammonia distribution between gas and liquid phases

The concentration of gaseous NH_3 in biogas is usually very low ($<0.1 \text{ mg}\cdot\text{m}^{-3}$). It may exceed $1 \text{ mg}\cdot\text{m}^{-3}$, and be present at a maximum of $1.5 \text{ mg}\cdot\text{m}^{-3}$. This occurs especially when excreta from poultry or special types of waste are fermented. Concentrations up to $150 \text{ mg}\cdot\text{m}^{-3}$ have been reported, which may impair the burn behavior and the life of the motors.

Figure 6.3 shows the ammonia distribution of the gas and liquid phases in an anaerobic system. Ammonia forms from the degradation of organic nitrogen compounds and is released into the liquid phase. Ammonia gas is easily dissolved into water and

reacts with H_2O , becoming a weak alkali environment. At the same time, under Henry's law, NH_3 gas escapes into air and keeps a dynamic equilibrium between gas and liquid phases. Considering the harmful effects of NH_3 gas in biogas, predicting the NH_3 concentration is meaningful.

According to Henry's law, NH_3 gas is the result of the liquid ammonia concentration, as indicated by the following equation.

$$P_{NH_3} = k_H \cdot [NH_3] \quad (6.17)$$

It is difficult to obtain an accurate value of k_H , because this factor is temperature-dependent. Sander (1999) used Eq. (6.18) to calculate Henry's law constant, K_H .

$$k_H = k_H^0 \times \exp\left(\frac{-\Delta \ln H}{R} \left(\frac{1}{T} - \frac{1}{T^0}\right)\right) \quad (6.18)$$

where H = the enthalpy of the solution, R represents the gas constant and T represents the absolute temperature. Here, the temperature dependence is

$$\frac{-d \ln k_H}{d(1/T)} = \frac{\Delta \ln H}{R} \quad (6.19)$$

The values in Eq. (6.19) have been provided in Sander's report as listed in Table 6.1. Using Eqs. (6.17–19) and Table 6.5, the k_H can be calculated. As listed in Table 6.5, each row gives the value of $\frac{k_H^0}{[M/atm]}$ and $\frac{-d \ln k_H}{d(1/T)}$ from different references. Nevertheless, these values were different. As a result, the relationship of K_H and T was certainly not fixed.

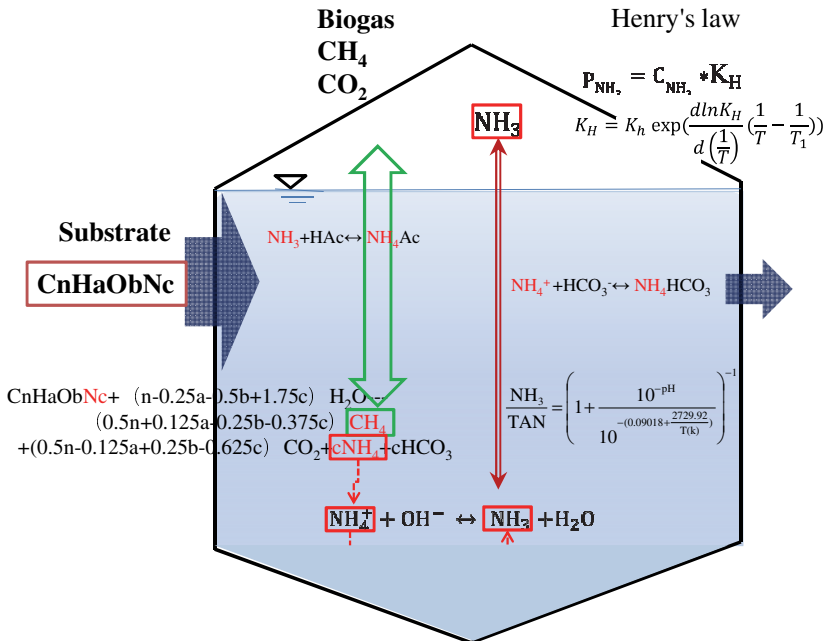


Fig. 6.3. Ammonia in the anaerobic system.

Table 6.5. Parameters of Henry’s law constants (Sander 1999).

$\frac{k_H^\theta}{[M/atm]}$	$\frac{-\ln k_H}{d(1/T)}$ [K]
5.9×10^1	4,100
5.7×10^1	4,100
1.0×10^1	1,500
6.1×10^1	4,200
7.6×10^1	3,400
5.8×10^1	4,100
7.8×10^1	
5.8×10^1	4,100
5.6×10^1	4,100
5.6×10^1	4,200
6.1×10^1	4,200
2.7×10^1	2,100
6.2×10^1	
5.4×10^1	
6.0×10^1	4,400

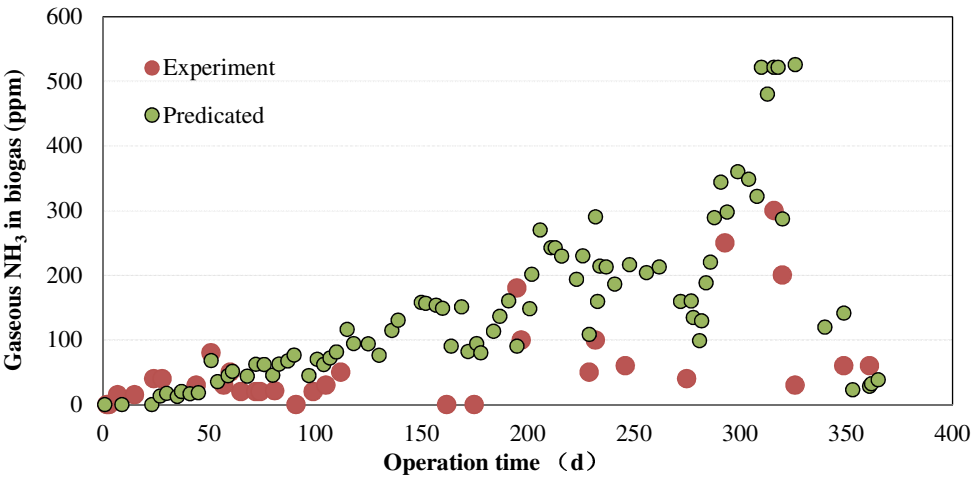


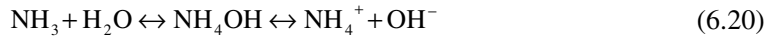
Fig. 6.4. A comparison of ammonia gas in biogas and the simulated data.

In Fig. 6.4, the ppm concentration of ammonia gas in biogas and simulated values calculated by Niu *et al.* (2013) are compared. The digester was used to treat chicken manure as sole-substrate in mesophilic temperature using a continuous flow stirred-tank reactor (CSTR). In the beginning of the experiment, the ammonium in the anaerobic system was not considered to be so high. The simulated values of ammonia gas are consistent with the results of gas chromatography. Throughout a long-term experiment over a wide range of ammonium concentrations, the simulated values were in good agreement with the test concentrations of ammonia gas. This allowed for the proposal of

a method for correlating the ammonia gas in biogas, molecular ammonia in digester aqueous and the amount of nitrogen in the substrate.

2.4. Distribution of molecular ammonia (NH_3) and total ammonia nitrogen in liquid phase

The reaction of NH_3 with H_2O in the aqueous phase can be described by Eq. (6.20), which is based on the principle of mass balance and a weak alkaline chemical equilibrium. The ratio of molecular NH_3 to total ammonia nitrogen ($\text{TAN} = \text{NH}_3 + \text{NH}_4^+$) can be calculated by Eq. (6.21). From the equations, the ratio can be determined and the effect of the pH can be calculated mathematically. Molecular NH_3 was distinguished as the cause of inhibition. The molecular NH_3 proportion under different temperatures and pH was represented in Fig. 6.5.



$$\frac{[\text{NH}_3]}{[\text{TAN}]} = \left(1 + \frac{10^{-\text{pH}}}{10^{\left(0.9018 + \frac{2729.92}{T(\text{K})}\right)}} \right)^{-1} \quad (6.21)$$

3. Ammonia Inhibition and Recovery

3.1. Effects of NH_3 and TAN on anaerobic processes

Ammonia is essential for maintaining a pH buffer in an anaerobic system. However, a high concentration of ammonia is toxic. As introduced by Speece (1996), ammonia toxicity may present a problem with feedstocks that contain high ammonia concentrations or its precursors, such as proteins. Inhibition is an especially significant problem when digesting swine or poultry manure, which often have total ammonium concentrations of higher than $4 \text{ g-N}\cdot\text{L}^{-1}$ (Hansen *et al.* 1998).

High TAN concentrations may inhibit methanogenesis: a high pH interacts with TAN because the toxic NH_3 dominates at high pH. The biogas process becomes more sensitive towards ammonia when the pH value increases, which again increases the concentration

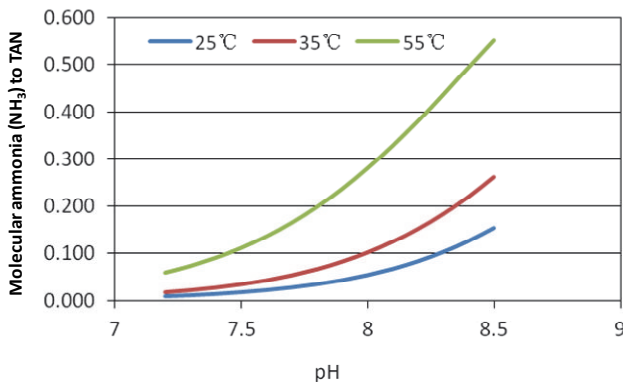


Fig. 6.5. Ratio of free ammonia to total ammonium depends on pH and temperature.

of NH_3 . Temperature is another important parameter that affects the occurrence of inhibitory effects. As reported by Angelidaki and Ahring (1994), when the NH_4^+ -N load was high, a reduction of the temperature below 55 °C resulted in an increase in the biogas yield and better process stability. Temperature reduction, therefore, had a positive effect on volatile fatty acid degradation by reducing the NH_3 concentration. Summarized by Hansen *et al.* (1998), based on previous studies, the molecular NH_3 concentration depends mainly on three parameters: the total ammonia concentration, temperature and pH.

With regard to the mechanism and inhibitory values of NH_3 and TAN, different results have been reported for different kinds of substrates. It was shown that NH_3 diffuses into microbe cell membranes and sequentially ionizes with H_2O to NH_4^+ and OH^- leading to a pH imbalance between the inside and outside of the bacterial cell. This pH change affects the transportation of the materials and leads to lower enzyme activity. However, inhibition might also be related to TAN concentration (Nielsen and Angelidaki 2007). Inhibition has been reported to start at a TAN level of 1.5–2.0 $\text{g}\cdot\text{L}^{-1}$. However, NH_3 tolerance of up to 3–4 $\text{g}\cdot\text{L}^{-1}$ for an adapted process has also been reported (Angelidaki and Ahring 1993). McCarty and McKinney (1961) found that the inhibitory level of molecular NH_3 was 150 $\text{mg}\cdot\text{L}^{-1}$. Molecular NH_3 concentrations of 700–1,100 $\text{mg}\cdot\text{L}^{-1}$ were shown to be capable of triggering inhibition in the anaerobic digestion of many kinds of substrates. An ammonium concentration of 4 $\text{g}\cdot\text{N}\cdot\text{L}^{-1}$ was shown to be inhibitory during the digestion of cattle manure (Angelidaki and Ahring 1993).

Fortunately, inhibition originated from both molecular NH_3 and TAN was not bacteriocidal. Speece and Parkin (1983) found that when 10,000 $\text{mg}\cdot\text{L}^{-1}$ NH_4^+ -N was added to methanogenic biomass at a neutral pH, the gas production rate gradually dropped to zero, as though the biomass was experiencing cell death. However, after about ten days of negligible gas production, biomass activity quickly recovered to 70% of the control rate within the first five days. In the anaerobic system, microorganisms have the potential to tolerate inhibition due to acclimation. As a result, the same concentration of ammonia that is inhibitory in a system may show no inhibitory effects on a properly acclimated biomass. The concept proposed by Speece (1996) is valuable when considering ammonia/ammonium inhibition in an anaerobic system treating animal manure. According to Koster and Lettinga (1988), after acclimation, it was possible for a process to tolerate levels of NH_4^+ -N ammonia concentration 6.2 times higher than the initial toxicity threshold level. When treating some toxicants, the system can be operated by carefully and slowly increasing the toxicant concentration to achieve successful acclimation. Therefore, the effects of ammonia from a long-term experiment were more informative for the design and management of an anaerobic system.

3.2. Effects of temperature on ammonia inhibition

The ratio of molecular NH_3 to TAN at room temperature (25 °C), under the mesophilic condition (35 °C) and under the thermophilic condition (55 °C) is shown in Fig. 6.5. From this figure, it can be seen that higher pH results in a significant increase of the molecular NH_3 in the anaerobic system. On the other hand, temperature can affect the

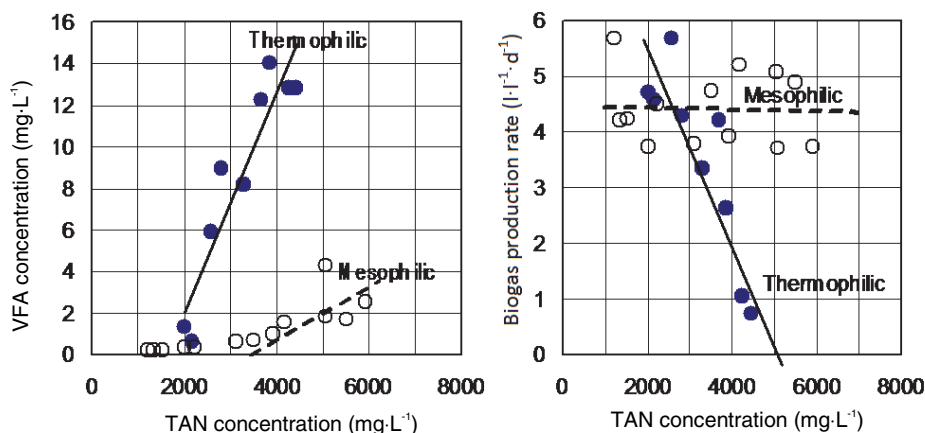


Fig. 6.6. Comparison of ammonia inhibition effects on thermophilic and mesophilic digesters (Li *et al.* 2004).

ratio. Thermophilic processes produced higher molecular NH_3 . From the perspective of the effect of ammonia inhibition, the mesophilic condition has the advantage of maintaining the process stability, or at least can tolerant higher levels of ammonia in the anaerobic system.

As illustrated in Fig. 6.6, under relatively high $\text{NH}_4^+\text{-N}$ concentrations, the mesophilic digester can produce biogas steadily. However, a significant decrease in the biogas production rate was observed in Fig. 6.6 (Right), when the $\text{NH}_4^+\text{-N}$ concentration exceeded $2,000 \text{ mg}\cdot\text{L}^{-1}$.

3.3. Recovery of ammonia-inhibited process

Niu *et al.* (2013) carried out experiments to investigate the effects of ammonia on anaerobic processes using chicken manure as the sole substrate. Through a long-term experiment (400 days), the feasibility of mesophilic fermentation was found at a TAN concentration lower than $5,000 \text{ mg}\cdot\text{L}^{-1}$. This value was higher than many previously reports, which were mostly based on batch experiments. VFA accumulation occurred in response to TAN higher than $5,000 \text{ mg}\cdot\text{L}^{-1}$. When the TAN exceed $10,000 \text{ mg}\cdot\text{L}^{-1}$, biogas production nearly ceased.

Since ammonia is not bacteriocidal, the recovery of inhibited anaerobic systems need further discussion. As reported by Niu *et al.* (2013), inhibition resulting from ammonia can be recovered by reducing the toxic compounds' concentration. As shown in Fig. 6.7, the seriously inhibited digester with a TAN of $16,000 \text{ mg}\cdot\text{L}^{-1}$ was recovered by diluting the digester and washing the inner sludge. Nielsen and Angelidaki (2007) investigated different strategies to recover an inhibited system. Active methane-producing biomass (digested cattle manure) was inhibited with NH_4Cl and subsequently, 3–5 days later, diluted with 50% water, 50% digested manure, or with 50% fresh manure or kept undiluted. It is possible to improve the recovery speed by dilution of the biomass with either water, reactor effluent or manure.

Methane fermentation of chicken manure produce much NH_4^+ which caused inhibition:

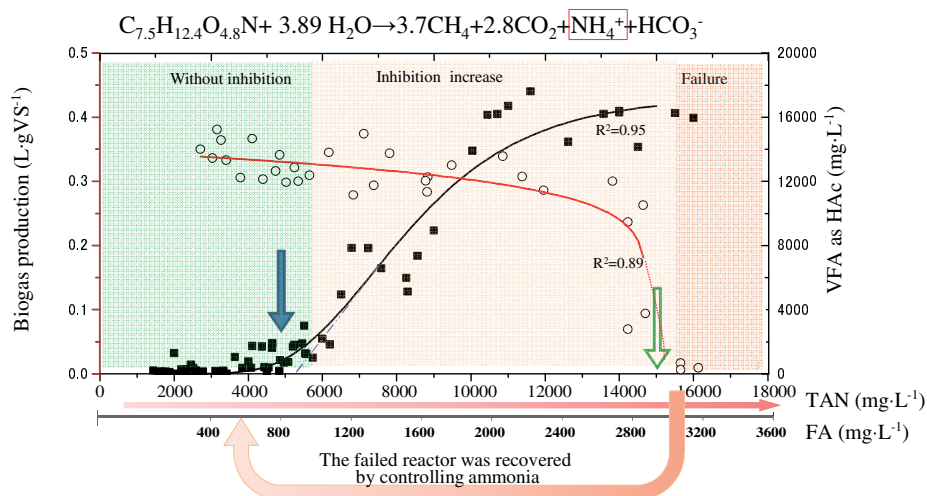


Fig. 6.7. Effects of ammonia on anaerobic digestion and digester recovery (Niu *et al.* 2013).

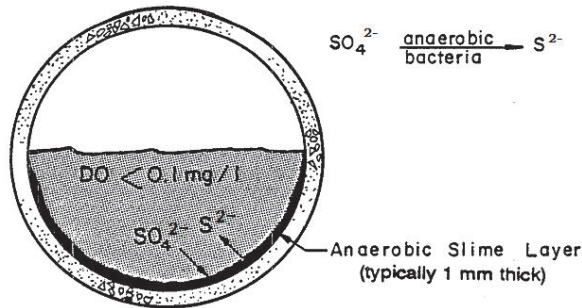
3.4. Effects of ammonia inhibition on microbial community dynamics of sole-chicken manure fermentation

As is generally known, methane fermentation is a microbial process achieved by the interaction between microorganisms within bacteria and archaea involving several consequent degradation phases, typically hydrolysis, acidogenesis and methanogenesis. Hydrolytic and acidogenic bacteria have been reported to have greater diversity and faster growth rates than methanogens. The inherently low growth rate of methanogens makes the anaerobic systems sensitive to environmental changes. The fermentation of high N-content chicken manure makes it susceptible to inhibition by ammonia.

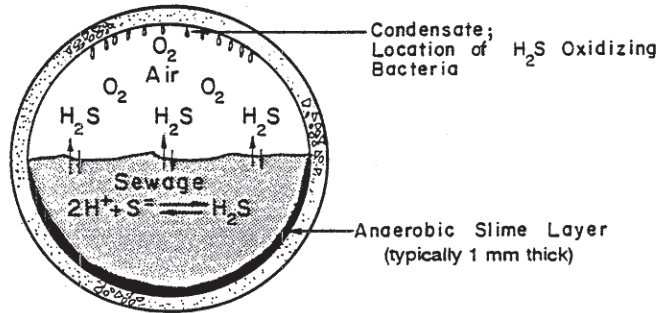
As recently reported by Niu *et al.* (2014), the effects of ammonia on microbial communities of mesophilic anaerobic digestion with total ammonia nitrogen with a range of 2,000–16,000 $\text{mg}\cdot\text{L}^{-1}$ were confirmed. 16S-rDNA cloning and TRLFP results revealed that the microbial community shifts significantly responded to TAN. Aceticlastic *Methanosarcina acetivorans* increased gradually from 17% in the steady stage to 72% in the recovered stage with high resistance compared with aceticlastic *Methanosaeta*, which almost disappeared at high TAN. In contrast, hydrogenotrophic *Methanoculleus* increased from 2% in the steady stage to 30% in the inhibited stage and decreased to 13% in the recovered stage. The results revealed that TAN has an obvious effect on microbial community shifts and the functional resilience of the process. The dominant microorganism of the high nitrogen substrate fermentation was shown in Fig. 6.8.

4. Effects of H_2S , S^{2-} and SO_4^{2-} on Anaerobic Processes

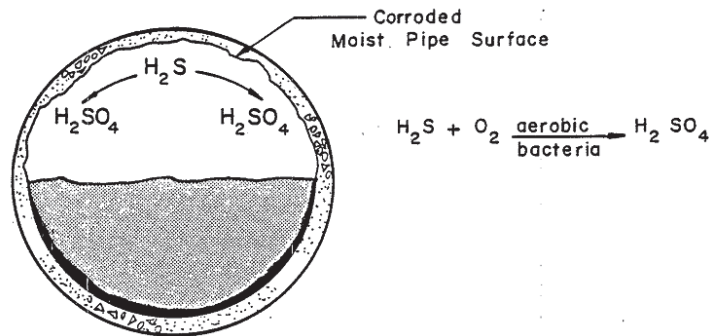
Sulfate reduction to H_2S is one of the most common and extensive microbiological processes on Earth. The environmental consequences of sulfate-reducing bacteria (SRB)



(A) Sulfate is biologically reduced to sulfide in the anaerobic slime layer on the submerged pipe wall.



(B) H_2S formed in the wastewater is released from solution as a gas and enters the sewer atmosphere.



(C) H_2S is oxidized to sulfuric acid by aerobic processes. Thiobacillus bacteria live on moist, non-submerged surfaces. Acid attacks concrete, causing corrosion.

Fig. 6.9. Mechanism of sulfide generation and corrosion in sewers.

can be devastating because H_2S is the precursor to biogenically produced H_2SO_4 in wastewater collection atmospheres, which causes many problems worldwide in corrosion. Hydrogen sulfide corrosion in wastewater systems often results in costly, premature replacement or rehabilitation of systems used in the transport and treatment of wastewater. Sewers designed to last 50–100 years have failed sulfate-reducing bacteria due to hydrogen sulfide corrosion in as short as 10v20 years. Electrical and mechanical equipment with an expected life of 20 years has required replacement in as short as five years (Speece 2008). During anaerobic digestion, H_2S is produced, especially when protein-rich substrates or sulfur-containing compounds are being treated. The concentration of H_2S in biogas varies from a few hundred ppm to ten thousand ppm

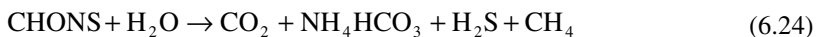
depending on the components of the substrate. The H_2S present in biogas has to be removed.

Hydrogen sulfide corrosion can occur by two mechanisms: 1) acid attack resulting from the biological conversion of hydrogen sulfide gas to sulfuric acid in the presence of moisture, and 2) direct chemical reaction with metals such as copper, iron and silver with hydrogen sulfide gas. The first mechanism is the one that is the principal cause of internal sewer corrosion, while the second can cause premature failure of electrical and instrumentation systems, and mechanical equipment used in the transport and treatment of wastewater (US EPA, 1991).

As represented in Fig. 6.9A, under anaerobic conditions, anaerobic bacteria reduce sulfate — one of the most common constituents in water and wastewater — to sulfide. In large sewers, this occurs primarily in slimes (0.25–3 mm, but typically 1 mm thick) attached to the submerged portion of the interior pipe surface (US EPA, 1991). Since H_2S is a weak acid, S^{2-} , HS^- and molecular H_2S present a dissociation equilibrium in liquid phase. Some metals, such as Ca^{2+} , Fe^{2+} and Fe^{3+} , would affect the distribution of S^{2-} in some degree due to chemical precipitation. As similar with CO_2 , the proportion of molecular H_2S in liquid and in gas phase is regulated by Henry's Law. The released H_2S in the gas phase would be further oxidized to H_2SO_4 under aerobic and suitable moist conditions, as shown in Fig. 6.9B. Figure 6.9C shows that the strong acid H_2SO_4 would cause the fallout of the concrete matrix and aggregate due to corrosion.

4.1. Formation of H_2S in an anaerobic system

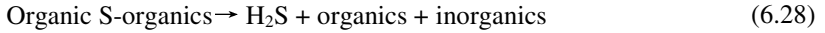
Wastewaters from the chemical industry often contain high concentrations of both sulfate and organic S compounds. When this kind of wastewater is treated with an anaerobic process, SRB would compete not only with methane-producing archaea (MPA), but also with other bacteria for electron donors. The consumption of some carbon sources by SRB often leads to a decrease in methane production and even failure of the treatment process. Most SRB grow significantly at 3–4 hours doubling time. In the presence of sulfate, acetate is the final product of the incomplete oxidation by SRB of some organic compounds such as lactate, propionate, butyrate and higher straight-chain fatty acids (Speece 2008). Many SRB strains can also consume H_2 . SRB therefore plays an important role in H_2 control and propionate degradation in anaerobic processes, especially in thermophilic processes, due to the tendency of propionate accumulation. In 1933, Symons and Buswell (1933) established Eq. (6.24) to quantify H_2S formation from the degradation of sulfur-containing substrates.



Sulfur-containing compounds can be present in the following forms (Deublein and Steinhauser 2012):

- sulfate and sulfite (in industrial wastewater in high concentrations)
- sulfide (H_2S , HS^- and S^{2-})
- undissociated molecular hydrogen sulfide in the liquid (most toxic)
- hydrogen sulfide in two dissociated forms: HS^- and S^{2-}

The empirical cell formulation of anaerobic cells considering sulfur is $C_5H_7O_2NP_{0.06}S_{0.1}$. The obligate requirement for sulfide has been identified in that formulation. The optimal growth of methane production is $0.01\text{--}1.0\text{ mg}\cdot\text{L}^{-1}$ sulfur as S. From Eqs. (6.25–28), H_2S is generated from the reduction of SO_4^{2-} or the degradation of protein and organic S-organics.



4.2. Ionization equilibrium of hydrogen sulfide: Molecular H_2S and S^{2-}

Concentrations of molecular H_2S in water can be calculated by the first-stage ionization equilibrium of hydrogen sulfide with water pH and dissolved sulfide concentration.



$$K_{a,1} = \frac{[HS^-] \cdot [H^+]}{[H_2S(aq)]} \quad (6.30)$$

$$\text{at } 35^\circ\text{C}, K_{a,1} = 14.9 \times 10^{-8} \text{ (Omil } et al. 1995) \quad (6.31)$$



$$K_{a,2} = \frac{[S^{2-}] \cdot [H^+]}{[HS^-]} \quad (6.33)$$

$$\text{at } 35^\circ\text{C}, K_{a,2} = 6.43 \times 10^{-16} \quad (6.34)$$

Within the well operated anaerobic system pH range of 7 to 8.



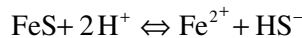
so that total dissolved sulfide = $HS^- + H_2S(aq)$

$$[H_2S(aq)] = \frac{\text{Total sulfide}}{1 + \frac{K_{a,1}}{10^{-\text{pH}}}} \quad (6.36)$$

Under anaerobic conditions, ferric ions are reduced to ferrous ions, which tend to precipitate due to their low solubilities. There are two dissolution equilibriums for ferrous sulfide, as follows.



$$K_{sp,1} = \frac{[Fe^{2+}] \cdot [H_2S]}{[H^+]^2} \quad \text{at } 35^\circ\text{C}, K_{sp,1} = 790.3 \quad (\text{Sun } et al. 2008) \quad (6.38)$$



$$K_{sp,2} = \frac{[Fe^{2+}] \cdot [HS^-]}{[H^+]} \quad \text{at } 35^\circ\text{C}, K_{sp,1} = 1.2 \times 10^{-4} \quad (\text{Chapelle } et al. 2009) \quad (6.39)$$

$$\frac{K_{sp,1}}{K_{sp,2}} = \frac{[HS^-] \cdot [H^+]}{[H_2S]} \quad (6.40)$$

$$\text{Total dissolved sulfide} = HS^- + H_2S(aq) \quad (6.41)$$

$$[H_2S(aq)] = \frac{\text{Total dissolved sulfide}}{\frac{K_{sp,2} \times 10^{pH}}{K_{sp,1}} + 1} \quad (6.42)$$

where K_{sp} is the solubility product.

The distribution of sulfide in an up-flow anaerobic sludge blanket (UASB) treating industrial wastewater containing high sulfate are illustrated in Fig. 6.10. H_2S was the final biological product generated by the activity of SRB. Within an alkaline pH system, the molecular H_2S concentration is significantly lower than the dissolved sulfide, and will react with cations, most typically with Fe^{2+} .

4.3. Competition between sulfate-reducing bacteria and methanogens: Theories and new findings

In anaerobic treatment processes, SRB and methane-producing archaea (MPA) always compete for a carbon source (Jing *et al.* 2013). In sulfate-rich wastewater digestion, SRB often outcompetes MPA, and produces corrosive and poisonous sulfide. The competition between SRB and MPA in digestion depends largely on the types of substrate and the COD/sulfate ratio. SRB can utilize many low molecular weight compounds, including butyrate, lactate, propionate, acetate, ethanol and methanol. Normally, SRB have an advantage over MPA during the utilization of such substrates due to their favorable kinetic properties and thermodynamic conditions (Mizuno *et al.* 1998). In low COD/SO_4^{2-} situations, SRB predominates in carbon source utilization and electron flow transmission, and suppresses the activity of MPA (Shin *et al.* 1997). It has been concluded that a COD/SO_4^{2-} ratio higher than ten is desirable to prevent the inhibition of methane production. In recent research, some findings indicate that lower COD/S ratios of wastewater can also be treated by the anaerobic process.

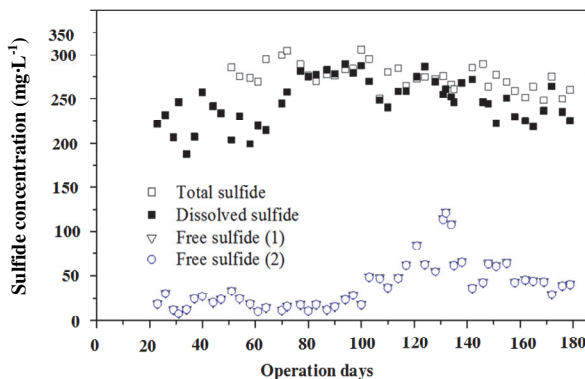


Fig. 6.10. The distribution of S species in the anaerobic system.

Jing *et al.* (2013) carried out a 180-day UASB experiment to investigate the competition between SRB and MPA using synthetic chemical wastewater containing $3,000 \text{ mg}\cdot\text{L}^{-1}$ of SO_4^{2-} , $1,000 \text{ mg}\cdot\text{L}^{-1}$ of ethanol and $1,000 \text{ mg}\cdot\text{L}^{-1}$ of acetate ($3,000 \text{ mg}\cdot\text{L}^{-1}$ of COD in total). The COD removal and methane yield was maintained at above 80% and a $0.18 \text{ L}\cdot\text{CH}_4\cdot\text{g}\cdot\text{COD}^{-1}$ with an HRT above 6 h and an OLR below $12.3 \text{ g}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$. When the HRT was further decreased, VFA accumulation resulted in COD removal deterioration, whereas sulfate removal was maintained at around 30%. At an HRT of 2 h, molecular H_2S increased to above $110 \text{ mg}\cdot\text{L}^{-1}$ and caused digestion inhibition. COD and electron flow were mainly utilized by MPA, and methane was generated by *Methanosaeta* with acetate as the substrate. SRB accounted for 17.6% of the bacteria and belonged to an incomplete oxidizer, which utilized ethanol rather than acetate in the sulfate reduction.

Li *et al.* (2015) used an UASB reactor to treat chemical synthesis-based pharmaceutical wastewater containing rich organic sulfur compounds and sulfate and established a feasible process. At a COD/ SO_4^{2-} of 8, for practical application, the optimum OLR was found to be $8 \text{ kg}\cdot\text{COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$, where a nearly 70% COD reduction occurred with biogas containing 63% methane. The distribution of the archaea and bacterial community varied greatly with altered OLRs. The S release from some organic sulfur compounds in the reactor was attributed, at least in part, to some species, such as *Lysinibacillus sphaericus* and *Clostridium cellulovorans*. Increasing the sulfate loading at a COD/ SO_4^{2-} ratio of up to 1.5 resulted in a light inhibition of methanogenesis due to the high sulfide concentration ($1,212 \text{ SO}_4^{2-}\text{-S mg}\cdot\text{L}^{-1}$) with no obvious suppression of sulfidogenesis.

4.4. In situ removing H_2S from biogas

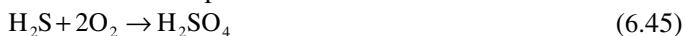
A combination of heat and power (CHP) is mainly used for the utilization of biogas. For trouble-free operation of a CHP, limit values of $100\text{--}500 \text{ mg}\cdot\text{Nm}^{-3}$ of H_2S cannot be exceeded (equal to 0.05% by volume), according to the recommendations of the manufacturer of CHP. The removal of H_2S is done nowadays mainly by biological desulfurization. The process is based on the oxidation of H_2S by injection of a small amount of air (2–5%) into the headspace of a digester.

The effects of injecting air into a bioreactor on the H_2S oxidation are described in Fig. 6.11 (Deublein and Steinhauser, 2012).

The oxidation of H_2S to sulfur and/or sulfate occurs according to the following reactions (Deublein and Steinhauser, 2012):



The direct reaction of H_2S to sulfate is also possible:



In an easy and economical manner, biological H_2S degradation can be accomplished by achieving immobilization inside the bioreactor on the wall and the cover surfaces above the substrate level. Particularly in smaller agricultural plants, this kind of immobilization is preferred. If this surface is not sufficient, plates or cloths have to be

hung in the upper space of the bioreactor. The air can be injected directly in the headspace of the digester, and the reaction occurs on the floating layer, on the reactor wall, and on other surfaces in the gas room.

For this kind of desulfurization, *Sulfobacter oxydans* bacteria must be present, to convert H_2S into elementary sulfur and sulfuric acid. For the desulfurization inside the digester, *S. oxydans* does not have to be added because it is present inside the digester (Weiland 2010).

4.4.1. Filamentous elemental sulfur (S^0) formation in digester

As mentioned by Speece (2008), the main problem of current hydrogen sulfide emission control technology is the cost. Since the formation of H_2S in anaerobic digestion processes cannot be completely eliminated unless oxic conditions can be constantly maintained in the environment, one should carefully choose the proper cost-effective method to control H_2S emission. There are different technologies of chemical (precipitation via the addition of Fe^{2+} or Fe^{3+}) and biological (actions of sulfide oxidation bacteria promoted by air injection to headspace) processes that can be used to remove H_2S from biogas. The biological process can achieve more effective H_2S removal than chemical processes because of extremely high substrate affinity of sulfide oxidation bacteria (SOB). The biological desulfurization has been applied in some biogas plants by injecting a small amount of air into the headspace of a digester to remove H_2S from biogas (Kobayashi *et al.* 2012). As discussed above, the products of sulfide oxidation can be elemental sulfur S^0 and sulfur acid. The sulfide oxidation level is regulated by the concentration of O_2 in the headspace. Kobayashi *et al.* (2012) have investigated the formation of S^0 in a full-scale biogas plant. They found that the S^0 formed in the ceiling and catwalks of the digester, present a filament shape with length of 100–150 μm . The S^0 attached the mats, which covered the entire headspace of the digester (Fig. 6.12).

The authors announced the first report of filamentous sulfur formation in a non-marine environment. Two SOB species related to *Halothiobacillus neapolitanus* and *Sulfurimonas denitrificans* were identified. Factors such as moisture content and

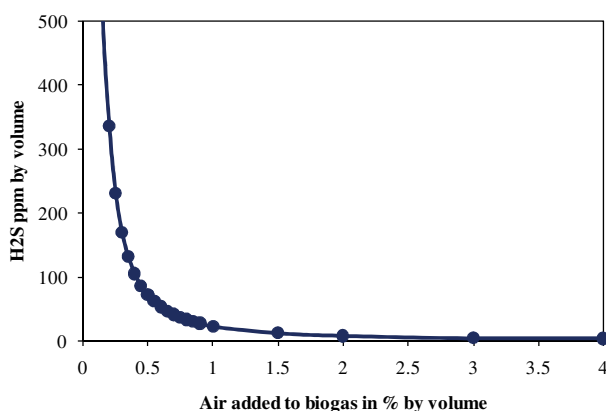


Fig. 6.11. Correlation between hydrogen sulfide content in the biogas and air flow into the bioreactor.

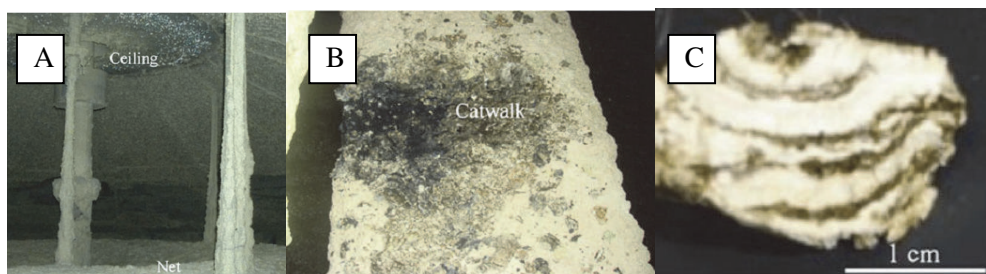


Fig. 6.12. S^0 formation in digester; (A) microbial mats developed in the ceiling, (B) and the catwalk, (C) a vertical cross section of the microbial mats consisting of white and black layers (Kobayashi *et al.* 2012).

nutrients of mats influence the formation of S^0 . In a digester, the water and nutrients were actually provided by the sludge droplets on the mats. As a result, more sulfur S^0 tended to form in the lower part of the headspace near the liquid level of the digested sludge. As concluded by the authors, the water and nutrients for the SOB provided by digested sludge droplets were the key factors controlling the activity and the microbial community for sulfide oxidation. In a full-scale biogas plant, it is possible to provide suitable area or place for the SOB growth by placing an immobilized carrier near the liquid level of digested sludge, which is the source of water and nutrients.

4.4.2. Self nutrient supplement reactor for H_2S oxidation

Bio-desulfurization is more cost, effective than chemical precipitation using Fe_2O_3 . The moisture and nutrient supplementation are the key factors that affect the H_2S removal efficiency. In a CSTR system, however, the normal operation of feeding and discharging keep a nearly constant liquid level within a digester. It was impossible to provide more and more places for SOB growth due to the deficiency of moisture and nutrients. It is reasonable to develop a reactor with a dynamic liquid level that could maintain the necessary conditions to ensure the activity of SOB. Under this point, Kobayashi and Li (2011) designed a novel anaerobic digester consisting of a container, partition plates and a U-tube.

The substrate was fed from port 3, and effluent including residual substrate and biomass produced was passed through a siphon outside of the reactor to the outlet in order to separate the biogas from the liquid (Fig. 6.13). The headspace of chamber 1 was closed, and the biogas produced was stored until being transported to chamber 2 via the U-tubes. The biogas produced in the reactor went out from the top of chamber 4 to a gas meter. The authors monitored the moisture content of the biofilm, which was around 75% during the experiment. That indicated the biofilm did not become drier during the long-term experiment of 220 days. The H_2S content of the biogas decreased from around 2,000 ppm on average to below 10 ppm on average with air addition. Actually, the average H_2S reduction ratio ($m^3-H_2S \cdot m^{-3}-O_2$ added) in the self-agitated reactor ranged from 0.33 to 0.46, while that in the CSTR was only 0.24. That indicated an improvement in

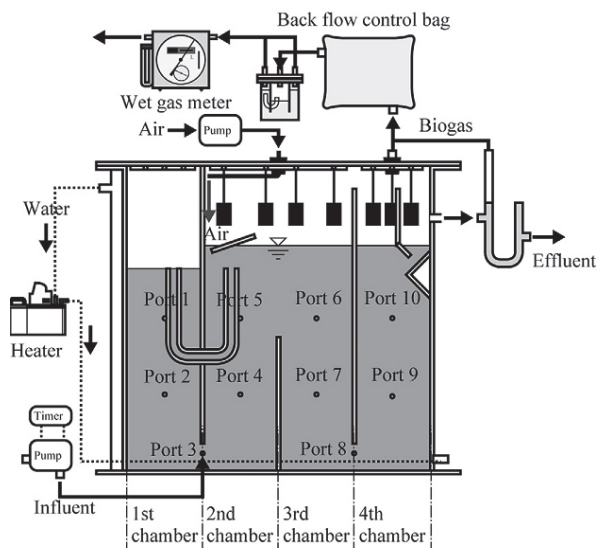


Fig. 6.13. Schematics of the reactor with dynamic liquid level (Kobayashi and Li 2011).

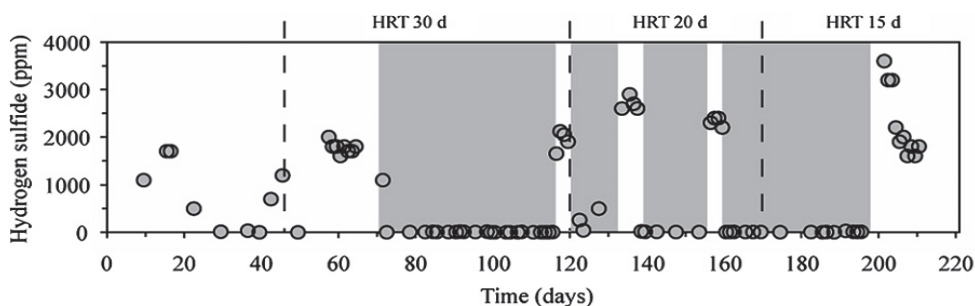


Fig. 6.14. H₂S removal with and without air addition (Kobayashi and Li 2011).

biofilm space for H₂S oxidation in this novel reactor. The H₂S in biogas with and without air addition into digester is illustrated in Fig. 6.14.

There was another potential benefit of batch stirring that was not discussed in this paper (Kobayashi and Li 2011). As mentioned by Speece (2008), the propionate degradation would be inhibited by continuous and intensive mixing due to the requirement of maintaining the aggregates' structure of syntrophic bacteria in digester. Specially, in thermophilic digestion, the propionate problem was more obvious. On the other hand, an unstirred reactor would reduce the contact of bacteria with substrate due to the existence of dead zones. As a result, there was a dilemma of whether to use stirring. As introduced in the self-agitated reactor system, the mixing of substrate and sludge was applied in a batch mode and depend on organic loading rate and biogas production rate. During an unstirred period, the syntrophic bacteria could aggregate. The batch mode stirring could eliminate the dead zones and improve the mass transfer of a digester.

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Chapter 7

Modelling Anaerobic Digestion Processes

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Anaerobic process modelling is rapidly expanding as new processes and reactor designs are developed, and a better understanding of the underlying processes is established. At the same time, new applications and demands on the art have been developed, particularly the generation of bioenergy and fuels, as well as plant-wide modelling of wastewater processes, and the integration of activated sludge and anaerobic models. This has required a rethink of what should go in the model, with a focus on the biochemical pathways (resulting in e.g., the IWA Anaerobic Digestion Model No. 1 — ADM1) shifting towards a better description of associated processes, such as chemistry, gas transfer, and organic solubilization, as well as new topics such as integration with hydrodynamics. In this chapter, we take a very broad view of anaerobic process modelling, including new applications, model development fit for applications, determining biochemical structure and potential new structures, as well as the identification and characterization of processes fit for modelling. While based on fundamentals, this leads to a practical view of how to best apply this diverse range of techniques to best solve real problems in anaerobic process modelling. A general approach to the problem of parameter and input characterization applicable to the broad range of processes and problems generally modelled is also included.

1. Introduction

Over the last ten years, there has been a substantial expansion of anaerobic digestion processes, broadly in the fields of solid waste digestion (Mata-Alvarez *et al.* 2000), digestion for energy production, and leveraging of existing assets through codigestion (Mata-Alvarez *et al.* 2011). It has also been more broadly applied in emerging areas, such as membrane bioreactors for industrial and sewage treatment (Liao *et al.* 2006), as well as applications across low-strength wastewater treatment and resource recovery (Batstone and Virdis 2014).

Anaerobic process modelling has classically focused on sewage sludge (activated and primary) treatment (Batstone *et al.* 2002), with the IWA Anaerobic Digestion Model No. 1 (ADM1) focused on sludge digestion using that as the target process. This has

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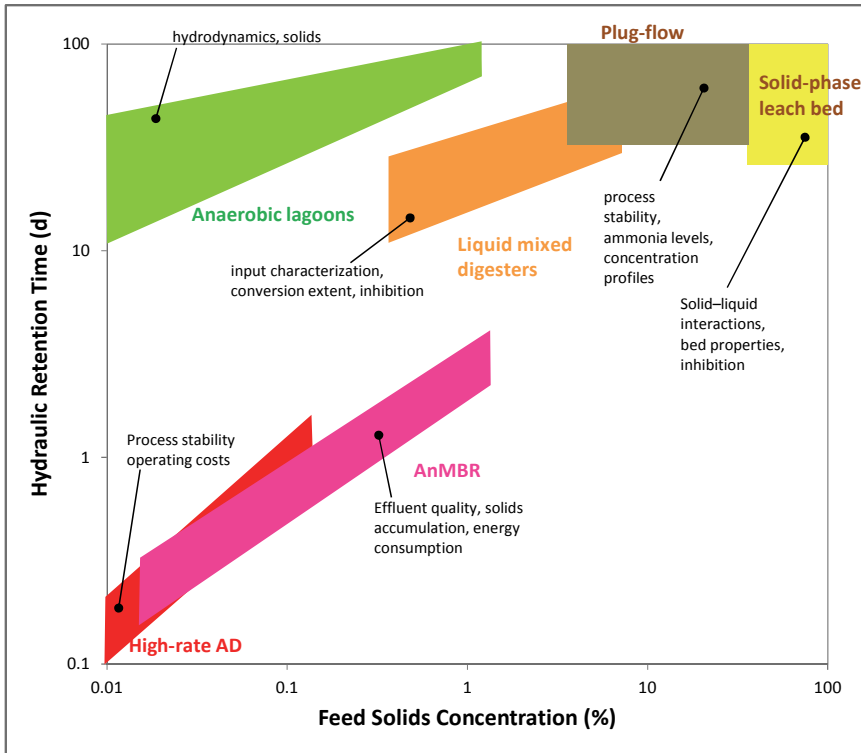


Fig. 7.1. Common anaerobic technologies, their fit-to-feed concentration and HRT, and common modelling objectives (modified from (Jensen 2014)).

driven a large proportion of the modelling developmental work, for example characterization models (Nopens *et al.* 2009) and the development of ancillary process models (Jeppsson *et al.* 2013). Other popular targets have included industrial wastewater (Batstone and Keller 2003), animal manures, and crop residues (Galí *et al.* 2009). As the applications for anaerobic processes expand, model objectives become more complex, and a wider range of approaches becomes necessary. This problem is formulated in Fig. 7.1, along with key objectives and potential approaches for process modelling.

At the same time, there has been increased acceptance and application of enhanced simulation tools, statistical methods, methods for model selection and translation, and methods for identifying the impact of uncertainty on process outcomes (Benedetti *et al.* 2012; Machado *et al.* 2014). There has also been development in tools to analyze anaerobic processes, particularly the microbial community in the context of reactor performance (Vanwonterghem *et al.* 2014) and methods to better link biological processes with complex factors, such as hydraulics (Van Hulle *et al.* 2014).

A shift in goals and requirements for increased capability has been matched by improvements in understanding of anaerobic process fundamentals, better tools to investigate anaerobic processes, and improvements in computing capacity and tools for conducting complex simulation scenarios. This chapter focuses on anaerobic process modelling within the context of these changing goals for model-based analysis.

2. Modelling Approaches

The normal approach to most waste(water) dilute stream modelling problems, including liquid stream anaerobic process modelling (Batstone *et al.* 2002) and activated sludge process modelling (Henze *et al.* 2000), is the dilute stream assumption. That is, hydraulic inventory is not a function of biological or chemical reaction. As such, for a generalized system balance, the reaction terms (defined by biochemical and chemical reactions) can be defined independently from advective and diffusive mass transfer terms. As a specific example, a generalized mass balance term for a dilute concentration state (particulate or soluble) in a mixed reactor is:

$$\frac{dS}{dt} = \sum \frac{q_{in,i}}{V} (S_{in,i} - S) + \sum r_{S,i} \quad (7.1)$$

Where S is the concentration of the state (S for soluble, X for particulate), $q_{in,i}$ is the mass or volumetric flow for stream i , V is the hydraulic volume or mass, and $r_{S,i}$ is the volume specific reaction rate for process i . Eq. 7.1 is used for fixed or variable volume reactors, with a state equation $\frac{dV}{dt} = \sum q_{in,i} - \sum q_{out,i}$ determining liquid volume in the latter case.

The context specific advective–diffusive implementation (term 1 in Eq 7.1) can be considered somewhat independently from the biokinetic model, with examples shown below (Table 7.1).

Indeed, popular platforms such as Aquasim (Reichert 1994), as well as many others implement advective–diffusive in hard-coding, or at a low level, and allow the biochemical and chemical rate components ($r_{s,i}$) to be implemented at the surface level. While it is important to be able to implement and solve full state-level models from first principle, this chapter focuses on the formulation and implementation of the reaction components. These components can be separated into biochemical (reactions mediated indirectly or directly by microbes) and chemical (chemical transformations, such as acid–base reactions) reactions that occur spontaneously.

Table 7.1. Reactor configurations.

Advective–Diffusive Model (compartment type)	Anaerobic Example	Governing Mechanism
Mixed tank	sludge digester AnMBR High–strength high-rate	hydraulic flow
Plug-flow	plug-flow solids digester anaerobic trickling filters low–strength high-rate	convective transport
Biofilm	anaerobic granule models	diffusion
Multi-phase (solid–liquid)	fixed bed/leach bed	convection–diffusion

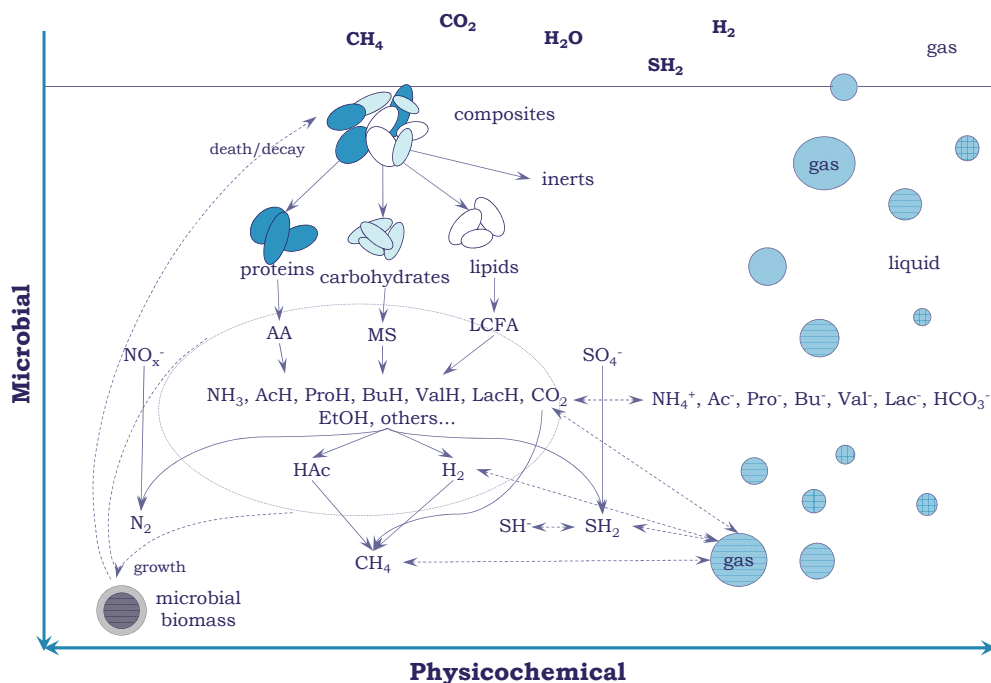


Fig. 7.2. Overview of microbial and physicochemical reactions taking place in anaerobic digestion (adapted from (Batstone *et al.* 2002)).

2.1. Microbial reactions and biochemistry

Numerous physicochemical and microbial processes take place simultaneously during anaerobic digestion (see Fig. 7.2). Microbial conversion processes (i.e. reactions catalyzed by microorganisms) are typically organized into so-called functional microbial groups for modelling purposes. Each microbial group activity is typically described by an averaged conversion stoichiometry (involving yields for substrates and products, as well as microbial biomass growth) and a kinetic expression (describing the rate as typically a function of substrates' concentrations and inhibitory components or conditions).

Model structure is discussed further in Section 3, but a common structure is one specific microbial group for the fast fermentation of soluble substrates into acetate, hydrogen, and carbon dioxide, and of specific microbial groups for methanogenesis (both from acetate and hydrogen). This basic level of model complexity is already capable of capturing dynamics between acido/acetogenic fermentation (fast) and methanogenesis (slow) (see Fig. 7.3), allowing for the prediction of process instability due to acidic accumulation (Bernard *et al.* 2001).

The recommended level of detail in the description of intermediate fermentation processes and components, (such as specific VFAs and other intermediates) heavily depends on the intended application of the model and on the requirements for the model outputs (see Section 3).

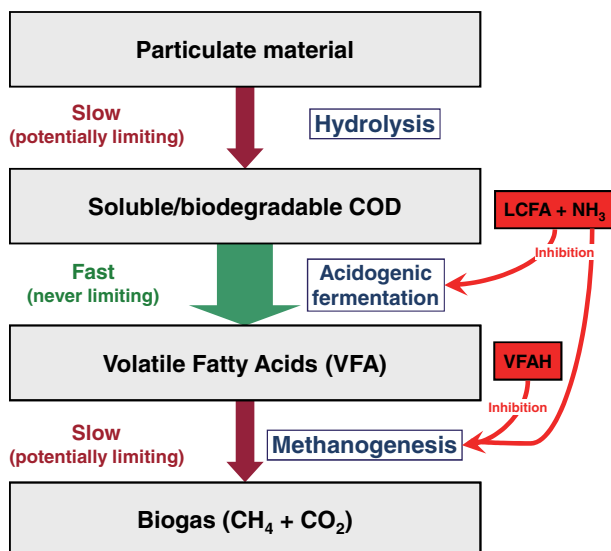


Fig. 7.3. Dynamics of the main steps during anaerobic digestion of solid substrates. Hydrolysis or methanogenesis can be limiting, but acido/acetogenic fermentation is always faster than methanogenesis.

Depending on the objectives and influent composition to the AD process, a model might require a more or less structured description of microbial groups to account for specific processes of relevance. An example is the case of sulfate or nitrate reduction that might or might not be relevant depending on the model objectives and the substrate composition. The inclusion of specific fermentable substrates/intermediates, such as lactate, ethanol, and others is also case-dependent.

Recent developments in anaerobic codigestion have brought up the need for the incorporation of specific substrates and their degradation. Recently, generalized approaches have proposed lumping all easily fermentable substrates under the same microbial groups (García-Gen *et al.* 2013). This approach works well, as long as the mass and electron equivalent (COD) balances are carefully maintained. Considering that the fermentative acidogenic steps are the fastest of the digestion process, if the substrates are solubilized, they are quickly converted into VFAs and later into acetate and hydrogen. This makes the accurate description of the exact transient intermediates of smaller relative importance in order to predict biogas and removal efficiency, as long as the electron (COD) and mass balances remain correct.

The microbial reduction of sulfate in anaerobic digestion can be important in specific cases. If sulfate-containing wastewaters or substrates are used, the accurate prediction of hydrogen sulfide might be of importance due to its effect on the quality of the biogas together with its malodorous, toxic, and corrosive nature, as well as inhibitory character for microorganisms. The presence of sulfate and organic matter leads to the growth of sulfate-reducing bacteria (SRB) under anaerobic conditions. Sulfate-reducing bacteria will preferentially utilize hydrogen as an electron source, but at very high sulfate:COD levels (Batstone 2006), they can compete with the other microorganisms for these

intermediates at the acidogenic, acetogenic, and methanogenic levels (Fedorovich *et al.* 2003).

Since SRBs can utilize multiple AD intermediates, including hydrogen, as electron donors, AD models incorporating sulfate reduction face the problem of accounting for all relevant electron donor combinations. However, if all possible electron donor microbial sulfate reduction processes are incorporated, the level of complexity and number of parameters can increase substantially. As stated in section 2, the application should define the level of detail required in the model. A number of sulfate reduction models and modifications, including some for the ADM1, have been developed (Barrera *et al.* 2013; Barrera *et al.* 2014; Batstone 2006; Fedorovich *et al.* 2003; Knobel and Lewis 2002). Differences between the approaches lie mainly in the number of electron donor substrates considered for SRBs and on the inhibition kinetic expressions for hydrogen sulfide.

Nitrate reduction by denitrifying microorganisms is another process not typically included in AD models. Considering that the product of nitrate reduction is not inhibitory nor toxic and does not have adverse effects other than biogas dilution, the impact of nitrate or nitrite reduction on the process is mainly limited to a strong competition for electron donors (as the respiration of nitrate is a highly energetic, high-yield metabolism), nitrate-reducing bacteria will outgrow and outcompete all others. Incorporation of nitrogen reduction poses similar problems as those of SRBs, as specific processes and kinetics would need to be included for each competing electron donor, adding largely to the amount of parameters to be determined. Some model attempts have been reported in line with this approach (Rousseau *et al.* 2008; Tugtas *et al.* 2006).

The inherent complexity in biological processes combined with the uncertainty, complexity, and variability of the influent substrates, with varying concentrations and compositions, makes the selection of the adequate level of complexity in a model critical. It will ultimately be the objective of the model and the amount and quality of information and data available that will define the optimum level of complexity of anaerobic digestion models. Optimal (minimum) model structure can be determined mathematically (Bernard *et al.* 2006), but model complexity generally exceeds the minimum requirements required to describe the basic kinetics in order to maintain mechanistic relevance (see section 3). It is commonly the modeller's expertise in view of the model objectives and quantity and quality of the information and data available that should determine the level of complexity most suited for a specific modelling application.

2.2. Physicochemical processes' ionic speciation and the role of pH

Acid–base speciation and the role of pH are critical in anaerobic digestion systems and their accurate modelling is a critical factor in obtaining accurate model predictions in both the liquid and gas phases. Besides its impact in the CO₂ gas–liquid equilibrium, affecting the biogas composition, the role of pH is of great importance due to its effect on the microbial reaction rates through specific inhibitions (e.g., on methanogenesis) as these can lead to process destabilization. Any model aiming at the prediction of acidification and destabilization should incorporate an accurate description of the acid–base speciation and pH.

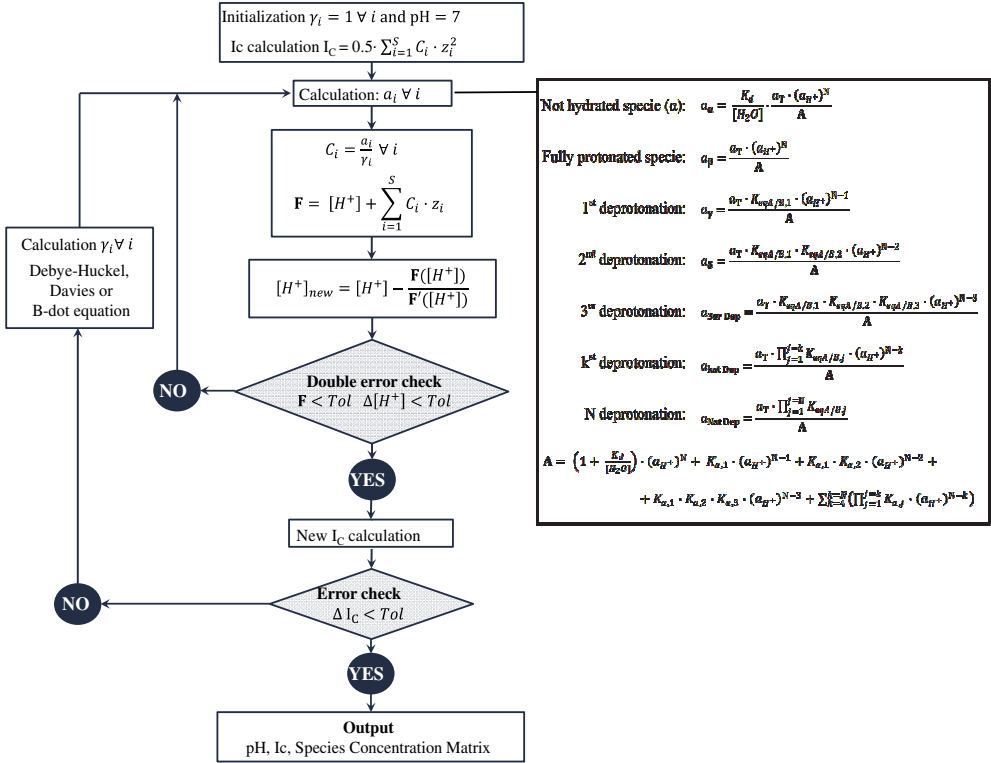
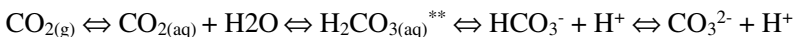


Fig. 7.4. Possible algorithm for pH and ionic speciation calculations accounting for component multiple deprotonations, ionic strength, and hydrations (e.g., CO_2) (González-Cabaleiro *et al.* 2013).

Acid–base reactions are known to reach equilibrium at rates that are orders of magnitude faster than other (bio)chemical reactions in the process. This allows for their algebraic modelling directly through equilibrium equations (i.e., by assuming instantaneous acid–base equilibrium in the reactor at all times) and by solving a charge balance. The ADM1 algebraic implementation of ionic speciation and pH (Batstone *et al.* 2002) is a representative framework from which more comprehensive physicochemical models have been developed. Recently, efforts have been directed to address the accuracy of the pH solvers by incorporating activity correction coefficients based on the ionic strength (Solon *et al.* 2015). Activity corrections generally require additional iterative effort due to the implicit relationship between ion activity and activity coefficient. Fig. 7.4 shows a possible generalized approach to solve pH and ionic speciation in aqueous systems accounting for multiple deprotonations, hydration (of CO_2), and ionic strength. Activity and ion pairing have generally been neglected in wastewater systems, with the excuse that they are relatively unimportant at low concentrations. However, the large variations in strengths that are seen in plant-wide wastewater treatment modelling, as well as the importance of non-ideal ion behavior in precipitation modelling (Batstone *et al.* 2012), mean that this is likely to be an important part of future wastewater and anaerobic process modelling.

2.2.1. Liquid–gas transfer

Two approaches can be followed to describe the transfer from liquid to gas in anaerobic digesters. The simplest approach is the modelling of gas product components as directly generated in the gas phase (e.g., methane or hydrogen), however, this carries the implicit assumption of liquid–gas equilibrium and neglects any mass transfer limitations and possible supersaturations. This approach can have very limited application because anaerobic digesters are known to show supersaturation of gas components in the liquid medium, including methane, hydrogen, carbon dioxide and others, that impact the process dynamics. In addition, components such as CO₂ and H₂S are of weak acidic nature with carbon dioxide quickly hydrating into carbonic acid and deprotonating into bicarbonate and carbonate depending on the pH. The distribution of these weak acid soluble gases can strongly affect and be affected by pH in the reactor, leading to significant inaccuracies in the model predictions of biogas composition if not properly accounted for.



Due to the strong pH-related interactions, the use of specific state variables for the concentration of dissolved soluble gases is normally recommended at least for CO₂ (and H₂S if modelled) in integration with the acid–base speciation. The modelling of dissolved methane and hydrogen should have a lower impact in biogas prediction, however, hydrogen in solution can affect the thermodynamics of some key biological reactions. On the other hand, dissolved hydrogen is also a variable prone to causing numerical problems during simulations. The typically small numerical value of dissolved hydrogen combined with the relatively large fluxes for its production (acidogenesis) and consumption (methanogenesis) makes its dynamic simulation highly stiff for most numerical solvers. Approaches have been suggested to eliminate the problem in large models, such as ADM1, by bringing the hydrogen modelling directly into the gas phase (Rosen *et al.* 2006). It will ultimately be the model application and objectives that defines the most adequate level of complexity and detail.

2.2.2. Solids precipitation

Solids precipitation is a critical element that describes solid–liquid interactions for key ions. It has generally not been included in anaerobic process modelling, with the key exception being the University of Cape Town group (Musvoto *et al.* 2000). It is particularly important to describe phosphorous kinetics, with key applications being descriptions of the complete phosphorous cycle in wastewater systems. Key compounds likely to precipitate (and interact through precipitation) are inorganic carbonates, sulfides, and phosphates on the anion side, and iron, calcium, and magnesium on the cation side, with many possible precipitates even in this subset (Batstone *et al.* 2012). However, while the range of precipitants is very complex, it appears possible to determine a relatively simple and broadly applicable kinetic relationship (Kazadi Mbamba *et al.* 2015), consisting of a fundamental equilibrium-driven process that is relatively tolerant of errors in assessing kinetics. As stated above though, most precipitation processes

require inclusion of non-ideal ion behavior, including ion pairing and ion activity, due to its increased impact on multi-valent ions.

2.2.3. Thermodynamics of AD systems

Most (bio)chemical anaerobic oxidation and methanogenic reactions taking place during anaerobic digestion are characterized by a low Gibbs energy exchange (ΔG), mainly due to the absence of strong external electron acceptors such as oxygen. This low-energy exchange makes some key reactions during AD take place in close proximity to thermodynamic equilibrium. This implies that only narrow ranges of concentrations of products and substrates allow for all reactions to remain within thermodynamic feasibility. A key component to this balance is the dissolved hydrogen involved in most of the reactions either as product or substrate. Dissolved hydrogen concentrations need to remain sufficiently low to keep the thermodynamics of e.g., butyrate and propionate oxidation in the negative ΔG , while at the same time, sufficient hydrogen must be present for hydrogenotrophic methanogenesis to proceed (both kinetically and thermodynamically). Interspecies electron transfer (by hydrogen or other mediators) in anaerobic digestion is a well-known phenomenon, but is yet to be fully understood (Batstone *et al.* 2006).

The basic approach to describing the inhibitory impact of hydrogen is non-competitive inhibition (Batstone *et al.* 2002), but still allows for (reduced) activity when activity should theoretically be zero. Alternative approaches can include more fundamental switch factors in kinetic expressions that stop the reaction rate when ΔG approaches zero (Batstone *et al.* 2006). This removes a parameter (since inhibition is related to the fundamentals of thermodynamics), but includes the assumption that the model hydrogen is the same as the *in situ* hydrogen experienced by the hydrogen producer, which may not be the case for microcosms within biofilms, and indeed, syntrophic activity is largely independent of bulk hydrogen (Batstone *et al.* 2006). At the moment, the empirical non-competitive function reflects experimental results, while being technically less satisfying.

2.3. Kinetic modelling of microbial reactions

The rate at which microbial conversions take place in AD is determined by a number of factors beyond simply the concentration of the required substrates. These factors include the activity of the microbial catalyst, as well as the presence of inhibitory components and environmental conditions (such as pH, temperature, salinity, etc.). The volumetric rate ($\text{gCOD} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$) of a microbial reaction is determined by the maximum metabolic activity of the active microorganisms together with the abundance (concentration) of those microorganisms, by the concentrations of the substrates and the affinity for them from the microorganisms, as well as by the presence of inhibitory components or environmental conditions that can lower the overall rate. Inhibitions can be diverse in their mechanisms, from reversible types of inhibitions primarily affecting the affinity between microorganisms and substrates, to inhibitions of more an irreversible nature, such

as toxicity that could e.g., increase cell maintenance or decay and hamper the microbial activity capacity in the long term.

Most rate expressions of microbially catalyzed reactions are defined in terms of a specific substrate or product conversion per volume of reactor and per time and are typically made up of the product of a biomass-specific substrate uptake rate times the activity (concentration) of the microbial biomass ($r_{Si} = q_{Si} \cdot X$; $g_{Si} \cdot L^{-1} \cdot h^{-1}$). Rate definitions in terms of units of biomass growth (on a specific substrate metabolism) per volume of reactor and time are also found; these are made up of the product of a specific biomass growth rate times the activity (concentration) of that microbial biomass ($\mu \cdot X$; $g_X \cdot L^{-1} \cdot h^{-1}$). Both expressions can be generally interchangeable through a yield parameter ($\mu \cdot X = Y_{XS} \cdot r_{Si}$), if constant stoichiometry metabolism is assumed.

The most detailed kinetic expressions to represent the reaction rate per unit of volume and time are commonly made up of four elements (Fig. 7.5): namely (i) a biomass specific maximum rate (defined typically as a parameter), which represents the maximum turnover or metabolic work that one unit of microbial catalyst can carry out; (ii) the activity (usually concentration) of that microbial (catalyst) biomass; (iii) a substrates', concentration-dependent term that slows the reaction when substrates become limiting; and (iv) inhibition terms typically included as factors multiplying the rate expression.

Comparison of thermodynamic and non-competitive inhibition functions have been discussed above, and are further outlined in (Batstone *et al.* 2002), but generally non-competitive functions are applied as they are simple, comparable to Monod kinetics, and have a meaningful parameter (K_I = level at which biomass is 50% inhibited). It should be noted, however, that observed inhibition in dedicated studies almost never follows a non-competitive form (e.g., see Pratt *et al.* 2012).

Kinetic expressions in bioprocess models including AD are typically the main source of parameters requiring calibration and identification. It is clear that the level of complexity of kinetic expressions in AD can become quickly unmanageable due to the number of processes involved and, for this reason, it is of great importance to assess the required level of complexity required when modelling kinetics in AD. The objective of the model and its frame of application will define which kinetic effects to incorporate in the rate equations (e.g., including or excluding rate effects by temperature, pH, and specific substrates or products, toxic components, salinity, bioactive components, LCFA, ammonia, hydrogen sulfide, etc.). It is here where the modeller must apply the simplest kinetic expression that serves the purpose of the model application.

$$\begin{array}{c}
 r_{S_i} = q_{S_i}^{\max} \cdot \left(\frac{g_X}{L \cdot h} \right) \\
 \text{OR} \\
 \mu \cdot X = \mu^{\max} \cdot X \cdot \prod_i \frac{S_i}{K_{S_i} + S_i} \cdot \prod_j I_j \left(\frac{g_{S_i}}{L \cdot h} \right)
 \end{array}$$

Maximum
specific
activity

Specific
biomass
concentration

Mass action
(Monod-like)
terms

Reversible
inhibition
terms

Fig. 7.5. Typical structure of kinetic expressions commonly found in anaerobic digestion models. Rate can be expressed either as substrate consumption or biomass growth per volume and time.

A common problem in mixed culture bioprocess modelling is the inherent difficulty in achieving a separate identification of the maximum specific uptake rates from the microbial biomass concentration. This is due to the extreme difficulty in measuring the actual concentration of specific microbial groups separately from each other (typically only total biomass concentrations are available from tests such as volatile suspended solids). Although developments using molecular techniques have addressed this problem (Vanwonterghem *et al.* 2014; Werner *et al.* 2011; Yu *et al.* 2006), gaps remain in how to quantitatively link DNA-based (16S, functional gene, or metagenomic) relative abundances and actual specific microbial activities and, to date, no good applicable techniques are available for specific active biomass quantification in AD systems.

Modellers must therefore provide a good estimate of the microbial specific concentrations in their biomass inoculum, especially if short-term data from the beginning of a simulation are to be used. Estimates of microbial groups' concentrations can be obtained by a combination of lumped measurements, such as volatile suspended solids with the use of estimated specific biomass growth yields. The differences in the energetics of the AD conversion processes lead to differences in growth yield among the microbial groups and these can be estimated (Kleerebezem and Van Loosdrecht 2010). By combining estimated (or measured) yields with long-term simulations, mimicking the source environment of the biomass (provided a sufficient characterization of the substrate in which that biomass developed is available), good working estimates of microbial groups concentrations can be obtained.

2.4. Unconventional and advanced modelling approaches

Although most models with proven practical process application in AD have been based on the above described approaches, i.e., a number of reaction processes of constant stoichiometry taking place simultaneously under specific rate expressions of higher or lower level of detail and complexity, a number of less conventional alternative approaches have been developed over the last years.

Some of these alternative modelling approaches are aimed at extending the application of AD models to other non-methanogenic (e.g., bio-hydrogen or VFA production) anaerobic systems while trying to run away from the inherent identifiability problems described above associated to the uncertainty in microbial biomass composition. Approaches have been developed to address the uncertainty in the microbial diversity in AD systems by incorporating kinetic differences between functionally redundant microbial species (Ramirez *et al.* 2009). In order to apply the ADM1 into non-methanogenic processes, variable stoichiometry approaches have been used for the acidogenic fermentation, well known to display non-constant product yields depending on environmental conditions (Penumathsa *et al.* 2008; Rodríguez *et al.* 2006). This approach does not serve effectively in methanogenic systems as the fermentation intermediates become converted quickly into acetate and hydrogen, but it is required in cases where e.g., VFAs and hydrogen are the final products of interest.

The increasing importance of AD of mixtures of substrates in anaerobic codigestion (AcoD) processes has brought up the important problem of how to deal with a large range

of substrates without increasing the number of processes and parameters of AD models to unmanageable sizes. Initial developments towards generalized modelling approaches to deal with the diversity and variability of substrate mixtures are available (García-Gen *et al.* 2013).

Other unconventional models have been developed with more fundamental research objectives. Among those, some models focusing on the detailed bioenergetic analysis of anaerobic microbial ecosystems have been developed (Rodríguez *et al.* 2008). These models attempt to bring light into the defining factors of specific fermentation products and their variability as a function of environmental conditions in anaerobic ecosystems (González-Cabaleiro *et al.* 2015a; Rodríguez *et al.* 2006). The reversibility of specific anaerobic pathways of interest has been also investigated using thermodynamics combined with basic physiological information (González-Cabaleiro *et al.* 2013). Bioenergetic modelling approaches have also recently provided insight into the possible ecological origins of fermentation product variability and of key microbial syntrophisms present in anaerobic ecosystems (González-Cabaleiro *et al.* 2015b).

3. Application of Models

The form of application of the model depends heavily on the modelling goals and system being analyzed. One of the key differentiators is whether a semi-mechanistic or fully empirical biochemical model is to be used. The first applies an empirical relationship (first-order, Monod, or other) model to biological activity, but then structures the model based on knowledge of the ecology as provided in section 2.1 (i.e., grey box). A fully empirical model applies empirical relationships (e.g., regressive or state-space or other) model to observed data only. We argue that generally, the semi-mechanistic or grey box approach as outlined in section 2.1 should be applied to anaerobic models, as it:

- (a) allows for continuity and parameter and state compatibility across varying levels of model complexity.
- (b) has some predictive capability outside the fitted range. Fully empirical models generally do not have any capability outside the data ranges evaluated (Hangos and Cameron 2001).
- (c) provides mechanistic interpretations of model outputs.

The main disadvantage is that mechanistic models generally have a lower level of identifiability (Dochain and Vanrolleghem 2001), since they generally include processes or mechanisms that are structurally redundant or not rate-limiting. The key exception is probably control applications, where model complexity needs to be limited. Some key applications and levels of model complexity are shown in Table 7.2.

While the basis of the model is the grey box mechanistic model based on biochemical structure (e.g., the ADM1), actual model complexity can range from very simple (e.g., first order) to more complex than the ADM1 (e.g., ADM1+extensions). It is possible to maintain parameter compatibility across a broad range of model complexities, particularly if the readily identified *controlling mechanism* (e.g., hydrolysis in a high-solids system) is specifically identified.

Table 7.2. Model complexity compared with model goals.

Goal	Model complexity	Ref.
Material characterization	If batch, low. If continuous, low to high.	1
Design and prototyping	Low to high (generally low for design, high for prototyping).	
Analysis/design of experiments	Low, particularly if focused on particular substrates or mechanisms; however, can isolate subset from more complex model (e.g., ADM1).	2
Ecological analysis	Low, particularly if focused on particular substrates or mechanisms; however, can isolate subset from more complex model (e.g., ADM1).	3
Process analysis	High, and standardized (e.g., ADM1).	4
Plant-wide modelling	High, and standardized (e.g., ADM1), but with other models (e.g., ASM1, etc.), particularly if used for control. May be low for scenario analysis/LCA.	5
Education/operator training	High, and standardized (e.g., ADM1).	
Control	Minimal.	6

1. Batstone *et al.* 2009; 2. Penumathsa *et al.* 2008; 3. Ho *et al.* 2014; 4. Hinken *et al.* 2014; 5. Nopens *et al.* 2010; 6. Dimitrova and Krastanov 2012.

3.1. Simple models of batch tests and continuous digesters

The most basic modelling of anaerobic digestion is done to characterize biochemical methane potential (BMP) tests (Jensen *et al.* 2011). In a batch system, with no flow, with a single, process first-order in degradable solids concentration (X_s), Eq. 7.1 can be simplified to:

$$\frac{dX_s}{dt} = -k_{hyd} X_s \quad (7.2)$$

where k_{hyd} is the hydrolysis coefficient (d^{-1}).

This can be solved analytically, and expressing it as a function of attainable methane yield ($B_0 - ml_{CH_4} \cdot g_{VS}^{-1}$) results in:

$$B = B_0 \left(1 - e^{-k_{hyd} t} \right) \quad (7.3)$$

where B_0 is the attainable methane potential.

This is a simple model with two parameters that can be fitted with parameter optimization (including estimation of parameter uncertainty — see section 3.4) — to obtain a best-fit model to the data (Fig. 7.6). This model can be readily extended to a 2-substrate system (Wang *et al.* 2013), particularly for substrates such as activated sludge, which often have both slowly and rapidly degradable fractions. Apparent degradability (f_d) can be estimated from the B_0 as:

$$f_d = \frac{B_0}{380 \cdot COD:VS} \quad (7.4)$$

where f_d is the degradability, or fraction of COD, that is degraded at infinite retention time, and COD:VS is the COD:VS ratio ($g_{COD} \cdot g_{VS}^{-1}$), and is approximately 1.42 for activated sludge (Batstone *et al.* 2009), and up to 1.8 for primary sludge.

It should be noted that f_d represents the total degradable fraction, and where carbohydrates, proteins, and lipids are used as primary substrates, is the fraction of total

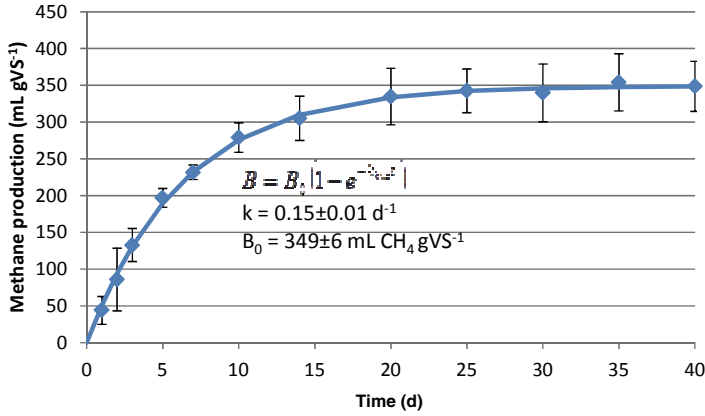


Fig. 7.6. Methane potential test and best-fit model (methane is at standard temperature and pressure). Error bars are two-tailed confidence intervals based on triplicate analysis.

feed that can be represented as these degradable substrates (the remainder is inert as X_I and S_I).

The parameters estimated using a batch test are generally conservative (in speed), or effectively underestimating k_{hyd} vs. a full-scale reactor (Batstone *et al.* 2009; Jensen *et al.* 2011), particularly with rapidly degradable substrates. However, they can be used directly for design purposes in continuous reactors by applying Eq. 7.1 in continuous steady state mode:

$$B = B_0 \left(1 - \frac{1}{1 + k_{hyd} t} \right) \quad (\text{mL}_{\text{CH}_4} \cdot \text{gVS}^{-1}) \quad (7.5)$$

Note that t in this case is average retention time (d) rather than batch time as in Eq. 7.3.

The same formula can be used to predict VS destruction in a continuous reactor:

$$\text{VSD} = f_d \left(\frac{1}{1 + k_{hyd} t} \right) \quad (\%) \quad (7.6)$$

As can be seen from a comparison of Eqs. 7.4 and 7.5, for a given retention time in a reactor, a batch process will achieve a higher extent of degradation for a given time t .

Alternatively, the parameters obtained from the batch test can be applied in a more complex model such as the ADM1 setting the k_{hyd} and f_d of the primary substrate appropriately (see below).

3.2. Modelling complex mixed continuous systems

Larger continuous anaerobic digester systems, in particular, full-scale systems can be regulated by multiple controlling mechanisms, including ammonia or other inhibition, substrate complexity, and physical–chemical interactions (Batstone *et al.* 2010; Pitk *et al.*

2014). The ADM1 was designed for application to complex processes where different controlling mechanisms may regulate under different conditions. The completely mixed (lumped parameter) form of the ADM1 is applicable to the following commonly applied systems:

Liquid–solids systems (<6% feed) (such as sludge digesters), where the controlling mechanism is either hydrolysis or inhibition of methanogens, may include activated and primary sludge digesters, where hydrolysis generally dominates (Batstone *et al.* 2009), or animal manure or codigestion plants, where inhibition and hydrolysis both dominate (Shi *et al.* 2014).

High-rate industrial anaerobic digesters, which generally treat highly soluble wastewaters. In this case, the controlling mechanism is generally the capacity of the retained methanogens and acetogens to convert intermediates to methane. Extensions may be required for the specific substrate (Hinken *et al.* 2014).

Low–strength domestic treatment systems may include domestic fed UASB reactors (Batstone 2006; Elmitwalli 2013), anaerobic membrane bioreactors (Benyahia *et al.* 2013), and anaerobic baffled reactors (Liu *et al.* 2014), though a multi-compartment model will generally be required for the latter. Controlling mechanisms in this case are degradation of particulates, gas–liquid solubility, and possibly generation of sulfides (Batstone 2006).

The parameter set applied with the ADM1 is generally effective for most of these systems, with appropriate extensions such as sulfate reduction (Batstone 2006). The largest initial challenge is probably feed characterization, particularly for complex substrates, such as primary or activated sludge. The ADM1 originally recommended use of the particulate complex state (X_c) for primary and activated sludges, but this has resulted in a number of disadvantages, including:

- (a) It does not allow for variation in the nitrogen and energy content, or degradability (fraction of X_c that degrades to non- X_I products) over time, as these are fixed in the X_c variable. In particular, primary sludge generally has higher energy content and is more degradable.
- (b) It results in a 2-step hydrolysis process, since both X_c and subsequent particulate substrates are subject to hydrolysis. To avoid 2-step, and excessively slow kinetics, the rates of carbohydrate, protein, and lipid hydrolysis must be set artificially high (e.g., 10 d^{-1}).

The most direct way to overcome this is to treat inerts, carbohydrates, proteins, and lipids as the primary input point (Fig. 7.7). Fractionation can be based on the following different methods:

- Fractionation to carbohydrates, proteins, and lipids (and inerts) based on primary measures (mostly food-based) for specific feeds (Buffiere *et al.* 2006).
- Model-based fractionation, which can be used for both primary and activated sludge (but is better suited to activated), where the inert part is based on the upstream model, and the degradable part is fractionated, based on preserving organic nitrogen continuity in proteins, and mass and COD continuity in carbohydrates and lipids (hence maintaining energy density (Nopens *et al.* 2009)).

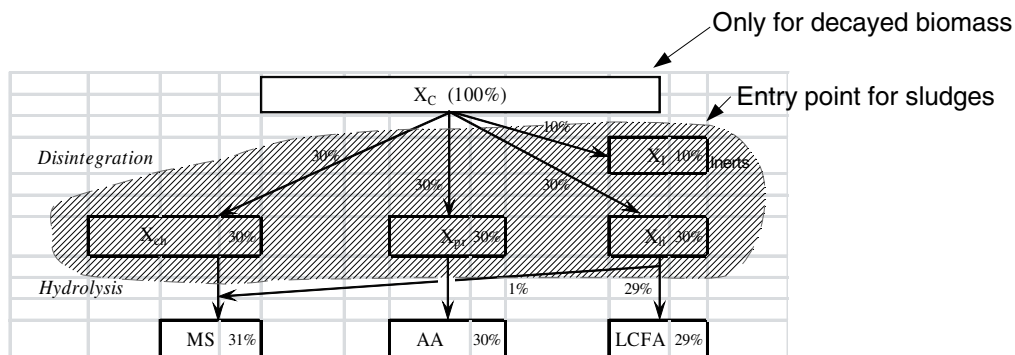


Fig. 7.7. Recommended use of the ADM1 primary substrate models, which is to define input materials (particularly primary and activated sludges) as a mixture of carbohydrates, proteins, lipids, and inerts. Nomenclature as for ADM1, including X_c ; complex, X_i ; particulate inerts; X_{ch} , carbohydrates; X_{pr} , proteins, and X_{li} lipids.

- Fractionation based on biochemical methane potential, with the degradable fraction (f_d) determined from the methane yield, protein from the ammonia release, and the carbohydrate and lipid fraction from the primary substrate COD:VS ratio (García-Gen *et al.* 2015).
- Methods that are chemical or hybrid (for example, determination of degradable fractions by sequential extraction and spectroscopy) (Jimenez *et al.* 2014).

All of these methods are aimed at fractionating, rather than determining kinetic coefficients, but particularly, the last two also provide hydrolysis coefficients. The ADM1 contains hydrolysis coefficients for carbohydrates, proteins, and lipids. The default approach for aggregate or composite materials is to set all three equal (and to the observed value), but hydrolysis can also be split into rapid or slow fractions (each of which will need carbohydrate, protein, and lipid fractions), or an additional (e.g., carbohydrate only) slow fraction can be defined (García-Gen *et al.* 2015) in some cases, where co-feeds are used and different hydrolysis coefficients can be applied. The default approach though is used to define an aggregate hydrolysis coefficient.

The issues around fractionation are common to most applications in mixed digester modelling (and indeed, distributed parameter systems; see below), and are probably the main challenges in modelling. Actual implementation apart from this is mainly the determination of modelling packages, and either adapting a base implementation to the desired application, or independently implementing the model of choice. The two most widely used (publicly available) version of the ADM1 are Aquasim 2.1d (Batstone *et al.* 2002) and Matlab Simulink (Rosen *et al.* 2006), both of which are available from the chapter author, but there exist implementations (or functionally similar implementations, e.g., Biowin's combined model) in a range of other packages. There are also a range of potential extensions, the two most important of which are likely sulfur (biological and physicochemical – see Barrera *et al.* (2014 for a recent review) and phosphorous, which is largely biological and associated with modelling phosphorous in environmental systems (see last section in this chapter).

The key outputs from anaerobic digester modelling are gas flow (or methane flow, which requires composition calculation) and solids content. The ADM1 as provided in the scientific and technical report does not provide solids as an output, but estimating it is relatively easy, as every component has an inherent COD:mass ratio (provided in the report), and hence the volatile solids concentration is the sum of these. Mineral solids also need to be tracked and can be simulated as a dynamic, but biologically inert state. Solids' concentration in the outlet allows for another output that can be used to fit relevant parameters, but also allows for the calculation of volatile solids destruction. It is, however, generally less dynamic compared with gas flow, mainly due to the large solids inventory in the digester (Fig. 7.8)

3.3. Complex (distributed parameter) systems

While there has been extensive work applying modelling in mixed digesters, the majority of reactor types as shown in Fig. 7.1 (and Table 7.1) are not effectively modelled using a lumped parameter approach, since there will be substantial variation in state variables across space. Particular examples where distributed parameter modelling is important include anaerobic biofilm reactors (including granular reactors), which can be

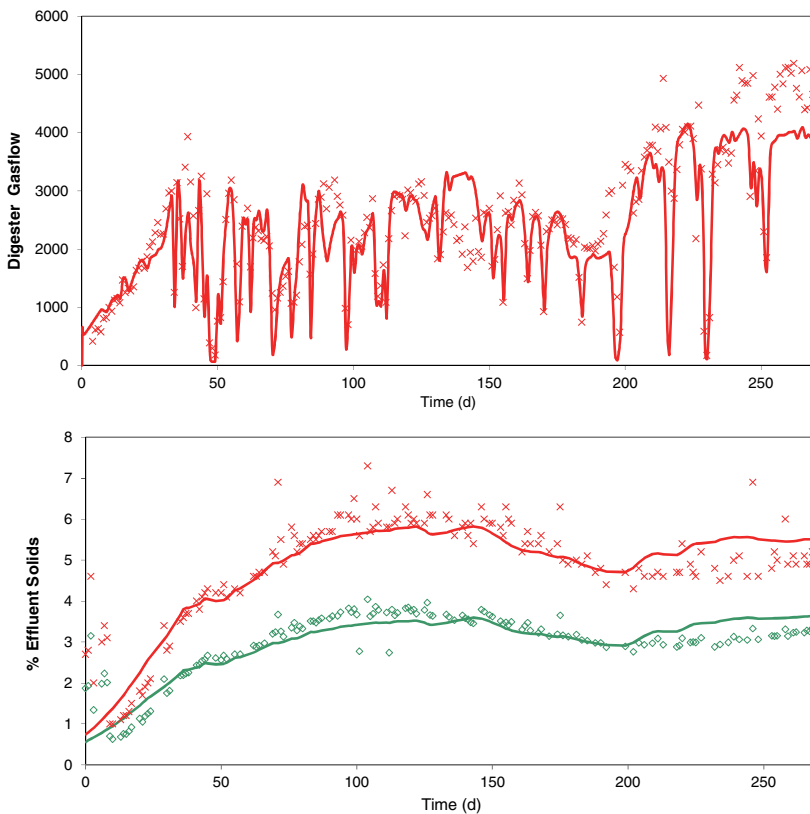


Fig. 7.8. Gas flow (top) and outlet solids (bottom) simultaneously predicted using the ADM1 (Batstone *et al.* 2009; Batstone *et al.* 2010).

modelled both in the micron scale through a biofilm model (Batstone *et al.* 2006), as well as through an advective–diffusive (possibly plug-flow reactor) on the reactor scale (Batstone *et al.* 2005), plug-flow semi-solid reactors (Dvorak 2012; Frear and Dvorak 2013), leach bed (including engineered landfill reactors) (Nopharatana *et al.* 2003), and anaerobic digestion and stabilization lagoons (Alvarado *et al.* 2012a; Alvarado *et al.* 2012b).

Even systems, which can be effectively represented by a single compartment from a biological perspective, may need a distributed parameter model for specific aspects, particularly those related to shear, mixing, or other operational considerations (Boyle-Gotla *et al.* 2014). Particularly interesting is the broader application of computational fluid dynamics in combination with biochemical reactions, with diffusive–advective–reactive mass equations being solved simultaneously with momentum equations (Gaden 2014). This can be very important, particularly in low-momentum systems (e.g., lagoons), where gas can impart mixing, or where the viscosity of the liquid is influenced by solids’ concentration. An alternative option is a hybrid approach, in which a complex CFD model is used to determine hydraulics, and this is translated to a simpler compartmental model for biochemical reactions (Alvarado *et al.* 2012a; Laurent *et al.* 2014).

The implementation of even very complex biochemical models within a distributed parameter environment is mathematically quite straightforward and involves the implementation of the parabolic partial differential equation for a reactive, diffusive mass state in an incompressible field:

$$\frac{\partial S_i(\vec{p}, t)}{\partial t} = D \nabla^2 S_i(\vec{p}, t) - \vec{v} \nabla S_i(p, t) + \sum r_i(p, t) \quad (7.7)$$

The mass state is intensive (Hangos and Cameron 2001), and is a function of the position (\vec{p}) and time (t). The first term represents diffusion (parameterized by the diffusion coefficient D), the second term represents convection (with velocity vector \vec{v}), and the last represents reactive terms (and inter-phase transfer terms), with these largely coming from the biochemical model (e.g., the ADM1). Particulate components that are not subject to diffusion have the first term excluded. Generally, either diffusion or convection will dominate (e.g., biofilm and plug-flow reactors respectively), and the equations can be further simplified.

The partial differential equations are generally represented as a number of ordinary differential equations through finite differencing or volume approximations, with boundary conditions required to fully specify the problem.

Modelling packages (e.g., Aquasim 2.1d) implement these equations, including boundary conditions for a variety of one-dimensional systems, including plug-flow and biofilm reactors, with the biochemical equations implemented by the user. However, they are also likewise implemented in multi-dimensional packages, such as Comsol and the Ansys packages. The main challenge in modelling large-scale distributed parameter problems is model solution (including discretization), rather than implementation.

Possibly one of the the most complex systems to simulate effectively is the leach bed system, which contains gas, liquid, and reactive solid phases, as well as complex

interactions between biologically active solids and liquid and gas flows (V_L , V_G). Particularly as a batch process, the hydraulics and effective volume of the solid bed can change, which then has an integrated impact on concentration in the liquid phase. This has been very sparsely addressed in the literature, but given the importance of engineered landfills and municipal solid waste bioreactors and their common operational difficulties (la Cour Jansen 2011), it is an important task.

Another system that is generally difficult to operate and is subject to distributed parameter effects, and which could benefit substantially from modelling, is the plug-flow bioreactor (Dvorak 2012). This can enable substantial intensification and relatively low-cost operation due to high-solids feed concentration, as well as enable cost-effective ammonia recovery (Frear and Dvorak 2013), but is subject to non-Newtonian fluid characteristics and related challenges in mixing and pumping and very high in-reactor ammonia characteristics, requires gas production to mix effectively, and is subject to propagation overload. The latter is a characteristic of plug-flow and batch systems, where a localized failure can propagate through space (along a plug-flow system) or time (along a batch) to cause whole-system failure.

Clearly, a range of reactor designs is subject to distributed parameter effects, and this justifies an increased focus in the application of anaerobic digestion in these environments. While there are particular challenges (e.g., solution of gas-liquid and large-scale algebraic problems), these are also common to other environmental modelling problems, and solution of these issues can help the field as a whole.

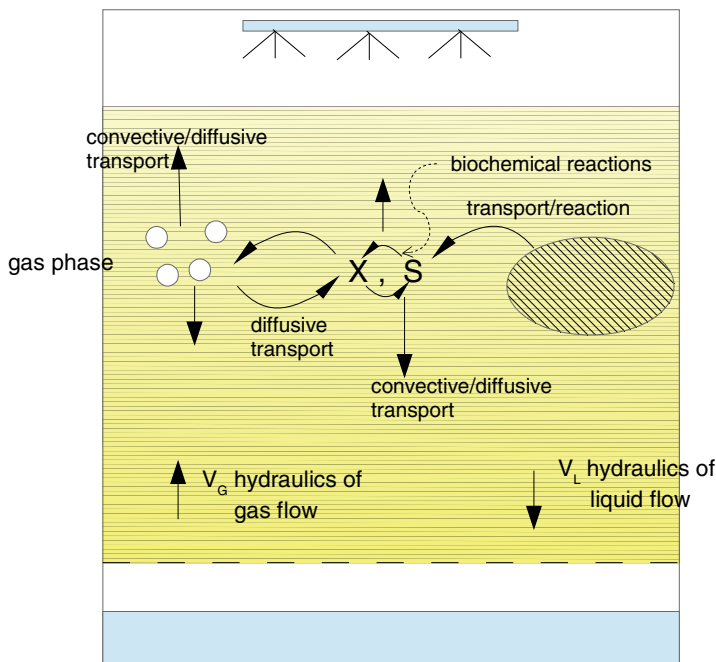


Fig. 7.9. Mechanisms for modelling in a leach bed system, including mass transport and multi-phase reaction.

3.4. Parameter estimation and uncertainty analysis

As stated above, effective parameter estimation, which includes input characterization, is probably the major challenge in the implementation of both simple and complex anaerobic process models. The statistics and basic approaches for parameter estimation has previously been covered extensively (Batstone *et al.* 2009; Dochain and Vanrolleghem 2001; Donoso-Bravo *et al.* 2011; Seber and Wild 2005) and is summarized here, but this section also covers strategy and generation of subsequent model confidence intervals (and application of the same principles to sensitivity analysis). It should be noted that most of this is relevant to lumped parameter or compartmentalized models only. The same inferential statistical methods are applied to distributed parameter models, but due to the simulation overhead, methods such as response surface methodology are normally applied to the identification of objective function regions.

3.4.1. Parameter estimation methodology and model confidence

The basic method of parameter estimation proposed here and elsewhere (and probably the simplest method that is statistically robust) is iterative optimization of an output to a model prediction by estimating parameters, with residual sum of squares as objective function ($J = RSS$). Alternative methods are given in Seber and Wild (2005).

The use of residual sum of squares is robust as it allows for the proportioning of variance (an essential statistical requirement for inferential analysis), while the use of machine fitting identifies the correct optimal parameter set (and optimal objective value). While the data fit must also be observed to identify that the model is suitable and that the optimization routine is not identifying a local minima, most of the procedures can be automated to identify optimal parameter set and parameter uncertainty. Issues such as residual autocorrelation and non-normality are inevitable in the application of non-linear modelling, and are a consequence of representing complex systems by simple models. Both aspects can be tolerated to a substantial degree and need not be corrected by transformation (e.g., as done in Batstone *et al.* (2003)), since the Central Limit Theorem applies to normalize aggregate parameters with sufficiently high n_{data} (Dudley 2014).

Depending on the problem, an unknown parameter set of number p (normally $p = 1-5$) will be defined as the parameter optimization problem. These may include hydrolysis parameters ($k_{hyd}, f_d - p = 2$), Monod parameters for a given process ($k_{m,ac}, K_{S,ac} - p = 2$), or a combination of inhibition and Monod parameters ($k_{m,ac}, K_{S,ac}, k_{I,NH3,ac} - p = 3$). While larger problems can be defined and solved, different outputs generally need to be scaled to a common order of magnitude, and it is easier to define discrete parameter–output sets.

For a given parameter estimation problem, p is estimated to obtain a minimum RSS_{min} . The uncertainty in p can be estimated in two ways, both of which are generally compatible for the majority of problems. If a gradient search technique is used, the parameter covariance matrix can be estimated from the final residual–parameter gradient (or Jacobian for the objective function RSS):

$$\text{cov}(p) = (jac' \cdot jac)^{-1} \frac{J_{opt}}{n_{data} - p} \quad (7.8)$$

where jac is the Jacobian, and J_{opt} is the optimal objective function value

The first term parameter–residual is the variance gradient, while the second term represents model mean squared error. Parameter variances are in the diagonals of this matrix, and the covariances are in the non-diagonal elements. Standard error in parameter(s) can therefore be estimated from the square root of the variances, and an estimate of the correlation coefficients can be calculated from the covariance divided by the two standard errors. Confidence intervals in parameters can then be estimated from standard error and a two-tailed t -test:

$$E_{95,p(i)} = s_{p(i)} t_{0.975,n-p} \quad (7.9)$$

As an alternative, where a better estimate of parameter confidence is required, where parameter identifiability is more difficult, or where a non-gradient parameter optimization technique is applied, the parameter confidence limits can be defined by a F -test (testing for the range of models that are not worse than the optimum), with this defined by the objective function value J_{crit} :

$$J_{crit} = J_{opt} \left(1 + \frac{p}{n_{data} - p} F_{0.95,p,n_{data}-p} \right) \quad (7.10)$$

An iterative search can find the surface p for which $J = J_{crit}$. This defines both vertical confidence intervals (as calculated in Eq. 7.8), as well as a “true” estimate of correlation coefficient. An example of both methods applied to the data and model in Fig. 7.2 is shown in Fig. 7.10. As is normal (and common to anaerobic processes when good data is obtained), the linear estimates are good predictors for true confidence, but correlation is lower in the true region, a result of the stability and non-linear characteristics of environmental, dilute systems.

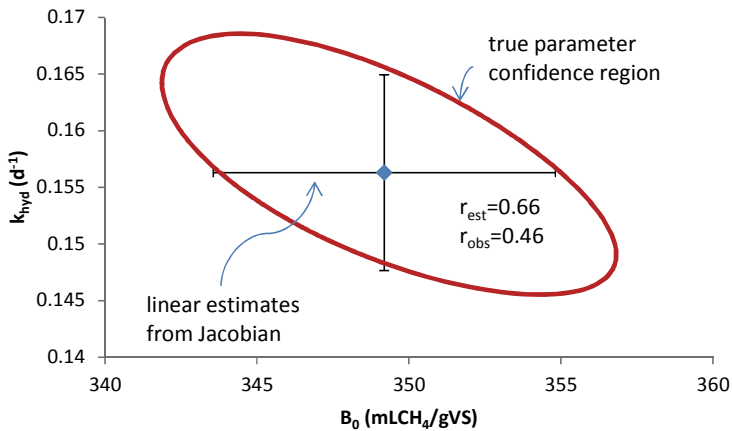


Fig. 7.10. Methane potential and hydrolysis coefficient for data shown in Fig. 7.2 (BMP data), showing linear estimates of parameter confidence (95% CI), and true parameter confidence region as estimated by F -test.

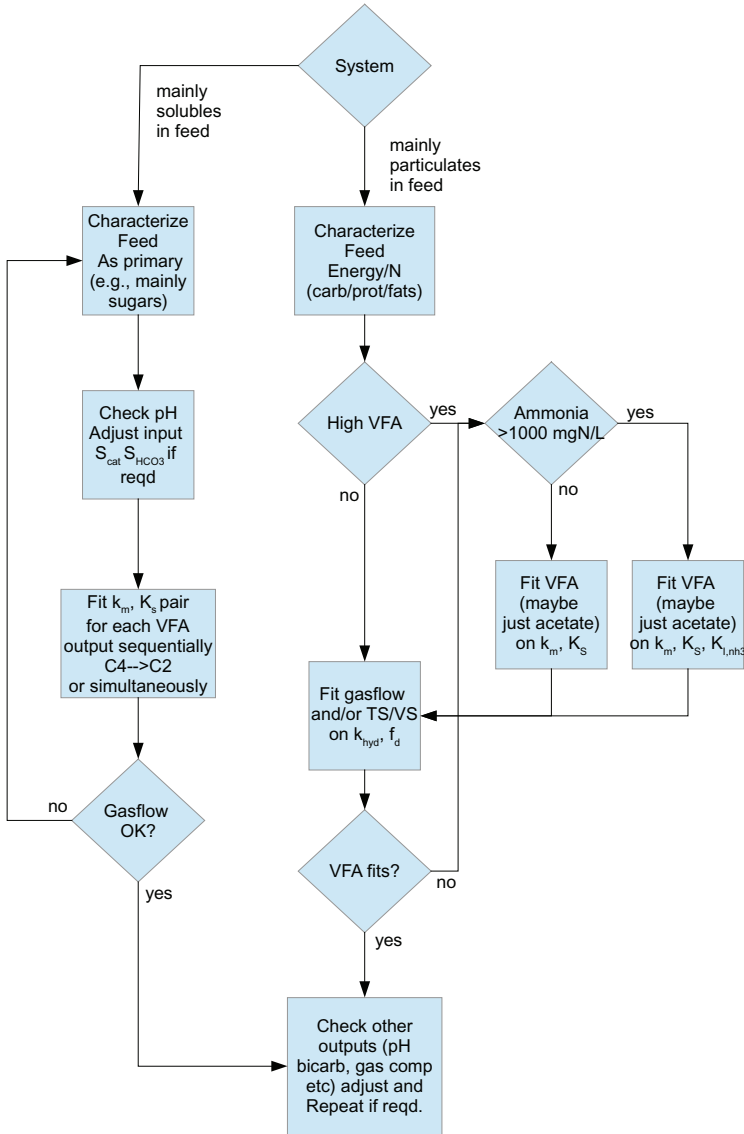


Fig. 7.11. Summary procedure for identifying models (individual parameter–output optimization problems to be solved using iterative search methods).

Non-linear model confidence intervals can be generated through the propagation of uncertainty principles as outlined in Ge *et al.* (2013):

- (a) The confidence interval in an optimized model can be determined by analytical propagation through the parameter covariance matrix, using a variation of Eq. 7.7. This is done (for example) through the Matlab command “nlpredci” (Seber and Wild 2005).

- (b) The parameter distribution can be propagated numerically through Monte-Carlo simulations and re-aggregation to generate model confidence intervals as in Benedetti *et al.* (2012) and Ge *et al.* (2013).

Either method can be used both to determine model confidence intervals (e.g., by taking inferred parameter values and variances), or sensitivity analysis (e.g., by imposing hypothetical parameter distributions), but Monte-Carlo will be more representative, since it does not apply linear approximation.

3.4.2. Parameter estimation strategy

As stated above, the range of contestable parameters (available to fit), even in complex models such as the ADM1, are limited. Stoichiometric parameters such as yields (product and biomass) are not contestable, unless there is an obvious basis for doing so (e.g., identifying carbohydrate, protein, lipid, and inert fractions in the influent). Decay rates should also not be varied unless there is an obvious mechanistic reason to do so due to high levels of correlation with uptake (or growth) rate coefficient (Batstone *et al.* 2006). The only inhibition parameters that are generally contestable are $K_{I,nh3}$, and if sulfide inhibition is included, parameters related to that. Most of the other inhibition parameters in the ADM1 are set low enough that they essentially act as switches.

Identification of fit parameter estimation and output targets depends heavily on the primary feed. A summary procedure is provided in Fig. 7.11. The primary differentiator is either:

- (a) solubles in feed (may be soluble sugars, proteins, or VFAs), where the optimization problem is (a) establishing feed pH and buffering by adjusting S_{cat} and $S_{HCO_3^-}$ ion concentration, and (b) fitting (through iterative optimization) k_m and K_S for the organic acids, either sequentially $C_4 \rightarrow C_3 \rightarrow C_2$, or all simultaneously. The pH should again be checked and adjusted if necessary.
- (b) particulates in feed, or a mix of particulates and solubles. While generally VFA will be low in both model and system effluent (particularly if not inhibited, or no solubles in feed), there is an iterative procedure between fitting organic acids and hydrolysis coefficients (k_{hyd} , f_d). This assumes lumping of the three hydrolysis coefficients ($k_{hyd,ch} = k_{hyd,pr} = k_{hyd,li} = k_{hyd}$).

4. Model Limitations and Future Directions

Application of anaerobic modelling to new reactor designs (and enhanced modelling of existing digesters) through distributed parameter modelling has been noted above as a key limitation. There has also been extensive work on biochemical extensions to the ADM1 to (for example) alternative electron acceptors, and alternative substrates and pathways.

The key outstanding limitation is probably in integrated application of anaerobic digestion models in larger environmental and engineered systems. A major goal is obviously plant-wide modelling of wastewater treatment plants, including activated

sludge and anaerobic digestion (and potentially extensions such as nutrient recovery). This has been addressed extensively for biological nitrogen removal systems, and as an example, the BSM2 was recently published. The approaches to this are either discrete models (e.g., ASM1, ASM2d, ADM1) connected by interfaces, as was done in the BSM2 (Gernaey *et al.* 2014), or the plant-wide supermodel, where all states and processes are tracked in all vessels (Grau *et al.* 2009). The advantage of the first method is that standardized, optimized models can be applied, and it is fast to solve. The advantage of the second method is that interfaces are not required. While the nitrogen cycle has been successfully applied to both methods, phosphorous modelling has been more difficult, and has only really been partially addressed, even in commercial software.

Part of the problem is the complexity of the whole-plant phosphorous system, which interacts with the sulfur, iron, calcium, and magnesium physicochemically (sulfur and iron also have biochemical reactive components) (Ekama *et al.* 2006; Ge *et al.* 2013). For example, phosphorous biologically binds with organics and iron in the activated sludge basin, is released due to biological phosphorous release and sulfide–iron precipitation in the anaerobic digester, and consequently precipitates with magnesium and calcium in the digester sludge. This describes both partial return through reject, as well as loss in the sludge. The problem is very complex, and requires the model to include ion activity correction and ion pairing (since phosphate is trivalent), as well as precipitation (Batstone *et al.* 2012). The University of Cape Town group has made this a focus, particularly on behavior in the anaerobic digester (Ekama *et al.* 2006). However the long-term goal needs to be the inclusion of comprehensive physicochemical models throughout the engineered water cycle, including sewers and drinking water systems (Pikaar *et al.* 2014; Sharma *et al.* 2013). A generalized and comprehensive physicochemical approach needs to be matched by the inclusion of appropriate biological processes for phosphorous, sulfur, and iron, which may need rethinking of elements of well-established standardized models, such as the ASM and ADM series.

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II. Applications

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Chapter 8

Microbial Fuel Cells: From Fundamentals to Wastewater Treatment Applications

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Microbial fuel cells (MFCs) have continued to offer the potential to increase treatment efficiency, power generation, and energy recovery during wastewater treatment, and have garnered considerable research interest over the past 15 years. For the time being, the potential of this technology remains unfulfilled as scaling-up MFC reactors has presented a significant engineering challenge. The recent development of materials and designs that reduce fabrication costs in addition to maintaining high performance at increased reactor sizes has brought MFCs a step closer to practical application. The optimization of operating conditions has led to additional improvements in performance and has further increased the feasibility of these technologies. Herein, the fundamental concepts governing MFC performance are detailed along with highlighting developments in materials, designs, and operating conditions with the hope of promoting future scale-up efforts and a more sustainable wastewater treatment infrastructure.

1. Introduction

Though wastewater itself is intrinsically rich in energy ($1.93 \text{ kWh}\cdot\text{m}^{-3}$), the energetic cost of wastewater treatment remains high (McCarty *et al.* 2011). Further developing wastewater treatment technologies that can enhance energy recovery in wastewater is an important step towards developing a more sustainable wastewater treatment infrastructure. Currently, well-developed technologies such as anaerobic digestion are able to recover some of the chemical potential energy in wastewater through the production of biogas. However, to convert biogas to a more practical form of energy such as electricity, combustion is required, and the low efficiency of combustion leads to a maximum 28–30% recovery of the total chemical potential energy in these systems (McCarty *et al.* 2011). Additionally, the large volumes of biogas required to run a

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generator combined with the cost of removing impurities in the biogas (siloxanes, H_2S , etc.) mean that electricity generation in these systems can only be feasible for large-scale operations. Developing technologies, such as microbial fuel cells (MFCs), that can generate electricity directly from the chemical potential energy in wastewater, as well as the ability to operate on varying scales, is critical in order to establish a sustainable wastewater treatment infrastructure for future generations.

MFCs utilize microbial catabolic activities to directly produce electricity from organic material in waste streams. This electrical power is produced through the liberation of electrons during the microbial oxidation of organic (and some inorganic) material in wastewater. As part of microbial metabolic processes, liberated electrons are transferred from the bacteria to the anode, the negative terminal. The electrons then flow through an external load to the cathode (positive terminal), where they combine with oxygen and protons that diffused from the anode, producing water (Fig. 8.1).

Theoretically, the direct conversion of the chemical energy in waste products to electricity by MFCs would allow for total energy recovery around 44% (McCarty *et al.* 2011). Currently, small MFC systems that are fed easily degradable substrates at high CODs have been observed to reach up to 35% energy efficiency with power densities reaching up to $4.30 \text{ W}\cdot\text{m}^{-2}$ ($2.87 \text{ kW}\cdot\text{m}^{-3}$) (Fan *et al.* 2012). Often, increased efficiencies are achieved using waste streams with low COD, though considerable power ($>1.0 \text{ kW}\cdot\text{m}^{-3}$) is normally only generated when high COD waste streams are utilized. Improvements in power and efficiency are both possible and necessary, especially at increased reactor sizes.

MFCs offer additional advantages if they were to be incorporated into wastewater infrastructure. Direct electricity generation in MFCs bypasses costly biogas scrubbing processes associated with anaerobic digestion. MFCs may also produce higher quality effluent than traditional anaerobic technologies due to the combination of anaerobic and aerobic processes associated with MFC operation (Wen *et al.* 2010). Also while MFCs

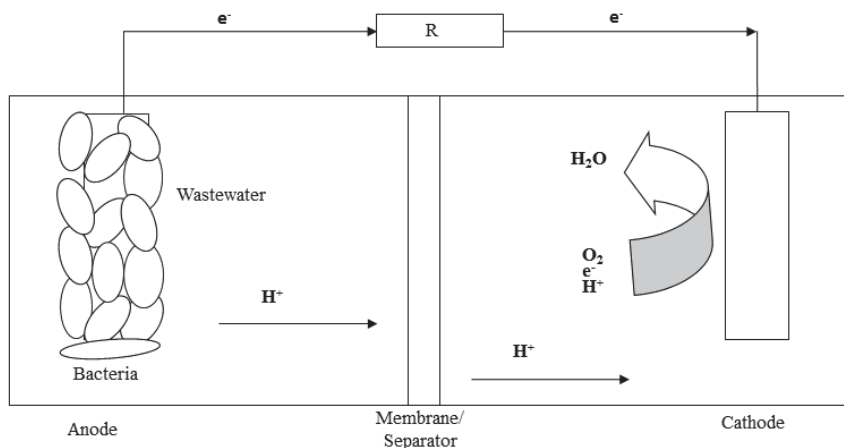


Fig. 8.1. Schematic diagram of an MFC. Organics in wastewater are oxidized by bacteria. Electrons are then passed to the anode from where they flow to the cathode, combining with oxygen and protons to form water.

are comparable to aerobic wastewater treatment on the basis of COD removal, they greatly limit sludge production (Huggins *et al.* 2013). Denitrification and the removal of ammonium also occur in MFCs (Clauwaert *et al.* 2007; Zhang and He 2012).

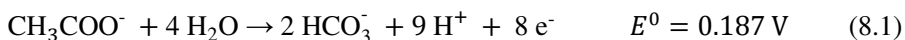
The potential advantages of MFCs will continue to drive research in the field, and although MFCs are still in the early stages of being scaled up for practical applications, many lessons have been learned and much experience has been gained. The goal of the current chapter is to present the fundamental concepts governing MFCs and highlight recent advances made in materials, fabrication, design, startup, and operation with the goal of promoting the further development of MFC technology.

2. Principles and Fundamentals

Similar to other types of electrochemical fuel cells, MFC performance is governed by the thermodynamics of the coupled redox half-reactions transpiring at the electrodes. Electricity in an MFC will only be generated if it is thermodynamically favorable, represented by a positive electromotive force between the anode and cathode. This can be determined through the difference in potentials between the electrodes. Limiting potential losses is critical in order to maintain high power outputs and efficiencies in MFCs.

2.1. Electrode potential

In order for an MFC to function, the reduction of the anode must occur simultaneously with the oxidation of the cathode. This maintains electron balance in the overall redox reaction. The ultimate source of the electrons in MFCs is the organic materials, but different organic compounds have different Gibbs free energies associated with their oxidation. These differences in oxidation favorability will be reflected by the standard potential of the reaction (E^0). A simple example is the oxidation of acetate (anode half-reaction) represented in Equation 8.1 (vs. NHE).



However, to determine the actual potential of the half-reaction occurring at the anode (E_{an}) under conditions relevant to an MFC environment, the Nernst equation is necessary (Equation 8.2).

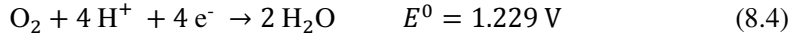
$$E_{an} = E^0 - \frac{RT}{8F} \ln \frac{[\text{HCO}_3^-]^2 [\text{H}^+]^9}{[\text{CH}_3\text{COO}^-]} \quad (8.2)$$

At room temperature, pH 7.0, and 10 mM for both acetate and bicarbonate, the potential of this reaction would be equal to -0.308 V (Equation 8.3).

$$E_{an} = 0.187 \text{ V} + \frac{8.31 \left(\frac{\text{J}}{\text{mol} \cdot \text{K}} \right) \times 298 \text{ K}}{8 \times 9.65 \times 10^4 \text{ C/mol}} \ln \left(\frac{10^{-2 \times 2} 10^{-7 \times 9}}{10^{-2}} \right) = -0.293 \text{ V} \quad (8.3)$$

The cathode half-reaction occurs at the cathode where a terminal electron acceptor is reduced. This terminal electron acceptor can be several different redox active species resulting in cathode potentials (E_{cat}) that typically range from 0.361–0.805 V. Potential

catholytes include ferric cyanide ($\text{Fe}(\text{CN})_6^{3-}$) ($E^0 = 0.361 \text{ V}$) and manganese dioxide (MnO_2) ($E^0 = 1.23 \text{ V}$). However, these electron acceptors must be regenerated or replaced, making the use of oxygen present in air the most practical electron acceptor for most designs. The oxygen reduction reaction (ORR) is represented in Equations 8.4 and 8.5 (Note: Not balanced against acetate oxidation reaction).



$$E = E^0 - \frac{RT}{4F} \ln \frac{1}{P_{\text{O}_2}[\text{H}^+]^4} \quad (8.5)$$

The partial pressures of O_2 and pH have a strong influence on cathode potentials associated with the ORR. Common conditions of $P_{\text{O}_2} = 0.2 \text{ atm}$ and $\text{pH} = 7$ will result in a cathode potential of 0.805 V , a value that will increase with decreased pH and/or increased partial pressure of oxygen.

2.2. Electromotive force

When two half-reactions such as those discussed here are connected in a closed electrical circuit, an electrochemical cell is formed. The electromotive force (E_{emf}) generated by this electrochemical cell can be determined by subtracting the anode potential (E_{an}) from the cathode potential (E_{cat}) (Equation 8.6).

$$E_{\text{emf}} = E_{\text{cat}} - E_{\text{an}} \quad (8.6)$$

Using the E_{cat} and E_{an} , calculated via the Nernst equation in the section above (0.805 V and -0.293 V , respectively), an E_{emf} of 1.10 V is determined for the overall reaction. The electromotive force of the reaction is positive, meaning that it will occur spontaneously in the given direction. The overall Gibbs free energy (ΔG_r^0) of the reaction can be calculated using Equation 8.7.

$$\Delta G_r^0 = -nFE_{\text{emf}}^0 \quad (8.7)$$

2.3. Potential losses

The open circuit voltage (OCV) of the MFC should be similar to the E_{emf} , but has only been observed at a maximum of 0.80 V without current flowing, and is generally below 0.62 V during current generation (Liu et al. 2005a; Rabaey et al. 2005b). These OCVs are significantly lower than the 1.10 V expected, meaning that large energy losses are occurring. These losses are due to the overpotentials of the anode and cathode, as well as ohmic losses. The overpotentials of both electrodes can be categorized as microbial metabolic losses, activation losses, and concentration/polarization losses.

In order to generate energy, bacteria need to be able to transfer electrons from the substrate at a low potential (e.g., acetate at -0.293 V) through its electron transport chain to an anode at a higher potential. The larger the difference is between the redox potential of the substrate and the potential of the anode, the higher the microbial metabolic loss. Large metabolic losses mean large metabolic gains for the bacteria, but reductions in the

maximum MFC voltage. The anode potential needs to remain as low as possible without inhibiting electron transfer through the microbial electron transport chain.

Activation losses occur at both the anode and the cathode. This portion of the overpotential is related to the transfer of the electron itself to or from the electrode surface and is current-dependent. These losses make up a large percentage of the total overpotential at low current densities where there are huge increases in activation losses as current increases, but make up a smaller percentage of the total overpotential at higher current densities as increases in activation losses become more gradual compared to increases in current (Laramie and Dicks 2003).

At high current densities-concentration or polarization losses become more pronounced. Concentration losses are related to the rate of mass transport of a species to or from the electrode. At high current densities, the ratio between reduced and oxidized species will shift in an unfavorable direction faster than the diffusion of those species away from the electrode can occur. These diffusion rates will then be the limiting factor in current generation rates. Well-mixed systems can prevent diffusional gradients from developing at the electrode and reduce concentration losses (Logan *et al.* 2006).

Ohmic losses associated with MFCs are related to the resistance to the flow of electrons through the electrodes and connections throughout the cell. This resistance includes the resistance to the flow of protons, generated at the anode, to the cathode where they are consumed. Membranes, faulty connections, low solution conductivity, and large electrode spacing can greatly increase ohmic resistances of the MFC.

Overall, the *OCV* of an MFC is a product of all the non-current-related overpotentials, while the internal resistance (R_{int}) includes the ohmic resistances in addition to the current related aspects of the anode and cathode overpotentials. The relationship between the actual cell voltage (V_{cell}), *OCV*, and R_{int} is shown in Equation 8.8.

$$V_{cell} = OCV - IR_{int} \quad (8.8)$$

2.4. Electrical power

When an external resistor connects the anode and cathode, an electrical current is generated. This current (I) is the product of the external voltage (V_{ext}) across the resistor divided by the resistance of the resistor (R_{ext}) (Equation 8.9). Power, a primary performance metric of MFCs, is the product of the current and the external voltage (V_{ext}) (Equation 8.10).

$$I = V_{ext}/R_{ext} \quad (8.9)$$

$$P = IV_{ext} \quad (8.10)$$

Power and current are both generally expressed as power and current densities normalized either to reactor volume (m^3) or cathode area (m^2) (the cathodic reaction is the limiting reaction in most MFC setups) (Oh and Logan 2006).

2.5. Coulombic and energy efficiencies

Along with power, coulombic and energy efficiencies are the primary performance parameters of MFCs. The efficiency with which the electrons that are liberated from the oxidation of the substrate are converted into electrical current is the coulombic efficiency (η_c), while energy efficiency (η_e) is the ratio of power produced by the cell compared to the heat of combustion of the substrate. Because biologies associated with MFC consume part of the substrate and energy for their own metabolic processes, these values are very different (decreased) compared to other electrochemical cells. These efficiencies should be optimized in treatment. BOD and COD are two wastewater parameters used to characterize the oxidative demand of the wastewater, and therefore, can be used to quantify how many electrons will be released following oxidation. These values can then be used to calculate both η_c and η_e . This is shown in Equations 8.11–12, where ΔCOD is the change of COD concentration ($\text{g}\cdot\text{L}^{-1}$), b_{O_2} is the number of moles of accepted electrons per reduction of one mole of oxygen, M_{O_2} is the relative molecular weight of oxygen, ΔG_r is the Gibbs free energy ($\text{J}\cdot\text{mol}^{-1}$) of oxidation reaction of organic substrates, and V is the volume of liquid in the anode compartment (L).

$$\eta_c = \frac{\text{Coulombs recovered in MFCs}}{\text{Coulombs released in substrate}} = \frac{\int_0^t I dt / F}{b_{O_2} V \Delta COD / M_{O_2}} \quad (8.11)$$

$$\eta_e = \frac{\text{Energy recovered in external loads}}{\text{Energy released in substrate}} = \frac{\int_0^t E_{ext} I dt}{-\Delta G_r V \Delta COD / M_{O_2}} \quad (8.12)$$

Energy efficiency can also be easily calculated by multiplying coulombic efficiency (η_c) and the quotient of external voltage (E_{ext}) over theoretical voltage (E_{emf}) (Equation 8.13).

$$\eta_e = \frac{E_{ext}}{E_{emf}} \eta_c \quad (8.13)$$

3. Microbiology

Microorganisms that inhabit MFCs are the catalysts in the conversion of the chemical energy in wastewater to electrical energy. The microbes oxidize organics in the wastewater, which serve the role of electron donors (lower potential). The electrons are then passed through the microbial electron transport chain at sequentially higher potentials and then ultimately transferred outside of the cell to an electrode acting as an electron acceptor (higher potential).

3.1. Extracellular electron transport and exoelectrogens

Electron flows are inherent to all metabolisms though the ability to transfer electrons extracellularly and reduce insoluble materials (e.g. metal-containing minerals, electrodes) is not. The reduction of insoluble compounds can only be performed by bacteria that have evolved to take advantage of less favorable electron acceptors available in anaerobic environments (Madigan *et al.* 2008). This ability to transfer electrons out of the cell is

known as extracellular electron transport, and the bacteria capable of this are known as exoelectrogens (Lovley 2012). The best recognized of these species are the *Deltaproteobacteria*, *Geobacter sulfurreducens*, and the *Gammaproteobacteria* *Shewanella oneidensis* (Ringeisen *et al.* 2006; Yi *et al.* 2009).

There are two primary mechanisms of extracellular electron transport — indirect and direct. Indirect electron transfer occurs through a soluble redox compound, organic or inorganic, being reduced or oxidized at the cell and diffusing towards either the electron acceptor or donor (Rabaey 2010). Many of these redox shuttles/mediators exist naturally in soils, including humic acids (Milliken and May 2007), though others such as pyocyanin may be produced by the bacterium itself (Rabaey *et al.* 2005a). Sulfur compounds and hydrogen can also be considered electron shuttles and play important roles in environments containing exoelectrogens (Rabaey 2010).

The alternate mechanism is that of direct electron transfer established through a physical electrical connection between the cell and the electrode. This mechanism has generated considerable interest in recent years, mostly focused on pili or pilus-like structures exhibiting high conductivity (Malvankar *et al.* 2011). While direct electron transfer in some species may be limited to enzyme complexes bound to or associated with the membrane, such as c-type cytochromes (Lovley 2012), the novelty of the production of conductive proteins by certain bacteria has sparked the imagination of researchers from both scientific and engineering backgrounds.

Geobacter-produced type IV pili, known as microbial nanowires, have demonstrated electrical conductivity, though the precise mechanisms and nature of this conductivity are still being debated (Malvankar *et al.* 2012b; Strycharz-Glaven and Tender 2012). One proposed model suggests that these pili proteins possess a metallic-like conductivity resulting from overlapping π - π orbitals of aromatic amino acids (Vargas *et al.* 2013). The other ‘superexchange’ model suggests that the conductivity is attributed to electron-hopping between pili-associated cytochromes (Bond *et al.* 2012). Regardless of mechanism, these pili confer conductivity to the entire biofilm, allowing exoelectrogenic bacteria to stack biomass and conserve energy during electron transfer (Kato Marcus *et al.* 2007). Even biofilms in which exoelectrogenic species only compose a fraction of the community have demonstrated high conductivity (Malvankar *et al.* 2012a). These electrical connections established through the production of microbial nanowires and conductive biofilms provide a means of communication between microbial cells, including communication between species in mixed communities (Reguera 2011).

3.2. Mixed species’ communities and interactions

Many of the highest performing MFCs do not contain axenic cultures of exoelectrogens, such as *Geobacter* sp., but consist of diverse communities (Lesnik and Liu 2014). The composition of these communities is shaped by the available carbon substrates (Torsvik and Øvreås 2002), though certain patterns have emerged. A consistent abundance of *Deltaproteobacteria*, *Clostridia*, and *Bacteroidetes* has been observed in MFCs utilizing easily degradable substrates, such as acetate (Ishii *et al.* 2014; Lesnik and Liu 2014). Increases in populations of *Gammaproteobacteria* and *Bacilli* have been observed when a

fermentable substrate, such as glucose, is present (Ishii *et al.* 2014). There is considerable evidence of syntrophy and other synergistic interactions that may result in increased power outputs and efficiencies observed in mixed-species MFCs (Parameswaran *et al.* 2010). The functional roles of members involved in these synergistic interactions are not well characterized though some have been previously hypothesized (Lesnik and Liu 2014). The *Deltaproteobacteria* in these communities, mostly *Geobacter* and *Desulfuromonas*, likely serve the role of anode-respiring bacteria, as several exoelectrogens in this phyla have been previously characterized (Logan 2009). Other phyla such as *Clostridia* and *Bacteroidetes* appear to play important roles as either hydrogen scavengers or fermentative bacteria (Parameswaran *et al.* 2010). Further elucidation and manipulation of these interactions may provide an alternate route to improving the performance of MFCs.

4. Materials/Fabrication

Several distinct components made out of a variety of materials are needed to fabricate a MFC. These components include the structural materials (typically plexiglas or PVC) (Liu and Logan 2004; Zhuang and Zhou 2009), the anodes, the membranes/separators, and the cathodes. Selecting electrode materials with properties capable of lowering activation energies and limiting potential losses is critical in order to further increase the power and efficiency of MFC systems. Properties such as surface area, conductivity, stability, and hydrophobicity can vary greatly between materials and have a large effect on MFC performance. In addition to improving performance, decreasing fabrication costs of MFCs is also essential, especially when scaling up reactor size towards practical applications is an objective. Recent advances towards these ends have been made, especially in reducing the large costs previously associated with cathode assembly. However, there is still considerable room for improvement in the material science of MFCs.

4.1. Anodes

The anode material provides the interface between the microorganisms in an MFC and the external circuit. This material provides a surface for attachment with biocompatibility being of primary concern. The need for biocompatibility differentiates anode materials of MFCs from other electrochemical cells without biological elements. Materials that make the best anodes are those with large specific areas that can support substantial growth over extended periods of time while also maintaining high conductivity (thereby limiting ohmic resistance). The capacitance of the anode materials is also important, with research showing that a lack of capacitance can lead to power overshoot, which can dampen performance (Peng *et al.* 2013b). Many different materials have been explored to find one to meet all these parameters, most of which are carbon-based (e.g. carbon cloth, carbon mesh), with a few others being metal-based (e.g. stainless steel mesh, Pt-coated titanium). Carbon-based materials have been found to be the most effective, and many have undergone further modification to improve performance.

Variations of carbon anodes include solid carbon materials (graphite rods (Liu *et al.* 2005a) and plates (Rabaey *et al.* 2003)), granular carbon materials (granular graphite (Bond and Lovley 2003) and activated carbon (Peng *et al.* 2012; Tender *et al.* 2002)), carbon-fiber materials (non-porous graphite (Ter Heijne *et al.* 2008), carbon cloth (Liu *et al.* 2005a), carbon paper (Jiang and Li 2009), and graphite fiber brushes (Logan *et al.* 2007)). Each of these materials has strengths and weaknesses.

Conductivities of solid carbon materials are high, but they make poor anodes in most settings due to their relatively small specific surface areas. Granular carbon materials are the opposite and present high specific surface areas but low conductivity. Carbon fiber anodes are a popular material choice as they represent an ideal tradeoff, with good conductivity and relatively large specific surface areas. Overall, carbon cloth anodes with a flat spatial structure appear to work best in many settings resulting in high power densities of $4.3 \text{ W}\cdot\text{m}^{-2}$ ($2.9 \text{ kW}\cdot\text{m}^{-3}$) (Fan *et al.* 2012).

Several additional modifications of carbon-based anodes have been explored. One of the most successful modifications was the use of an ammonia pre-treatment to carbon cloth and carbon fiber brushes (Cheng and Logan 2007; Logan *et al.* 2007). This approach increased the power density from 1.64 to $1.97 \text{ W}\cdot\text{m}^{-2}$ in designs with carbon cloth anodes (Cheng and Logan 2007), and from 0.75 to $1.20 \text{ W}\cdot\text{m}^{-2}$ in designs using carbon fiber brush anodes (Logan *et al.* 2007). The ammonia treatment process also appeared to promote bacterial attachment to the anode, resulting in the decrease of acclimation times (Cheng and Logan 2007).

Nanostructure modifications have also been effective at increasing performance. Anodes decorated with FeOOH (Peng *et al.* 2013a), gold, and palladium nanoparticles have been shown to be at highly increasing performance (up to 20 fold) (Fan *et al.* 2011). However, the most promising nanostructure modifications are carbon nanotubes, as they provide excellent specific surface areas, good thermostability, and high conductivity (Qiao *et al.* 2007). The presence of carboxyl groups in carbon nanotubes can also enhance the electron transfer capability of exoelectrogens and decreases in anode resistance have been observed. Power outputs have also been increased from 0.47 – $2.6 \text{ mW}\cdot\text{m}^{-2}$ to $42 \text{ mW}\cdot\text{m}^{-2}$ through the use of carbon nanotube–polyaniline (PANI) composite anodes (Qiao *et al.* 2007). Polypyrrole-coating and the electrochemical oxidation of carbon nanotubes/ graphite has similarly been shown to improve kinetic activity by a factor of 58.8 times (Lowy and Tender 2008).

4.2. Cathodes

The cathodic reaction is often the rate-limiting step of electrical current generation in MFCs. This makes improving the cathode one of the clearest ways to further increase MFC performance. As previously described, oxygen is the most popular terminal electron acceptor in MFC designs; thus, cathode studies are mainly focused on improving the ORR reaction while keeping fabrication costs down. In order for the ORR reaction to occur, oxygen, protons, and electrons all need to be present at the active catalytic sites of the cathode. A conductive base material/current collector is responsible for supplying electrons, while protons diffuse across from the anode, and oxygen diffuses across the

cathode that is typically exposed to air. This diffusion across the cathode without the solution leaking across is managed through the application of a hydrophobic diffusion layer. The catalyst, the current collector, and the diffusion layer are all critical parts of low-cost, high-performance air-cathodes.

4.2.1. Catalyst layer

The catalyst layer is perhaps the most important part of the cathode due to its impact on the ORR. It has also traditionally been the most expensive part due to the use of Pt for its noble metal catalytic ability. Due to the high cost of Pt, significant research has been conducted in order to optimize the Pt-loading based on cathode surface area (Cheng *et al.* 2006a; Santoro *et al.* 2013), in addition to identifying alternative catalysts. Results using FePc and CoTMPP as catalyst alternatives to Pt have been mixed; they have similar performance to Pt, but are still too expensive for large-scale application (Cheng *et al.* 2006a). However, the promising performance of carbon-based materials utilized as catalysts for air-cathodes has led to breakthroughs in reducing fabrication costs associated with MFCs. Early carbon-based materials used in this role were nitric-acid-activated graphite granules, with results demonstrating comparable power to Pt cathodes (Erable *et al.* 2009). More recently, activated carbon has proved to be an excellent catalyst for air-cathodes (Dong *et al.* 2012). These activated carbon cathodes are able to exhibit high performance even over extended periods of operation, with studies spanning as long as one year (Zhang *et al.* 2009a).

4.2.2. Binders

Because catalysts generally come in powder form that cannot conglomerate or stick to a conductive base material, a binder is necessary to integrate the carbon powder/catalyst with the base material/current collector. Binders are typically mixed with the catalyst and then either cold-pressed, hot-pressed, or rolled onto the base materials. Nafion has been effectively used in this role, but is too expensive to be applied on a large scale. Due to its comparably low cost, polytetrafluoroethylene (PTFE) has been tested as an alternative to Nafion. The initial power generation of MFCs with PTFE cathodes were reported to be lower than those with Nafion cathodes, possibly due to ionic gradients forming on the PTFE, thereby hindering charge transfer (Cheng *et al.* 2006a; Saito *et al.* 2010). However, longer term studies (40 days) indicate PTFE cathode performance was comparable to that of Nafion cathodes (Saito *et al.* 2011). Other low-cost compounds, such as polydimethylsiloxane (PDMS), have also provided positive results (Zhang *et al.* 2012).

4.2.3. Diffusion layer

A diffusion layer (gas diffusion layer) is critical in the cathode fabrication process to provide channels for oxygen diffusion to the reaction sites, while preventing water leakage under high hydrostatic pressure. A thick diffusion layer is necessary to withstand

the hydrostatic pressure, but too thick of a diffusion layer can significantly decrease oxygen diffusion and limit cathodic reaction rates.

PTFE is commonly used as a diffusion layer in MFC cathodes. Four layers of PTFE were found to be optimal for MFC performance, resulting in a 42% increase in maximum power density ($0.766 \text{ W}\cdot\text{m}^{-2}$) and a 171% increase in the η_c (32%) compared to no diffusion layer. Additional layers of PTFE improved the η_c primarily by restricting superfluous oxygen flux into the system. For continuous flow MFCs with increased hydrostatic pressure, the number of PTFE layers should be optimized to allow for ideal oxygen diffusion, while allowing for appropriate water pressure capacity. Carbon/PTFE composite diffusion layers have been used in fuel cells to increase the electrical conductivity and cell performance (Han *et al.* 2006; Luo *et al.* 2011; Zhang *et al.* 2010a).

A major drawback of using PTFE is that it requires high temperatures to cure after the application of each coating (330–380 °C) (Cheng *et al.* 2006b; Han *et al.* 2006). This step is both time- and energy-intensive, especially if applied on a large scale. Alternate diffusion layers have begun to be explored such as PDMS. PDMS is both cheaper and does not require such as high temperatures to cure (25–80 °C). Very recently, cheap poly (vinylidene fluoride-co-hexafluoropropylene) (PVDF-HFP) has also been used as a type of diffusion layer. A maximum power density of $1430 \text{ mW}\cdot\text{m}^{-2}$ (31% higher than Pt catalyst with PTFE diffusion layer) was achieved using PVDF-HFP (Yang *et al.* 2014).

4.2.4. Base material/current collector

The conductive base material and/or current collector of cathodes are typically carbon- or metal-based. Carbon cloth and carbon paper were initially used as current collectors, though their relatively high cost and limited conductivity drove the development of other suitable materials (Zhang *et al.* 2010b). One of the more economical current collectors that has been developed is a non-conductive canvas cloth coated with a conductive paint (nickel-based or graphite-based), though performance was not suitable for the development of high-power systems (Zhuang *et al.* 2010; 2009). Metal meshes made of nickel and copper have also been tested in conjunction with activated carbon, yielding similar performance to the cheaper stainless steel mesh ($\sim 1.2 \text{ W}\cdot\text{m}^{-2}$) (Zhang *et al.* 2009b). Metal meshes increased the conductivity of the cathode and provided better mechanical support in large-scale applications. However, studies have also reported corrosion of the mesh, warranting further research attention (Logan 2010; Wang *et al.* 2013). These corrosion issues often lead researchers to choose the more expensive, less rigid carbon cloth for several designs, even at increased reactor sizes.

4.3. Membranes/separators

Membranes or separators may be used in some MFC designs to isolate the anode and cathode. This isolation may be used to separate the anode chamber from the cathode chamber when a catholyte, such as ferricyanide, is used. It may also be used to prevent oxygen diffusion to the anode chamber, to prevent biofouling on the cathode, or to prevent short circuits when there is minimal spacing between the electrodes. In any case,

a good membrane/separator should still allow for the efficient transfer of ions necessary to complete the electrical circuit (Zhang *et al.* 2009a).

Varying designs (discussed in the following section) require different properties from membranes and separators. The earliest MFCs were designed similar to other electrochemical cells, which have distinct anode and cathode chambers. Selective membranes that allow for the diffusion of certain ions across the membrane, while preventing the diffusion of other compounds are required in these instances. These membranes can be either cation exchange membranes (CEMs) or anion exchange membranes (AEMs) (Min *et al.* 2005a). Nafion is a common CEM that has been widely used as a separator in chemical fuel cells and early MFC studies. However, research has indicated that AEMs were able to improve proton transfer and decrease internal resistance versus Nafion (Kim *et al.* 2007). Both AEMs and CEMs are expensive though and following the successful development of low-cost, high-power, high-efficiency membrane-less MFCs, research surrounding AEM development has waned.

In some designs, oxygen diffusion towards the anode has become a significant problem. Oxygen diffusion can result in significant biofouling and lead to decreases in power generation and coulombic efficiency. This effect becomes even more pronounced if the electrode spacing is small. Even though this was recognized as a problem, researchers have been reticent to put membranes back in designs due to the large increases of internal resistance associated with them, and simple separators have been developed to slow oxygen diffusion while maintaining efficient proton transfer. Polycarbonate, cellulose nitrate, and nylon were initially utilized as separators and not only prevented the oxygen diffusion, but allowed the electrode spacing to be minimized without the occurrence of short circuits (Zhang *et al.* 2010c). Later, a more ubiquitous J-cloth was reported as a good separator (Fan *et al.* 2007), with the use of the non-woven fabric yielding increases in power outputs ($4.3 \text{ W}\cdot\text{m}^{-2}$) and coulombic efficiency (83.5%) (Fan *et al.* 2012). Biodegradation and biomass accumulation is an issue with some separators like J-cloth, and glass fibers may provide significant benefits in these regards (Zhang *et al.* 2009b).

5. Design/Scale-Up

Numerous MFC configurations and designs have been explored over the last decade. The majority of these designs have only been tested on the millimeter scale, and still remain in the lab. Several designs from recent years have begun to attempt this jump to pilot-scale systems and special attention in this section will be paid to those designs. Past small-scale designs that have informed design recommendations are also discussed.

5.1. Double-chamber MFCs

Most early MFCs followed a more traditional electrochemical design consisting of separate anode and cathode chambers. The anode chamber would be anaerobic providing ideal exoelectrogen growth conditions, while the cathode chamber contained a catholyte

such as ferricyanide. These chambers could then be either connected by salt bridges or separated by various types of membranes (see Section 4.3).

5.1.1. Early stage double-chamber MFCs

The advantage of double-chamber designs is the ability to control anode and cathode conditions more readily allowing for more controlled studies of electron donors, electron acceptors, and anode-associated bacteria. However, scale-up of this type of design is not feasible due to the difficulty of assembly, cost of membranes, and high internal resistance. The high internal resistance is a product of the electrode spacing combined with the proton diffusion resistance of the PEM/AEM (contributing to 38–86% of total internal resistance) (Fan *et al.* 2008). The use of a catholyte that would either need to be replenished or rejuvenated or one that would be dependent on oxygen solubility distinctly narrows the possibility of significant improvement in performance and commercial applications.

5.1.2. Up-flow/Down-flow double-chamber MFCs

Because of the inherent difficulties associated with the scale-up of double-chamber MFCs (Fig. 8.2a), modifications were made to the design resulting in continuous flow MFCs. Up-flow MFCs (Fig. 8.2b) have a flow pattern that goes from the porous anode chamber at the bottom through a PEM to the cathode chamber at the top. Similarly, down-flow MFCs are designed with the flow going from the top of the anode chamber to the bottom. The porous anode of both the up-flow and down-flow MFCs allows for optimal substrate contact with the active biomass, resulting in BOD removal similar to those of efficiencies biologically aerated filters (Greenman *et al.* 2009).

Up-flow MFCs have been scaled up to a reactor volume of 2.4 liters (Zhang *et al.* 2010a), substantially larger than many traditional double-chamber designs. However, the primary disadvantage of these systems is similar to that of 2-chamber systems: large

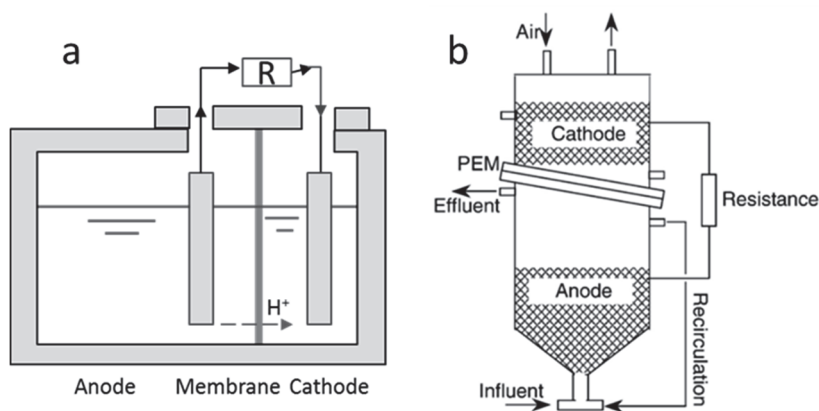


Fig. 8.2. Schematic diagrams of (a) early-stage double-chamber MFC (Adapted with permission from Logan *et al.* 2006; copyright 2006, American Chemical Society), and (b) double-chamber up-flow MFC (Adapted with permission from He *et al.* 2005; copyright 2005, American Chemical Society).

internal resistance due to the membrane and large distance between anodes and cathodes. In the scaled up design, a proton transfer resistance of $84\ \Omega$ was observed, even larger than that of double-chamber MFCs, resulting in low power densities ($170\ \text{mW}\cdot\text{m}^{-2}$) and coulombic efficiencies (from 0.7–8.1%). Though modification of the cathode chamber shape was able to decrease the internal resistance to $17.13\ \Omega$ (He *et al.* 2006), the use of an expensive membrane in up-flow MFCs combined with poor power generation continue to limit the practicality of these designs over the liter scale.

5.2. Single-chamber MFCs

Initial MFC designs assumed that a membrane was a critical component of these systems, though it was known to increase internal resistance. The first step towards addressing this issue was a design that incorporated the membrane into the cathode design by hot-pressing it to carbon cloth, forming a membrane electrode assembly (MEA). This advance enabled the combination of the separate anode–cathode chambers, creating a single-chamber MFC. Following iterations of this design removed the membrane completely leading to the standard single-chamber MFC design widely used in MFC studies today (Fig. 8.3a).

5.2.1. Single-chamber membrane-less MFCs

In smaller reactors (Fig. 8.3a), the removal of the membrane was shown to increase power generation by 88.5% compared to reactors with a proton exchange membrane (Liu and Logan 2004). However, when a larger (1.5 L reactor volume) continuous single-chamber membrane-less design was used, a power density of only $0.38\ \text{W}\cdot\text{m}^{-2}$ was generated, even though COD removals remained over 80% (Jiang *et al.* 2011). The internal resistance of this design was reported to be $120\ \Omega$, increasing to over $260\ \Omega$ during 15 weeks of operation.

5.2.2. Membrane electrode assembly

Membrane electrode assembly (MEA) designs incorporate the membrane into the electrodes and have been widely utilized in chemical fuel cells. A membrane cathode assembly (MCA) specifically incorporates the membrane into cathodes. The first MCA MFC was a cylindrical, continuous flow MFC with eight graphite rod anodes placed concentrically around an air-cathode, reaching a maximum power density of $26\ \text{mW}\cdot\text{m}^{-2}$ (Liu *et al.* 2004). This assembly yielded internal resistances of between 10 – $30\ \Omega$ during steady-state operation, significantly better than previous double-chamber and single-chamber designs. The compact nature of the MEA/MCA MFCs make them ideal for scale-up and several larger MEA/MCA configurations have been attempted (Ge *et al.* 2013; Kim *et al.* 2011; Li *et al.* 2008).

A 1.5-liter MEA MFC design was reported to generate power densities of $0.58\ \text{W}\cdot\text{m}^{-3}$ (Li *et al.* 2008). The design was then modified to include more electrode area (25 – $320\ \text{cm}^2$) and a further increase in power was observed ($2.02\ \text{W}\cdot\text{m}^{-3}$). Though power densities

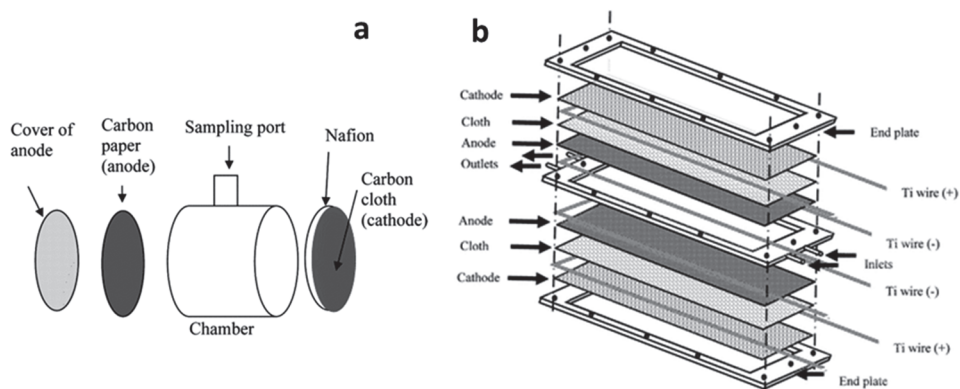


Fig. 8.3. Schematics of (a) single-chamber MFC (Adapted with permission from Liu *et al.* 2004; copyright 2004, American Chemical Society); (b) larger reactor with double CEAs (Adapted from Fan *et al.* 2012).

remained low, enhancing the surface area to volume ratio was shown to increase the power output by 3.2 times, with concurrent COD/BOD removals above 70% (Jiang *et al.* 2011, 2010). However, the costs associated with adding enough electrode area (and membranes) to be able to generate modest performance was a detriment to further development of this particular design.

A tubular MEA MFC was also constructed, consisting of concentric cylindrical MEA structures capable of being stacked together. The anode was located at the center of the cross-sectional area, separated from the MCA by the flow path. This design offered advantages in permitting good spatial distribution of the anode relative to the cathode and the ability to be easily modularized (Kim *et al.* 2011).

A similar tubular MEA MFC design was then used to treat activated sludge, resulting in an energy generation of $23.22 \text{ kWh}\cdot\text{m}^{-3}$, comparable to the $10.73\text{--}38.06 \text{ kWh}\cdot\text{m}^{-3}$ typically observed during anaerobic digestion. However, most of the energy was from methane collection, with only a minor portion of the energy coming from MFC-generated electricity (Ge *et al.* 2013). While this MEA MFC was constructed by wrapping the membrane and carbon cloth cathode around the anode carbon brush, other configurations such as wrapping and bolting membrane to different types of electrode materials have also been explored (Clauwaert *et al.* 2009; Zhang *et al.* 2013). The primary drawback to all these designs is the expense of the membranes used, making large-scale applications uneconomical.

5.2.3. Cloth electrode assembly/cloth cathode assembly

Cloth electrode assembly (CEA) and cloth cathode assembly (CCA) MFCs are similar to the MEA MFC, except for the replacement of the membrane with a cheaper separator. The separator is not ion-selective and allows for the diffusion of all solubilized compounds, but also has less resistance to the diffusion of protons and their carriers across it, thus decreasing internal resistance compared to membrane MFCs (see

Section 4.3). The highest performing separators are made of a non-woven cloth sandwiched between the electrodes used in CEA configuration. This configuration greatly decreased electrode distance to around 1 mm, resulting in a power density of $627 \text{ W}\cdot\text{m}^{-3}$ when operated in fed-batch mode and $1010 \text{ W}\cdot\text{m}^{-3}$ in continuous-flow mode, the highest reported power density for MFCs at the time (Fan *et al.* 2007). Due to high performance at low costs, a larger CEA MFC reactor was designed and tested. The larger reactor had a total volume of only 30 ml, but a total effective surface area of 200 cm^2 . The exceptional surface area to volume ratio was achieved by placing CEA structures on both the top and the bottom of the flow chamber, as seen in Fig. 8.3b. The reactor produced a power density of $2.87 \text{ kW}\cdot\text{m}^{-3}$ ($4.3 \text{ W}\cdot\text{m}^{-2}$), more than double the $1.8 \text{ W}\cdot\text{m}^{-2}$ produced in the 12-ml fed-batch MFCs with similar reactor architecture and buffer strength (Fan *et al.* 2007). High coulombic efficiency (83.5%) and COD removal rate ($93.5 \text{ kg}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$) were also achieved using this reactor design (Fan *et al.* 2012). Additional studies are needed to demonstrate CEA performance at further increased reactor sizes.

A similar, larger design CCA MFC (170 ml) using a canvas cloth separator and MnO_2 as a catalyst was able to lower the total cost of the reactor to less than 5% of MCA. Under batch mode, this configuration reached a power density of $86.03 \text{ mW}\cdot\text{m}^{-2}$ ($9.87 \text{ W}\cdot\text{m}^{-3}$) and 95% COD removal, with a coulombic efficiency of 30.2% (Zhuang *et al.* 2009).

5.3. Stacked/Modular MFCs

Individual cells can be stacked together to reach higher power output or better treatment efficiency. Recent progress that has been achieved in single MFCs can be easily applied to stacked designs. For example, based on the CCA design, Zhuang *et al.* (2012a) built a 10-liter stacked MFC to treat brewery wastewater. The COD removal rate was $4.9 \text{ kg}\cdot\text{COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ with 77.1% COD removal and 80.7% $\text{NH}_4\text{-N}$ removal at an organic loading rate (OLR) of $1.06 \text{ g}\cdot\text{COD}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$. Adding additional modules and adjusting loading rates could further increase the effluent quality and energy recovery (Zhuang *et al.* 2012b). For scaled up stacked MFCs, the highest power density was reported to reach up to $144 \text{ W}\cdot\text{m}^{-3}$ using a bipolar plate design with a 2-m^2 CEM (Dekker *et al.* 2009).

6. MFC Operation

With the experience of scaling up MFCs have come insights into how to quickly start up and operate the reactors. Over the last few years, studies focusing on the optimization of operational conditions in MFCs, specifically ones involving larger reactors, have informed the most efficient ways to do both of these tasks.

6.1. Start-up procedure

The start-up of the MFC reactor is geared around the development of a mature biofilm capable of efficiently breaking down the organics fed into the system and expelling electrons that are then converted into electricity. The first step towards cultivating these mature biofilms is inoculation. Following initial inoculation, the

microbial population is primarily composed of planktonic microbes. As more microbes adhere on the electrode surface, the abundance of exoelectrogens increases along with the power output, eventually reaching a steady state. Inoculation with an increased number of exoelectrogens and subsequent encouragement of their growth is the key to faster startups.

Typically, MFCs are inoculated with sludge that contains a highly diverse community, though different sources of inoculum can be highly dissimilar to each other. Even with these dissimilar inoculum sources, communities have been shown to converge to a similar composition with comparable power outputs over a period of two months (Yates *et al.* 2012). Other studies have shown that using MFC reactor effluent, biofilms reach a stable composition two weeks after initial inoculation (Lesnik and Liu 2014). Typically, the effluent from MFC reactors contains a higher percentage of exoelectrogens than sludge itself, resulting in these faster start-up times. On a small scale in laboratory settings, the fastest way to start up an MFC has been to rub new anode materials with an anode containing a mature biofilm on its surface. This process transfers the bacteria to the new anode and stable power outputs are generally reached around five days following the insertion of the new anode into the MFC. However, for large-scale systems, this may not be practical. A more practical route is to inoculate using mixed-species communities cultured from a high-power MFC, but grown in media with electron acceptors (e.g. Fe(III), fumarate) preferred by exoelectrogens. This procedure has been shown to improve start-up times; however, the composition of a community cultured in media will still be vastly different than the composition of a biofilm community grown on a MFC anode (unpublished results). Developing a method to cultivate high-power communities in a composition similar to the mature MFC biofilm structure is a key challenge currently being faced by MFC researchers.

There are, however, additional methods to improving start-up times not based around optimizing inoculum composition; most of these methods are geared towards promoting the growth of exoelectrogens following inoculation. One of the ways to encourage growth of exoelectrogens is to supply them with a preferred electron donor, such as acetate. Likewise, this amendment of wastewater with acetate ($400 \text{ mg}\cdot\text{L}^{-1}$) has been shown to decrease start-up times over the use of fumarate ($600 \text{ mg}\cdot\text{L}^{-1}$), glucose ($350 \text{ mg}\cdot\text{L}^{-1}$), or wastewater (Liu *et al.* 2011). Another method to improve start-up times may be to operate the reactor in continuous flow mode, thereby encouraging the adherence of bacteria to the electrode in addition to preventing localized depletion of electron donors by planktonic cells (Heffernan *et al.* 2009). Adding phosphate buffer to increase the conductivity and limit the growth of non-exoelectrogens has also been shown to be effective (Logan *et al.* 2007; Min *et al.* 2008; Nam *et al.* 2010), though increasing the buffer beyond 100 mM has demonstrated a negative impact on start-up times (Liu *et al.* 2011). Adding activated carbon powder will also lead to increases in solution conductivity, as well as providing a surface for the aggregation of microbes and substrate adsorption. Following the addition of activated carbon powder, start-up times were observed to be 30 hours (32%) less with concurrent voltage outputs up to 200 mV greater than MFCs without the addition in a fluidized bed MFC (Wang *et al.* 2014). Operating MFCs at appropriate voltages (e.g. 0.30–0.35 V for air-cathode MFCs) may also allow

for the establishment of suitable anode surface potentials and encourage the growth of bacterial clades associated with faster biocatalytic rates (Ishii *et al.* 2014).

6.2. Environmental and operational conditions

Power generation of an MFC can be affected by many factors, including physical and chemical characteristics, such as substrate type and concentration, pH, temperature, and ionic strength (Liu *et al.* 2005b). Hydraulic and electrical parameters, such as OLR, hydraulic retention time (HRT), and operating voltage, also greatly impact MFC performance. The optimization of these conditions is the key to successful operation of MFCs.

6.2.1. Substrate/Wastewater type

The substrates present in various wastewaters serve as the primary energy source in addition to the carbon source. Substrates therefore play a direct role in determining MFC performance from both power and efficiency perspectives. Several different substrates and wastewaters have been previously tested in MFCs (Pant *et al.* 2010), and though most MFC studies have used pure, easily degradable compounds, such as acetate or glucose, wastewaters typically contain complex mixtures of compounds. Both the composition and concentration of the wastewater are critical determinants of MFC performance, and identifying the appropriate wastewaters whose organics can be effectively degraded and converted into electrical energy will decide the economic viability of MFC technology.

Integrating MFCs into the current centralized wastewater treatment infrastructure would be a rapid way for MFCs to have a large effect on wastewater treatment and has been a research focus for several studies. However, MFCs utilizing domestic wastewater have only been able to generate power densities ($25\text{--}146\text{ mW}\cdot\text{m}^{-2}$) that are a fraction of what comparable designs fed acetate could generate ($506\text{ mW}\cdot\text{m}^{-2}$) (Liu and Logan 2004; Liu *et al.* 2005a), while moderate power outputs of $330\text{ mW}\cdot\text{m}^{-2}$ could be reached in scaled up designs using 100-mM buffer (Cheng and Logan 2011). Low-power outputs when fed domestic wastewater can be related to both the complexity of the organics within the waste and the low concentrations of them ($200\text{--}600\text{ mg}\cdot\text{L}^{-1}$ COD).

The treatment of sewage sludge has also been explored, but MFCs' inability to handle solids has meant that scaled up designs had to be modified, leading to low electricity generation ($130\text{ mW}\cdot\text{m}^{-2}$) even with buffer added (Ge *et al.* 2013). However, positive results from the treatment of fermented sludge supernatant, with power outputs reaching up to $1200\text{ W}\cdot\text{m}^{-2}$, suggest that there may be a way to incorporate MFCs into centralized wastewater treatment (Abourached *et al.* 2014).

The first commercial applications of MFCs are likely to be industrial, and wastes from several different types of industries have been tested. The most promising industry for initial application of MFCs is food-processing facilities due to their high carbohydrate concentrations and limited abundances of inhibitory compounds. Brewery wastewater has been tested using multiple designs, with use in a single-chamber design (50-mM phosphate buffer) resulting in power densities comparable to acetate MFCs ($438\text{ mW}\cdot\text{m}^{-2}$)

(Feng *et al.* 2008). A CEA design with no buffer, and fed brewery wastewater, produced less than half the power of acetate (638 vs. 1,994 $\text{mW}\cdot\text{m}^{-2}$), yet was significantly greater compared to outputs of other designs. Using this same setup, wastewater from a fruit, processing facility generated similar power outputs to brewery wastewater (777 $\text{mW}\cdot\text{m}^{-2}$), while power from potato-processing wastewater was slightly higher (910 $\text{mW}\cdot\text{m}^{-2}$). COD of all wastewaters tested were 3,000–6,000 $\text{mg}\cdot\text{L}^{-1}$, and were reduced by at least 88% in all instances (unpublished results).

Swine wastewater has also been used in single-chamber MFCs (with buffer), resulting in decent power (261 $\text{mW}\cdot\text{m}^{-2}$), though the high ammonium concentrations in animal wastes could become a problem (Min *et al.* 2005b). Lignocellulosic materials would be a cost-effective feedstock, though microorganisms in MFCs have not been shown to be able to directly degrade cellulosic biomass, and extensive pre-treatments would be necessary (Ren *et al.* 2008). Landfill leachate, composed of organic matter along with inorganic macrocomponents and heavy metals, has seen use in a few designs, but has yet to demonstrate significant energy production (Greenman *et al.* 2009).

6.2.2. Physical and chemical parameters

Compared with chemical fuel cells, MFCs must operate under mild conditions that are suitable for microbial growth. Optimal temperature for MFC operation ranges between 30–37 °C (Liu *et al.* 2005b). Decreasing the temperature below 32 °C to 20 °C has led to 9–17% decreases in performance (Feng *et al.* 2008; Liu *et al.* 2005b). This is important to consider when planning operation outdoors in climates that are consistently significantly below ideal operating temperatures. In these settings heating elements would likely be required.

pH is another critical component. Many wastewaters have pH outside what is suitable for most microbial growth, requiring the addition of acid or base to adjust in an appropriate range of pH 6–8. However, adjusting wastewater pH before biological treatment is a common practice. In order to maintain the neutral pH environment, a buffer is sometimes required, and in the absence of buffering, a relatively large pH change (from pH 7 to 8.5) is observed in single-chamber MFCs due to the consumption of weak acid substrates (such as acetate, etc.) (Nam *et al.* 2010).

Buffers can also have a large impact on internal resistance. Adding phosphate buffered saline (PBS) to brewery wastewater can increase the solution conductivity from 3.23 $\text{mS}\cdot\text{cm}^{-1}$ to 7.65 $\text{mS}\cdot\text{cm}^{-1}$ (50 mM PBS) and 14.6 $\text{mS}\cdot\text{cm}^{-1}$ (200 mM PBS). As a result, the maximum power density was increased 136% (483 $\text{mW}\cdot\text{m}^{-2}$) for 50 mM PBS added, and 158% (528 $\text{mW}\cdot\text{m}^{-2}$) for 200 mM PBS (Feng *et al.* 2008). Though buffer concentration can maintain a stable pH and strongly affect power generation by influencing solution conductivity, the addition of buffers to real waste streams is impractical due to associated costs.

New designs with reduced electrode spacing, such as CEA MFCs, lessen the importance of solution conductivity, and may limit the need for external buffers. Similarly, a self-produced bicarbonate buffer produced by CO_2 generation of the bacteria has been shown to be an effective proton carrier, and can lower the internal resistance and

improve the power output (Fan *et al.* 2007; 2012). Adding NaCl can increase the solution conductivity and power densities, but is not as effective as adding buffers (Liu *et al.* 2005b; Nam *et al.* 2010).

6.2.3. Hydraulic parameters

Hydraulic parameters such as ORL, HRT, and recirculation rate affect the power output and treatment efficiency, especially in larger-scale MFCs. Increasing the HRT results in decreased ORL, with subsequent increases in COD/BOD removal and coulombic efficiency due to a more complete consumption of substrates by the bacteria. However, since the substrate concentration and rate of substrate consumption is decreased during longer HRTs, decreased power outputs are observed (Li *et al.* 2008; Liu *et al.* 2004). For example, when the feeding rate of a continuous flow MFC was increased from 0.13 to 1.20 mL·min⁻¹, the HRT decreased from 2.56 to 0.278 h. As a result, the BOD removal decreased from 93.7% to 30.8%, and coulombic efficiency decreased from 97.2% to 77.5%, too (Fan *et al.* 2012). This was due to the incomplete fuel consumption under a ORL (low HRT) (Chang *et al.* 2004). In some cases, when the influent wastewater is not deoxidized, decreasing the HRT will increase the DO in the anode chamber, leading to a voltage drop. In this situation, the HRT could be further optimized for maximum power generation by increasing the HRT and decreasing the DO load (Li *et al.* 2008).

Recirculation is an important hydraulic component when operating MFCs and is used to ensure mixing of the substrates to increase treatment efficiency. Recirculation rates can be normalized to anode liquid volume (ranging from several times to several hundred times reactor volumes) (Pham *et al.* 2008; Zhang *et al.* 2010a). When the recirculation rate of an up-flow MFC was increased from nil to 140 mL·min⁻¹ and 500 mL·min⁻¹ (normalized to 3.5 and 12.5 times of anode liquid volume per hour, respectively), the maximum power density increased from 3.74 W·m⁻³ to 4.36 W·m⁻³ and 7.11 W·m⁻³, respectively. COD removal was also increased from 54.4% to 67.6% and 83.7% (Zhang *et al.* 2010a).

In order to reach a better treatment result and energy recovery, a larger MFC should be operated with a longer HRT and a lower OLR (Xiao *et al.* 2014). Thus, depending on substrate type and concentration, HRT, OLR, and recirculation rate should be optimized to meet the treatment requirements. However, more energy is needed for pumping the recycle flow at a higher recirculation rate. Thus, a balance needs to be reached between energy input and production.

For stacked MFCs, reactors can be connected hydraulically in series or parallel to enhance the overall power output and wastewater treatment efficiency or capacity. The serial stacks will result in a varying substrate concentration and composition within the stacked system, and cells at the end of the stacked system would have a reduced power output due to lower substrate concentration. The function of these stacks would be primarily for polishing effluent opposed to power generation (Gálvez *et al.* 2009). Few studies have used stacks hydraulically connected in parallel, which should be further explored.

6.2.4. Electrical parameters

Varying operating voltages can greatly affect energy recovery of MFC systems. In some high-performing systems, operating the MFCs at maximum power corresponds to a voltage efficiency (operating voltage divided by E_{emf}) of around 25%, resulting in an energy efficiency of around 21%. If operated at a higher voltage, the energy recovery can be greatly increased due to increases in voltage efficiency. However, the tradeoff is that power generation will be reduced (Fan *et al.* 2012).

The electrical connection between cells in stacked and modularized systems is also important in order to establish desired voltage or current outputs. Connecting cells in parallel leads to similar power densities and voltages as seen in the individual cells (Zhuang *et al.* 2012b). However, since the voltages of most MFCs are relatively low and generally unable to power many electrical devices, connecting several cells serially for an additive voltage effect is often warranted. In serially connected systems, the stacked MFCs had a similar power density to the individual MFCs, but a fold-increased total voltage depending on the number of stacks (Zhuang *et al.* 2012b). However, substrate depletion during high current conditions can often lead to voltage reverses of an individual cell (Kim *et al.* 2011; Oh and Logan 2007). When this occurs in a serially connected system, the OCV of the serially connected cells can become low and possibly close to zero resulting in a complete loss of power generation. This damaging effect can be minimized by applying external circuits with capacitors to the stacked MFCs (Kim *et al.* 2011). Some evidence has suggested that a combination series-parallel configuration is the most efficient, and has been observed to result in a 1.2-fold higher power density than serially connected MFCs and 30% higher than a parallel configuration (Ieropoulos *et al.* 2008).

6.2.5. Long-term operation

Several studies have shown a decrease in performance over extended periods of time, with deterioration of cathode performance believed to be the primary cause. Power generation has been reduced ranging from 22% to 60% in different studies over a period ranging from a year to 180 days (Zhang *et al.* 2011; Zhuang *et al.* 2012a). Decreases in cathode performance may be due to cathode alkalization by alkali salts (MnO_2 as catalyst) or clogging of pores by the biofilm or organic matter (activated carbon as catalyst) (Zhang *et al.* 2011). Nearly instantaneous recovery of performance can be achieved by simply rinsing the cathode with deionized water, re-applying the cathode catalyst, or replacing the cathode (Zhang *et al.* 2011; Zhuang *et al.* 2012a). However, several studies showed that the biofilm would appear again after the cleaning (Jiang *et al.* 2011; Zhang *et al.* 2013). Maintaining the stability of MFC performance over long-term operation is critical and should be one of the MFC focuses of future research.

7. Outlook

Recently, significant progress has been made in two areas that have previously limited the economic feasibility of MFCs: the huge cost associated with MFC construction and

the inability to generate meaningful power outputs, particularly at increased reactor sizes. Some of the fabrication costs have been overcome through the replacement of expensive catalysts and membranes with cheap materials, such as activated carbon and cloth separators. Additionally, improved performance at increased reactor sizes has been demonstrated by newly developed CEA MFC designs.

However, in order for MFC technology to achieve practical application in wastewater treatment, feasibility must be demonstrated on larger scales. To this point, the majority of MFC research has been performed on a millimeter scale, though there have been several attempts at increasing reactor size from 1 liter to 1000 liters (Janicek *et al.* 2014). Each step in the progression from laboratory to pilot-scale systems has exposed potential issues that must be addressed before full-scale systems are implemented. Many scale-up attempts have not proved successful in making the jump to commercial settings, but have contributed to the advancement of the technology as they have guided the optimization of microbial communities, materials, designs, and operating conditions of MFCs.

The promise and potential of microbial fuel cells will continue to spur their development. If energy recovery in MFC systems could approach the 44% value theoretically attainable, the effect on sustainability of the current wastewater treatment infrastructure would be immense. A process that consumes 3% of the total electrical load in developed countries could go from an energy-intensive process to possibly an energy-neutral or energy-positive one. There have been and will continue to be significant engineering challenges surrounding the practical application of MFCs, but the potential impact is too great to not continue the development of this technology.

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Chapter 9

Development and Applications of Anaerobic Membrane Bioreactor in Japan

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This chapter summarizes recent progresses on anaerobic membrane bioreactors' (AnMBR) technology and its applications. The historical and technological background of the AnMBR are first discussed followed by development of the process, configurations, type of membrane and performance in the treatment of municipal and industrial wastewaters, as well as solid wastes. Information related to the pilot experiment and full-scale application of AnMBRs is also provided with emphasis on the experience developed in Japan.

1. Overview of the Anaerobic Membrane Bioreactor (AnMBR)

1.1. *Historical background*

The conventional aerobic activated sludge process not only consumes tremendous amounts of energy, but also produces large quantities of residual sludge that require further disposal. Anaerobic treatment technology is a promising alternative because it does not need energy for aeration and produces substantially less sludge. Moreover, the methane-rich biogas generated from the treatment can be used as an energy source. In recent years, an anaerobic process has been developed using the membrane bioreactor (MBR) technology.

MBRs were first introduced to the aerobic treatment of municipal wastewater (Smith *et al.* 2012). Membranes in such bioreactors allow for better solid–liquid separation than the conventional gravity-based sedimentation, producing higher quality effluents. Furthermore, MBRs may be operated at higher biomass concentrations than the conventional processes, also resulting in better degradation efficiency. Overall, the aerobic MBR process offers the following advantages over the conventional activated

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sludge process: high removal efficiency and loading rate, better control over solids retention time and hydraulic retention time (HRT), and high biomass concentrations allowed in the reactor (Aquino *et al.* 2006; Akram and Stuckey 2008a; Van Zyl *et al.* 2008; Yuan *et al.* 2008).

Grethlein H.E. (1978) was the first to introduce membrane separation to anaerobic treatment in septic tank. In the early 1980s, researchers of Dorr-Oliver in UK tested this concept in full scale using an anaerobic membrane bioreactor (AnMBR) system, which was composed of a completely stirred tank reactor (CSTR) and an external cross-flow membrane filtration module (Li *et al.* 1985), for the treatment of wheat starch processing effluent. However, this work received little attention and not much follow-up has taken place for years.

In the late 1980s, Japan became interested in the AnMBR technology. A national research project — *Aqua Renaissance '90* — was kicked off, followed by many other projects on the application of membrane technology in the anaerobic treatment of various types of wastewater. At about the same time, Ross *et al.* (1990) in South Africa developed the anaerobic digestion with ultra filtration process combining an anaerobic CSTR reactor with a cross-flow ultra filtration module. Later, Kubota Corporation in Japan developed the submerged AnMBR process using the submerged flat-sheet membrane module, and Biothane developed another system with the external tubular membrane module. Since then, the development and applications of AnMBRs have attracted much global attention. Visvanathan and Abeynayaka (2012) reported that the number of publications on AnMBR had been mounting and exceeded those on UASB in 2009. AnMBR systems have now become accepted by the industry for the treatment of food wastes and many industrial wastewaters due to its simplicity, compactness and stable performance.

1.2. Technological background

1.2.1. Process configuration

In MBRs, a membrane is used to facilitate the solid–liquid separation so as to retain biomass in the reactor at high concentrations and to produce high-quality effluent. During the treatment process, sludge gradually builds up a cake on the membrane surface. The fouling on the membrane surface results in an increase of trans-membrane pressure (TMP) and the reduction of the effluent discharge. Figure 9.1 illustrates the three common configurations of which membrane modules are incorporated with MBR (Liao *et al.* 2006). For the external configuration shown in the Fig. 9.1a, the membrane module is located outside the anaerobic reactor, making it easily accessible for replacement. Membrane surface fouling may be reduced by increasing the circulation of mixed liquor, which may result in a substantial increase of operational cost. In the submerged configuration in Fig. 9.1b, the membrane module is immersed inside the reactor, in which the membrane surface is cleaned by recirculating the biogas from the headspace. Figure 9.1c shows an external submerged configuration, which combines both features in Figs. 9.1a and 9.1b. Among the three, most studies of AnMBRs were conducted for the submerged configuration (Van Zyl *et al.* 2008; Hu and Stuckey 2006; Akram and Stuckey 2008b; Bohdziewicz *et al.* 2008; Walker *et al.* 2009).

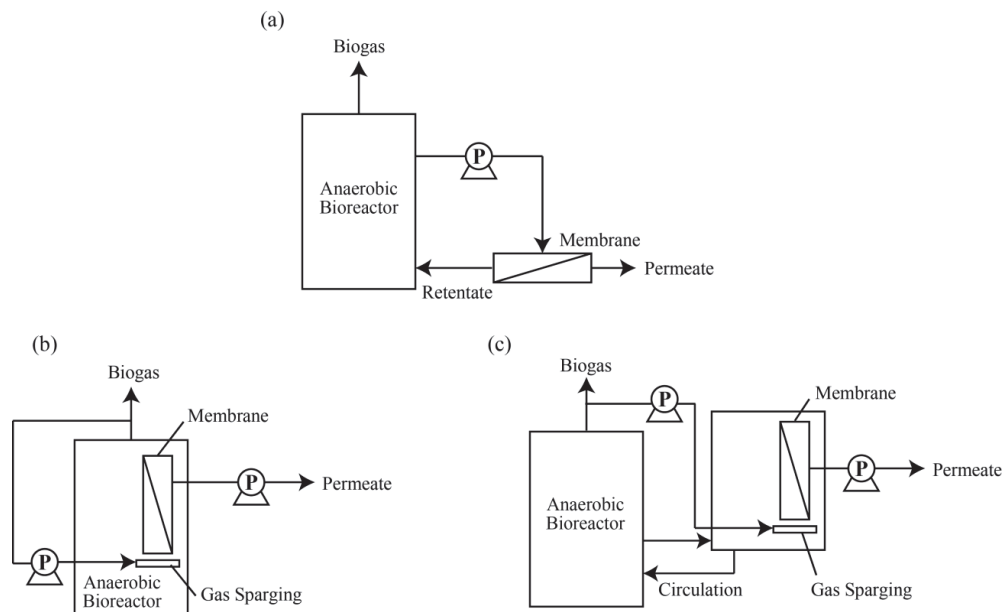


Fig. 9.1. Schematic diagrams of AnMBR configurations: (a) External AnMBR; (b) Submerged AnMBR; (c) External submerged AnMBR.

1.2.2. Types of membranes

Membranes are commonly classified, according to the pore size, into four categories: microfiltration for pore size ranging 0.05–10 μm , ultrafiltration 0.002–0.01 μm , nanofiltration 0.001–0.002 μm and reverse osmosis <0.001 μm . Among these four, microfiltration and ultrafiltration are most commonly used for MBRs and AnMBRs. Membranes may also be classified, according to their size and shape, into three types: flat-sheet, tubular and hollow fiber. Flat-sheet membranes are often used in laboratory studies for their stability and ease of cleaning and replacement (Kim *et al.* 2007; Kocadagistan and Topcu 2007; Lin *et al.* 2011; Kanai *et al.* 2010). Some also used tubular membranes, which are less vulnerable to fouling (Ho *et al.* 2007; An *et al.* 2009; Calderón *et al.* 2011; Herrera-Robledo *et al.* 2011; Salazar-Pelaez *et al.* 2011). However, hollow fiber modules are the most widely used in practice for submerged AnMBR treatment (Chu *et al.* 2005; Lew *et al.* 2009; Kim *et al.* 2010; Yoo *et al.* 2012; Liu *et al.* 2013) because of their cost-effectiveness resulting from their intrinsic feature of high membrane area per unit volume.

1.2.3. Control of membrane fouling

Membrane fouling would increase the extra cost required for membrane cleaning and replacement. Thus fouling control has attracted much research attention. Fouling begins when foulant, either organic or inorganic, deposits onto the membrane surface. The deposited foulant may either penetrate into the membrane interior or build up on the

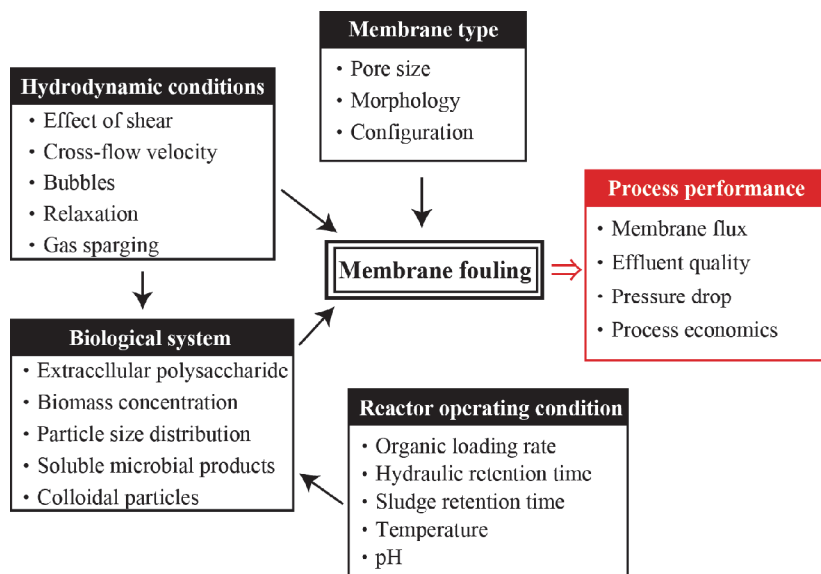


Fig. 9.2. Factors affecting membrane fouling.

surface forming a cake, both resulting in the decline in flux and the increase in TMP, as well as shortening of the lifetime of a membrane.

Figure 9.2 summarizes the factors affecting membrane fouling. Hu and Stuckey (2007) studied the effects of powdered activated carbon and granular activated carbon (GAC) on the fouling in AnMBRs using gas sparging for surface cleaning. They found that, compared with the control reactor, both powdered activated carbon and GAC reduced the TMP and increased the flux. Kim *et al.* (2010) added GAC into an anaerobic fluidized-bed membrane bioreactor and controlled membrane fouling using recycling liquid instead of biogas sparging. Similarly, Gao *et al.* (2014b) found that GAC may reduce the fouling in a fluidized-bed AnMBR. They found that the increase of GAC dosage resulted in greater removal of protein deposits on the membrane surface by carbon adsorption. This may attribute to the positive effect of GAC dosing.

Membrane fouling may be cleaned by increasing the turbulent flow shearing the membrane surface. This may be achieved by recirculating the mixed liquor or sparging with biogas. Xie *et al.* (2010) reported that the membrane flux of a submerged AnMBR increased and the fouling rate decreased when the biogas sparging rate was increased from 0.3 to 0.75 L·min⁻¹ in treating wastewater from the food industry. Zhang *et al.* (2007) found that, although cross-flow velocity is critical to reduce the fouling in AnMBRs, unnecessarily high flow velocity may break down the microbial flocs in the mixed liquor. These smaller flocs may either penetrate deeper into the membrane interior or form a denser cake on the membrane surface, reducing the flux as a result.

2. Organic Solid Waste Treatment

Two case studies on the application of AnMBRs in Japan for the treatment of high-strength organic solid wastes, i.e. ground coffee wastes and food wastes, are presented in

this section. Both processes were operated under very high organic loading rates (OLRs) with short HRT, as compared to those that used the conventional CSTR process for the digestion of organic solid wastes.

2.1. High-rate coffee grounds treatment using thermophilic AnMBRs

Ground wastes of coffee are rich in lipids and are difficult to be degraded under anaerobic conditions. Thermophilic digestion at 55–57 °C is considered to have an advantage of improving hydrolysis rate and dispersion of lipid; however, there has been no report on successful operation of coffee grounds treatment. AnMBRs are effective at retaining biomass and improving stability under the high OLR of coffee grounds (Qiao *et al.* 2013). Figure 9.3 illustrates the experimental apparatus that was used at first to treat coffee grounds alone as substrate. Results in Fig. 9.4 show that the AnMBR process performed satisfactorily for OLRs at 2 and 4 kg-COD·m⁻³·d⁻¹, but the performance failed when OLRs reached 6 kg-COD·m⁻³·d⁻¹. On the other hand, in the subsequent experiments using coffee grounds plus waste activated sludge as co-substrate, the process performed satisfactorily ranging 2–12 kg-COD·m⁻³·d⁻¹, the variation of which was controlled by changing the influent TS concentration from 25 to 150 g·L⁻¹ at 20 days of HRT. Results showed that 67.4% of the influent COD was converted to methane, and the final effluent, i.e. permeate collected from the membrane module, increased with OLR from 2.5 g·L⁻¹ at 2 kg-COD·m⁻³·d⁻¹ to 15 g·L⁻¹ at 12 kg-COD·m⁻³·d⁻¹. The membrane filtration flux was 5.1 L·m⁻²·hr⁻¹, which was not affected by the increase of biomass concentration in the mixed liquor, which eventually reached 75 g·L⁻¹.

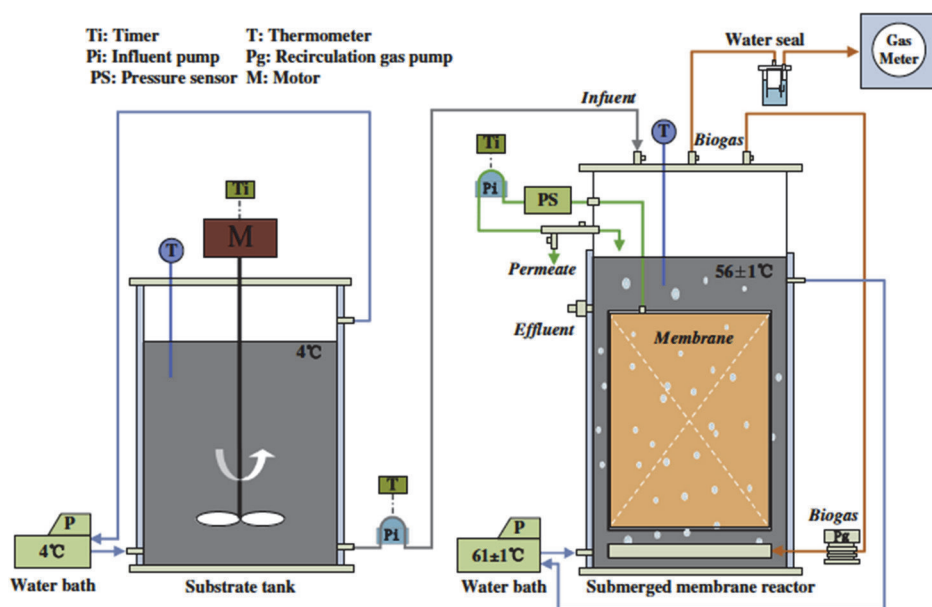


Fig. 9.3. Experimental set-up of AnMBR (Qiao *et al.* 2013).

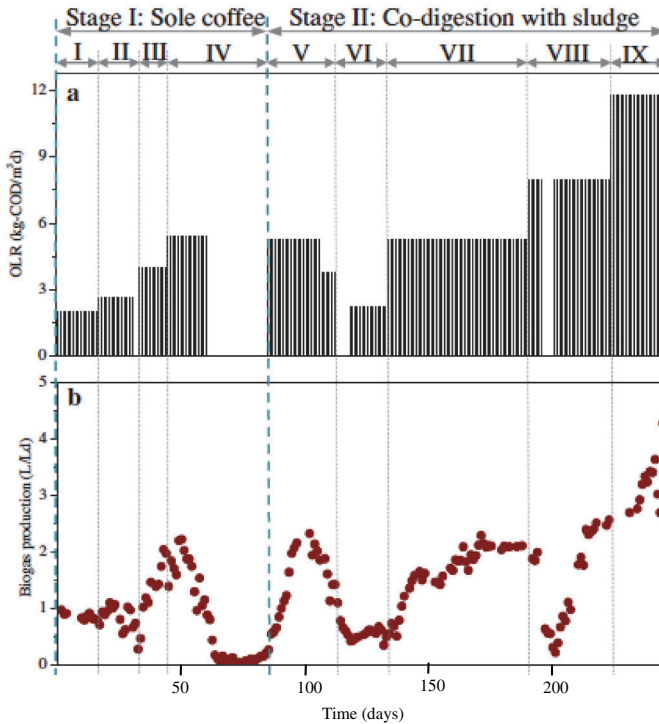


Fig. 9.4. Time course of AnMBR operation fed with coffee grounds and waste activated sludge: (a) OLR; (b) biogas production rate (Qiao *et al.* 2013).

2.2. Bio-hydrogen production from food waste at short HRT and high OLR

The AnMBR system has been successfully used in continuous fermentation for the production of bio-hydrogen. Shortening HRT is important in the hydrogen production of easily degradable organic wastes, such as those from the food industry. A short HRT helps the process of washing out the hydrogen-consuming microorganisms, such as methanogenic archaea and homoacetogenic bacteria, from the reactor, and of reducing the reactor pH to the preferable level of pH 5.5 for hydrogen production; in addition, a shorter HRT also means smaller reactor size. In the conventional CSTR system, washout of hydrogen-producing biomass at short HRTs may result in the reduction of overall reactor performance. This problem may, however, be overcome in AnMBR systems, in which biomass is retained by the membrane, independent of HRT. We thus conducted a continuous experiment for hydrogen production at 55 °C at high OLR using an AnMBR (Lee *et al.* 2014) similar to the one in Fig. 9.3. The substrate was food waste slurry with 45 g·L⁻¹ of TS, and the OLR level was step increased from 70 kg-COD·m⁻³·d⁻¹ to 125 kg-COD·m⁻³·d⁻¹ by shortening the HRT from 18.7 to 10.5 h. Figure 9.5 shows the OLR, biogas production rate and the biogas composition of H₂ and CO₂ throughout the experiment. It illustrates that the biogas production rate increased correspondingly with the increase of OLR, and the H₂ content in the biogas was consistently around 45%

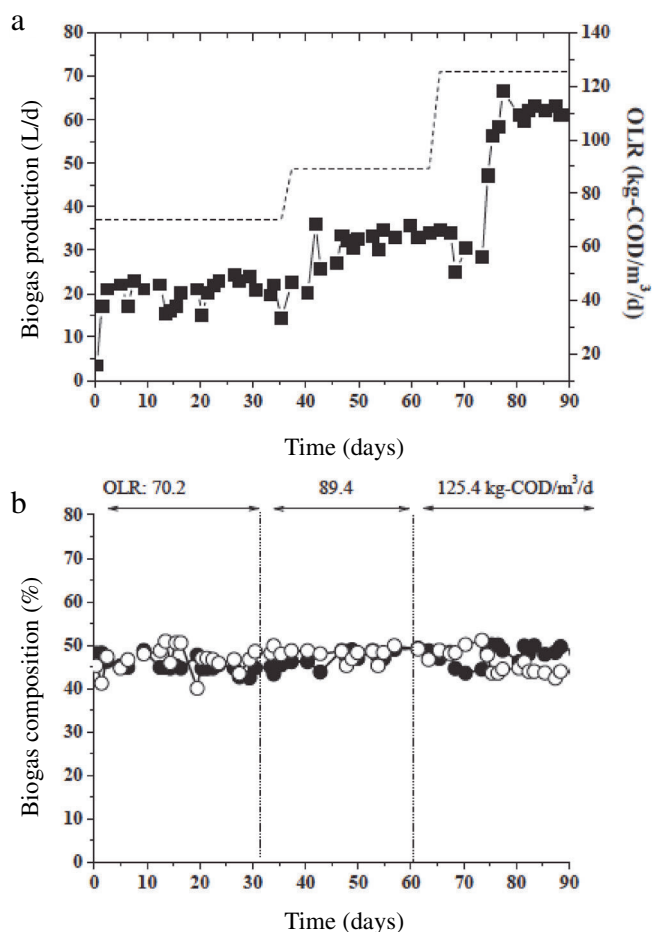


Fig. 9.5. Biogas production and composition in hydrogen-producing MBR: (a) biogas production ■, OLR ---; (b) H₂ content ●, CO₂ content ○ (Lee *et al.* 2014).

throughout the experiment. Over 97% of carbohydrate in the substrate was converted, and yet there was no methane produced in this study. Hydrogen yield, ranging 1.2–2.2 mol-H₂-mol-hexose⁻¹, was comparable to that reported by other researchers using food waste as a single feedstock (Chu *et al.* 2008; Wang and Zhao, 2009). This study demonstrates that hydrogen may be effectively produced from food wastes in AnMBRs operated at OLRs as high as 125 kg-COD·m⁻³·d⁻¹, corresponding to a short HRT of 10.5 h.

3. Municipal Wastewater Treatment

3.1. Types of membranes tested against synthetic and actual wastewaters

A number of studies have been conducted using AnMBRs for the treatment of municipal wastewater. Two of such studies using synthetic, instead of actual, wastewater are summarized below. Hu and Stuckey (2006) conducted a test for 100 days using both

hollow fiber and flat-sheet membranes made of polyethylene with 0.4- μm pores at HRT ranging 3–48 h. They found that both membranes were able to remove over 90% of soluble COD at HRT as low as 3 h. However, the system using flat-sheet membrane had on average a higher effluent flux ($11.74 \text{ l}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$) than the one using hollow fiber membrane ($9.74 \text{ l}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$) (Saddoud *et al.* 2007). Chen *et al.* (2014) tested the performance of a submerged AnMBR with a forward osmosis membrane at 25 °C. The process removed 96% of COD, nearly 100% of total phosphorus and 62% of ammonia-nitrogen.

Many other AnMBR studies were also conducted using actual municipal wastewater (mostly after primary settling). Operational conditions and reactor performance are summarized in Table 9.1. All of these studies used AnMBR of submerged configuration with either hollow fiber or flat-sheet membranes. Pore size of the membrane was mostly in the range of 0.1–1.0 μm , with some exceptions as large as 12 μm and 61 μm (Hu and Stuckey 2006; Ho *et al.* 2007; Zhang *et al.* 2010; Zhang *et al.* 2011). Giménez *et al.* (2011) reported that COD removal averaging 90% over a 140-day testing period using a 2.1- m^3 reactor with a hollow fiber membrane module (pore size 0.05 μm ; surface area 30 m^2). Similar performance was reported by Martinez-Sosa *et al.* (2012) using a 350-L reactor with flat-sheet membrane (pore size 0.038 μm ; surface area 3.5 m^2). Over 90% of COD was removed from the actual municipal wastewater tested.

3.2. Influence of temperature

Most of the AnMBR treatment studies of municipal wastewater were conducted at either mesophilic (30–37 °C) or ambient temperatures (20–30 °C). It is generally agreed that mesophilic treatments performed better with higher COD removal and may be operated at higher OLR and/or shorter HRT (Ho *et al.* 2007; Cerón-Vivas *et al.* 2012; Ho and Sung 2010). Kim *et al.* (2010) reported a COD removal of 99% at 35 °C with an OLR of 4.4–6.2 $\text{kg}\cdot\text{COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. Similarly, Gao *et al.* (2010) found a COD removal of 96% at 30 °C with an OLR of 5.1 $\text{kg}\cdot\text{COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. Both studies were tested against synthetic wastewater composed of easily biodegradable organic compounds. AnMBR performance was not as good, however, when tested against actual wastewater. Lin *et al.* (2011) found a COD removal of only 90% at an OLR of 1.0 $\text{kg}\cdot\text{COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ at 30 °C. Kocadagistan and Topcu (2007) reported a COD removal efficiency of 88% at 37 °C and at an OLR of 0.2–2.0 $\text{kg}\cdot\text{COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$.

Yoo *et al.* (2012) reported that COD removal was 84% at 25 °C with OLR of 3.9–4.7 $\text{kg}\cdot\text{COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. Based on COD mass balance, only 22% of theoretical methane production was collected in the biogas, while 38% of the methane was dissolved in the water and lost in the discharged effluent. Ho and Sung (2009) reported 94% of COD removal at 25 °C and HRT ranging 6–12 h with OLR ranging 1.0–2.0 $\text{kg}\cdot\text{COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. Decrease of HRT resulted in the increase of soluble COD in the reactor and the lowering recovery of methane from 48% to 35%. Sunaba *et al.* (2012) found that shortening HRT from 12 h to 6.0 h at 25 °C resulted in the lowering of COD removal from 94% to 87%; at HRT of 12 h methane recovery was 72%.

Table 9.1. Performance of laboratory AnMBR treating real municipal wastewater in the previous studies.

Volume (liter)	Configuration	Membrane (type, pore size, area)	Temperature (°C)	OLR (kg-COD m ⁻³ ·day ⁻¹)	HRT (h)	Influent COD (mg·L ⁻¹)	Effluent COD (mg·L ⁻¹)	COD removal (%)	Reference
5.8	submerged	hollow fiber, 0.4 µm, 0.19 m ²	15–35	0.75–0.95	6	247–449	n.a.	51–74	Gao <i>et al.</i> 2014a
5	submerged	flat-sheet, 0.45 µm, 0.118 m ²	25–30	1.02±0.14	10	427±59	60	86	Huang <i>et al.</i> 2013
0.442	submerged	hollow fiber, 0.1 µm, 0.0215 m ²	25	3.9–4.7	1.75	152±27	25±8	84	Yoo <i>et al.</i> 2012
60	submerged	flat-sheet, 0.6 µm	30	-1.0	10	342–527	40	90	Lin <i>et al.</i> 2011
45	submerged	flat-sheet, 61µm	10–30	n.a.	8.0	298±76	105±32	75	Zhang <i>et al.</i> 2011
10	side-stream	0.1 µm, 0.1 m ²	n.a.	0.03–0.16	12–48	38–131	18–37	72	Baek <i>et al.</i> 2010
45	n.a.	flat-sheet, 61µm	10–15	3.9–4.7	8.0	302±88	121±34	63	Zhang <i>et al.</i> 2010
12.9	submerged	tubular, 0.64 µm, 0.98 m ²	15–20	2.36	2.6	260±344	76±30	84	An <i>et al.</i> 2009
50	cross-flow	UF, 100 kDa, 1 m ²	37	0.23–2.0	15–60	685±46	88±6	88	Saddoud <i>et al.</i> 2007
50	cross-flow	flat-sheet, 0.2 µm, 0.003 m ²	35	n.a.	16	350–500	<30	98	Kocadagistan and Topcu 2007

Gao *et al.* (2014a) studied the performance of a novel process using an anaerobic fluidized-bed membrane bioreactor treating actual municipal wastewater at three temperatures. Results showed that at 35 °C, the process removed 74% of COD recovering 53% of methane; the corresponding values were 67% and 55% at 25 °C, and 51% and 39% at 15 °C. They also found that biofouling of membrane was more severe at lower temperatures, probably due to the increased deposition of tein, which may be alleviated by the dosing of GAC.

Smith *et al.* (2013) operated an AnMBR treating synthetic municipal wastewater at 15 °C and HRTs of 16–24 h (OLR ranging 0.4–0.7 kg-COD·m⁻³·d⁻¹), and achieved a COD removal of 92%. Zhang *et al.* (2010) treated actual municipal wastewater using an AnMBR at 10–15 °C and HRT of 8.0 h (OLR ranging 3.9–4.7 kg-COD·m⁻³·d⁻¹), and obtained a COD removal of 63%. An *et al.* (2009) reported that 84% of the COD removal was obtained in treating actual municipal wastewater at 15–20 °C and HRT of 2.6 h (OLR ranging 2.4 kg-COD·m⁻³·d⁻¹). Zhang *et al.* (2011) treated actual municipal wastewater at 10–30 °C and HRT of 8.0 h with a COD removal of 75%.

Figure 9.6 illustrates the effect of temperature on COD removal from municipal wastewater based on literature data for both conventional anaerobic treatment and AnMBR treatment. Results showed that COD removal efficiency in general decreases with the lowering of temperature; however, the effect is less noticeable in the AnMBR treatment systems. Figure 9.6b also shows that AnMBR could remove over 85% of COD from municipal wastewater at temperatures as low as 15 °C.

4. Full-Scale Applications and Pilot-Scale Experiments of AnMBRs

The AnMBRs process has become increasingly popular for the treatment of industrial wastes and wastewater in the past decade. Out of the 38 full-scale worldwide AnMBR installations known to us, 26 plants use the submerged configuration with flat-sheet membrane modules; and among those operated in North America, ten plants use tubular membrane modules. Feedstock treated are those having high SS and lipid contents, which make them difficult to be treated by the conventional UASB process. These feedstock

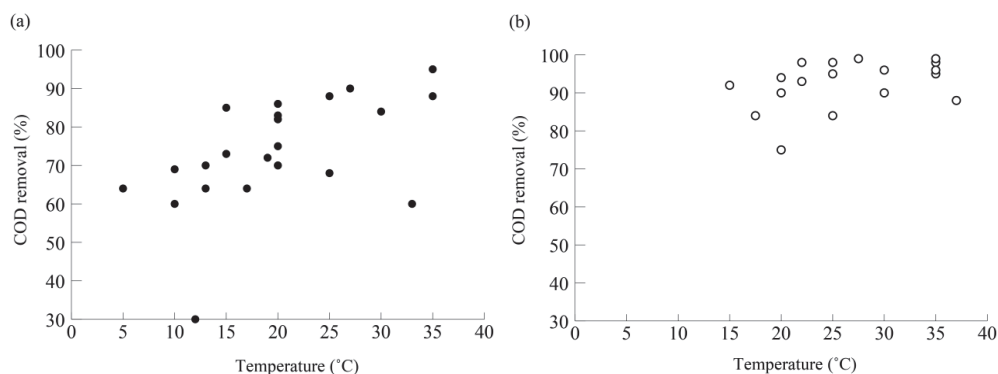


Fig. 9.6. Relationships between temperature and COD removal: (a) conventional anaerobic treatment; and (b) AnMBR.

include municipal solid wastes and wastewaters from alcohol, palm oil and food industries. Table 9.2 summarizes the 26 full-scale submerged AnMBR systems installed by the Kubota Corporation of Japan since 2000. Two case studies are presented below using the submerged AnMBR process for the treatment of wastewater from a dairy plant and a palm oil plant.

Table 9.2. Full-scale AnMBR installed by Kubota (Japan).

No.	Plant	Countries	Wastewater	Year
1	A Palm Oil Com. (T Mill)	Indonesia	POME 420 t·day ⁻¹	2014
2	A Palm Oil Com. (U Mill)	Indonesia	POME 420 t·day ⁻¹	2014
3	A Palm Oil Com. (B Mill)	Indonesia	POME 420 t·day ⁻¹	2014
4	A Palm Oil Com. (N Mill)	Indonesia	POME 420 t·day ⁻¹	2014
5	A Palm Oil Com. (G Mill)	Indonesia	POME 420 t·day ⁻¹	2014
6	B Palm Oil Company	Malaysia	POME 936 t·day ⁻¹	2013
7	Y Inc.*	USA	Confectionery 160 t·day ⁻¹	2012
8	K Company*	USA	Bakery 93 t·day ⁻¹	2012
9	Plant A*	USA	Supermarket Food Waste 200 t·day ⁻¹	2011
10	MT Shochu Distillery	Japan	Ethanol stillage 35 t·day ⁻¹	2010
11	T Shochu Distillery	Japan	Ethanol stillage 6 t·day ⁻¹	2010
12	Sa Shochu Distillery	Japan	Ethanol stillage 65 t·day ⁻¹	2009
13	K Inc.*	USA	Salad dressing WW 475 t·day ⁻¹	2008
14	M Company	Japan	Dairy 30 t·day ⁻¹	2008
15	F Shochu Distillery	Japan	Ethanol stillage 12 t·day ⁻¹	2008
16	Ho Shochu Distillery	Japan	Ethanol stillage 60 t·day ⁻¹	2008
17	D Shochu Distillery	Japan	Ethanol stillage 20 t·day ⁻¹	2007
18	Y Shochu Distillery	Japan	Ethanol stillage 20 t·day ⁻¹	2007
19	S Shochu Distillery	Japan	Ethanol stillage 60 t·day ⁻¹	2006
20	H Shochu Distillery	Japan	Ethanol stillage 15 t·day ⁻¹	2006
21	B Company	Japan	Syrup and jelly 6.5 t·day ⁻¹	2004
22	P Company	Japan	Potato 5.2 t·day ⁻¹	2003
23	Association of N sanitation	Japan	Municipal solid wastes 5 t·day ⁻¹	2003
24	Association of K sanitation	Japan	Municipal solid wastes 16 t·day ⁻¹	2003
25	I Company	Japan	Red beans, etc. 2 t·day ⁻¹	2002
26	Association of S sanitation facility	Japan	Municipal solid wastes 8 t·day ⁻¹	2000



Fig. 9.7. AnMBR treatment of dairy wastewater.

4.1. Dairy processing wastewater

Dairy processing wastewater is lipid-rich and, thus, very difficult to be treated by conventional anaerobic processes like UASB. Recently, dairy processing plants have begun to use the AnMBR process. An AnMBR was installed to the plant's manufacturing caffè latte and concentrated coffee solutions (Wakabayashi and Wakahara, 2009). Wastewater containing 2% lipids and 7% COD was treated at the flow rate of $30 \text{ t}\cdot\text{d}^{-1}$ with an OLR of $10.6 \text{ kg}\cdot\text{COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ in a system consisting of an acidogenic reactor and two methanogenic AnMBRs, each with a working volume of 100 m^3 (Fig. 9.7). Figure 9.8 shows the time course of the COD loading rate throughout the experiment. The submerged MBR has 150 flat-sheet membranes (0.8 m^2 each) (Fig. 9.9). The substrate to biomass concentration ratio was kept at 2.3. Each day, 13 tons of sludge were withdrawn from the reactor and 17 tons of effluent were collected from the membrane modules. The waste sludge was dewatered, dried and finally used as fuel for boilers. The system was designed to remove 71% of COD and to produce $880 \text{ m}^3\cdot\text{d}^{-1}$ of biogas, and the residual COD (29%) mainly consisted of the undegraded concentrated coffee and the excessive

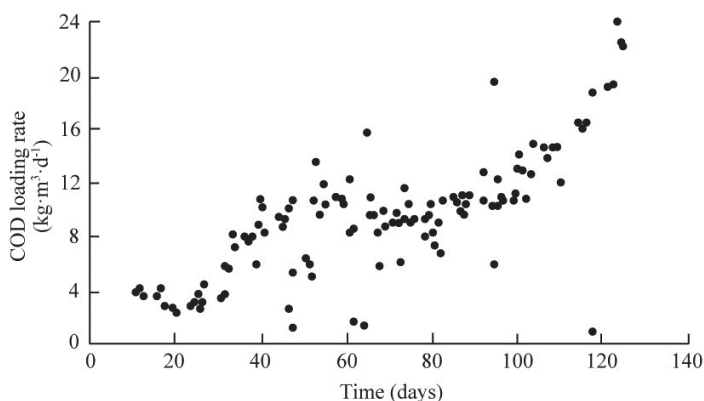


Fig. 9.8. Example of COD loading rate during a consecutive experiment.

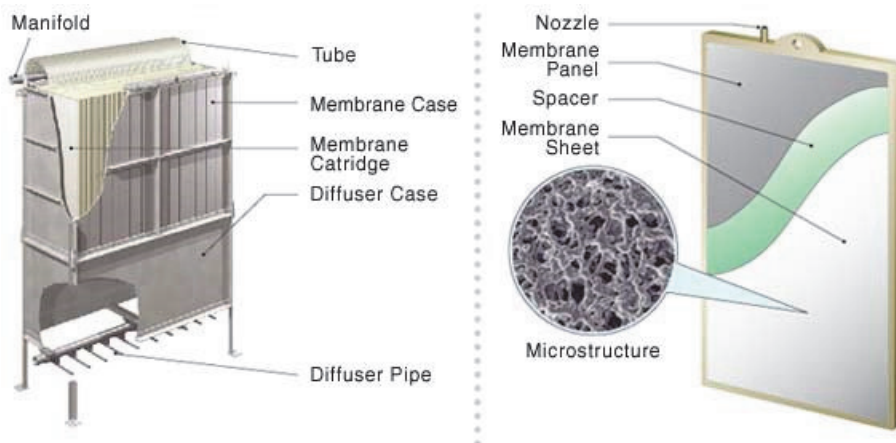


Fig. 9.9. Structure of KUBOTA submerged membrane unit.

sludge withdrawn. The BOD in the permeate throughout the membranes was $1,290 \text{ mg}\cdot\text{L}^{-1}$. After desulfurization, the biogas was used as fuel for boilers, saving $95 \times 10^3 \text{ l}$ of A-grade oil.

4.2. Palm oil mill effluent (POME)

Palm oil production is a big business in southeast Asia. Malaysia, which produces half of the palm oil in the world, has established a stringent effluent quality standard of $20 \text{ mg}\cdot\text{BOD}\cdot\text{l}^{-1}$ for the palm oil mills. As POME contains high levels of lipids and COD, the AnMBR process is expected to perform well and to produce effluent meeting the discharge standards. A pilot-scale experiment of AnMBR was investigated for high-rate treatment of POME in Malaysia (Fig. 9.10) (Kato *et al.* 2011). As shown in Fig. 9.11, the process consisted of a 2-stage treatment first in a thermophilic AnMBR (51°C), followed by an aerobic MBR.

Table 9.3 shows the characteristics of POME, including $67,500 \text{ mg}\cdot\text{L}^{-1}$ of COD and $6,600 \text{ mg}\cdot\text{L}^{-1}$ of oil and grease. Influent POME was diluted with effluent (membrane permeate and sludge) and then introduced into the AnMBR (Fig. 9.11). The AnMBR reactor was semi-continuously operated for 250 days. Thereafter, membranes were removed and the reactor was operated without membrane for another 220 days so that the effect of membranes could be evaluated. Figure 9.12 illustrates the performance of AnMBR only in COD removal, plus HRT, SRT and COD loading rate throughout the experiment. With the membrane, the AnMBR removed nearly 100% of soluble COD (S-COD) and about 80% of total COD. By contrast, the reactor without a membrane removed only about 70% of total COD. The SRT of AnMBR was 3–4 times the HRT. When the AnMBR was operated at a very short HRT of five days, corresponding to loading rates of $10\text{--}20 \text{ kg}\cdot\text{COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$, it removed 81.4% of total COD and 97% of S-COD. In order to achieve the same performance, the same reactor without a membrane would need to operate at a HRT of 16 days. This result suggested that AnMBRs may require only about one third of the volume of a normal CSTR without membranes.

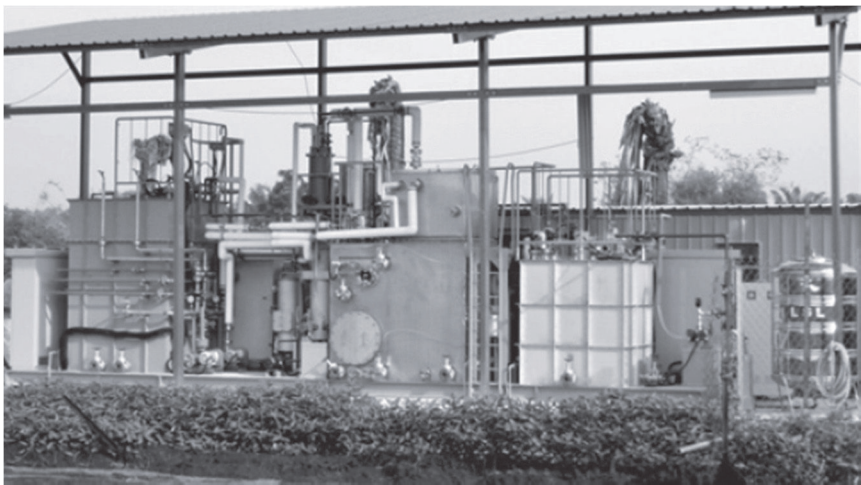


Fig. 9.10. Testing plant of AnMBR for POME treatment (capacity: 0.4 t·d⁻¹).

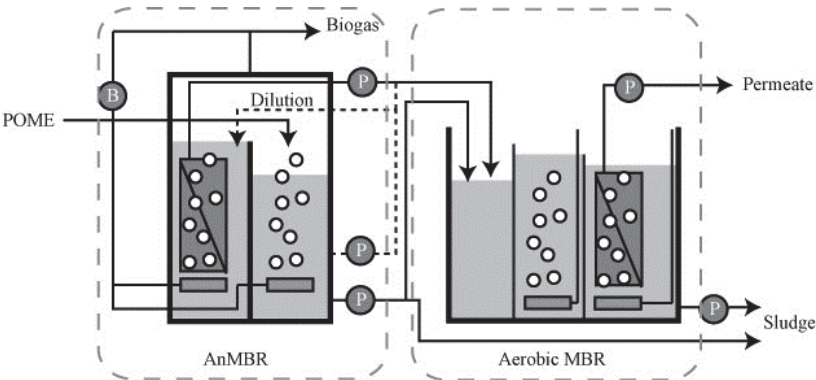


Fig. 9.11. Schematic diagram of the anaerobic–aerobic MBR process used for POME treatment experiment.

Table 9.3. Characteristics of POME fed to the AnMBR.

Parameter	Average
pH	4.5
TS	39,800 mg·L ⁻¹
COD	67,500 mg·L ⁻¹
BOD	31,100 mg·L ⁻¹
Oil and grease	6,600 mg·L ⁻¹
Solids (>0.5 mm)	75 mg·L ⁻¹
T-N	827 mg·L ⁻¹
NH ₄ ⁺ -N	197 mg·L ⁻¹
T-P	161 mg·L ⁻¹
Sulfide	212 mg·L ⁻¹
K	3,110 mg·L ⁻¹
Alkalinity	197 mg-CaCO ₃ ·L ⁻¹

The permeate and excess anaerobic sludge were then treated in the downstream aerobic MBR. Table 9.4 summarizes the effluent quality from aerobic MBR. It shows that the final effluent meets all of Malaysia's discharge limits for POME. As a result of this pilot study, a number of full-scale plants have been installed using this combined scheme of AnMBR and aerobic MBR for POME treatment in Malaysia and Indonesia. Figure 9.13 shows such a plant in Malaysia treating $900 \text{ m}^3 \cdot \text{d}^{-1}$ of POME. The AnMBR reactor was operated at $50\text{--}55^\circ\text{C}$ with over 80% of influent COD converting to methane and a final effluent BOD below $20 \text{ mg} \cdot \text{L}^{-1}$.

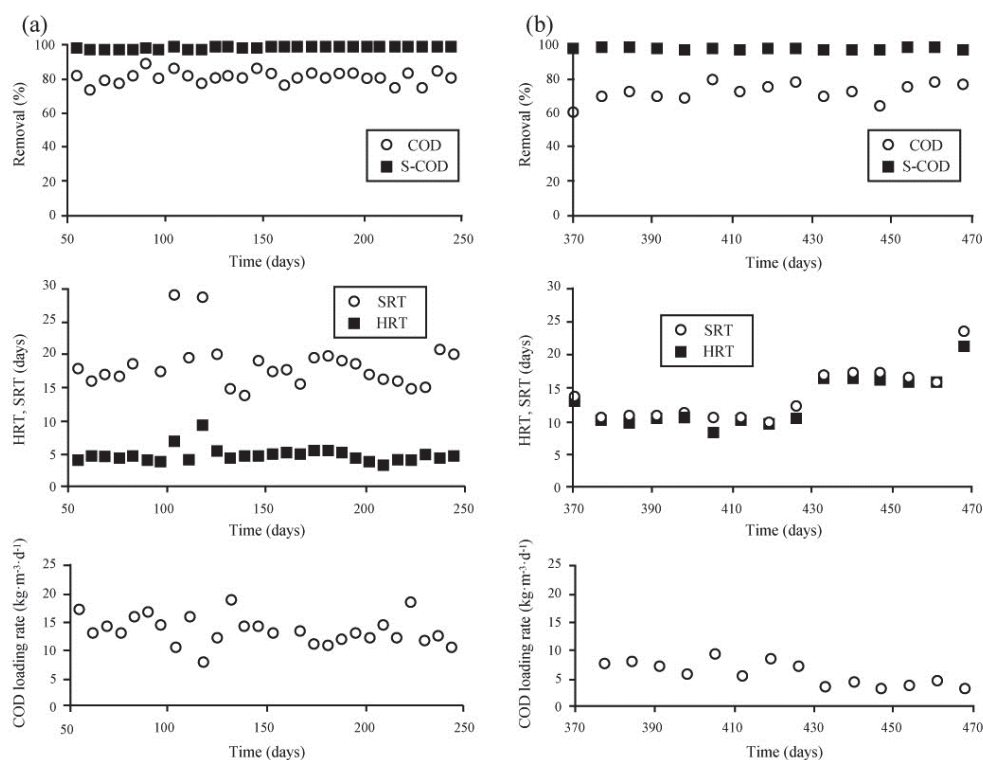


Fig. 9.12 Single-stage anaerobic COD removal, HRT, SRT and COD loading rate : (a) AnMBR, and (b) same anaerobic reactor without membranes.

Table 9.4. Effluent quality from aerobic MBR.

	Discharge limits for POME	Final effluent (100-day average)
BOD	$100 \text{ mg} \cdot \text{L}^{-1}$ ($20 \text{ mg} \cdot \text{L}^{-1}$ since 2013)	$7.4 \pm 1.9 \text{ mg} \cdot \text{L}^{-1}$
SS	$400 \text{ mg} \cdot \text{L}^{-1}$	$0 \text{ mg} \cdot \text{L}^{-1}$
Oil and Grease	$50 \text{ mg} \cdot \text{L}^{-1}$	$0.48 \pm 0.7 \text{ mg} \cdot \text{L}^{-1}$
$\text{NH}_4^+ \cdot \text{N}$	$150 \text{ mg} \cdot \text{L}^{-1}$	$0.42 \pm 0.07 \text{ mg} \cdot \text{L}^{-1}$
T-N	$200 \text{ mg} \cdot \text{L}^{-1}$	$151 \pm 35 \text{ mg} \cdot \text{L}^{-1}$
pH	5–9	8.72 ± 0.14
Temperature	45°C	$30.6 \pm 0.76^\circ\text{C}$



Fig. 9.13. AnMBR treatment of POME in Bintulu city, Sarawak state, Malaysia (capacity: $900 \text{ m}^3 \cdot \text{d}^{-1}$).

5. Conclusions

AnMBR has recently attracted much attention due to its high efficiency, such as very short HRT and good effluent quality in treating solid wastes and industrial wastewaters. A number of full-scale treatment systems have been in successful operation in Japan and south Asia. The application of this technology is still in its infancy, and has tremendous potential for further growth. On the other hand, there are challenges remaining for further broadening of the applications of this technology. These include steady performance in treating wastewater at low temperatures, and the control and reduction of membrane fouling.

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Chapter 10

Anaerobic Fluidized Bed Membrane Bioreactors for the Treatment of Domestic Wastewater

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The anaerobic fluidized bed membrane bioreactor (AFMBR) is a new energy-neutral methanogenic biological treatment system that combines an anaerobic fluidized bed reactor (AFBR) and internal membranes. It is unique in that the fluidized granular particles, while serving as a surface for active biofilm attachment, rub against membrane surfaces, resulting in a reduction in fouling with low energy expenditure. Fluidized particles denser than water permit good mass transfer, and allow for the maintenance of a large biological population, even at the short hydraulic retention time as required for dilute wastewater treatment. Membranes prevent the escape of volatile suspended solids so they can be biodegraded adequately and removed separately for subsequent disposal. The AFMBR can be used alone for wastewater treatment or as the second reactor in a staged treatment system. Laboratory and pilot studies with domestic wastewater with a staged AFBR–AFMBR system, both containing granular activated carbon particles and operated at total HRT near 6 h and temperatures down to 8 °C, have demonstrated high organic removal efficiency, low waste biosolids production (0.05 kg·kg-COD⁻¹), micro-contaminant removal efficiencies better than conventional aerobic activated sludge treatment, and a very small footprint. Further research on best approaches for dissolved methane capture and nutrient removal and recovery are ongoing.

1. Introduction

The continued growth in world population and economic well-being are combining to create a rapidly increasing demand for water and other natural resources. Another important factor affecting water is climate change caused by growing fossil fuel usage (IPCC 2007), which is already leading to less water in many areas already under

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deficiency and more water in other areas where already plentiful (Milly *et al.* 2005), changes that are very difficult to predict and thus to address. Among the various steps necessary to help mitigate the growing harm of such changes to human's and the world's ecosystems is to reduce both energy and water usage, and wherever possible, to switch from fossil fuels to renewable forms of energy. A step in this direction for the environmental engineering field would be changing from reliance on aerobic treatment, which uses considerable energy, to anaerobic treatment, which instead produces energy in the form of methane gas (McCarty 1964a; Lettinga *et al.* 1983). Additionally, the very costly and difficult problem of biosolids production and disposal is greatly reduced through anaerobic treatment (McCarty 1964a). Such change to anaerobic treatment has already taken place to a major degree in the treatment of more concentrated organic solid wastes and industrial wastewaters (Fang 2010; Speece 2008). However, with membrane bioreactors that have been developed in recent decades, it is now possible to efficiently treat domestic and other dilute wastewaters anaerobically, even at temperatures down to the 10 °C range, an achievement not thought possible until recent years.

Anaerobic treatment of domestic wastewater is not a new concept — indeed its use for this purpose preceded the development of aerobic treatment (McCarty 1981). Cesspools, septic tanks, and Imhoff tanks were among the earliest technologies used, but their low removal efficiency for BOD and SS was insufficient to meet growing environmental needs. In recent years, improved anaerobic treatment systems, such as the anaerobic baffled reactor, the anaerobic filter (AF), and most importantly, the up-flow anaerobic sludge blanket (UASB) reactor and its various modifications have been widely used (Speece 1983; Fang 2010), but still, treatment efficiency often is not adequate to meet environmental needs, especially in temperate regions (Lettinga *et al.* 1983). Even in tropical areas, subsequent treatment by aerobic or other processes is often required (von Sperling and Oliveira 2009). The major problem identified many years ago is not the slowness of methanogenic organisms, but rather the slow rate of hydrolysis of the light volatile suspended solids (VSS) present in domestic wastewater (Lettinga 1981), which quickly pass through typical anaerobic biological reactors and pass to the effluent. The advantage of the membrane bioreactor is not only that it prevents the loss of the active methanogenic culture, but also prevents the escape of the VSS as well, so that they can be held within the reactor and become biodegraded (Shin *et al.* 2014).

Membrane bioreactors can be characterized by the location of the membranes, the type of membrane, and the method used to prevent excessive membrane fouling (Brindle and Stephenson 1996; Visvanathan *et al.* 2000; Lin *et al.* 2013). Membranes may be located externally or internally to the biological reactor. Fouling control in external membranes relies on the shearing away of foulants by the recycling and fast movement of water by the membrane. The energy requirement for this is quite high and so internal membranes are generally the preferred type for dilute wastewaters. Internal membranes come in direct contact with the wastewater treating microorganisms. Here, fouling has generally been controlled by gas sparging — the rapid bubbling of air or recycled biogas below the membranes. This is more energy efficient than with external membranes, but the energy cost can still be quite high, 0.5–5 or more kWh·m⁻³ of wastewater treated (Martin *et al.* 2011).

A method of controlling membrane fouling that appears to have a much lower energy cost and has been demonstrated at pilot scale is the anaerobic fluidized bed membrane bioreactor (AFMBR), the subject of this chapter. Here, particles of sand, granular activated carbon (GAC), polymer beads or other particulate materials are fluidized by the external recycle of water from the top to the bottom of the reactor with a rapid upward movement of water within the reactor (Kim *et al.* 2011). In this process, the fluidized and moving particles rub against the membrane surfaces, and in this manner, remove the foulants. Both flat-plate and hollow fiber membranes may be used in this system, and membranes may be of many different compositions. In the many studies conducted to date (Shin *et al.* 2014; Kim *et al.* 2011; Yoo *et al.* 2012; Bae *et al.* 2013; Bae *et al.* 2014; Yoo *et al.* 2014), the AFMBR has been preceded by an anaerobic fluidized bed bioreactor (AFBR) without membranes, resulting in a reduced organic loading to the AFMBR. Details of both the AFBR and the AFMBR, their modes of operation, and efficiencies in the treatment of domestic wastewater as observed through lab-scale and pilot-scale studies over a range of wastewater temperatures are provided in the following.

2. Anaerobic Fluidized Bed Reactor

2.1. Characteristics and advantages of AFBRs

While the anaerobic fluidized bed reactor is a well-established process for anaerobic treatment of industrial wastewaters (Speece 2008; Fang 2010), use for treatment of dilute domestic wastewaters is relatively new and more limited. The main components of the AFBR are a long cylindrical reactor, a recirculation pump, a recirculation line, and fluidized support materials, as illustrated in Fig. 10.1.

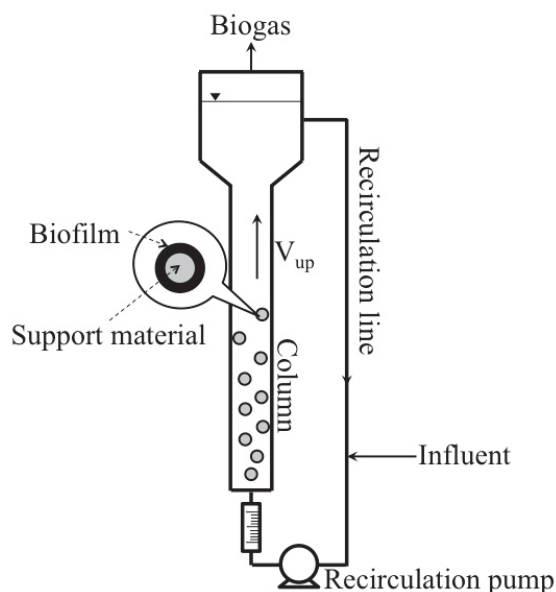


Fig. 10.1. A schematic figure of the AFBR.

The active biofilm is attached to the support material in the AFBR, which is fluidized by the upward liquid flow of recirculated reactor fluid. This is a similar mechanism found in other granular reactors, such as the expanded granular sludge bed (EGSB) reactor, but here, the support media has a higher density and thus requires a higher up-flow velocity (V_{up}) to achieve fluidization with bed expansion of 25–100% of the static reactor bed height (Khanal 2008). A generally applied up-flow velocity of 1.8–38 m·h⁻¹ is typical (Heijnen *et al.* 1989). This high velocity results in high shear stress on the support materials, resulting in a dense biomass that can yield a long solids retention time (SRT) as needed for efficient operation. Petrozzi *et al.* (1991) investigated the use of different types of fluidized material, and concluded that lighter support material tended to develop a thicker, but lighter, biofilm with greater tendency to wash out from the system or migrate through the recirculation pump. Hence, heavier and denser (1.5–2.7 g·cm⁻³) particles have been recommended for support material, such as sand, GAC, or silica. Moreover, the size of support material was indicated to be another important factor in reactor performance. Although some studies indicated that smaller particles required a shorter start-up period and gave better performance than larger particles (Heijnen *et al.* 1989; Petrozzi *et al.* 1991), there still exists controversy on the best particle sizes to use. Prakash (1995) recommended an optimum support material size to be 0.1–1 mm diameter.

Albeit the AFBR was not as widely used in the past for treating low-strength wastewaters as the UASB and EGSB reactors, the unique characteristics of the AFBR provide advantages for this purpose as summarized in Table 10.1. Switzenbaum (1983), Borja and Banks (1995) compared the performance between an AF and the

Table 10.1. Advantages of the AFBR for treating low-strength wastewaters.

Factor	Advantage	References
High up-flow velocity	high mass transfer of substrate to the biofilm (thin thickness of biofilm is another factor)	Switzenbaum (1983)
		Sanz and Fdz-Polanco (1989)
		Borja and Banks (1995)
		Buffière <i>et al.</i> (1995)
	no problems of channeling and clogging	Borja and Banks (1995) Rittman and McCarty (2001)
Heavy support material	maintaining biomass at short hydraulic retention time (HRT) even less than an hour	Shin <i>et al.</i> (2012)
Long cylindrical column shape having high biomass concentration	requiring small area of land (small footprint)	Heijnen <i>et al.</i> (1989)
Immobilized biomass	increasing process stability and toxic shock resistance	Sanz and Fdz-Polanco (1989) Bohlmann and Bohner (2001) Rittman and McCarty (2001)

AFBR for treating dilute wastewaters. Both concluded that the AFBR had better removal efficiency because of a lower diffusion limitation and better mass transport from liquid to biomass. In another comparison, Sanz and Fdz-Polanco (1989) concluded that the AFBR has a higher COD removal efficiency than the UASB when treating domestic wastewater at 15–25 °C, because the turbulence created around particles in the AFBR increases substrate transfer. Buffière *et al.* (1995) also claimed that the thin biofilm on the fluidized media yielded a higher activity and removal efficiency, which was directly related to good mass transfer. As another factor of importance, the high upward liquid velocity in the AFBR reduced the occurrence of channeling or clogging (Borja and Banks 1995; Rittmann and McCarty 2001).

Borja and Banks (1995) evaluated the relative effect of organic loading rate (OLR) between the AF and the AFBR. Here, the AF was unable to operate with a higher OLR than 20 kg-COD·m⁻³·d⁻¹ without causing a clogging problem, while the AFBR performed quite well without operational problems at 40 kg-COD·m⁻³·d⁻¹. The AFBR gave stable maintenance of biomass and support material even at very short HRTs. Indeed, Shin *et al.* (2012) applied the AFBR for the treatment of synthetic wastewater mainly composed of acetate and propionate at an HRT of only 17 min and achieved almost complete removal of these fatty acids. Such a short HRT is likely to pass out granular biomass in the USAB and other reactors that do not contain dense support media for organism attachment. Another advantage of the AFBR is its small and compact area requirement (small footprint) due to the high biomass concentration it can maintain at short HRTs, and the high column-shaped reactor that can be used (Heijnen *et al.* 1989). The AFBR also has stability, even with fluctuations in influent concentration (or OLR) and the presence of toxic compounds. Immobilized biomass in the AFBR was reported to be resistant to antibiotics 500 times higher than with suspended-type cultures (Costerton *et al.* 1985). Such stability from toxic materials was also found by Bohlmann and Bohner (2001) and by Sanz and Fdz-Polanco (1989).

2.2. General performance of the AFBR for low-strength wastewater

Various studies have been conducted to evaluate the treatment of low-strength wastewater with the AFBR, as summarized in Table 10.2. With the various support materials that have been used, results generally indicate that quite high COD removal efficiencies can be obtained. Chen *et al.* (1985) studied a range of influent wastewater concentrations of 500–9,000 mg·L⁻¹ at short HRT. Although the OLR was as high as 50 kg·m⁻³·d⁻¹, the COD removal efficiency was still higher than 90%. Sanz and Fdz-Polanco (1990) studied the treatment of raw domestic wastewater under psychrophilic conditions. At short HRT of 1.7–2.3 h and low temperature (10 °C), the COD removal efficiency still averaged higher than 70%. Low temperature had little adverse effect on performance. Additionally, stable COD removal efficiency was obtained even with great fluctuations in influent characteristics, which would be advantageous when treating domestic wastewater. Tseng and Lin (1994) reported a 72% COD removal at 21 °C when treating domestic wastewater with an AFBR. With operation at temperatures higher than 30 °C, COD removal efficiency was greater than 86%. Another performance of the AFBR

Table 10.2. Reported conditions and performance of the AFBR in treating low-strength wastewater.

Influent		Operating condition					COD	References
Type	COD conc. (mg·L ⁻¹)	Media	V _{up} (m·h ⁻¹)	HRT (h)	Temp. (°C)	OLR (kg·m ⁻³ ·d ⁻¹)	Removal (%)	
Glucose	500–9,000	carbon	-	0.5–8	35	50	90	Chen <i>et al.</i> (1985)
Raw domestic wastewater	150–590	arlita	6	1.7–2.3	10	8.9	>70	Sanz and Fdz-Polanco (1990)
Raw domestic wastewater	395	activated carbon	-	1.5–6	21	1.6–6.3	72–81	Tseng and Lin (1994)
Glucose beef extract	250–800	activated carbon	-	1.5–12	35	0.5–12.8	86–98	Tseng and Lin (1994)
Yeast extract + sucrose	560	activated carbon	24	18	30	0.7	88–96	de Oliveira <i>et al.</i> (2010)
Settled sewage	235–300	GAC	43.3	1	10–25	1.2–2.3	58–72	Yoo <i>et al.</i> (2014)
Acetate + Propionate	130–2,010	GAC	24 - 66	0.7–11.5	35	4.2–4.4	82–98	Shin <i>et al.</i> (2012)

treating sewage in mesophilic to psychrophilic condition (10–25 °C) was found in a staged process (AFBR+AFMBR) study by Yoo *et al.* (2014). The COD removal efficiency of the first stage AFBR alone was 58% to 72% at a quite short HRT of one hour. Although removal efficiency decreased with temperature, the BOD₅ removal efficiency was still 71–80%, indicating that the AFBR removed biodegradable compound stably even at 10 °C.

Even though several researchers have demonstrated that the AFBR can effectively treat low-strength wastewater at very short HRTs, there still has been a basic misunderstanding about the ability of methanogens themselves to achieve low effluent concentrations. To examine this factor, the performance of the AFBR was tested at short HRTs (Shin *et al.* 2012). The synthetic influent was a mixture of acetate and propionate. The acetate and propionate effluent concentrations were always lower than the detection limit of 0.4 mg·L⁻¹ with influent COD concentrations ranging 45–2,010 mg·L⁻¹, OLR ranging 4.2–18 kg-COD·m⁻³·d⁻¹, and HRT ranging 0.28–11.5 h. This value for acetate is well below most of the previously reported so-called threshold values resulting under non-fed organism starvation conditions (no data of propionate threshold value has been reported for comparison). This unexpected result appears to be related to the combined AFBR advantages and the particular bacterial species present. Microbial analyses indicated that *Methanosaetaceae* (previously known as *Methanothrix*) were in clear dominance among the acetate-using methanogens, certainly a requirement for very low acetate concentrations. The results provided clear evidence that methanogenesis is not a rate-limiting factor for dilute wastewater treatment.

2.3. Operational concerns with the AFBR: DO and bed stratification

A possible concern when treating dilute wastewater anaerobically is the effect of dissolved oxygen (DO), which may be present in influent wastewaters. Methanogenic activity is susceptible to oxygen, which can have an adverse effect on methane production, and could cause the accumulation of volatile fatty acids (VFAs) (Whitman *et al.* 1992). In contrast, others have demonstrated a tolerance of methanogenic activity towards the presence of oxygen. Kato *et al.* (1994; 1997) indicated that an influent DO of 3.8 mg·L⁻¹ had no detrimental effect on either UASB or EGSB treatment of synthetic wastewater containing 200–400 mg·L⁻¹ COD. Shen and Guiot (1996) varied the O₂ loading rate of 0.03–0.4 kg-O₂·m⁻³·d⁻¹ and DO concentration of 0.5–8.1 mg·L⁻¹ by supplying DO in the recirculation line, while maintaining an OLR of about 3.5 kg-COD·m⁻³·d⁻¹. They found that COD removal efficiency was not affected by DO level; however, the CH₄ yield gradually decreased as influent DO increased. This indicated that with higher DO, a larger portion of the influent organic substrate was consumed by aerobic microorganisms.

However, the above studies did not demonstrate the effect DO may have with very diluted wastewaters. To investigate this, Shin *et al.* (2011) treated a low-strength synthetic wastewater (47–266 mg·L⁻¹ of COD) with and without DO (<1–6 mg·L⁻¹). No significant adverse effect on methanogenic activity was found with 130 mg·L⁻¹ of influent COD and 6.7 mg·L⁻¹ of DO. However, with only 52 mg·L⁻¹ of influent COD, a rapid

growth of oxygen-consuming zoogloal-like organisms resulted. After operating efficiently for a while, effluent COD concentration suddenly soared to $260 \text{ mg}\cdot\text{L}^{-1}$ due to a washout of a large growth of organisms that resulted from aerobic organic oxidation. Also, the methanogenic activity was adversely affected, resulting in a long recovery period to regain the lost methanogenic activity. Concluded was that the DO/COD ratio was a crucial factor for maintaining adequate anaerobic performance. As long as the influent DO/COD ratio did not exceed 0.05, there should be no significant adverse effect on performance. However, if the ratio were higher than 0.12, the system could experience problems.

Another concern for AFBR operation is bed stratification, which can occur if there is uneven biofilm formation on support materials throughout the reactor. This problem is especially severe when there is a large amount of biomass covering the fluidized media. This reduces the overall density of the media, thereby increasing its buoyancy and tendency to be washed out from the system. Although this problem could be serious, research has suggested an easy solution. Applying a high shear stress (high liquid velocity) can make the biofilm thinner and more homogeneous (Zhang and Bishop 1994; Schreyer and Coughlin 1999). Further, Andrews and Tien (1979) indicated that by using a uniform size of support media, the potential for this problem was reduced.

3. Anaerobic Fluidized Bed Membrane Bioreactors

3.1. *Characteristics of the AFMBR*

The AFMBR combines an AFBR with submerged membrane filtration (Kim *et al.* 2011). The AFMBR includes biochemically active organisms, fluidized particles serving as the support media for active microorganisms attachment, and membranes that permit treated effluent (permeate) to pass, but not the organisms or other suspended material. The AFMBR is advantageous for anaerobic treatment particularly of dilute wastewater, such as domestic wastewater, because the membrane effectively prevents washout of the slow-growing methane-forming anaerobic microorganisms, thus providing a long SRT as needed to maintain a large population level, while allowing operation at short HRTs as required to reduce reactor size and cost. Since the membranes submerged in the AFMBR are operated under suction pressure, low-pressure driven membranes, such as microfiltration or ultrafiltration membranes that can reject particulate and colloidal materials, are generally used.

The most important need in the AFMBR is to effectively control membrane fouling, a long-standing problem with membrane operation. Membrane fouling results from the deposition of foulant materials within membrane pores and/or on membrane surfaces, resulting in pore blockage and cake formation, respectively. Membrane fouling reduces membrane permeability, increasing the resistance to water flow, which sometime requires frequent chemical cleaning, thereby shortening membrane life-time and creating operational nuisance. Currently, the common approach used to control fouling in anaerobic MBR technology is gas sparging, or the introduction of biogas below membranes to create a high upward shear rate or cross-flow velocity across the

membrane surfaces (Beaubien *et al.* 1996; Choo and Lee 1998; Elmaleh and Abdelmouni 1997; Hu and Stuckey 2007). Although this method is effective, it requires much energy (Martin *et al.* 2011). As an alternate approach to biogas-sparging to reduce membrane fouling, granular materials such as GAC that are somewhat heavier than water, can be added to the membrane bioreactor, and fluidized by recycling bulk reactor fluid from the bottom of the reactor, causing a high upward water velocity. While being fluidized, the moving materials come into physical contact with the membranes, during which both physical movement of particles and biodegradation help to reduce membrane fouling at relatively little energy cost. The beneficial effect of media fluidization on fouling reduction has been confirmed to be the physical contact of media with membrane surfaces, as the recirculation flow rate itself is much too low to prevent fouling (Kim *et al.* 2011). When adsorbent materials are used as the fluidized media, some organic fouling reduction by adsorption can be expected, further increasing treatment stability and efficiency (Aslam *et al.* 2014; Akram and Stuckey 2008).

3.2. Types of membranes

In anaerobic MBR technology, both flat-sheet and hollow fiber membranes, having different hydrophobic natures and nominal pore size ranging 0.1–0.01 μm (Akram and Stuckey 2008; Liao *et al.* 2006), can be applied. With hollow-fiber membranes, compact microfiltration or ultrafiltration modules can be fabricated with high packing density of 1,000–10,000 $\text{m}^2\cdot\text{m}^{-3}$. Plate-and-frame units made up of stacked flat-sheet membranes can ensure mechanical support for the membranes, and at the same time, can provide for the removal of membrane permeate. Because membrane permeate is here collected from individual support plates, this also makes determining the location of faulty membranes a simple matter (Mulder 1998). Tubular membranes can be used in anaerobic MBRs, but these pressurized-encased membranes would not be suitable for use in an AFMBR. Hydrophobic membranes consisting of polyethersulfone, polyethylene, polyvinyl chloride, and polyvinylidene difluoride are commonly used in anaerobic MBRs. They are more suitable than hydrophilic membranes for an AFMBR, mainly due to their better mechanical strength and resistance against chemicals (Kim *et al.* 2011; Aslam *et al.* 2014; Liao *et al.* 2006; Aquino *et al.* 2006; Jeison *et al.* 2008). Also, hydrophobic membranes are generally more prone to organic fouling, and thus the membranes are sometimes modified with hydrophilic additives (Sainbayar *et al.* 2001). Ceramic membranes consisting of inorganic materials (i.e., TiO_2 , Al_2O_3), on the other hand, could be of interest for trial applications in an AFMBR. These membranes are not yet widely available on a commercial scale for anaerobic MBRs, but have excellent chemical and thermal resistances when compared with polymeric membranes (Ng 2014).

Pore size is another important feature in membranes. Larger membrane pores should have lower membrane resistance, requiring lower suction pressure to achieve a desired permeate flux than membranes having smaller denser pores. However, they can lead to higher chances of pore blocking. Pore blocking reduces pore area for water passage, causing progressive cake layer formation on the membrane surface. Since media fluidization serves as an external functionality to control membrane fouling, it may be

best to use membrane materials that have a lower pore fouling potential. There is much yet to be learned about the best membranes for AFMBR use.

3.3. Fouling control mechanism

As media needs to be fluidized sufficiently in the AFMBR to cover the whole membrane surface to adequately reduce fouling, recirculation flow rate must be sufficient to achieve this, while not allowing overflow of media from the reactor. Fluidized media rubs against the membrane fouling layer, displacing it from the membrane. The movement of the media can also generate secondary hydraulic flows that promote shear conditions on membrane surfaces, also achieving some fouling reduction. Such scouring or mechanical cleaning with granular materials has been reported (Huang *et al.* 2008; Siembida *et al.* 2010). For example, in a lab-scale AFMBR study, when fluidization was stopped, suction pressure increased from 0.04 to 0.24 bar within 4 h of operation (Kim *et al.* 2011). When GAC fluidization was restarted, the suction pressure dropped rapidly so that within 2 h, the trans-membrane pressure (TMP) returned to a level similar to that with distilled water (Fig. 10.2). Thus, GAC fluidization is an effective scouring tool for fouling reduction in an AFMBR, effectively controlling the fouling by colloids and suspended particles. With recirculation alone and no GAC, fouling was reduced somewhat but nowhere near that achieved when GAC was present (Kim *et al.* 2011). Higher GAC concentrations

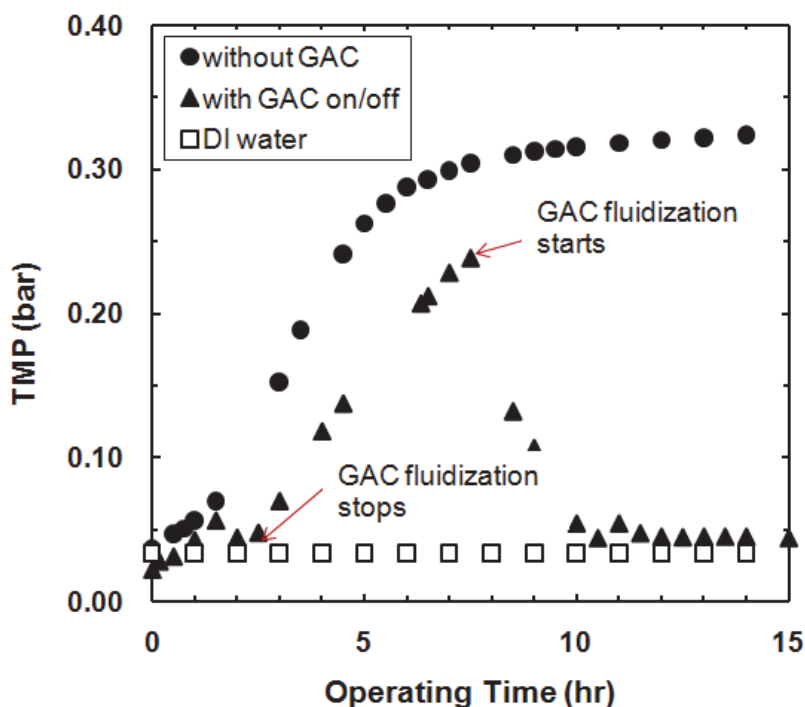


Fig. 10.2. Effect of GAC fluidization on TMP in the AFMBR at permeate flux of $10 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ (LMH) and recirculation rate of $1.4 \text{ L}\cdot\text{min}^{-1}$ (Kim *et al.* 2011).

resulted in marginally lower TMP change, but higher concentrations resulted in higher material costs.

During long-term operation with a lab-scale AFMBR treating synthetic wastewater (Kim *et al.* 2011), TMP was found to increase rapidly to 0.32 bar within 0.5 day when GAC fluidization was stopped. However, with continuous GAC fluidization using a set-point flux of 10 LMH, the TMP stabilized at about 0.1 bar for about 100 days without the need for chemical cleaning and/or backwashing.

3.4. *Effect of media characteristics on fouling control and energy consumption*

The extent of fouling in the AFMBR can be affected strongly by the physicochemical properties of the fluidized media. GAC particles can reduce membrane fouling both by adsorption of foulants and by their scouring action along membrane surfaces. Sorption was found to be the dominant process with fresh GAC, and here, smaller particles having a higher adsorption capacity than larger particles can initially play an important role in fouling control. However, after sorption capacity has been exhausted through longer-term operation, membrane scouring dominates the fouling control. In that case, the larger GAC particles are more favorable for reducing membrane fouling (Aslam *et al.* 2014). Thus, the relative benefit of smaller versus larger GAC particles (adsorption vs. scouring) depends upon the length of time of GAC usage.

The energy requirement for controlling membrane fouling is a crucial aspect of AFMBR operation. The overall energy requirement per unit volume of membrane permeate for the AFMBR is determined dominantly by the product of the recirculation flow rate and the pressure head against which this flow rate is pumped, all divided by the permeate flow rate. In turn, the three variables are interdependent. Membrane fouling is a function of the amount and extent of media fluidization by the recirculating fluid, as is the recirculation head loss. The energy requirement for fluidization in the AFMBR is closely related to the mass of media added (or packing ratio) and the recirculation flow rate. As packing ratio increases, the media mass requiring fluidization increases, which in turn, increases the hydraulic pressure head loss through the reactor.

Mechanical scouring with the same type of media tends to be more effective with larger media than with smaller media, but the energy cost is higher. The additional energy input with larger particles enhances the shear rate and scrubbing action along the membrane surface (Huang *et al.* 2010). In comparative studies, non-adsorbing fluidized media, such as sand particles and polyethylene terephthalate (PET) beads, were found to have similar behavior to pre-adsorbed GAC particles; that is, a lower fouling rate is achieved by larger media size (Aslam *et al.* 2014). However, membrane fouling was relatively lower with larger PET beads than with heavier but smaller silica particles. An effectiveness of fluidized media type on fouling control has been well demonstrated, but better understanding of this overall is still needed.

Another important factor for AFMBR design is the packing density of the membrane module. The denser the membrane packing with a given membrane flux, the higher will be the reactor flow rate and the lower will be both the HRT and reactor cost. However,

membrane fouling is controlled dominantly by the mechanical cleaning action of the fluidized media along membrane surfaces. If the packing density is too high, good contact with the fluidized media may not be obtained, and net reactor flux would decrease and fouling rate would increase. Here, it is important to optimize the design to obtain the highest packing density that is still compatible with good fouling control. Also important is design of the recycled water distribution system so that good contact of the media with the whole effective membrane surface area is assured. Another important factor in AFMBR design is the packing ratio of the fluidized media (Kim *et al.* 2011; Aslam *et al.* 2014), which is the relative depth of the unfluidized media in the reactor. In general, the higher the packing ratio of GAC up to 50% of the reactor height, the lower was the fouling rate. A similar finding was found when silica particles were used (Aslam *et al.* 2014). However, a packing ratio higher than 50% reduced the media scouring functionality, thus reducing membrane fouling control (Kim *et al.* 2011).

Figure 10.3 shows that a lower packing ratio was beneficial with respect to energy requirements, but in turn, was associated with an increase in the fouling rate. For non-adsorbing silica medium and with a given energy requirement to achieve 100% fluidization, the fouling was significantly lower with smaller media that had the higher packing ratio. With the same packing ratio, the larger media reduced fouling rate better than did the smaller media. However, the larger media required much more energy for 100% fluidization due to the higher recirculation flow rate required. For example, with a 50% packing ratio for GAC particles, the energy requirement for fluidization increased exponentially with particle size (Aslam *et al.* 2014). The interdependent relationships between particle size, particle density, packing ratio, recirculation head loss, recirculation

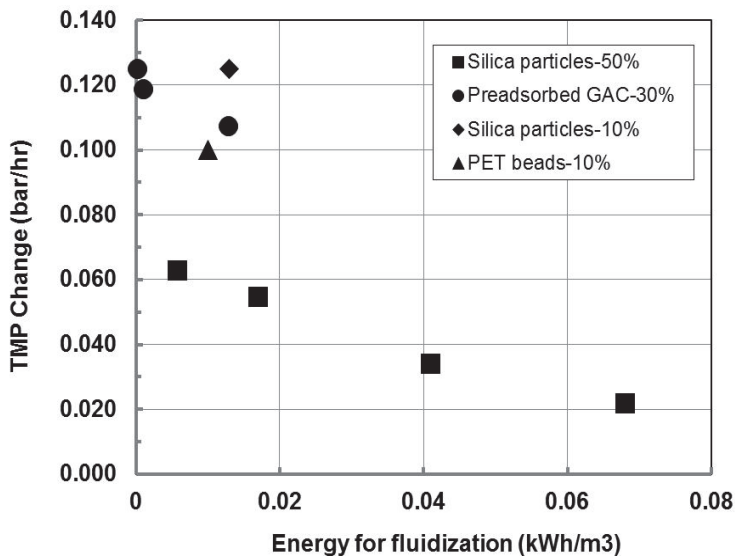


Fig. 10.3. Relationship between TMP change and energy for fluidization for different non-adsorbing fluidized media and packing ratio at set-point flux of 50 LMH. Specific gravity/size for GAC, silica, and PET beads are 2.3/1–2mm, 2.7/1–2mm and 1.3/3mm, respectively (Aslam *et al.* 2014).

rate, and permeate flux rate are complicated, but need to be well understood for effective AFMBR design.

Better understanding of other aspects of fluidized media is also needed. In polydisperse suspensions, selective deposition of foulants may occur because each foulant has its own response to media fluidization. In addition, larger particles may enhance the transport of smaller particles by scouring and sweeping them away from the membrane by shear-induced conditions (Chellam and Wiesner 1997). Also observed was that GAC fluidization in the AFMBR disaggregated microbial flocs, producing smaller compressible materials in the AFMBR, in the range of 0.1–1.2 μm , than were originally present in the feed suspension (Lee *et al.* 2015). Similar results were found in another type of submerged aerobic MBR system with polypropylene materials added as suspended carriers (Huang *et al.* 2010). A significant portion of the floc-size particles entering the AFMBR reactor appear to become disaggregated by fluidization, and perhaps biodegradation, to produce the smaller cellular and colloidal debris that are generally characterized as more effective foulants. The result of the interactions between foulant materials and types of media on membrane fouling needs to be much better established.

4. The Staged Anaerobic Fluidized Membrane Bioreactor (SAF-MBR) System

The staged anaerobic fluidized bed membrane bioreactor (SAF-MBR) consists of an AFBR followed by an AFMBR (Kim *et al.* 2011). The AFBR, used before the AFMBR, is capable of efficient organics removal at short HRT and high OLR (Shin *et al.* 2012). The AFBR effluent organics are further treated in the AFMBR, and additional methane is produced. One of the major advantages of using the AFMBR after the AFBR is its ability to retain the VSS longer, effecting their further degradation, as already discussed.

A lab-scale SAF-MBR produced highly efficient COD and BOD removals when treating domestic wastewater primary effluent under temperatures from 10 to 25 °C (Yoo *et al.* 2014). Obtained were COD and BOD₅ removals higher than 90% and 94%, respectively, even at 10 °C, and with total HRT of 2.3 h. Biosolids production was 0.04 g-VSS·g-COD⁻¹, far less than that with typical aerobic treatment. The system was operated for 310 days at a fixed membrane flux of 9 LMH without the need for back flushing or chemical cleaning of the membranes. A pilot-scale SAF-MBR has also been operated for comprehensive evaluation of system performance when treating settled domestic wastewater, reflecting the diurnal and seasonal changes occurring in influent characteristics and temperature. The following section focuses on the performance of this pilot system over a period of 600 days.

4.1. SAF-MBR Pilot Plant

4.1.1. SAF-MBR configuration

A pilot-scale SAF-MBR system was installed at the Bucheon wastewater treatment plant (WWTP) (Fig. 10.4), Republic of Korea, as described in detail by Shin *et al.* (2014). The 3.0-m tall stainless steel AFBR had a reactor volume of 0.99 m³. A 0.73-m³ settling tank

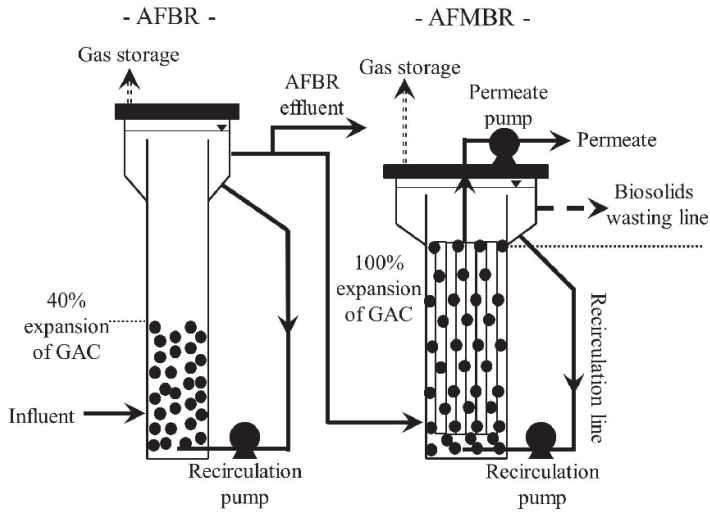


Fig. 10.4. Configuration of the SAF-MBR (Shin *et al.* 2014).

with a submerged weir was installed at the top of the reactor column to prevent GAC overflow into the recycle line. The AFBR contained 139 kg of GAC (Calgon F300 USA) with effective size of 0.8–1 mm, occupying 25% of the reactor column volume without fluidization. The GAC was fluidized to about 40% of the reactor height by recycling reactor effluent at a $0.15 \text{ m}^3 \cdot \text{min}^{-1}$ flow rate (upflow velocity of $27 \text{ m} \cdot \text{h}^{-1}$).

The rectangular-shaped AFMBR was 0.9 m by 0.4 m in area, and had a working volume of 0.77 m^3 . A settler with a total liquid volume of 1.4 m^3 was connected on the top of the AFMBR to prevent GAC loss from the reactor. Five membrane modules, each holding 1.85-m long hollow fiber polyvinylidene fluoride membranes with pore size of $0.03 \text{ } \mu\text{m}$, and total membrane surface area of 39.5 m^2 (provided by Cheil Industries, Republic of Korea), were installed in the AFMBR. Effluent was vacuum withdrawn from the AFMBR through the membranes. The AFMBR contained 264 kg of GAC, occupying 60% of the reactor volume when settled. The recycle flow rate of $0.53 \text{ m}^3 \cdot \text{min}^{-1}$ ($75 \text{ m} \cdot \text{h}^{-1}$ upflow velocity) expanded the GAC to 100% of the reactor volume so that all of the membrane surfaces came into contact with the fluidized GAC particles in order to prevent membrane fouling.

4.1.2. Operating conditions

The AFBR was seeded with 100 L of digested sludge containing $47 \text{ g} \cdot \text{L}^{-1}$ total suspended solids and obtained from a Bucheon WWTP anaerobic digester. After 85 days of operation at HRT of 4.8–11.1 h, the AFBR HRT was reduced to 2.0 h, with corresponding flow rate of $12 \text{ m}^3 \cdot \text{d}^{-1}$. The operation of the AFMBR began 95 days after operation of the AFBR began, but only with microorganisms carried over from the AFBR, and this is termed day zero of system operation. Biosolids were wasted daily from the AFMBR recycle line, which contained no GAC, so little of the active microorganisms attached to the GAC were lost by this procedure. Thus, the retention time of wasted

biosolids and that of the active organisms could be controlled independently. The bulk biosolids wasting ratio, defined as the wasting flow rate divided by influent flow rate to the AFMBR, was maintained at 0% until day 100, 5% for days 101 to 340, and then was reduced to 1% after day 350 for the remainder of the study.

In order to prevent an excessive TMP increase from occurring after 107 days of AFMBR operation, a membrane relaxation approach was used. Here, the permeation pump was turned off for 5 min in every 30 min, which effectively changed the net membrane flux from 7.5 to 6.2 LMH and HRT from 2.6 to 3.1 h. The net flux was further reduced to 4.1 LMH in the second winter when the relaxation period was changed to 10 min off during each 30 min period, yielding an HRT of 4.8 h. Total HRT of the system was thus varied over the course of evaluation from 4.6 to 6.8 h.

The pilot-scale SAF-MBR system was fed with 2 mm screened primary effluent with COD and BOD₅ of 300 ± 60 and 160 ± 45 mg·L⁻¹, respectively. The SAF-MBR system was operated without temperature control, yielding wastewater temperature ranges of 15–25 °C during spring and fall, 25–30 °C in the summer, and 8–15 °C in the winter.

Samples, taken 2–3 times per week, were obtained in two different ways. From days 1 to 339, grab samples were taken around 9 a.m. From day 340, composite samples were prepared by mixing samples taken every 0.5 h to provide more representative wastewater characteristics for the system. However, differences between the 9 a.m. grab samples and the composite samples were less than 10% to 20%.

4.2. Performance of the pilot-scale SAF-MBR

4.2.1. Organic removal and biosolids production

Removals of COD and BOD₅ together with their effluent values during each seasonal period are illustrated in Fig. 10.5. The average COD removal during the first fall and winter was higher than 80%, even though the system had not yet been well acclimated due to slow microbial growth at low wastewater temperatures of 8–20 °C. Such high COD removal is likely partly explained by organic sorption onto the fresh GAC. Average COD removal increased to 89% during the first spring, and then increased even further to 94% in summer with temperature reaching a high of 30 °C. As the system became well acclimated by the summer, average COD removal efficiency in the second winter was still a good 90%. The trend in BOD₅ removal was quite similar to that of COD, with BOD₅ removal in the second winter being higher than 95%. With such high organic removals, the average permeate COD remained below 30 mg·L⁻¹ even during the second winter, a value that is comparable to that obtained with conventional aerobic processes. Corresponding average permeate BOD₅ was below 10 mg·L⁻¹, also during the coldest winter months. Average VFA concentrations in the AFMBR permeate were below the 0.4 mg·L⁻¹ detection limit in the second fall and winter periods when temperature was below 15 °C. Such low VFA concentrations confirm that methanogenesis is not the limiting step at low temperatures with domestic wastewater.

Sulfate reduction, a strong competitor for methane production, was less complete during the winter. Sulfate removal in the AFBR decreased from 94% during the summer

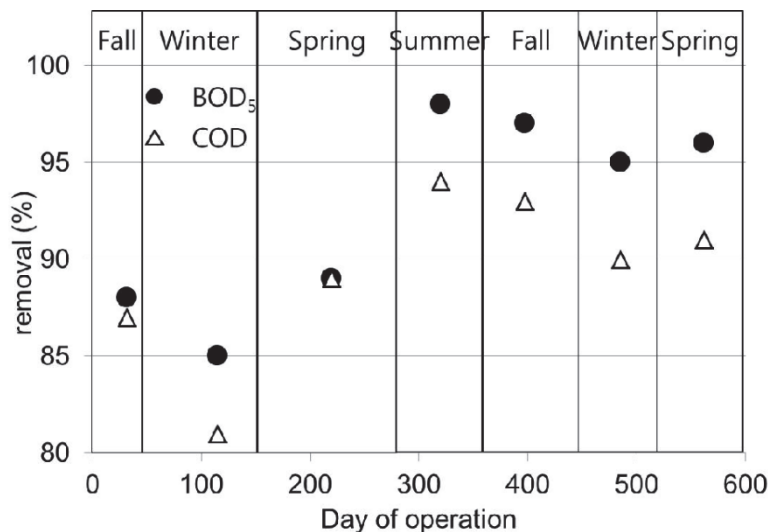


Fig. 10.5. Removals of COD and BOD₅ during the SAF-MBR pilot study.

to a low of 48% during the coldest winter period. Sulfate reduction by the overall SAF-MBR system decreased from 100% during summer to a low of 62% when the temperature was 9–11 °C. Temperature thus had a large impact on sulfate removal, allowing for higher conversions of organics to methane during the colder winter months. COD removal associated with sulfate reduction was less than 13%.

With a bulk wasting flow rate equal to 1% of the AFMBR influent flow rate as used after the summer period, the bulk VSS concentration remained 600–1,200 mg·L⁻¹, which is significantly lower than that generally found with gas-sparged anaerobic MBRs treating domestic wastewater. This results because VSS retention time can be maintained significantly lower than microorganism retention time in the AFMBR, one of its important advantages. The wasted-biosolids COD represented about 10% of the influent COD, and was calculated to represent a VSS SRT of about 36 d, which was sufficient for VSS destruction of 90%. Biosolids production was only 0.05 kg-VSS·kg-COD⁻¹. Additionally, the removed biosolids were already digested, meaning no further biological treatment would be required for their disposal, and their mass represented less than one quarter of that normally obtained from aerobic treatment.

Effluent concentrations of total phosphorous and nitrogen did not change significantly during treatment, averaging 3.7 ± 0.5 and 34.0 ± 3.6 mg·L⁻¹ throughout the study period, respectively.

4.2.2. Biogas production and distribution

Reactor gas composition was quite different from that normally obtained with high-strength wastewater or conventional anaerobic sludge digestion. Figure 10.6 illustrates computed variations in the composition of produced gas as a function of influent BOD_L (ultimate BOD), assuming the produced gas is 65% methane and 35% carbon dioxide, and equilibrium between the gas and liquid phases at 20 °C. With low BOD_L, gaseous N₂

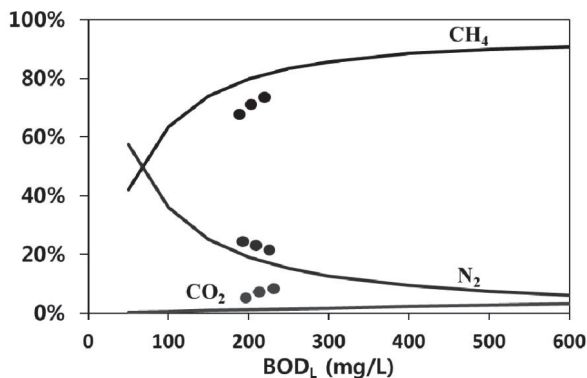


Fig. 10.6. Variations in gas composition as a function of influent BOD_L.

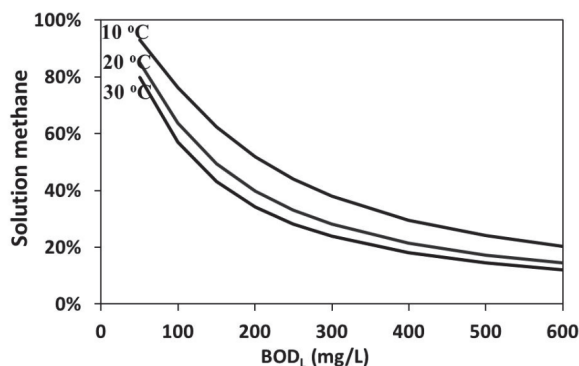


Fig. 10.7. Estimated effects of BOD_L and temperature on the fraction of total methane production that will be dissolved in the effluent.

composition is high due to stripping of dissolved N₂ normally present in the influent wastewater. In contrast, CO₂ in the gas phase is much lower than 35%, due to its high solubility and low relative production rate. Measured gas compositions of N₂, CO₂, and CH₄ of the pilot-scale plant at 20 °C, represented as circles, are in general agreement with these estimates, except for the usual observation that methane tends to be supersaturated in anaerobic effluents, a fact not considered in the calculations.

The resulting low CO₂ in the gas phase is beneficial in terms of pH control. For conventional anaerobic treatment with high COD, bicarbonate alkalinity higher than 2,000 mg as CaCO₃·L⁻¹ is generally necessary for adequate pH control (McCarty 1964b). However, for dilute wastewater, the low CO₂ reduces the alkalinity needed to maintain adequate pH. During the whole period of SAF-MBR operation, no alkalinity addition was required to control pH. The pH and alkalinity within both the AFBR and AFMBR remained in the narrow ranges of 6.6–6.8 and 220–260 mg·L⁻¹ as CaCO₃, respectively.

Figure 10.7 illustrates the computed effects of BOD_L (biodegradable COD) and temperature on the amount of dissolved methane in the effluent, assuming water/gas

equilibrium and no sulfate reduction. Assuming BOD_L to be $200 \text{ mg}\cdot\text{L}^{-1}$, the percentage of total CH_4 that would be dissolved is estimated to be 50% at 10°C . Actual measured dissolved methane during the second fall was 28% of the total methane produced when temperature was about 20°C and BOD_L without sulfate reduction was $175 \text{ mg}\cdot\text{L}^{-1}$, and during the second winter was 60% with temperature ranging $9\text{--}15^\circ\text{C}$ and BOD_L without sulfate reduction was $187 \text{ mg}\cdot\text{L}^{-1}$. This confirms that the fraction of dissolved methane is much higher in winter than in summer. This dissolved methane must be recovered, not only to gain its energy content, but also because it is such a strong greenhouse gas.

4.2.3. Membrane fouling control

Figure 10.8 illustrates the variations in TMP and flux in the AFMBR throughout the experimental period, during which time no chemical cleaning of membranes took place. The AFMBR flux was reduced from 7.5 to 6.1 LMH during the year as otherwise without cleaning the TMP would increase excessively. Additionally, a relaxation period began to be used, which decreased the net flux even more, from 7.5 to 4.1 LMH. The TMP could be maintained below 0.1 bar without relaxation during the first fall, but then increased to 0.27 bar partly because of increased water viscosity with the temperature drop in winter, as well as a slow increase in membrane fouling. To prevent further TMP increase after day 107, a 5-min period of membrane relaxation with the permeation pump turned off was used once in every 30 min. This resulted in a reduction in the net flux from 7.5 to 6.2 LMH, which kept the TMP below 0.24 bar during the remainder of winter. The net flux had to be decreased further from 6.2 to 5.6 LMH at 307 d in order to maintain the TMP level under 0.3 bar. However, the TMP increased further to 0.5 bar at 455 d as temperature decreased in the second winter, but stayed at that level until the end of the second spring. With the increase in temperature during the second summer, TMP decreased to 0.35 bar (data not shown). Although relaxation did help to control the TMP increase, it came at the expense of a reduced flux. Thus, although the AFMBR was actually operated for longer than 600 d without shutting down for chemical cleaning or

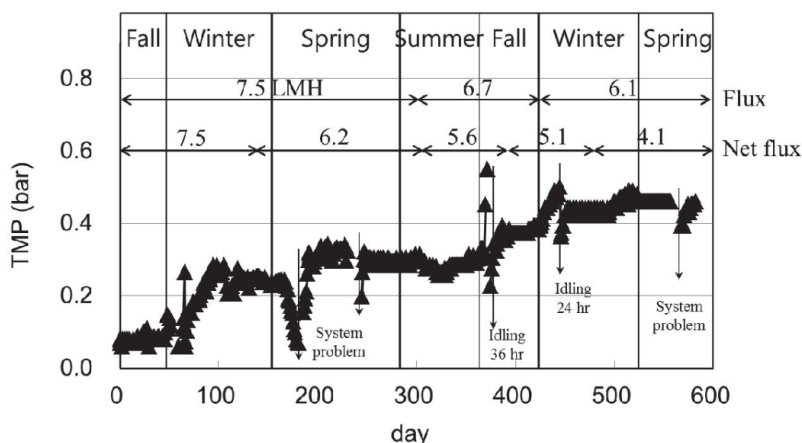


Fig. 10.8. Variations with time in TMP and flux in the AFMBR (Shin *et al.* 2014).

backwashing of the membranes, it is apparent that periodic membrane chemical cleaning will be required if a higher flux is to be used and maintained.

It is expected that the low VSS concentration that can be maintained in the bulk liquid of the AFMBR compared with the much higher concentrations in the 6,000–14,000 mg·L⁻¹ range (Huang *et al.* 2013) normally found in gas-sparged anaerobic membrane bioreactors helps to reduce membrane fouling. The ability to independently control the bulk VSS concentration from active microorganism concentration allows the AFMBR to be operated at very short HRTs, such as the total of 2.3 h found suitable for efficient treatment in laboratory SAF-MBR studies (Yoo *et al.* 2012 and 2014). If HRT is reduced in gas-sparged systems, the bulk VSS concentration must increase in direct proportion in order to maintain a long organism SRT. In the SAF-MBR system, the active microorganisms are maintained separate and are concentrated on the GAC particles. Their concentration measured as GAC VSS at day 380 was 0.036 g-VSS·g-GAC⁻¹. This yields an averaged reactor microbial VSS concentration of 11,000 mg·L⁻¹, or an order of magnitude higher than the VSS in the bulk liquid that was subject to daily wasting.

4.2.4. Micropollutants removal

A question of interest in wastewater reuse is the potential for removal of micropollutants by the SAF-MBR system. Research to date has indicated that the system is excellent for removal of many micropollutants, and indeed in many cases, its removal capability exceeds that by conventional aerobic activated sludge systems. For example, Dutta *et al.* (2014) reported that a lab-scale SAF-MBR system effectively removed the 20 pharmaceuticals they detected in primary settled domestic wastewater with medium removal of 96% and a range between 86–100% except for diclofenac (78%). In a comparable study by Yang *et al.* (2011) using aerobic activated sludge treatment followed by membrane filtration for the treatment of primary treated domestic wastewater effluent, the median removal of 16 pharmaceuticals and personal care products was much less than with the anaerobic system or 79%, with a broad range of 0–100%. Many of the compounds found in the two studies were different, so the overall studies cannot be compared directly. However, eight of the compounds found in the two studies were the same. For these, the median removal by the aerobic membrane bioreactor was only 57% with a range of 9–100%, while for the SAF-MBR system, it was 91% with a range of 78–100%, a large significant difference. Interestingly, the activated sludge treatment coupled with microfiltration was then followed by separate GAC treatment. With the additional GAC process, the median removal then became similar to that of the SAF-MBR system with a median removal of 94% and range of 71–100%. Based upon this comparison, the SAF-MBR system was better than the combined aerobic-settling-membrane system for microcontaminant removal, but about equivalent to that of the aerobic membrane system followed by GAC treatment.

A more recent direct comparative microcontaminant removal study between SAF-MBR and aerobic activated sludge treatment was conducted with the pilot SAF-MBR system at the Bucheon WWTP in South Korea (McCurry *et al.* 2014). The full-scale aerobic treatment system there included primary clarification and 4-stage biological

nutrient removal (anoxic, anaerobic, anoxic and aerobic sections), with a total HRT of 9.9–11.4 h. Here, both disinfection byproduct production potential and micropollutant removal were evaluated. For the 11 representative micropollutants evaluated, removals by the SAF-MBR pilot system were better for ten of them than with full-scale activated sludge treatment. Better removals were obtained for two antivirals (acyclovir, lamivudine), three beta-blockers (atenolol, metoprolol, propranolol), a beta-blocker aerobic biotransformation product (metoprolol acid), an anti-convulsant (carbamazepine), two antibiotics (sulfamethoxazole, trimethoprim), and *N*-nitrosomorpholine. Also, the SAF-MBR was better for removal of the precursors of *N*-nitrosodimethylamine. Additionally, concentrations of trihalomethane precursors were lower in SAF-MBR effluent in two of the three samples.

These results confirm that the SAF-MBR system is in general much better at micropollutant removal than that obtained by conventional aerobic activated sludge treatment, and comparable to that obtained by an activated sludge treatment coupled with microfiltration followed by activated carbon adsorption. In the SAF-MBR system, GAC is a component of the system, and used primarily as a platform for the anaerobic microorganisms and to reduce membrane fouling. In both the laboratory and pilot SAF-MBR studies, the systems were operated for over a year without GAC replacement before the micropollutant evaluations were conducted, so the GAC adsorption capacity might be expected to have been exhausted. Although biological regeneration of GAC might be a possible mechanism, the actual removal mechanisms are currently unknown. This is thus an area needing further attention.

4.3. Energy balance and footprint

An energy balance for the pilot-scale SAF-MBR system is summarized in Table 10.3. The energy requirement was calculated from measured hydraulic head losses through the reactors (mainly for GAC fluidization) and recycle lines, and the flow rates associated with them (Kim *et al.* 2011). The total estimated energy requirement for the pilot system operation was 0.23 kWh·m⁻³, most of which was for the AFMBR.

The energy requirement to achieve a high up-flow water velocity in the AFBR was only 0.016 kWh·m⁻³, which agrees with previous laboratory reports (Yoo *et al.* 2012). For the AFMBR, the energy requirements in the main reactor and bulk liquid recirculation line were 0.103 and 0.108 kWh·m⁻³, respectively. This relatively higher energy consumption was mainly associated with the higher up-flow velocity required for fluidizing a greater mass of GAC to 100% of the membrane height. This required an up-flow velocity of 75 m·h⁻¹, yielding a recycle to influent flow rate ratio of 127 or three times that for the AFBR.

Energy requirements for the AFMBR can be reduced with better design. For example, the energy consumption for the recirculation line could be reduced from 0.108 to 0.011 kWh·m⁻³ by doubling the diameter of the recycle line, as head loss is proportional to the square of water velocity, but velocity is inversely proportional to the square of the pipe diameter. This change in diameter would reduce the total energy requirement significantly. By doubling the AFMBR membrane density, the energy requirement could be reduced

Table 10.3. Energy balance for the pilot-scale SAF-MBR system ($\text{kWh}\cdot\text{m}^{-3}$) (Shin *et al.* 2014).

	Energy consumption			CH ₄ Energy**
	Main reactor*	recycle line	Total	Potential
AFBR	0.016	-	0.016	
AFMBR	0.103	0.108	0.211	
Total	0.119	0.108	0.227	0.139

*Head loss for GAC fluidization, friction loss in reactor, and membrane losses

**Electrical energy from methane combustion, assuming 35% conversion efficiency

almost by half, as would be the HRT. Occasional chemical cleaning of membranes would also reduce energy requirements and/or allow operation at higher flux.

Energy can be obtained by converting the methane produced to electricity through cogeneration. The theoretical energy available from the methane produced ($121 \text{ mg}\cdot\text{L}^{-1}$ as COD equivalent) is $0.42 \text{ kWh}\cdot\text{m}^{-3}$ (1 mole of CH₄ or 64 g-COD equivalent = 0.222 kWh). Considering an energy transfer efficiency of 33% in the conversion of methane to electricity, the potential electrical energy production from the pilot SAF-MBR would be $0.139 \text{ kWh}\cdot\text{m}^{-3}$. This is sufficient to satisfy an energy requirement of $0.13 \text{ kWh}\cdot\text{m}^{-3}$ obtained through a modification of the AFMBR recycle line. Although there would be an additional energy requirement for the recovery of dissolved methane, further improved design of the SAF-MBR system could yield a net energy-positive SAF-MBR system, even with a significant loss of methane from sulfate reduction. Significantly more energy could also be obtained using the methane produced from primary sludge digestion. This, together with the recommended modifications, achieves a net positive energy for the treatment of domestic wastewater.

Another advantage of the SAF-MBR is a small footprint. The AFBR could be a tall reactor, perhaps as much as 10 m tall, thus saving on footprint. As the AFMBR does not require a sedimentation basin or multi-media filtration, an additional saving in footprint is possible. A further improvement in plant footprint can be achieved by increasing the operating flux of the AFMBR with possible adoption of regular chemical cleaning or reducing the HRT of the AFMBR by increasing membrane density, as already discussed. A great additional savings in footprint also results from the low biosolids production of the system, biosolids that are already treated by anaerobic digestion in the SAF-MBR system. Thus, area requirement for the SAF-MBR system can be much less than with a conventional aerobic treatment system.

4.4. Extended applications of the SAF-MBR system

Extended applications of the SAF-MBR system need to be explored. One possible benefit might come from the elimination of the first AFBR, in effect combining both reactors into a single reactor. Bae *et al.* (2014) compared the performance of a single AFMBR with that of the SAF-MBR with lab-scale systems. Although the single AFMBR was found to be an effective alternative to the SAF-MBR system based on COD removals and TMP variations, a pilot study of the single AFMBR system would be beneficial. A single

AFMBR may also be used for post-treatment of effluent from other anaerobic processes. For example, Ren *et al.* (2014) proposed the use of the AFMBR for post-treatment of microbial fuel cell (MFC) effluent. They treated domestic wastewater having a COD of $210 \text{ mg}\cdot\text{L}^{-1}$ at a total HRT of 9 h, 8 h for the MFC, and 1 h for the AFMBR. The AFMBR removed 43% of the influent COD, which the MFC did not remove, and the resulting effluent COD was below $20 \text{ mg}\cdot\text{L}^{-1}$. Likewise, the AFMBR can be used for the post-treatment of UASB, anaerobic baffled reactor, or other anaerobic reactors for which organic removal has not been sufficient.

5. Future Research

There are several areas where research could be most beneficial prior to full-scale application of the AFMBR. The design of the AFMBR needs optimization. Also, although efficient removals of COD and BOD have been achieved, challenges with the post-treatment of the AFMBR effluent to meet more stringent discharge limits have not yet been well addressed. Important is that, while the effluent contains no SS, it does contain low concentrations of reduced inorganic compounds (ammonium, phosphorus, sulfide, and methane), and perhaps some organics (BOD and/or COD) with different characteristics than found with aerobically-treated wastewater. This suggests that new or modified technology development is needed. This section also provides potential strategies for post-treating the effluent from the AFMBR.

5.1. Design optimization

With respect to design, optimization of membrane density and energy minimization associated with GAC fluidization needs to be addressed. Operation of the SAF-MBR at higher flux would reduce the construction and operating costs with the reduction of HRT. Methods of chemical washing without adverse effects on biomass on GAC to increase the membrane flux could increase the economics of the SAF-MBR system. The comparative value of using a flat-sheet membrane instead of a hollow fiber membrane needs to be evaluated, as does the use of other membrane materials than those so far used in feasibility studies conducted to date. The laboratory and single pilot studies conducted so far have indicated the significant potential usefulness and efficiency of the SAF-MBR system for domestic wastewater treatment. Following just the first pilot SAF-MBR system studied, there is a high likelihood that it can be greatly improved in cost, reliability, and effectiveness. Subsequent studies in this direction are warranted.

5.2. Dissolved methane recovery

A significant need is to find the best process for recovery or removal of dissolved methane as pointed out by Liu *et al.* (2014) and others. Not only is the dissolved methane a potential energy source and should be used, loss otherwise to the atmosphere is highly undesirable as it is a strong greenhouse gas. Furthermore, methane oversaturation generally occurs in anaerobic membrane bioreactor effluents, especially with plug-flow systems when operated at a short HRT (Hartley and Lant 2006; Smith *et al.* 2011).

Hypotheses for oversaturation are the non-equilibrium status of the methane liquid–gas phase, high water pressure in reactors, and micro-size biogas-induced high solubility (Pauss *et al.* 1990; Kim *et al.* 2011; Smith *et al.*, 2012). As methane is a poorly soluble gas, several proven technologies are available for its removal.

Although few field applications of dissolved methane recovery from anaerobically treated effluents have been reported, there are many available dissolved methane removal technologies such as commercial systems commonly used for removing methane from contaminated groundwaters. Potential approaches for recovery are as follows:

Stripping tower: The phase-induced shift via air stripping has been widely applied, e.g. ammonium stripping. Simply, the stripping efficiency can be increased by generating an uneven air flow over rough materials (Tchobanoglous *et al.* 2014). Stripping towers have been applied for methane removal from leachate.

Ventury and inverted syphon: Here, water flow through siphon pipes allows a sharp pressure drop as it rises up to the desired elevation, causing the dissolved methane to release into a gas phase for recovery (Finnemore and Franzini 2002). Commercial air conduction methods using venturi have been used for the removal of dissolved methane from groundwater (US water fusion open air system, US water systems). Additionally, the same concept of using vacuum towers to decrease N_2 from wastewater (Maciejewski *et al.* 2011) could also be applied for dissolved methane removal.

Membranes: Gas permeable membranes are also commercially available and are widely used for the removal of oxygen from waters, a gas that is similar in solubility to methane. Bandara *et al.* (2011) proposed using a degassing membrane for methane recovery, and achieved over 90% methane recovery with this system. Another approach used to achieve a higher methane concentration is to induce a vacuum over the membranes. Using this approach with a poly-di-methyl-siloxane membrane contactor, methane recovery was achieved with an intermediate liquid velocity (Cookney *et al.* 2012). However, the high energy requirement found is not eco-friendly, and further modifications towards economic feasibility are needed.

Poly-3-hydroxybutyrate (PHB) production: PHB, a bio-derived and biodegradable plastic, can be produced through dissolved methane oxidation by PHB-producing methanotrophic bacteria (Pieja *et al.* 2012). The dissolved methane in the effluent from the AFMBR would be a perfect substrate for such PHB production.

Such technologies (except membranes and PHB production) are likely to use less than $0.05 \text{ kWh}\cdot\text{m}^{-3}$ for efficient dissolved methane removal. Effluent dissolved methane concentration in AFMBR effluent is typically in the range of $1 \text{ kWh}\cdot\text{m}^{-3}$ containing about $0.22 \text{ kWh}\cdot\text{m}^{-3}$, which is much higher than $0.05 \text{ kWh}\cdot\text{m}^{-3}$, but approximately $0.07 \text{ kWh}\cdot\text{m}^{-3}$ of electricity could be obtained from this recovered methane through cogeneration. Thus, while sufficient electrical energy could be obtained from the methane to pay the energy cost of its removal, lowering the removal energy input is desirable in order to obtain greater net electrical energy benefit. Furthermore, cogeneration also produces heat energy, and the potential benefit of this should not be ignored.

5.3. Ammonia and phosphate management

Ammonia in wastewater is commonly removed as N_2 gas by nitrification/denitrification, which requires the input of much aeration energy, as well as energy contained in organic or inorganic electron donors. In addition an expansion of the number of treatment units and associated detention times will result. Phosphate removal can be obtained biologically or chemically. However, phosphorus is one of the fast depleting resources, and thus recovery of it or its direct use in wastewater reuse schemes for irrigation are more desirable outcomes. Reuse of the wastewater for irrigation would also reduce the need for removing ammonia, and indeed would be a beneficial use of the water in water short areas.

Nutrient recovery for use as fertilizers: Ammonium and phosphorous in anaerobically treated AFMBR effluent is an appropriate liquid fertilizer that may be used directly through plant irrigation. While the potential risks of trace contaminants in the effluent need to be evaluated before soil application (Kinney *et al.* 2006; Musson *et al.* 2010; Smith *et al.* 2012), the significant removals by the SAF-MBR system mentioned previously are encouraging (Dutta *et al.* 2014). While further investigation may be warranted if water reuse for irrigation is to be considered, it should be noted that aerobically treated, filtered, and disinfected wastewater is already widely applied and proven safe for irrigation, even of crops eaten raw.

Although nutrient recovery or direct use of wastewater for irrigation would be a better option, there is still need to seek the best technologies for nutrient removal from anaerobically treated effluents as often required for surface water disposal. In the following, approaches that are suitable for dilute-strength AFMBR effluent are reviewed.

Biological nitrogen removal processes — nitrification–denitrification, Anammox, CANDO: A difficulty in applying the usual nitrification–denitrification approach for the post-treatment of AFMBR effluent lies in its low COD/BOD content. Thus, an external electron donor would need to be applied, e.g. methanol, acetic acid, glucose, sulfur, thiosulfate, hydrogen sulfide, hydrogen, dissolved methane, etc. (Straub *et al.* 1996; Park and Yoo 2009; Shao *et al.* 2010). However, this would increase the operational costs. An alternative is to couple nitritation and anaerobic ammonia oxidation (Anammox) (Mulder *et al.* 1995; Jetten *et al.* 2001). Such application with dilute wastewaters at ambient and low temperatures has been widely studied in the lab (De Clippeleir *et al.* 2011; Kartal *et al.* 2010; Kwak *et al.* 2012; Lee *et al.* 2013; Ma *et al.* 2011 and 2013; Winkler *et al.* 2012; Gilbert *et al.* 2014), but pilot-scale to full-scale studies are needed. Most recently, a newer process termed “Coupled Aerobic-Anoxic Nitrous Decomposition Operation (CANDO)” enables power yield by converting ammonium into N_2O for combustion. This leads to the novel concept of turning ammonium into an energy source (Scherson *et al.* 2013).

Physical nitrogen removal processes — Ion exchange, sorption: Ion exchange, by strong acid cation and weak basic anion resins, is a mature technology developed decades ago, but its high cost may prohibit economic implementation (Koon and Kaufman 1975; Leakovic *et al.* 2000; Jorgensen *et al.* 2003; Thornton *et al.* 2007; Malovanyy *et al.* 2013). However, cheaper absorbents (e.g. banana peels, modified biosorbents, zeolites,

and clinoptilolite) have been used at full scale for ammonium recovery. These absorbents are reported to be cost-effective, with high exchange capacity and fast adsorption and desorption kinetics (Jorgensen *et al.* 1976; Huang *et al.* 2010; Li *et al.* 2011; Milán *et al.* 2011; Deng *et al.* 2014).

Chemical phosphorous removal processes — chemical precipitation: Coagulants can and have been used to remove phosphorus. Various materials/chemicals, e.g. uncalcined synthetic hydrotalcite, hydrous ferric oxide resin, and ferrous or ferric coagulant, have been proven to be effective for phosphorous removal (Terry 2009; O’Neal and Boyer 2013; Tchobanoglous *et al.* 2014).

Currently, physical or chemical removal processes have robustness for nutrient removal/recovery from AFMBR effluents, while emerging biological processes require further investigation.

5.4. Hydrogen sulfide

Hydrogen sulfide is produced by sulfate reducers, and causes odor, corrosion, and health problems. Therefore, removal of hydrogen sulfide is commonly practiced from biogas plants. Here, processes for the removal or utilization of dissolved hydrogen sulfide from AFMBR effluents are discussed.

Biological removal processes — denitrification: As addressed under biological nitrogen removal, sulfide could serve as an electron donor for denitrification. But, unless influent sulfate concentration is quite high, the sulfide produced anaerobically may not be enough to meet discharge limits, thus additional electron donors may need to be added.

Physical removal processes: Sulfide can be removed as a gas by processes similar to those used for dissolved methane as discussed previously.

Chemical removal processes — Microaeration, $FeCl_3$, $FeCl_2$: Microaeration of bioreactors via on-line DO or ORP control can oxidize sulfide into elemental sulfur without negative effects on methanogenic treatment of high-strength wastewaters (Chen *et al.* 2010; Lohwacharin and Annachhatre 2010). This is a potential strategy for sulfide control in AFMBR effluents. With chemical precipitation, ferrihydrite, and ferric and ferrous coagulants can not only remove phosphorus, but can also remove sulfide efficiently (Wang and Pei 2012; Tchobanoglous *et al.* 2014).

Energy generation — sulfide fuel cell: Power can be generated through sulfide drawn at the anode of a fuel cell. By means of this electrochemical reaction, energy could be produced through sulfide oxidation (Dutta *et al.* 2008 and 2009). However, how best to remove the elemental sulfur then deposited on the anode is a question needing to be resolved.

5.5. Life-Cycle Sustainability Assessment (LCSA)

Post-treatment processes that might be developed for the AFMBR require comprehensive informative assessment to select the ones that best meet the specific application needs. LCSA that includes economic costs and energy balance missing in life-cycle assessment (LCA) should be applied for this need. With it, the goal of a paradigm shift from pollution control to resource recovery ensures better quality and transparency of

the compressive information for decision-makers or/and process designers (Corominas *et al.* 2013).

6. Summary

The AFMBR has good potential for energy-efficient treatment of domestic wastewater while providing effluent BOD₅ and COD concentrations comparable to that from filtered conventional activated sludge treatment. Here, wastewater is treated anaerobically by biofilms attached on fluidized granular media and membrane filtration. Membrane fouling is uniquely controlled at low energy expenditure by movement of the fluidized GAC along the membrane surfaces. The membranes also capture wastewater influent VSS, and hold them for sufficient time to allow for adequate biodegradation. They can then be removed for disposal at a rate independent of the rate at which the attached biofilm microorganisms are lost, another advantage of this system over gas-sparged systems. Not only is this system near energy-neutral in operation, but the reactor waste biosolids are already treated and their production is only about 0.05 kg·kg-COD⁻¹, less than one quarter of that from the typical aerobic treatment system. This, combined with absence of the need for final clarifiers, multi-media filters, and external biosolids treatment and disposal facilities, leads to a relatively small footprint for the AFMBR system.

The capabilities of the AFMBR have been assessed through both lab- and pilot-scale SAF-MBR systems operation, where the AFMBR has generally been used as the second stage following AFBR treatment of primary treated domestic wastewater. Effluent COD and BOD₅ concentrations less than 25 mg·L⁻¹ and 10 mg·L⁻¹, respectively, have been obtained with wastewater temperatures ranging 8–30 °C and short HRT of 2.5–6.8 h. The SAF-MBR has been operated for over a year without the need for membrane chemical cleaning. However, periodic chemical cleaning would likely increase the sustainable membrane flux. The best method for doing this needs evaluation in future studies.

The fouling rate in the AFMBR is an important variable that is dependent upon the physical properties of the fluidized media and the packing ratio. With non-adsorbing media, lower fouling is obtained with larger media, but here the energy requirement is also higher. Increasing the packing ratio up to 50% lowers the fouling rate, but also increases the energy requirement. In general, better fouling control tends to be associated with an increase in the energy requirement for media fluidization. However, better understanding of the overall complex interactions between foulants, fluidized media and membrane types, and energy requirements is needed for improvement in the operation of AFMBR systems and to better optimize system performance.

With the growing increase in wastewater reuse, the need is likely to increase for knowledge concerning the removal of organic micropollutants and precursors of disinfection by-products from wastewater. For the list of micropollutants so far studied, the SAF-MBR system with GAC fluidization in general shows better removal of micropollutants and precursors of disinfection by-products than comparable aerobic treatment systems. Research to evaluate removal capabilities for a broader list of micropollutants, and to better understand the removal mechanisms and possible intermediate products formed through AFMBR treatment is yet needed.

There are other important issues concerning AFMBR operation that need to be addressed. Included are knowledge of the best procedures for recovery of dissolved methane and the management of nutrients in AFMBR effluents. Effluent dissolved methane must not be allowed to escape to the atmosphere for it is a strong greenhouse gas. Methane is a poorly soluble gas, and several proven technologies are available for its removal, but knowledge of the best cost- and energy-efficient method for doing so is yet needed. How to best address wastewater nutrients is another important issue. Nutrients may not need to be removed and indeed could add value when wastewater is reused for irrigation. For nitrogen and phosphorus removal, when necessary, there are traditional methods that might be used, but there are several emerging technologies that have been or are being developed that are much less costly and energy-consuming. These deserve exploration. Ultimately, the AFMBR system needs to be evaluated in a LCSA, to ensure better quality and transparency for decision-makers and/or process designers.

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Chapter 11

Development and Application of Anaerobic Technology for the Treatment of Chemical Effluents in Taiwan

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Over the last 30 years, more than 80 full-scale anaerobic reactors have been installed in Taiwan. With sizes ranging from 80–12,000 m³, they are used to treat high-strength wastewater from the petrochemical industry, which produces chemicals used in manufacturing products, such as phenolic resin, polyester resin, adhesive, petrochemical fiber, and nitro fiber. Three types of anaerobic bioreactors have been widely implemented in Taiwan: anaerobic filter, up-flow anaerobic sludge blanket, and anaerobic fluidized bed. Efforts to develop anaerobic biotechnology in Taiwan began with academic programs and tremendous advancement has been made to improve the treatment efficiency of these three types of anaerobic bioreactors through full-scale applications to treat wastewater generated from the manufacturing of polyethylene terephthalate and purified terephthalic acid. In these applications, bioreactor start-up and operation were observed to highly influence the anaerobic treatment performance. These three types of anaerobic bioreactors perform differently based on loading capacity, acclimation procedure, and treatment operation.

1. Introduction

Anaerobic technology plays a significant role in the treatment of industrial wastewater in Taiwan. For over 40 years, the country's petrochemical industry has been growing rapidly, producing chemicals that are widely used in various consumer products. The manufacturing processes associated with this industry generate high-strength wastewater, which calls for highly efficient treatment capable of degrading recalcitrant compounds. Anaerobic biological processes have been applied widely in the treatment of this industrial wastewater due to the ability of these systems to effectively handle high concentration and loading rate of organics. Compared to the conventional aerobic treatment of activated sludge, anaerobic treatment provides advantages attractive to the

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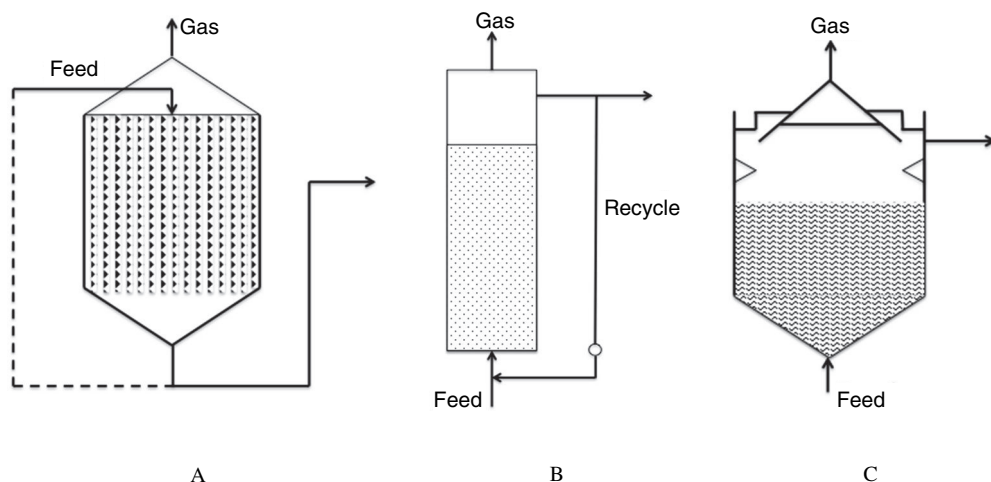


Fig. 11.1. Schematic diagram of: A) Down-flow Anaerobic Filter (DAF), B) Anaerobic Fluidized Bed (AFB), and C) Up-flow Anaerobic Sludge Blanket (UASB).

Taiwanese industries, including its capacity to manage higher volumetric loading, recover methane for energy, generate less sludge, and utilize less land.

In Taiwan, three types of anaerobic bioreactors have been developed and widely used: anaerobic filter (AF), up-flow anaerobic sludge blanket (UASB), and anaerobic fluidized bed (AFB). As shown in Fig. 11.1, the AF bioreactor is packed with plastic media, providing surface area for microbial attached growth, and the system can be operated in both the up-flow and down-flow directions. The biomass acclimated in the system is retained in the pores of the filter as well as on the surface of the plastic media. UASB, the second type of bioreactor discussed, is the most common anaerobic treatment found in many applications worldwide. The system makes use of the sludge blanket formed by the acclimated microbes to degrade organics in the wastewater influent. Lastly, AFB is a technology that utilizes fluidized media for biomass attachment and degradation of refractory compounds. The applications of these three anaerobic bioreactors are proven to be effective in the petrochemical industry in Taiwan.

In anaerobic bioreactors, there are generally a few key microbial steps occurring. Hydrolysis, the first of these steps, converts insoluble organic polymers, such as proteins, carbohydrates, and fats, into simple soluble monomers. The products of the hydrolysis process are then utilized by the fermentative bacteria in the second stage of anaerobic process — acidogenesis — producing volatile fatty acids. Further conversion occurs when these volatile fatty acids are consumed to produce acetic acid, carbon dioxide, and hydrogen in the process of acetogenesis. The fourth and last step of the anaerobic process — methanogenesis — takes and converts acetates to methane and carbon dioxide by consuming hydrogen. A successful final stage of anaerobic process is the result of syntrophic cooperation that is facilitated by the hydrolytic fermentative, acidogenic, and acetogenic bacteria, as well as the methanogens involved in this chain of microbiological processes. In-depth analysis of microbial groups and their functions in anaerobic bioreactors are further described in Chapter 2 by Kamagata.

In Taiwan, local researchers at the Industrial Technology Research Institute (ITRI) and professors from different local universities have devoted more than 30 years to the research and development of anaerobic biotechnology. Along with many environmental engineering companies, ITRI has developed their own anaerobic biotechnology to treat several types of industrial wastewater other than petrochemical, including effluents from yeast fermentation, winery/brewery, fine chemical, food processing, synthetic polyester fiber, phenolic resin, and photo-electron industries. Full-scale implementation of anaerobic biotechnology is expected to continue progressing in Taiwan.

This chapter reviews the progress and development of research and pilot studies of anaerobic technology in Taiwan. Industrial applications of the three types of anaerobic bioreactors aforementioned above are also discussed later in this chapter.

2. Anaerobic Biotechnology Development in Taiwan

The application of anaerobic processes in industrial wastewater treatment is distinct from its traditional treatment areas like sludge digestion, night-soil fermentation, and solid waste composting. Unlike traditional wastewater sources, which are consisted of easily biodegradable substrates as the main components, industrial wastewater often contains various synthetic chemicals, including synthetic polymer, aromatics, and heterocyclic compounds that are relatively less biodegradable. These synthetic chemicals tend to inhibit microbial growth and therefore contribute to the challenges in the development of anaerobic biotechnology in industrial wastewater treatment. To treat various types of industrial wastewater, academic programs in Taiwan have focused on improving the application of anaerobic biotechnology. The efforts were mainly on the understanding of down-flow anaerobic filter (DAF), UASB, and AFB to treat industrial effluents, and on the implementation of these anaerobic technologies to treat industrial wastewater.

2.1. Development of anaerobic biotechnology in academic programs

2.1.1. AF bioreactors

In 1973, lab-scale AF bioreactors were first used and successfully demonstrated to treat the effluent discharged from a yeast production factory (Tsai and Kao 1973). Similar to a trickling filter, AF employs a type of media for attached microbial growth. The void spaces created by the type of media also allow for anaerobic microorganisms to accumulate and, as a result, the reactor influent comes into contact with substantial active biomass as it passes through the filter media. The capacity of this bioreactor to retain biosolids is beneficial from treatment efficiency and maintenance points of view (Cheng 1984). The knowledge gained was further applied to treat various types of industrial wastewater. Later, a 2-phase AF process was evaluated for its potential to remove antibiotics from wastewater (Hsu and Young 1991). To support these studies, two kinetic models were developed to understand the methanogenesis process within the attached biofilms, and its inhibition by substrates in AF bioreactors (Chou *et al.* 2008). As AF bioreactors were not effective in refractory substrates such as starch and cellulose, the study and application of UASB reactors to treat industrial wastewaters have started.

2.1.2. UASB bioreactors

UASB treatment utilizes the formation of granular sludge blanket that is suspended in the anaerobic reactor. Reactor influent enters in the up-flow direction as wastewater components are degraded by the methanogenic archaea. The UASB bioreactor, capable of generating biogas, is the most popular type of anaerobic treatment and is seen in various applications worldwide. Anaerobic microorganisms are grown and granulated by the means of well-controlled organic and hydraulic loading. Syntrophic microbial species can be accumulated together and formed in a spherical structure with a diameter of 0.1–2.0 mm (Cheng *et al.* 1990; Cheng *et al.* 1997a,b); Wen *et al.* 1994; Fang *et al.* 1996; Liu *et al.* 2000; Wu *et al.* 2001a,b,c). Even distribution of influent substrate provides uniform hydraulic flow to float the granular sludge in the bioreactor bottom. Extremely high density and concentration of biomass can be maintained in the bottom half of the sludge bed to effectively degrade the influent substrate. The new growing microbes form a layer of sludge blanket above the sludge bed of granulated biomass. The fermented biogases of CO₂ and CH₄ flow upward and lift the sludge blanket up to the top of bioreactor (Lay and Cheng 1998). Thus, different types of 3-phase separators have been designed and installed at the top of bioreactor. The bioparticles flowing upward impinge the inclined plate and the attached biogas is separated and released to the headspace of gas collector. Meantime, the bioparticles settle downward to the sludge blanket and the effluent liquid overflows the top weir. The patented separators have been developed and applied internationally.

Achieving good sludge granulation to increase biomass accumulated in the bioreactor is a common challenge among all UASB studies. The quantity of anaerobic biomass is usually insufficient to handle shock loading and refractory substrates. This observation led to a study investigating the characteristics of different syntrophic bacteria and methanogens in a UASB developed for high organic loading (Chen and Tseng 1984). It is understood that the integrated network structure necessary for anaerobic sludge granulation is provided by the filamentous *Methanosaeta* species. However, it was shown through this study that the predominant *Methanosarcina* grew more rapidly than *Methanosaeta* in a reactor treating influent with a relatively high acetic acid concentration of 6,000 mg·L⁻¹ and pH below 6. Additionally, two separate growth phases of syntrophic bacteria and methanogens result in higher specific growth rate and desired kinetic characteristics, such as inhibitory allowance of heavy metal, thermophilic fermentation of biological sludge, and 2-stage fermentation of cellulose. However, phase separation of syntrophic bacteria and methanogens in UASB bioreactors could not be established strictly by process control or substrate fractionation.

2.1.3. AFB bioreactors

The mechanism of contaminant degradation in AFB has been studied in Taiwan partly through the analysis of the media used for attached growth in the bioreactor. The fluidized bed in AFB is packed with a type of carrier, which supports the growth of biofilm necessary for organic removal. Due to its adsorptive capacity, large surface area, and advantages over the UASB process, the most popular medium used in AFB is

granular activated carbon (GAC). Its high adsorptive capacity can increase the removal of aromatic compounds and subsequently dampen shock loading, while its large surface area improves microbial attachment (Cheng *et al.* 1997a,b). The fluidization of GAC also eliminates mechanical attrition between particles (Lazarova and Manem 2000). Optimization of the use of GAC in AFB includes studies of carbon adsorption mechanisms, desorption and bioregeneration, and analysis of attached biomass.

Studies of AFB in Taiwan have been focused on addressing the AFB's disadvantage related to hydrodynamics. Recirculation of the treated effluent in a GAC-AFB process provides a high dilution rate with the influent substrate concentration, and subsequently reduces potential inhibition on substrate utilization and enhances the rate for substrate diffusion into the biofilm for further degradation (Liang *et al.* 1995). However, the hydrodynamic regime is extremely difficult to control in a full-scale bioreactor. Hydrodynamic adjustment in the field and power consumption are the major disadvantages of an AFB. In order to address the shortcomings of the technology, studies have been performed not only on the hydrodynamics itself, but also in the areas of process start-up, biofilm growth, and fluidization control. Additionally, there are other studies that investigated the reactor's performance when operated under distinct conditions. For example, the feasibility of GAC-AFB has been evaluated for the treatment of low substrate concentration and wastewater at ambient temperature. Anaerobic biodegradation of chlorophenols and nitrophenols in AFB with an emphasis on kinetic modeling of phenolic inhibition has also been studied to improve AFB operation (Chang *et al.* 2004).

While most studies on AFB have been executed at a lab-scale level, a pilot-scale study was successfully implemented to observe the technology's performance treating food-processing wastewater with moderate COD concentrations (Industrial Technology Research Institute 1992). This cooperative study, completed by the researchers at ITRI, made use of a 5-ton AFB bioreactor with a height of 21 m, providing sufficient fluidization of the GAC bioparticles. Low concentration of volatile fatty acids contributed to the predominant growth of *Methanosaeta*, resulting in a dense filamentous network that enhanced the biofilm attachment on the GAC surface. However, observed from the long lag-phase of microbial growth, low organic loading lowered the system's start-up performance. Insufficient hydraulic retention time also prevented complete biodegradation of high-molecular-weight organics, such as proteins and lipids present in the wastewater. Reactor operation at start-up is therefore one of the critical aspects of the AFB process.

2.1.4. DAF bioreactors

DAF is a U.S.-patented process that was developed by the research group at Amoco Petrochemical Corporation in Chicago, U.S. The system consists of plastic filter media that support microbial growth as attached biofilm. Pall rings, rocks, and polyvinyl plastics are some of the typical packing materials used as filter media in DAF (Cheng 1984). The system anaerobically degrades contaminants in the wastewater as the influent moves in a down-flow direction. Compared to the other type of AF, an up-flow anaerobic

filter, DAF may offer greater solids removal efficiency and sulfide stripping in the upper part of the reactor. However, loss of biosolids is an important aspect of this technology that may require better control.

2.2. Anaerobic biotechnology development in research institutes

Significant progress in the development of anaerobic biotechnology has also been made by several research institutes in Taiwan, including ITRI, the Refining & Manufacturing Research Center of the China Petroleum Corporation (RMRC), and the Research Institute of Taiwan Sugar Corporation. These institutes mainly identified critical works needed for advancing anaerobic technology in Taiwan. For example, the R/D program at ITRI aims at developing a series of commercial biotechnology, including various patents for UASB and AFB bioreactors. With regards to this goal, ITRI published a study on a lab-scale anaerobic filter process treating wastewater from glutamine fermentation, and their finding indicated that low anaerobic biodegradability could be achieved due to the high concentrations of sulfate and sodium chloride present in the wastewater (Chang *et al.* 1986). Thus, the removal efficiency of the existing biotechnology needed to be further optimized to treat this type of wastewater. In addition, RMRC has also studied the anaerobic process of UASB at a lab-scale level. The institute designed a bioreactor with a volume of 16 L to treat wastewater from single-cell protein manufacturing processes. Following the success of this laboratory-scale study, RMRC designed a full-scale UASB bioreactor with a volume of 250 m³ to treat food-processing wastewater. After appropriate start-up, the process successfully handled a volumetric organic load of 3.0 kg-BOD·m⁻³·d⁻¹.

Research institutes in Taiwan have further focused their R/D programs to improve existing anaerobic biotechnologies. In 1985, the Research Institute of Taiwan Sugar Corporation developed its own anaerobic biotechnology with two large UASB bioreactors, each with a volume of 5,000 m³ and a special phase separator. The reactor was used to treat saline yeast fermenting wastewater containing high concentrations of refractory compounds and sulfate. Due to the sulfate reduction and hydrogen sulfide production, further developments were pursued to improve hydrogen sulfide removal from the liquid phase. Furthermore, ITRI also established its own anaerobic biotechnology development group. As a long-term project, the research institute further investigated the ability of anaerobic bioreactors to degrade winery wastewater (Owen *et al.* 1979). The research institute established procedures to determine the biogas production and measure the coenzyme F₄₂₀ activity of the methanogens present in the reactor. The research efforts were expanded to treat wastewater containing antibiotics and waste from glutamine fermentation processes.

The research institutes have further focused on their efforts in pilot-scale studies. Pilot-scale evaluation is a critical step of anaerobic technology development that involves reactor start-up to enrich necessary microbial populations to degrade different synthetic chemicals. For example, ITRI conducted a pilot study at two wineries using UASB bioreactors, each with a volume of 1.0 m³ and a height of 4.5 m, to treat a high COD influent concentration of 12,000 mg·L⁻¹. The maximum volumetric loading rate of

30 kg-COD·m⁻³·d⁻¹ was achieved after 1.5 years with a reduction in COD greater than 80%. After four pilot studies, researchers from ITRI and engineers from CTCI Corporation scaled up the UASB bioreactors to a volume of 120 m³ and successfully constructed 32 bioreactors to treat wastewater discharged from seven wineries. Customized software was also employed to provide a good control for pH adjustment as well as reactor start-up.

3. Full-Scale Design and Operation of Anaerobic Biotechnology

3.1. UASB

In Taiwan, 30 sets of modified UASB bioreactors, each with a volume ranging from 100–200 m³, have been designed by ITRI and CTCI Corporation so far. The units were tested using wastewater from six wineries that produce a variety of commodities such as rice wine, fruit wine, brandy, and Kao-Liang liquor. Some of the wastewater streams contained extremely high concentration of COD, and dilution is required before entering the UASB unit. Most of the winery wastewater was treated with 2-stage anaerobic processes to carry out acidogenesis and methanogenesis, and polished with an activated sludge process to meet discharge permit requirements (Cheng *et al.* 1990). Table 11.1 summarizes the full-scale UASB reactors that ITRI has successfully implemented.

ITRI researchers have extended the use of UASB to treat wastewater discharged from different chemical industries. In 1987, an UASB reactor with a volume of 600 m³ was designed and successfully implemented to treat wastewater from adhesive tape manufacturing processes. The UASB process was also applied to treat polyester-containing wastewater streams discharged from different synthetic fiber manufacturing plants. During this period, the ITRI researchers gradually scaled up the volume of the UASB bioreactors to 1,500 m³ and then to 3,500 m³. The reactor height was also expanded to 11 meters. The most important modification was the addition of inclined plates and tubes to the 3-phase separator. These improvements of reactor design enhanced the process performance of biomass entrapment and COD removal efficiency in spite of incomplete sludge granulation during the start-up period.

Wastewater characterization of three synthetic fiber manufactures had been investigated through laborious instrumental analysis, bench-scale unit operation, and pilot-scale experiments for two years. Simple compounds present in the fibrous wastewater, such as acetic acid, ethylene glycol, formaldehyde, acetaldehyde, and methanol, were the major constituents and easily degraded by anaerobes (Liang *et al.* 2003). On the other hand, aromatic compounds with different functional groups exhibited different degrees of biodegradability to anaerobic microbes. Aromatic compounds, such as terephthalate, *p*-toluic acid, and even benzoate, were refractory to the unacclimatized anaerobes (Li *et al.* 1995; Cheng *et al.* 1997a, b; Liang *et al.* 2002a,b; Liang 2006). However, the anaerobic bacteria in the UASB system could be acclimated to degrade these aromatic compounds after a long duration of microbial adaptation (Kleerebezem 1999; Wu 2001) (see Chapter 3 by Narihiro *et al.* for detailed description).

Table 11.1. List of full-scale UASB bioreactors implemented by ITRI.

Company	Date	Volume (m ³)	Reactor Number	Industry	Flow (CMD)	COD _{in} (mg·L ⁻¹)	COD _r (%)
Asia Chemical Co.	1988	700	1	Adhesive Chemical	400	9,000	98
		300	1				
Kuang Cheng Chemical	1991	80	1	Plastic	80	8,000	95
Taiwan Tobacco and Winery Co.	1992	100	30	Winery, Distillery	60–200	4,000–30,000	90
Hwalon Tofen I	1992	450	2	Polyester	800	5,000	90
Hwalon Tofen II	1993	500	2	Polyester	1,000	5,000	90
Changdchun petrochemical	1993	1,350	2	PVA, TMP, PVB	1,200	4,200	≥70
Hwalon Tofen III	1994	600	2	Polyester	1,000	5,000	90
Fushin airline	1995	135	1	Food Processing	250	2,000	≥65
Taiwan Tobacco and Winery Co.	1995	1,300	1	Distillery	1,300	10,000	≥70
Hwaline							
Far-Eastern Co.	1995	3,500	1	Polyester	4,000	2,500	≥70
Hwalon Malaysia I	1995	600	2	Polyester (A/B)	1,200	5,000	90
Hwalon Malaysia II	1996	600	2	Polyester (C/D)	1,200	5,000	90
Dain Chemical I	1996	500	1	Plastic Stabilizer	200	8,000	≥70
President Hsinsih I	1997	800	2	Food Processing	4,000	2,500	90
President Yanmei I	1997	200	1	Food Processing	1,000	2,500	90
President Yanmei II	1998	260	2	Food Processing	2,500	2,500	90
Taiwan petrochemical Co.	1999	800	1	Petrochemical/ Maleic Acid/Anhydride	125	12,000	80
Dain Chemical II	1999	900	1	Fatty Acid	400	8,000	80
President Mardou	2002	350	350	Food Processing	2,000	1,000	90

Company	Date	Volume (m ³)	Reactor Number	Industry	Flow (CMD)	COD _{in} (mg·L ⁻¹)	COD _r (%)
Chengia Co.	2002	1,000	2	Paper	3,200	7,400	80
Changchun II	2003	1,350	2	Petrochemical	2,000	3,000	70
Kimen winery	2003	1,000	1	Distillery	650	3,000	90
President Hsinsih II	2002	390	2	Food processing	2,000	2,500	90
Kwangfei LCD	2004	1,335	2	TFT-LCD	3,400	4,500	70 (TOC)
Tungbao LCD	2004	720	2	TFT-LCD	1,400	8,000	70 (TOC)
China LCD	2005	1,000	4	TFT-LCD	5,000	3,500	70 (TOC)
FN&N COCA COLA - Malaysia	2005	200	1	Beverage	400	3,000	75
Liter LCD SSP	2005	200	1	TFT-LCD	200	5,000	70
Chimei LCD II	2005	1,000	1	TFT-LCD	1,600	1,500	70 (TOC)
Chimei LCD VII	2005	900	4	TFT-LCD	6,000	2,500	70 (TOC)
President Hsinsih III	2006	1,200	1	Food processing	4,000	1,500	90
Formosa Chemical Co. Mainland China	2006	1,200	2	Petrochemical/ Polyester	4,000	2,500	80
Glow tec, Vietnam	2007	1,200		Yeast Fermentation	1,000	6,500	80
Oriental Union CP	2007	1,000	1	Petrochemical /EG	600	8,500	70
F&B Dairy, Malaysia	2008	180	1	Dairy	600	2,200	85
Mc Food, Malaysia	2009	180	1	Food	560	3,000	85
KFC, Malaysia	2009	180	1	Food	400	3,000	85
Far Eastern (OPTC)	2010	4,000	1	PTA	2,400	6,000	75 (Revamping)
Far Eastern (OPTC)	2010	5,000	1	PTA	3,000	6,000	75 (Revamping)
FN dairy, Malaysia	2011	560	1	Dairy	500	2,400	85
Taiwan Bayer (Changhwa)	2013	128	2	Alkyd Resins	10	90,000	60

3.2. AFBs

AFBs developed by ITRI utilize a fluidized bed containing media that provides surface area for bacteria attachment and contaminant degradation. Wastewater flows in the upward direction with a velocity high enough to keep the height of the bed constant. The high porosity of the fluidized bed prevents clogging and therefore has an advantage over fixed bed reactors. Performance of several bench-scale AFB reactors has been proven successful for the treatment of wastewaters from industries such as food-processing, dairy, synthetic fiber, and plastic resin manufacturing.

The AFB is a relatively new treatment process with great potential. To keep the bioparticles in the bioreactor from hydraulic washout, a relatively heavy and dense particle medium is used as the support particle to improve the attachment of microbial biomass. Media such as sand and GAC are employed. The attached biofilm accumulates denser biomass on the particle surfaces. These bioparticles are then fluidized along the bioreactor height, with the recirculation of effluent providing appropriate hydraulic velocity (Holst *et al.* 1997). To achieve better treatment efficiency, it is necessary to have good biofilm attachment, which can further promote syntrophic growth among anaerobic microbes and ensure fluidization of the bioparticles without particle attrition (Lazarova and Manem 2000).

Rigorous pilot studies of AFB have served as a strong base to the subsequent design, construction, and operation of seven full-scale AFB bioreactors in Taiwan. These reactors (Fig. 11.2) were successfully implemented in the treatment of organic chemical wastewater from the manufacturing processes of latex, sizing agent, and nitrocellulose (Table 11.2). Although the performance of the bioreactors shows promising effluent quality, there are still technology limitations that must be addressed. Such challenges include washout of carrier associated with biomass due to high load of biogas production, and variation of wastewater composition, which is highly dependent on the product production output.



Fig. 11.2. Anaerobic fluidized beds for chemical wastewater treatment in Taiwan.

Table 11.2. Full-scale AFB bioreactors developed by ITRI.

Factory name	Year	Reactor size	Products	Design criteria
En Hou Polymer Chemical Co.	1992	58 m ³	Size for textile industry	HRT=12 h CODr= 75%
		1.9 m(ϕ) \times 20.5 m (H)		Loading = 8 kg-COD·m ⁻³ ·d ⁻¹
Chan Sieh Enterprises Co.	1994	86 m ³	Polyester resin & latex	HRT=10 h CODr= 75%
		2.5 m(ϕ) \times 17.5 m(H)		loading=4 kg-COD·m ⁻³ ·d ⁻¹
T.N.C. Industrial Co. (denitrification)	1995	192 m ³ \times 2	Nitrification fiber	HRT=3 h loading=6 kg-NO ₃ ⁻ ·m ⁻³ ·d ⁻¹
		3.5 m(ϕ) \times 20 m(H)		Nitrate removal: 98%
Lidyc industrial Co.	1999	192 m ³	Polyester resin	HRT=12 h CODr= 80%
		3.5 m(ϕ) \times 20 m(H)		loading=4 kg-COD·m ⁻³ ·d ⁻¹
Baw Sieh industrial Co.	1999	275 m ³	Phenol-	HRT=24 h CODr= 80%
		5 m(ϕ) \times 14 m(H)	formaldehyde resin	loading=4 kg-COD·m ⁻³ ·d ⁻¹
Eternal chemical industrial Co.	1999	320 m ³ \times 2	Phenol, amino,	HRT=24 h CODr= 80%
		4.5 m(ϕ) \times 20 m(H)	alkyd resin	loading=4 kg-COD·m ⁻³ ·d ⁻¹
Far Eastern Textile Co.	2004	470 m ³ \times 2	PET fiber	HRT=4 d CODr= 75%
		5 m(ϕ) \times 24 m(H)		loading=5 kg-COD·m ⁻³ ·d ⁻¹
IDS Co. (Malaysia)	2010	170 m ³ \times 2	Beverage industry	HRT=3.5 h CODr= 75%
		3.25 m(ϕ) \times 22 m(H)		loading=10 kg COD·m ⁻³ ·day ⁻¹
CMO solar industry	2010	52 m ³ \times 2	Ammonia waste from solar battery,	HRT=3 h Nitrate= 90%
		2.3 m(ϕ) \times 13 m(H)	denitrification	loading=3 kg NO ₃ ⁻ ·m ⁻³ ·day ⁻¹
GenTech Co.	2012	78 m ³ \times 1	Solar battery chip waste, denitrification	HRT=2 h Nitrate= 80%
		2.5 m(ϕ) \times 16 m(H)		loading=3 kg NO ₃ ⁻ ·m ⁻³ ·day ⁻¹

4. Application of Full-Scale Anaerobic Bioreactors for Industrial Wastewater Treatment

4.1. Wastewater from PTA manufacturing processes

4.1.1. DAF

Amoco researchers and engineers designed and constructed the first two DAF bioreactors, each with a volume of $10,000 \text{ m}^3$, for China American Petrochemical Co. Ltd. (CAPCO) in Kao Hsiung, Taiwan. The DAF bioreactors were designed to treat PTA industrial wastewater in series with an activated sludge reactor. Recirculation of the DAF effluent back to the inlet of the reactors was incorporated to further enhance the removal efficiency. The two processes are followed by a sedimentation tank, where the treated wastewater is discharged and the sludge is wasted or recycled to the inlet of the activated sludge tank. A schematic diagram of the wastewater treatment implemented by CAPCO is shown in Fig. 11.3.

The CAPCO wastewater treatment plant is capable of handling a wastewater flow rate of $8,000 \text{ m}^3 \cdot \text{d}^{-1}$ with an influent TOC concentration of $4,000 \text{ mg} \cdot \text{L}^{-1}$ or a COD concentration of $10,000 \text{ mg} \cdot \text{L}^{-1}$. Thus, the two DAF reactors combined have the capacity to receive a COD daily load of approximately $80,000 \text{ kg}$. With a moderate volumetric organic loading rate of $4 \text{ kg} \cdot \text{COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$, the reactors are operated with a hydraulic retention time (HRT) of 2.5 days (Amoco Chemical Company 1991). Recirculation and gas stripping of the DAF effluent further reduce the influent COD concentration and carbonic acidity, which improves the anaerobic biodegradation of aromatic compounds.

The stepwise acclimation of CAPCO's DAF bioreactors provided a smooth start-up for the system by allowing it to reach an optimum performance at a certain organic loading and ramped up its capacity in stages. The acclimation resulted in the growth of different anaerobic microorganisms on the attached biofilm capable of degrading different organic compounds present in the PTA industrial wastewater. The attached biofilm growth on the plastic filter media in the bioreactors could degrade most of the

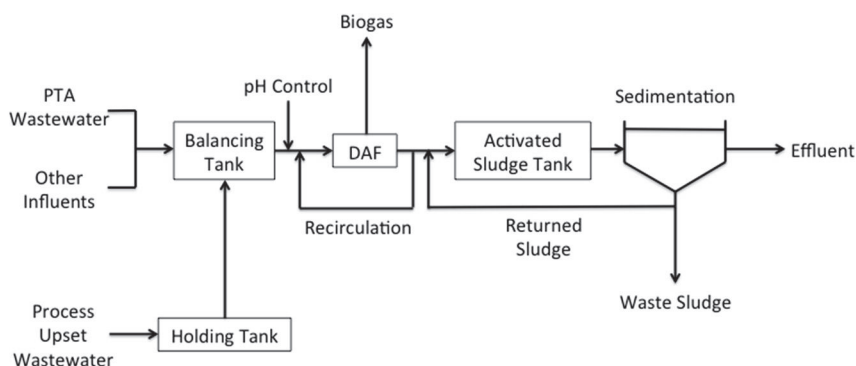


Fig. 11.3. Schematic flow diagram of CAPCO's wastewater treatment plant with anaerobic filter and activated sludge bioprocesses.

organic constituents in the wastewater, including acetate, benzoate, phthalate, and *p*-toluic acid, while achieving high COD/TOC removal efficiency >85%. The successful performance of DAF attracted environmental engineers around the world and this particular project received the Kirkpatrick Chemical Engineering Achievement award in 1991.

After several years of steady performance, the influent characteristics of the PTA wastewater treated by the DAF reactors and the activated sludge tank were investigated, particularly in terms of specific aromatic compounds. Three major aromatic acids, identified as terephthalic acid (TA), *p*-toluic acid (*p*-Tol), and benzoic acid, are shown to be the predominant refractory wastewater components in the plant influent (Table 11.3). The three aromatic acids were of high concentrations both in the influent and the effluent of the DAF. In addition to aromatic compounds, traceable concentrations of heavy metals were also detected.

The process effluent qualities for both DAF and activated sludge units at CAPCO's wastewater treatment plant are presented in Table 11.4. Among the wastewater constituents, acetic acid and benzoate were degraded effectively by DAF, while TA and *p*-Tol had more refractory effects on the anaerobic biodegradability of the compounds with removal efficiencies of 67.3% and 84%, respectively. The COD removal efficiency of 76.8% by DAF was also low compared to BA and HAc. Further treatment by activated sludge significantly reduced, if not completely eliminated, all five compounds monitored.

Table 11.3. Characteristics of CAPCO wastewater.

Wastewater Constituent	Influent Concentration (mg·L ⁻¹)			
	Anaerobic Filter		Activated Sludge	
TOC	2,763	4,325	423	1,031
acetic acid	1,866	3,935	12	261
trimellitic acid	137	1,123	11	94
<i>o</i> -phthalic acid	49	466	8	58
4-(hydroxymethyl)benzoic acid	152	325	4	20
terephthalic acid	1,367	2,587	183	1,271
<i>m</i> -phthalic acid	138	440	19	97
4-carboxy benzaldehyde	7	23	trace	
benzoic acid	481	1,350	24	148
<i>p</i> -toluic acid	346	704	78	346
Co	16	38	1	15
Mn	13	36	5	8
Br ⁻	20	64	20	49

Table 11.4. Performance of CAPCO's wastewater biological treatment unit.

Compounds	AF _{inf.}	AF _{eff.}	AST _{eff.}	Removal Efficiency (%)	
				AF	AST
CODs	8,264	1,917	80	76.8	95.8
TA	920	301	5	67.3	98.3
Benzoate	991	26	ND.	97.4	100
<i>p</i> -Tol	488	78	ND.	84.0	100
Acetate	2,166	63	ND.	97.1	100

Note: (a) AF: Anaerobic Filter, AST: Activated Sludge

(b) unit: mg·L⁻¹

Performance evaluation of DAF at the CAPCO wastewater treatment plant shows successful removal of refractory compounds present in the PTA wastewater. Although some compounds were not effectively removed by DAF without further treatment by activated sludge, the technology offers significant reductions in treatment cost, as well as operation efforts. Some of technology's drawbacks include the potential of filter clogging due to the accumulation of solids and long acclimation time.

4.1.2. UASB

In Taiwan, UASBs are commonly applied in the treatment of wastewater from PTA and polyethylene terephthalate (PET) manufacturing processes. Four sets of patented UASB bioreactors, each with a volume of 1,600 m³, were designed by Grontmij Consulting Engineers and constructed by Hepe Engineering to treat wastewater discharged from the PTA manufacturing plant operated by Tuntex Tetrochemicals Inc. (Pereboom *et al.* 1994). After the UASB treatment, the effluent was further polished by an aerobic post-treatment. A schematic treatment diagram of the treatment plant at Tuntex Tetrochemicals is shown in Fig. 11.4. The plant layout is shown in Fig. 11.5.

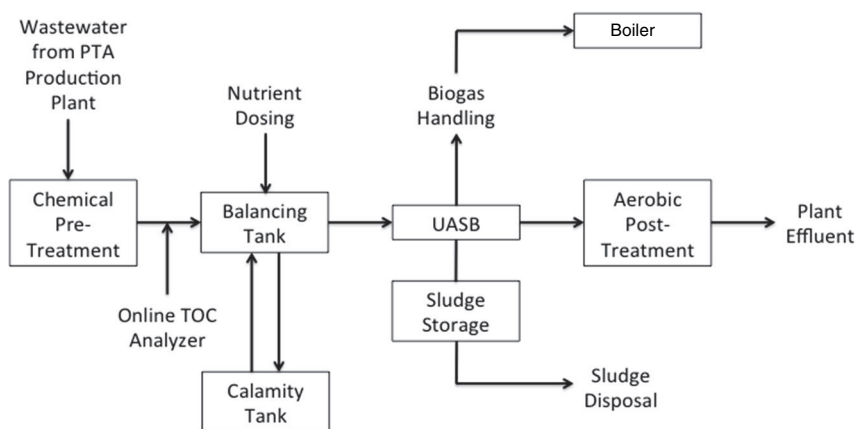


Fig. 11.4. Schematic treatment diagram of the PTA wastewater treatment plant at Tuntex Tetrochemicals.

- Legend
- 1)chemical pre-treatment
 - 2)balancing tank
 - 3)calamity tank
 - 4)UASB-reactor
 - 5)biogas compressor
 - 6)anaerobic sludge tank
 - 7)aerobic treatment
 - 8)sludge handling
 - 9)control room

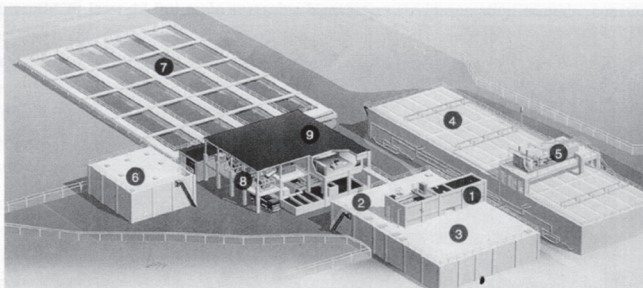


Fig. 11.5. Tuntex Petrochemical's PTA wastewater treatment plant in Tainan, Taiwan.

Table 11.5. Composition of wastewater from a PET-manufacturing factory.

COD	pH*	Acetate	Propionate	Benzoate	Ethylene glycol	Acetaldehyde
5,590	7.63	113	70	740	1,540	1,830

*All units are $\text{mg}\cdot\text{L}^{-1}$, except pH.

After two years of acclimation promoted high bioactivity and the growth of acetate-utilizing methanogens could be promoted, the aromatic compounds present in the wastewater inhibited the growth of certain anaerobic microbes, which consequently limited the process removal efficiency. The problem was mitigated by lowering the sludge loading rate during start-up and limiting the food to mass ratio (F/M) to less than 0.2 d^{-1} . This led to significant improvements in aromatic compound degradation and enhanced removal efficiency.

4.2. Wastewater containing acetaldehyde

Industrial processes involved in the manufacturing of PET in Taiwan generate wastewater containing high concentrations of ethylene glycol and acetaldehyde, along with some intermediate components. The COD concentration of this waste stream ranges from $4,500\text{--}8,600\text{ mg}\cdot\text{L}^{-1}$. Table 11.5 lists average concentrations of the main wastewater components resulting from PET manufacturing processes. With a higher average concentration than ethylene glycol, acetaldehyde is shown to be the major component of wastewater from PET manufacturing plants.

A UASB reactor with a volume of 600 m^3 was constructed and evaluated as a treatment technology aimed at degrading acetaldehyde in PET wastewater. Figure 11.6 depicts the treatment processes commonly implemented for this wastewater treatment. Before the wastewater is pumped to the UASB reactor, steady flow is achieved through the use of an equalization tank, while its pH is adjusted to around 7. The effluent from the anaerobic process then flows into an aerobic bioreactor for further treatment. The UASB reactor was seeded with sludge obtained from several pig manure digesters. The seed sludge volume was approximately 50% of the reactor volume and its concentration was about $15,000\text{ mg}\cdot\text{VSS}\cdot\text{L}^{-1}$. The performance of the UASB reactor treating acetaldehyde was assessed based on its start-up process and effluent quality.

4.3. Wastewater containing phenol and formaldehyde

Manufacturing processes of resins generate wastewater streams that are treatable by AFB. Table 11.6 shows the approximate concentrations of COD, phenol, and formaldehyde as the main components of wastewater from the different resin-manufacturing processes. Phenol and formaldehyde, found in wastewater from manufacturing processes of phenol-



Fig. 11.6. Process diagram for the treatment of wastewater containing acetaldehyde.

Table 11.6. Components of wastewater from different resin-manufacturing factories.

Source of Wastewater	Reaction Compounds	COD mg·L ⁻¹	Phenol mg·L ⁻¹	Formaldehyde mg·L ⁻¹
Phenol-formalin resin	Formalin			
	Phenol	105,000 ^a	17,000	8,300
	Para tert-butyl phenol	(22,000) ^b	(1,500)	(1,550)
Amino resin	Sodium salt, Urea			
Alkyd resin	Phthalic acid	85,000		
	Adipic acid	(12,000)	-	-
	Ethylene glycol			
Mixture		97,000	11,000	5,200
		(17,000)	(1,900)	(1,200)

^a: average; ^b: standard deviation

formalin and amino resins, are toxic to microorganisms. Thus, pre-treatment is required before wastewater enters the AFB bioreactors.

Figure 11.7 shows the processes involved in the treatment of wastewater containing formaldehyde. AFB is followed by another biological process involving the aerobic biofilter. As the wastewater enters the treatment process, flow is equalized in an equalization tank and diluted with recycled effluent from the aerobic biofilter. Thus, the circulation rate is set between the final settling tank and the point prior to the AFB reactor. The pH of the wastewater is also adjusted to neutral before it is pumped to the reactor. The effluent then goes to the aerobic biofilter for further treatment.

The AFB reactor used in the treatment process described in Fig. 11.7 was designed based on results obtained from a series of pilot studies (Liang *et al.* 1995). The reactor, 4 m in diameter and 20 m in height, utilizes GAC with a diameter ranging from 0.5–2.0 mm as carriers. The volumetric loading was set at 3.0 kg-COD·m⁻³·d⁻¹ and the process could achieve a COD removal rate of up to 70% (Fig. 11.8). As shown in Fig. 11.9, this specific application of AFB can significantly reduce the concentrations of phenol and formaldehyde. The toxicity of these two compounds inside the reactor also reaches far below the threshold as it is exposed to high mixing conditions. Furthermore, dilution of influent by a portion of the treated effluent allows for the complete degradation of formaldehyde, such that the exposure of microorganisms to formaldehyde is harmless. Phenol is less toxic to methanogens than formaldehyde and thus, even without complete removal, the resulting low concentration of the compound is below the toxic threshold for the microorganisms present in the reactor.

The performance of AFB in the treatment of wastewater containing formaldehyde was first evaluated by Liang *et al.* (2002b). The most recent study assessed the technology performance based on a higher influent concentration of formaldehyde but lower COD and phenol influent concentrations than the previous study. The process described in Fig. 11.7 results in a lower effluent concentration range for phenol, while the reactor efficiency of formaldehyde removal is improved (Table 11.7).

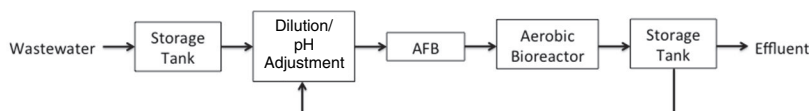


Fig. 11.7. Process diagram for the treatment of wastewater containing formaldehyde.

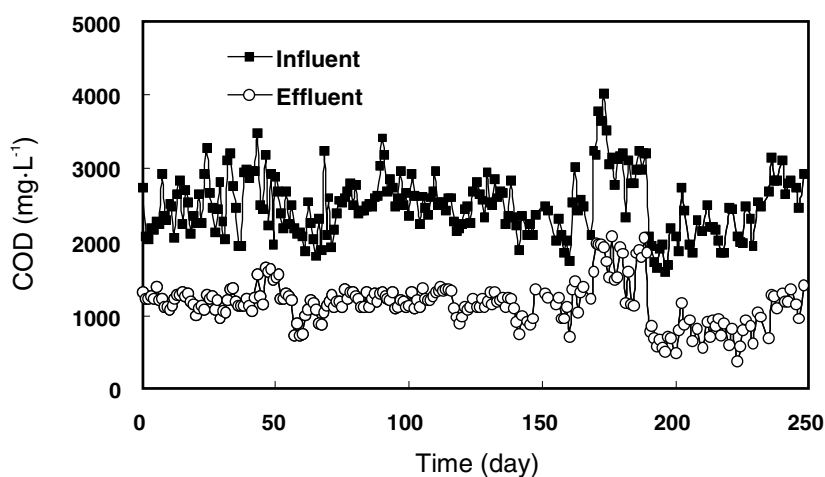


Fig. 11.8. Performance of the AFB reactor.

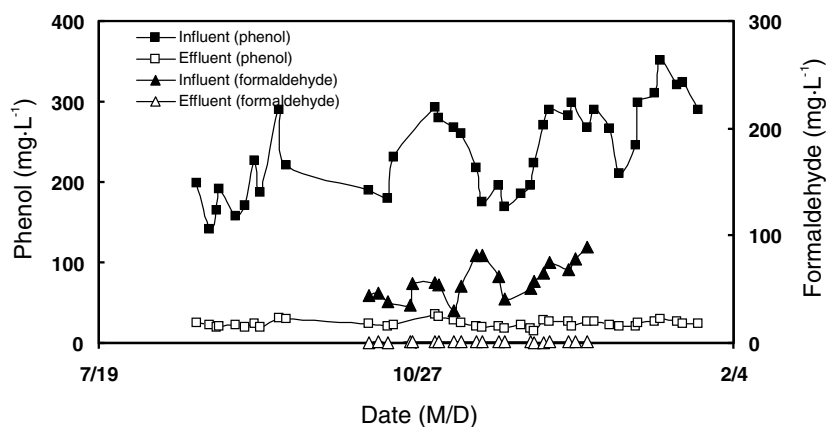


Fig. 11.9. Removal of phenol and formaldehyde in the AFB reactor.

Table 11.7. Performance of AFB reactors and aerobic biofilters treating wastewater containing formaldehyde.

Reference	Item	AFB influent	AFB effluent	Aerobic effluent
Previous (Liang, 2002b)	COD	2,000–3,500*	100–350	40–60
	Phenol	500–1,000	10–50	0.05–0.5
	Formaldehyde	40–60	1–5	ND
This study	COD	1,800–2,800	800–1,200	80–130
	Phenol	180–320	10–30	0.05–0.1
	Formaldehyde	70–100	1–5	ND

* All units are in $\text{mg}\cdot\text{L}^{-1}$

Table 11.8. Comparison of performance indicators between AFB and UASB.

Products/Wastewater	Reactor size (m ³)	Flow rate (m ³ ·d ⁻¹)	COD _{in} (mg·L ⁻¹)	COD _r (%)	Process
Size for textile industry	65	100	12,000	85	AFB
Polyester resin & latex	86	86	4,000	70	
Polyester resin	192	400	3,000	80	
Phenol-formaldehyde resin	300	5	150,000	90	
Phenol, amino, alkyd resin	300 × 2	15	100,000	70	
Rice wine	3,000	1,000	30,000	90	UASB
Dairy, beverage	1,680	4,000	2,500	90	
Tape	1,000	400	9,000	95	
PVC stabilizer	80	80	8,000	95	
PVA, TMP, PVB	1,350 × 2	3,500	4,200	70	
Polyester fiber & yarn	600 × 2	1,200	5,000	90	
Polyester fiber	3,300	4,000	2,500	65	
Plastic additive	500	200	8,000	70	
Terephthalic acid	1,600 × 4	6,000	5,000	70	
Maleic acid/anhydride	800	124	12,000	80	

4.4. Performance comparison

Both UASBs and AFBs have been proven to effectively treat industrial wastewater in Taiwan. Except for the large implementation of DAF by Amoco and CAPCO in the treatment of wastewater from PTA manufacturing processes, the UASB is the most commonly used anaerobic bioreactor in various industries. Table 11.8 shows the different wastewater-generating industries that UASB and AFB have successfully been implemented in. Performance of each technology can be affected by different factors. For example, the response of the reactor to shock loading gives a good comparison between reactor performances. For UASBs, higher substrate concentrations and denser granules were observed at the bottom of the reactor. Sudden shock loading would drastically impact the anaerobes located at the bottom of the reactor and lower the UASB performance. In contrast, for AFB, the inflow mode used in the effluent recirculation provides a rapid up-flow velocity and a high dilution rate for the refractory wastewater. Thus, the effect of shock loading on the performance of an AFB reactor could be reduced.

5. Conclusion

Thirty years of research and development programs were pursued in Taiwan to develop three different anaerobic technologies (i.e., DAF, UASB, and AFB). Implementation of full-scale bioreactors has been shown to be effective in treating high-strength industrial wastewater in various industries. DAF and UASBs were proven to be highly effective in treating wastewater from the manufacturing processes of PTA. The UASB is also effective in treating wastewater from the PET industry and the AFB in treating industrial wastewater containing phenol and formaldehyde. The size of the full-scale anaerobic bioreactors treating various types of wastewater ranges from 80–12,000 m³. To optimize

process performance, these anaerobic bioreactors generally required a long acclimation process approximately six months or longer. Different levels of operating parameters and performance indicators should be established to maintain the syntrophic microbial community needed for the treatment process of various industrial wastewaters.

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Chapter 12

Anaerobic Sewage Treatment in Latin America

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In Latin America, the interest in high-rate anaerobic (pre-)treatment of sewage using UASB reactors is steadily growing since its introduction in the mid-1980s. A 2012 survey showed that 17% of 2,734 sewage treatment plants of six countries in Latin America used UASB technology, including 32% of the 702 plants in Brazil alone for sizes up to one million population equivalent. The main advantage of UASB technology is the very low or even zero energy demand, leading to an up to 10-fold drop in operational costs compared to activated sludge. In fact, anaerobic systems produce energy-rich biogas, which, however is rarely used as an energy source thus far. A compact UASB system can be implemented in a decentralized way, also in the urban area, which makes it very cost-effective compared to pond systems, for which much longer conveyance systems are required. Modern high-rate UASB reactors have a standardized design with hydraulic retention times of 6–10 hours, and BOD removal efficiencies reaching up to 70–80%. As anaerobic treatment only removes the organic pollutants, any additional requirement, e.g. for nutrient removal, requires a second treatment step. At present, many different combinations have been investigated of which several are successfully applied at full scale. Moreover, novel research results even show further perspectives for the development of cost-effective integrated anaerobic–aerobic systems for complete treatment. This chapter describes the current state-of-the-art anaerobic sewage treatment in Latin America and provides some outlooks for upcoming developments.

1. The Role of Anaerobic Sewage Treatment in Latin America

With the emergence of the up-flow anaerobic sludge blanket (UASB) technology in the 1980s, several Latin American countries began to adopt anaerobic sewage treatment technology to the flowsheets of sewage treatment plants (STPs). Anaerobic sewage

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treatment, in various cases followed by units of aerobic post-treatment systems, was regarded as an alternative to the traditional wastewater treatment systems used historically, such as the mechanized activated sludge and the land-based lagoon systems. This new trend of using combined (anaerobic/aerobic) sewage treatment systems, due to investments in research and development and favorable climate conditions for use of anaerobic systems, led Latin America, notably Brazil, Colombia and Mexico, to become a frontrunner in the use of UASB reactors for domestic wastewater treatment.

In Brazil, the use of UASB reactors for wastewater treatment was introduced in the early 1980s, when research by several groups of academics and engineers in the area of wastewater treatment started. While during the introduction, the inappropriate use of UASB reactors had damaged the credibility of this technology within state water companies and environmental protection agencies, this has been restored in recent decades as a result of the intensification of studies and research in the area, and also due to the experience gained in the operation of full-scale plants. Undoubtedly, a great contribution to the consolidation and dissemination of the anaerobic technology for the treatment of domestic sewage in Brazil came from the National Research Programme on Basic Sanitation (PROSAB), which was carried out from 1997 to 2007 (Chernicharo *et al.* 2001).

A recent survey in the Latin American region (Noyola *et al.* 2012) identified three major technologies for municipal wastewater treatment: stabilization ponds, activated sludge (extended aeration and conventional processes) and UASB reactors. A total survey of 2,734 treatment facilities was obtained from six countries in the region (Brazil, Colombia, Chile, Dominican Republic, Guatemala and Mexico). The distribution by number of these three technologies are 38, 26 and 17%, corresponding altogether to 81% of the surveyed facilities. It is worth noticing that the UASB system, although a newcomer in the field of municipal sewage treatment and within this specific application a history after market introduction of only 25 years, takes the third place, behind more than century-old processes. However, this picture changes when the technologies in Latin America are ordered by treatment capacity (design flow). In such cases, both versions of activated sludge turn out to be the most important, followed by stabilization ponds, enhanced primary treatment and UASB in the fourth place, i.e. 58, 15, 9 and 7% of the total design flow in the sample. It is clear that stabilization ponds, and even UASB, are widely applied in the region, but in small facilities. In fact, the survey also found that 67% of the STPs in Latin America are small, with design flows of less than $25 \text{ L}\cdot\text{s}^{-1}$, and 34% are very small, having flows of less than $5 \text{ L}\cdot\text{s}^{-1}$.

UASB reactors used for the treatment of domestic wastewater are now considered a consolidated technology in Latin America, where several large full-scale plants, treating a population equivalent up to 1,000,000 inhabitants (Onça STP, Belo Horizonte, Brazil), have been in operation for more than ten years. The costs of a treatment plant with a UASB reactor followed by aerobic biological treatment usually allow CAPEX savings in the range of 20–50% and OPEX savings above 50%, in comparison with a conventional activated sludge plant (von Sperling and Chernicharo 2005; Chernicharo 2006). This is considered to be one of the reasons for the increase of wastewater treatment coverage in Latin America. Table 12.1 summarizes the recent literature reports on the performance of full-scale municipal anaerobic sewage treatment plants, notably employing UASB reactors.

Table 12.1. Performance of the more recently installed full-scale anaerobic sewage treatment plants treating municipal sewage in different Latin American countries.

Location	STP	Effluent concentration			Removal efficiency			Population equivalent (inhabitants)	Reference
		COD (mg·L ⁻¹)	BOD (mg·L ⁻¹)	TSS (mg·L ⁻¹)	COD (%)	BOD (%)	TSS (%)		
Brazil	Septic tank + Anaerobic filter	473	-	190	39	-	36	2,141	Silva <i>et al.</i> (2013)
Brazil	UASB	283	-	132	58	-	49	3,047	Silva <i>et al.</i> (2013)
Brazil	UASB	114	38	132	79	84	59	70,000	Rosa <i>et al.</i> (2012)
Brazil	UASB	251	98	85	65	74	71	24,000	Oliveira and von Sperling (2011)
Colombia	UASB	-	60	-	-	77	-	320,000	WERF (2010)
Brazil	UASB	170	66	75	58	68	56	544,000	Franco (2010)
Brazil	UASB	247	97	112	62	67	54	-	Van Lier <i>et al.</i> (2010)
Brazil	UASB	190	70	60	60	65	61	1,000,000	Chernicharo <i>et al.</i> (2009)
Colombia	UASB	144	-	81	58	-	65	-	Peña <i>et al.</i> (2006)
Brazil	UASB	181	75	127	64	74	51	24,719	Baréa and Alem Sobrinho (2006)
Brazil	UASB	106	69	-	72	72	-	150,000	Carraro (2006)
Brazil	UASB	161	66	-	77	78	-	-	Tachini <i>et al.</i> (2006)
Brazil	UASB	237	64	127	60	69	52	3,808	Busato (2004)
Brazil	UASB	202	-	80	67	-	61	18,000	Florencio <i>et al.</i> (2001)
Colombia	UASB	177	69	72	66	78	69	9,000	Peña <i>et al.</i> (2000)

According to the survey of Noyola *et al.* (2012) and considering that their sample of 47% of the estimated total facilities in the six selected countries being representative, Brazil has the highest percentage of UASB reactors treating municipal sewage (32%), followed by Colombia (25%), Dominican Republic (20%), Guatemala (19%) and Mexico (11%). Chile has not adopted this technology for municipal wastewaters, as activated sludge (64%) and aerated ponds (35%) cover the demand (the remaining 1% are trickling filters). This data is presented in Table 12.2.

As mentioned, facultative ponds and UASB reactors in Latin America are used mainly in small- to medium-sized treatment plants. A further analysis of the size distribution shows that UASB reactors represent 18% and 19% of the small facilities, with design flows lower than 25 L·s⁻¹ and between 25–250 L·s⁻¹, respectively, and 13% and 16% of bigger ones, with flows between 250–2,500 L·s⁻¹ and higher than 2,500 L·s⁻¹, respectively. As it can be seen, the fraction of UASB reactors in the sampled facilities by Noyola *et al.* (2012) does not vary significantly with the STP size (13–19%).

That survey showed that 67% (1,842) of the facilities in the sample are small STPs, with design flows lower than 25 L·s⁻¹. Furthermore, 34% (919) are very small with design flows lower than 5 L·s⁻¹. This data is depicted in Fig. 12.1, showing details of the size

distribution of those small STPs for the two biggest countries in the region, Brazil and Mexico.

Table 12.2. Total number of sewage treatment plants (STPs) in six Latin American countries, sample sizes and number of UASB reactors in the sample (Data from Noyola *et al.* 2012)

Country	Estimated total STP in the country	STP in the sample	Percentage of estimated total facilities (%)	UASB facilities in the sample	UASB percentage in the sample (%)
Brazil	2,985	702	24	225	32
Chile	263	177	67	0	0
Colombia	583	139	24	35	25
Dominican	56	31	55	6	20
Guatemala	87	32	37	6	19
Mexico	1,833	1,653	90	188	11
Total	5,807	2,734	47	460	17

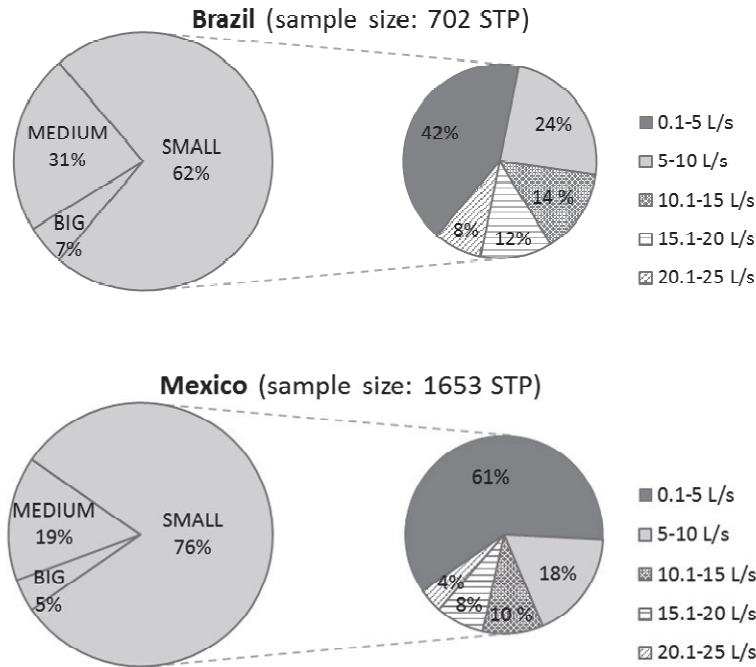


Fig. 12.1. Size distribution of the STPs in the sampled facilities in Brazil and Mexico with a zoom on smaller installations; big flow: higher than 250 L·s⁻¹, medium flow: 25–250 L·s⁻¹, small flow: lower than 25 L·s⁻¹.

The fraction of small STPs are bigger in Mexico (76%) than in Brazil (62%). Moreover, of those small facilities, the smallest ones are also predominant in Mexico (61%) if compared to Brazil (42%). This data suggests that both Brazil and Mexico are addressing the demand of sanitation and sewage treatment with small facilities rather than with centralized big STPs. However, there is evidence that many smaller facilities do not meet operation and effluent quality standards, due to improper management (Oliveira and von Sperling 2011; Noyola *et al.* 2012). In such cases, the environmental impacts generated by many small STPs could be higher than building a flow-equivalent properly operated big wastewater treatment plant (Lundin *et al.* 2000). On the other hand, the natural attenuation capacity of the environment or water bodies receiving the small (treated) decentralized flows might be just enough to deal with the (residual) pollutants. In contrast, the discharge of huge flows of partially treated urban sewage will certainly lead to an environmental overload, leading to environmental problems and human health constraint. Moreover, centralized STPs require huge sewerage networks and pumping stations, and thus require large time frames for realization, as listed in Section 3.1. Nonetheless, the survey indicates that smaller facilities are also associated with higher energy consumption per treated cubic meter (Noyola *et al.* 2012), being a concern about the sustainability of smaller versus bigger treatment facilities. The latter formulates a clear message to dedicate special attention to the operational and management issues of small STPs in order to achieve the improvement and the beneficial aspects related to decentralized and small STPs. Of course, actual impacts can only be assessed in a case-by-case basis, considering the whole system, from the collecting system to final disposal.

Specifically among the STPs, including UASB reactors in Brazil, the Onça sewage treatment plant, located in the city of Belo Horizonte, Minas Gerais state, has been considered the largest STP in Latin America to use this technology. That facility has capacity to treat $1,800 \text{ L}\cdot\text{s}^{-1}$ of sewage (population equivalent of 1.0×10^6 inhabitants), with overall organic matter removal efficiency of around 90%, including the contribution of the post-treatment with trickling filters.

It is worth mentioning that the high number of STPs with anaerobic reactors are installed in the states of Paraná and Minas Gerais, Brazil, as indicated in Fig. 12.2. A notable and remarkable feature is the predominance of anaerobic reactors in the treatment process scheme of the STPs operated by two of the most important state sanitation companies of Brazil (SANEPAR and COPASA). The figure shows that around 88 and 75% of the STPs operated by SANEPAR and COPASA, respectively, adopt anaerobic reactors (especially UASB).

2. State of the art of Anaerobic Wastewater Treatment

2.1. Current design criteria

Given the increasing importance of the UASB reactor for sewage treatment in the Latin American region, several measures should be taken in relation to the adequate design and operation of the system. One of the most important aspects of the anaerobic process applying UASB reactors is its ability to develop and maintain sludge with excellent

settling characteristics. Therefore, it is important to mention the recent publication of the technical standard “Hydraulic and sanitary engineering design for wastewater treatment plants” by the Brazilian Association of Technical Standards, under the code ABNT NBR 12209:2011. This standard was updated and for the first time included hydraulic and process engineering design criteria for UASB reactors, among other technologies. This standard was developed by a study committee made up of representatives of the private sector, public sanitation companies and universities, through national consultation prior to its publication. Specifically in the case of UASB reactors, the main design criteria can be summarized as shown in Table 12.3.

Verifications made in projects already implemented have indicated that the hydraulic retention times (HRTs) for average flows are not always within the standardized range.

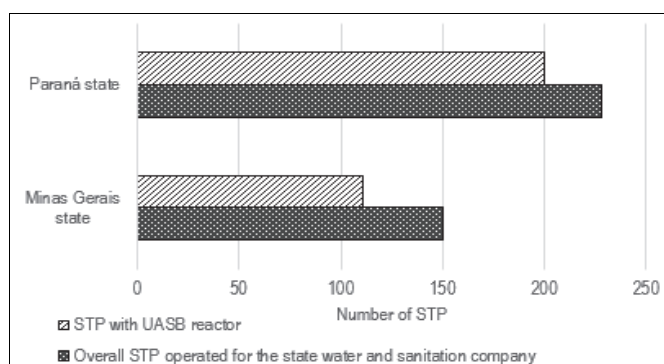


Fig. 12.2. Predominance of anaerobic reactors in STPs of two Brazilian states.

Table 12.3. Main design criteria adopted in Brazilian UASB reactors, according to national standard ABNT NBR 12209:2011.

Parameter	Unit	Value	Comment
Hydraulic retention time at average flow	h	6–10	6 h for sewage temperature > 25 °C 7 h for sewage temperature 22–25 °C 8 h for sewage temperature 18–21 °C 10 h for sewage temperature 15–17 °C
Upflow velocity at average flow	m·h ⁻¹	≤ 0.7	Less than 1.2 m·h ⁻¹ for the maximum peak flow
Useful depth	m	4–6	The minimum useful depths of digestion and settling compartments are 2.5 and 1.5 m, respectively
Feed inlet density	m ² per feed point	2.0–3.0	The minimum inlet pipe internal diameter shall be 75 mm
Angle of gas collector	degrees	≥ 50	The UASB reactors must have scum removal device

For reactors fed by pumping stations, the HRT tends to be even more reduced, sometimes reaching only 0.5 hour in the settler compartment, when there are two or more pumps in operation (von Sperling and Chernicharo 2005). Therefore, the recent publication of the standard brings an important aspect: in the case of feeding by pumping stations, the maximum pumping flow rate should not exceed more than 25% of the maximum sewage influent flow. Therefore, the use of pumps with variable speed drives or a minimum of three pumps, one for backup and rotation, is recommended.

Regarding the excess sludge withdrawal, the aforementioned standard recommends at least one discharge point per 100 m² bottom area. In addition, there should be discharge pipes with a minimum diameter of 100 mm at two different heights, close to the bottom and between 0.8 m and 1.3 m above the bottom.

With respect to the management of biogas, it is recommended that STPs with average flow capacity above 250 L·s⁻¹, without gas utilization, must have at least two incinerators, one as backup. The biogas pipeline must be designed with a maximum velocity of 5 m·s⁻¹ from the average gas flow, and a minimum diameter of 50 mm.

2.2. Current post-treatment facilities

Considering the intrinsic limitations associated with the anaerobic systems and the need to develop technologies that are more appropriate to the reality of developing countries, it is imperative to include a post-treatment stage for the effluents generated in anaerobic reactors. This stage has the purpose of polishing not only the microbiological quality of the effluents, in view of the public health risks and limitations imposed on the use of treated effluents in agriculture, but also the quality in terms of organic matter and nutrients, in view of the environmental damages caused by the discharge of these remaining pollutants into the receiving surface water.

The so-called combined systems (anaerobic/aerobic), using UASB reactors as the first biological treatment stage, allow for the achievement of the necessary efficiencies to comply with the discharge standards in most Latin American countries. The flowsheets of the most frequent post-treatment alternatives being applied are presented in Fig. 12.3.

3. Centralized versus the Decentralized Approach

Historically, sewerage systems were constructed to convey sanitary flows and urban spills away from populated areas. In the many expanding cities of the 19th and 20th century, this indeed improved the hygienic conditions considerably, leading to a drastic drop in waterborne diseases. The collected sewage was subsequently discharged to surface waters, threatening the environmental health of the receiving water bodies. The latter, however, was not yet part of governmental regulations. In the industrialized countries of Western Europe and Northern America, environmental regulations were only implemented in the last 3–4 decades of the past century. The large cities, which were already served with extensive sewerage systems, were also targeted to be the first served by STPs. The huge sewage flows of these cities had a tremendous impact on the environmental health of the recipient water bodies. In most cities, the first STPs were

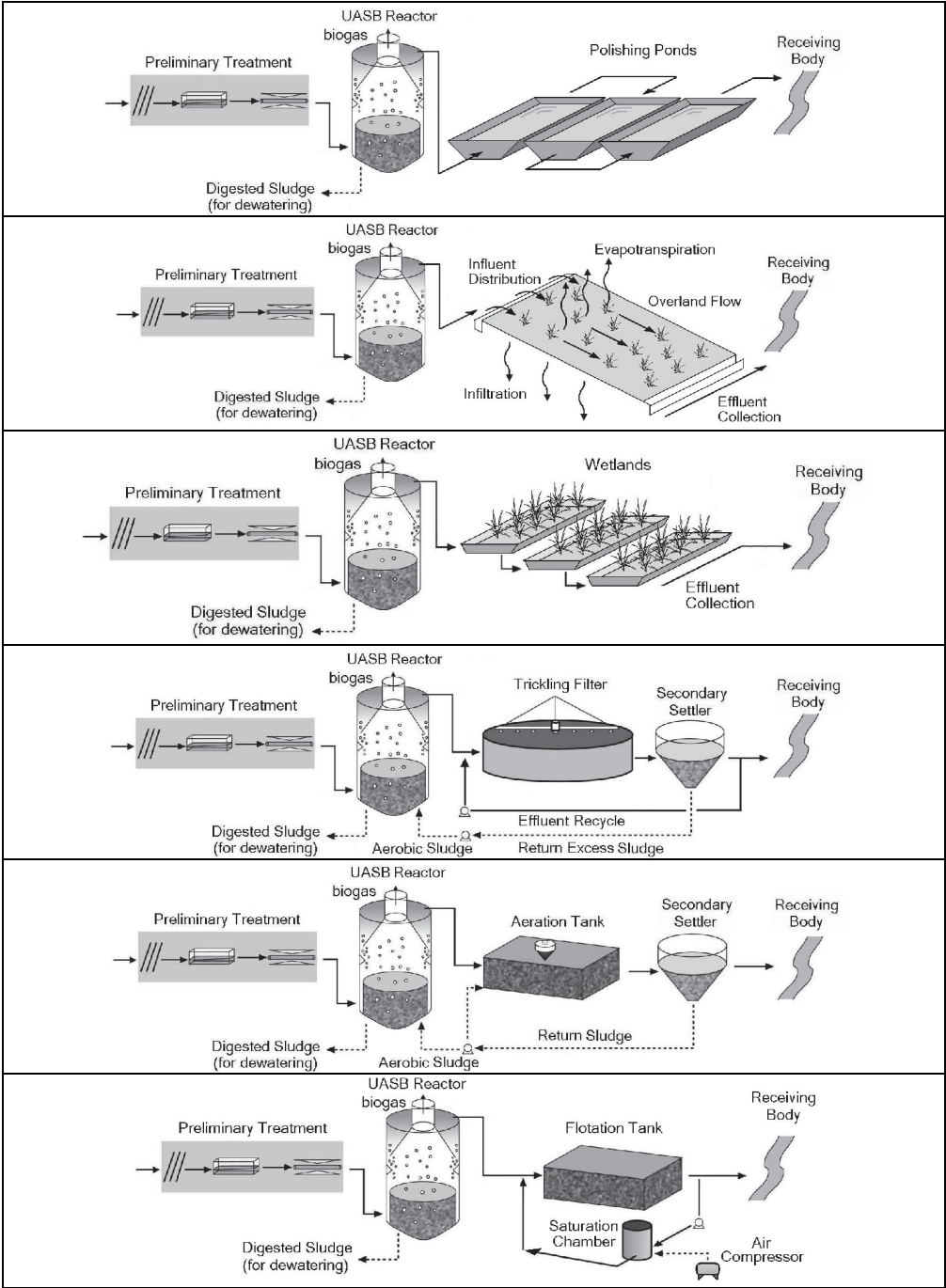


Fig. 12.3. Flowsheets of the most common combined UASB/post-treatment systems applied in Latin America (von Sperling and Chernicharo 2005).

located at the central outfall of the sewerage prior to discharge to open surface waters. By addressing this large point source, the environmental impact could be reduced by implementing a single STP. As such, centralized sewage treatment was borne, being a logical consequence of historic developments. However, this centralized approach also puts a financial burden to authorities for constructing, maintaining and extending these services to all citizens (Lettinga *et al.* 2001).

The centralized treatment approach, with its advantages of economy of scale, has developed as a kind of blueprint for sanitary systems, sewerage and treatment. Particularly in hilly areas, the centralized sewerage systems require pumping stations and siphons, as well as large trunk sewers in order to collect all the sewage from the expanding cities. With the full coverage by multi-tap drinking water supplies at the household level and the increase in drinking water consumption, the sewage outfalls became huge and so also the required STPs. The latter became industrial complexes consisting of advanced technology, requiring highly qualified personnel. The discrepancy between the served large areas in industrialized countries and non-served areas in the less prosperous countries became larger and larger. Up to date, the centralized approach is more than often considered as the blueprint for adequate sanitation and environmental protection. This has resulted in situations where governments pursue centralized sanitation and high-level treatment, but is not able to implement this owing to huge financial constraints (van Lier and Lettinga 1999). Painful examples can be found in Latin America where stringent environmental laws are indeed met at very few centralized treatment plants in large urban areas, whereas the majority of communities are not even served by primary treatment.

Recognized constraints of the centralized approach are:

- High investment costs for (trunk) sewers, pumping stations and siphons. Regular maintenance is indispensable and renovations are required every 60–70 years.
- Limited flexibility owing to long planning horizons. Difficult to anticipate on large demographic changes.
- Central outflow (even if treated) pose a high load of pollutants to the environment. As such, more advanced treatment is required with a higher degree of centralization.
- Gravity flow sewer systems require minimum flow conditions to prevent sewer clogging. In (semi-) arid climate countries, which suffer from limited tap water supply, minimum flows are not guaranteed.
- Centralized systems generally consist of sewers that carry both urban sanitation and urban drainage of pluvial waters. This approach results in large flows of contaminated water.
- Extensive combined sewerage networks have limited hydraulic capacity. Exceeding this capacity results in sewage overflows, contaminating the environment.
- Extensive sewerage systems are vulnerable for ruptures and cracks, particularly in seismic sensitive areas, which may result in severe pollution of water reservoirs and aquifers.
- Urban populations sense little ownership of centralized services, possibly resulting in discharges of hazardous compounds into the sewer by residents, industries, etc. (“out

of eye, out of concern”). Toxic discharges will constrain the STP and the possible reuse of treatment by-products.

- Combined centralized sewer systems in relation to a fully paved urban environment results in the possible exportation of rainwater from the residential areas, leading to decreasing groundwater levels in the urban area.

3.1. *Application of decentralized systems in Latin America*

Local conditions fully determine what the most proper sanitation approach will be taking socio-economic and environmental constraints into account. Proper sanitation is a function of mass flow per area per time unit, in which socio-economic factors determine the pallet of sanitation solutions (Letema *et al.* 2014). Sanitation option criteria will finally determine which solution is most adequate at a specific location (Malekpour *et al.* 2013). Although the coverage by sewer systems is steadily increasing, the population growth in Latin America and the Caribbean has surpassed the capacity of national and local governments in order to meet the demand for water supply and sewerage (Noyola *et al.* 2012). Moreover, disposal of the collected sewage is merely uncontrolled. This situation can be verified with data from the Water and Sanitation Program that established that Latin America and the Caribbean countries reached 91 and 79% coverage of water supply and sewerage, respectively, but provided treatment to just 15% of municipal wastewater (WSP 2007). Lack of integrated planning is a major obstacle. The construction of sewerage systems follows urban growth without planning proper disposal of the collected urban wastewaters. Moreover, major cities have the prime attention; meanwhile, the devastating impact of the uncontrolled discharge of decentralized collected flows seems to be ignored.

However, progress has taken place in Brazil with the project called ReNTED — National Network of Decentralized Sewage Treatment, which brings together 13 important Brazilian universities. The general goal is to develop local and decentralized systems of sewage treatment, including sustainability aspects and management of liquid, solid and gaseous by-products.

4. Current Limitations and Constraints

4.1. *Preliminaries*

Although the application of anaerobic technology has significantly expanded in Latin America in the last 10–15 years, some limitations and constraints still need to be solved and have guided the investigations of many research institutions and operators, as discussed in this section. The design, operational and managerial aspects of UASB reactor systems especially need improvements, since the further expansion of the technology and its wider acceptance in the near future can be significantly hindered by sub-optimal functioning UASB.

Research has been focused on topics aiming at improving the design and operation of UASB reactors. Particularly, research related to scum accumulation, biogas and waste gas

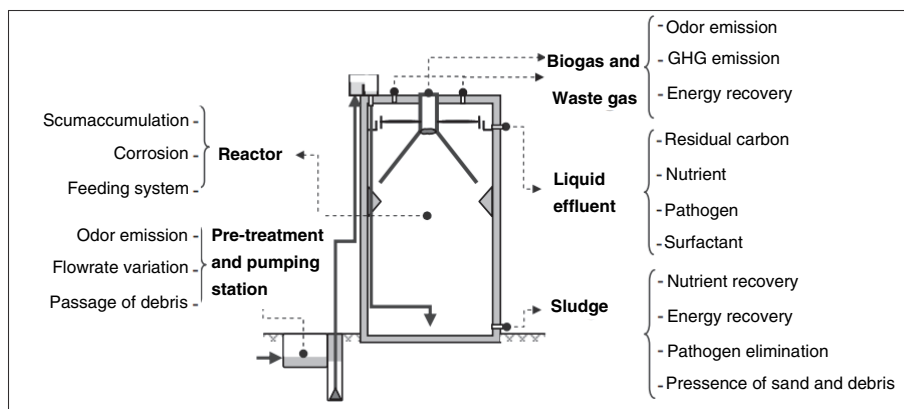


Fig. 12.4. Topics of interest for improvements in UASB reactors treating domestic wastewater.

management, post-treatment and energy recovery, received the most attention, as highlighted in Fig. 12.4. The main constraints remain on the potential odor problems and difficulties associated with it, but also on nutrient removal in the treatment scheme, as well as on the operational constraints as discussed in Section 4.5. The overall advantages and constraints of anaerobic sewage treatment in comparison to activated sludge processes are listed in Table 12.4.

4.2. Restrictions for nutrients

When nutrient removal is required to meet the quality standards of the receiving water body, the use of anaerobic processes preceding a complementary aerobic treatment for biological nutrient removal should be carefully analyzed. Anaerobic systems present good biodegradable organic matter removal, but the concentrations of N and P in the effluent might even be higher than in the influent. When considering conventional nutrient removal techniques, the sole removal of BOD in the anaerobic reactor certainly causes a negative effect on biological treatment systems aiming at nutrient removal. Notably, the effluent from the anaerobic reactor will have N/COD and P/COD ratios much higher than the values desired for the good performance of the mentioned conventional biological nutrient removal processes.

When nitrogen removal has to be accomplished, the application of conventional nitrification–denitrification processes are so far selected to complement the UASB reactor. In such cases, the anaerobic reactor should only treat a part of the influent raw sewage (possibly no more than 50–70%). The remaining part (30–50%) should be directed to the complementary biological treatment, aiming at nitrification and denitrification, so that there is enough organic matter for the denitrification step. In this case, the big advantage of the use of the anaerobic reactor is to receive and stabilize the sludge generated in the complementary treatment, eliminating the need for an anaerobic sludge digester. For concentrated sewage, as observed in the Middle East, the recycle of nitrified effluents to the UASB reactor for combined nitrification–denitrification can be

Table 12.4. Advantages and constraints of high-rate anaerobic sewage treatment systems over aerobic processes.

Advantages	Constraints
<ul style="list-style-type: none"> • Substantial (reaching 90%) savings in operational costs as no energy is required for aeration • Potential reductions in investment cost considering that primary clarification, the bioreactor, secondary clarification and the sludge digester are combined into one tank: the UASB reactor. However, the UASB reactor needs to be extended by a post-treatment step to reach effluent requirements • The produced methane (CH₄) is of interest for energy recovery or electricity production • The technologies do not make use of high-tech equipment, except for main headwork pumps and fine screens. The treatment system is less dependent on imported technologies • The process is robust and can handle periodic high hydraulic and organic loading rates • The system is compact with HRTs of 6–9 h, and is, therefore, suitable for applications in urban areas, minimizing conveyance costs • Small-scale applications allow for decentralized treatment, making sewage treatment less dependent on the extent of sewage networks • The sludge production is low, well stabilized and easily dewatered; consequently, it does not require extensive post-treatment • The valuable nutrients (N and P) are conserved, which give the treated wastewater a high potential for crop ferti-irrigation 	<ul style="list-style-type: none"> • Anaerobic treatment converts most of the influent COD into CH₄. However, the extent of organic matter removal is less than the activated sludge processes, requiring in most cases, adequate post-treatment to meet the discharge or reuse criteria • The produced CH₄ is partially dissolved in the effluent (depending on the influent COD concentration and the applicable hydraulic flow). So far no measures are applied in full-scale plants to prevent CH₄ escaping to the atmosphere • The collected CH₄ is often not utilized for energy generation and in some cases not even flared (contribution to greenhouse gas emissions) • There is little experience with full-scale application at moderate to low temperatures • Reduced gases, like H₂S, that are dissolved in the effluent may escape causing odor problems • High influent sulfate concentrations may limit the applicability of sewage treatment as it results in the conversion of organic BOD/COD to inorganic BOD/COD, meaning that organic matter gets degraded while the sulfate gets reduced to the odorous and corrosive sulfide

considered (Kassab 2009; Kassab *et al.* 2010). However, in Latin America such a concept is of much less relevance since the UASB reactor design is already determined by the hydraulic flow. A recycle would immediately require a larger reactor volume. Considering that the main focus in Latin America is merely the removal of organic matter and suspended solids, there are a few plants designed for nitrogen removal, being more common to find STPs with only ammonia removal (nitrification). In this sense, the main experiences have been with the application of activated sludge plants and, more recently, biological trickling filters filled with sponge-based packing media (demo-scale), which achieved about 80–90% ammonium-N removal, associated with low excess sludge production (Almeida *et al.* 2013). Demo-scale experiences in India even showed complete ammonium-N removal as well as 30–40% total N removal operating a sponge bed trickling filter following a UASB reactor (Uemura and Harada 2010).

As the requirements of environmental agencies will become more restrictive in the near future, the development of research on post-treatment of UASB effluent should be emphasized. The efforts may be on: (i) the simultaneous removal of ammonia and nitrate in structured bed reactors with intermittent aeration (Gadelha *et al.* 2013); (ii) the simultaneous removal of ammonia and nitrate in tertiary aerobic–anoxic fixed-bed reactor using biogas as an electron donor (Pantoja Filho *et al.* 2013); (iii) the use of electron donors present in the liquid and gaseous phases of the anaerobic chamber for denitrification in an anaerobic–anoxic reactor coupled with a nitrifying reactor (Morgan-Sagastume *et al.* 1994; Souza and Foresti 2013; Okada and Foresti 2013); and (iv) the use of partial nitrification to nitrite combined with ammonium oxidation to nitrogen gas, the so-called Anammox reaction (Sánchez Guillén *et al.* 2014, 2015).

Also, the application of biological phosphorus removal is constrained with the use of an anaerobic reactor for the main treatment step, for two main reasons: i) the effluent from the anaerobic reactor presents a P/COD ratio higher than that of the raw sewage, which harms the performance of the biological phosphorus removal system; and ii) if the phosphorus-rich sludge generated in the biological phosphorus removal treatment is directed to the anaerobic reactor for stabilization, the phosphorus incorporated to this sludge will be released under anaerobic conditions and leave with the effluent from the anaerobic reactor. Both will significantly hamper the application of a conventional Bio-P system as post-treatment step. At present, phosphorus removal in treatment plants using anaerobic reactors seems to only be effective if chemical products are used for P precipitation (iron or aluminum salts).

With regard to P removal, the application of dissolved air flotation processes in some large sewage treatment plants in the Brazilian state of Paraná and the Federal District is worth mentioning. An interesting example is the Samambaia (D.F.) STP (population equivalents: 180,000 inhabitants), which has a high-rate anaerobic reactor incorporated into a facultative pond system. The monitoring by the sanitation company (unpublished results) has demonstrated total phosphorus removals of around 95%.

4.3. Restrictions for pathogens and microbiological indicators

As with most secondary treatment methods, compact anaerobic processes are not efficient at eliminating pathogenic organisms from the effluents and, as a result, require a post-treatment stage if pathogen removal is pursued. For small systems and under proper conditions, polishing ponds can be a very effective method for improving the microbiological quality of anaerobic effluents (von Sperling *et al.* 2004). If properly designed and implemented, they can achieve very high levels of pathogen removal, with virtually 100% helminth eggs and protozoan cysts removal, and 3–6 log units removal for bacteria and viruses (von Sperling and Chernicharo 2005). In addition, the ponds also polish the anaerobic effluent in terms of organic matter and oxidize ammonia. Alternatively, the solubilized ammonia can be removed mainly through algal uptake and also volatilized in the form of ammonium (NH_3), due to the high pH as a result of an intense phototrophic activity (Camargo Valero and Mara 2007).

In situations when land availability is limited, a compact disinfection process, such as chlorination, UV radiation and ozonation, should be regarded as options for the post-treatment, as means of improving the overall efficiency of pathogen removal, especially bacteria and viruses. However, with regard to chlorination, the risk of the formation of disinfectant by-products is very high, owing to the relatively high concentrations of residual organic matter in the UASB effluents.

Cost-effective pathogen removal along with extensive aeration of residual compounds was obtained in the so-called down-flow hanging sponge (DHS) system in combination with a UASB pre-treatment (Uemura and Harada 2010; Tandukar *et al.* 2005). The DHS is in fact a biotower trickling filter with reinforced polyurethane as packing material. Owing to the open structure of the DHS, the effluent is passively fully aerated by improved convective airflows. The developing aerobic biomass appeared to be a very successful scavenger of colloidal pathogenic biomass.

A recent study (unpublished results) carried out in a UASB reactor (16.8 m³) followed by a pilot-scale trickling filter also filled with polyurethane media (Centre for Research and Training in Sanitation UFMG/COPASA, Belo Horizonte, Brazil) showed similar good results regarding the removal of *Escherichia coli* and total coliforms (approximately 3 log units). Additionally, this system has operated without secondary clarifiers due to the low excess sludge production, which is an important operational strategy in the simplification of the conventional flowsheet of this technology.

4.4. Odor nuisance

Odorous emissions are a huge concern in anaerobic reactors treating domestic sewage, which certainly may hamper the diffusion of the technology in Latin America. To avoid the population's complaints, several sewage treatment plants have been employing considerable amounts of resources with the application of chemical products, with the goal to minimize or mask the hydrogen sulfide and/or other odorous emission in the vicinity. In most cases, there is no clear identification of the emission's source, which may be related to the influent sewage characteristics, reactor performance or the turbulent discharge of the effluent.

Methane and carbon dioxide are the main gaseous products of the anaerobic digestion; nevertheless, depending on the nature of the incoming precursors, pH and redox potential, different odor-related substances may be biologically formed in anaerobic reactors. Most of the odorous compounds are reduced sulfur and amino compounds, such as sulfides, mercaptans and amino-sulfides. Hydrogen sulfide, resulting from the de-assimilative reduction of sulfates or thiosulfates, is the most common compound associated with the sewage odors, but other sulfur compounds may also contribute (van Langenhove and de Heyder 2001). In the sewer networks and interceptor sewers, most of the generated sulfide occurs in the biofilm layer fixed on the walls of pipes or sludge deposits at the bottom of the pipes (WEF 2004). Table 12.5 shows the typical H₂S concentrations in the atmosphere of different units of sewage treatment plants and sewerage system.

Although there are several alternatives for the control of odorous emissions, the selection criteria for the most proper alternative depends on two main criteria: gas flow and odorous gases concentration.

Regarding odor control in anaerobic reactors, several other criteria should be considered (Burgess *et al.* 2001; Kennes *et al.* 2001), e.g.:

- odorous gases' biodegradability;
- local characteristics, including human resources;
- source of emissions and design aspects related to gas capture and conveyance;
- the relative concentration of H₂S/CH₄;
- energy recovery plans;
- treatment goals.

The decision on which alternatives should be considered for the control of odorous emissions must result from a balance between technical, economic and environmental criteria. This analysis should take into account the quantitative and qualitative aspects of each alternative, as shown in Fig. 12.5. Developing countries such as those in Latin America can be regarded as being in an intermediate or transition situation, and solutions should be assessed case-by-case.

Table 12.5. Typical atmospheric concentrations of H₂S in different units of sewage treatment plants and sewerage systems.

System Unit	Average concentrations or range variations		Reference
	mg·m ⁻³	ppm	
Sewerage	0 to 417	0 to 300	Matos and Aires (1995)
Pumping	0.57	0.4	Silva <i>et al.</i> (2007)
station	1 to 3	0.7 to 2.0	Antunes and Mano (2004), Silva <i>et al.</i> (2007)
Waste gas ¹	0 to 73	0 to 50	Pagliuso <i>et al.</i> (2002), Souza (2010), Souza <i>et al.</i> (2010)
Waste gas ²	146 to 730	100 to 500	Pagliuso <i>et al.</i> (2002), Souza (2010)

¹ Gas from the settling zone of the UASB reactor; ² Gas from the dissipation chamber downstream the UASB reactor

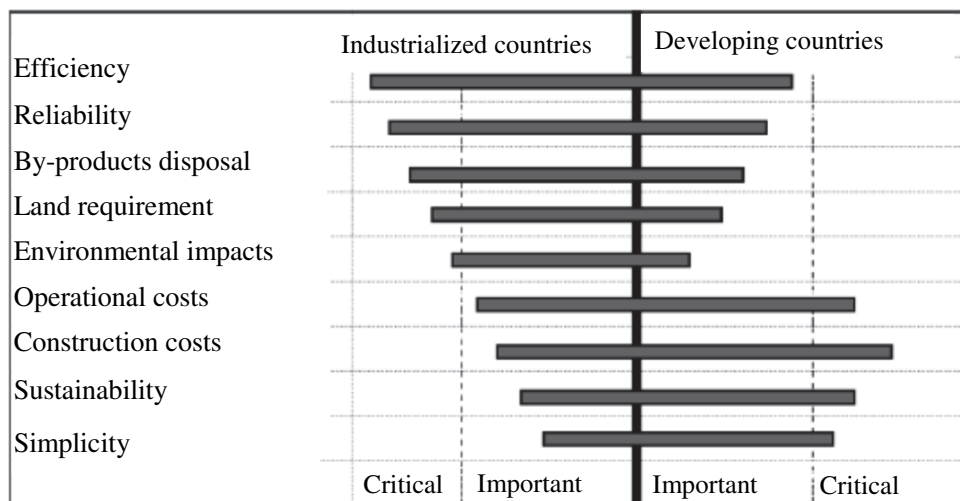


Fig. 12.5. Aspects to the alternative selection of odor treatment. Adapted from von Sperling (1996).

In a qualitative analysis of the main features of each method for the treatment of odorous emissions from sewage treatment plants, Chernicharo *et al.* (2010) indicate that direct combustion and biochemical methods, and particularly the biofilters, are bringing together the largest number of advantages for the treatment of waste gases. This results from considering the critical and priority factors in developing countries, like sustainability, simplicity and low implementation and operational costs.

Removal of H_2S in the biogas stream can be achieved by thermal oxidation, using methane as fuel. However, there may be incomplete combustion of the H_2S in the case of conventional burners (open), leading to the formation of sulfuric acid. To ensure complete sulfide combustion, sealed burners must be used, with combustion chambers. The biogas energy recovery for more valuable purposes (e.g., a vehicular fuel, injection into the natural gas line) generally require methods that enable the selective removal of H_2S and the enrichment of methane in the biogas.

Different alternatives can be considered for removing the dissolved sulfide in the reactor effluent, such as stripping in a dissipation chamber, as reported by Souza *et al.* (2012) and Glória *et al.* (2014), followed by a treatment step (for example, using a biofilter). Additionally, important reductions of hydrogen sulfide (>80%) dissolved in the treated effluent were observed after crossing the discharge hydraulic structure of the demo-scale UASB reactor (pipeline and splitting box), largely due to high turbulence causing the emissions into the atmosphere. This indicates that dissolved H_2S can possibly be entirely released from the effluent in the case of more turbulent or long discharge pipelines. This also calls for attention to the proper design of these discharge structures. Notwithstanding, if the STP has some liquid phase aerobic system as a post-treatment of the anaerobic effluent, this is the simplest and most cost-effective for the treatment of odors, since the biochemical oxidation of H_2S can be easily reached in these systems. A

number of dedicated technologies are on the market to remove sulfides from anaerobic effluents and the produced biogas (Lens and Pol 2000; Noyola *et al.* 2006).

4.5. Operational constraints

4.5.1. Process operation with low skilled personnel

Lack of qualified operators seems to be one of the major problems in the sewage sector in Latin America and countries like India (van Lier *et al.* 2010). In Brazil, only by the end of the last decade, sewage treatment started to become a priority by local, state and federal authorities and, therefore, the effects of new policies in the sanitation sector are still to come. Although new investment plans in the last decade facilitated the construction of several treatment plants in all Brazilian regions, there is still a clear lack of qualified personnel to work in these newly constructed plants. The result is that various plants are poorly operated, especially regarding the correct management of excess sludge and scum in UASB reactors.

In this respect, in order to avoid the unwanted loss of solids in the final effluent and the problems associated with the non-removal of scum on a regular basis, as discussed later in this section, there is a strong need to establish operational routines for excess sludge management and scum removal from the inner part of three phase separators. So far, only a few plants in Brazil have these routines adequately implemented, either due to design constraints or to sole availability of low-skilled personnel.

4.5.2. Design and construction

Although the design and construction of UASB reactors have experienced improvements in the last decade, there are some constraints that still affect the proper operation of UASB reactors. In Brazil, the following problems have been reported in some plants:

- use of inadequate materials and coatings, as these can cause corrosion problems in concrete and metal structures;
- use of inadequate dewatering systems, as this can negatively impact the excess sludge management. For instance, the use of mechanized dewatering systems is difficult to be maintained in continuous operation in many plants, even in large ones;
- lack of or inadequate scum removal devices, as this can pose serious difficulties to the adequate management of the scum that accumulates inside the three phase separators of UASB reactors. This can be a major problem in various UASB reactors that were designed and constructed in previous years. Only more recently, the reactors have been designed with proper scum removal devices;
- use of inadequate hydraulic profiles along the treatment train, as these can seriously impact the management of dissolved gases, especially methane and hydrogen sulfide, that can be released into the atmosphere;
- installation of unlevelled collection weirs, as this can lead to problems of preferential fluxes inside the settler compartment of UASB reactors, as well as to scum accumulation near the weirs that are positioned at higher levels, due to flux reduction or even cease.

4.5.3. Sludge withdrawal

One of the main features of UASB reactors is their great capacity for biomass retention when operated under suitable operating conditions, resulting in high sludge ages and conferring a greater degree of sludge stabilization. The excess sludge can be dried in sludge beds and no smell is expected to arise (van Lier *et al.* 2010). Even though UASB reactors present a high capacity of biomass retention, excess sludge withdrawal must be performed regularly and in a suitable way, otherwise it may cause excessive solids loss to the settling compartment. The consequences of this operational failure are a deterioration of the effluent quality and the occurrence of operational problems in the post-treatment unit, notably in the case of trickling filters (Chernicharo and Almeida 2010).

Although the establishment of proper operational routines for excess sludge management is known as one of the most important points to be improved in sewage treatment using UASB reactors (Chernicharo *et al.* 2014), this is far to be achieved, especially in small-scale plants, where the lack of qualified personnel is more notorious.

4.5.4. Scum removal

A major operational limitation reported in most full-scale plants is the removal of scum that accumulates inside the 3-phase separators. The accumulation and irregular removal of scum leads to blockage of the natural passage of gas, which in turn impairs its collection and imposes hurdles for energy recovery. This problem has been addressed by many authors (Halalsheh *et al.* 2005; Souza *et al.* 2006; Pereira *et al.* 2009).

Advances in scum removal from UASB reactors have been experienced through the design of hydrostatic removal devices (Chernicharo *et al.* 2013), which are based on the control of the water level within the 3-phase separator. It is achieved by increasing or decreasing the pressure in the gas line situated between the 3-phase separator and a water seal located on the top of each reactor. Controlling the water level within the gas chamber allows the scum to pour into a weir, routing the material to disposal. Tests conducted in a full-scale UASB reactor (Laboreaux STP, City of Itabira, Brazil) indicated that the appropriate adjustment of pressure in the gas line and the resulting level of scum within the gas chambers enabled the effective removal of scum (Fig. 12.6).

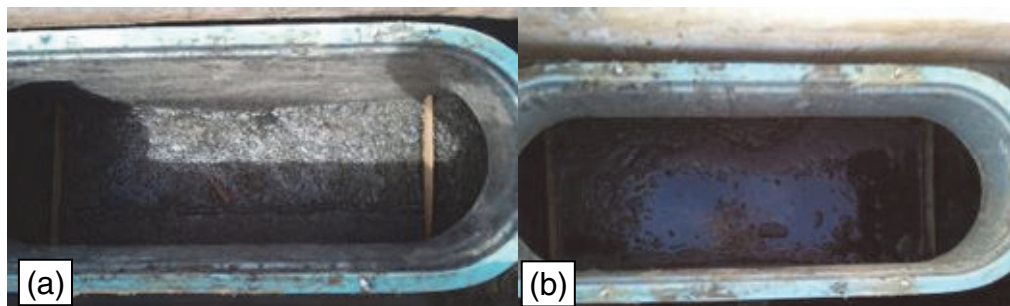


Fig. 12.6. Visual inspection of the scum layer under the hatch of a 3-phase separator: (a) before (thick scum layer) and (b) after (virtually no scum) the removal test. Source: Chernicharo *et al.* (2013).

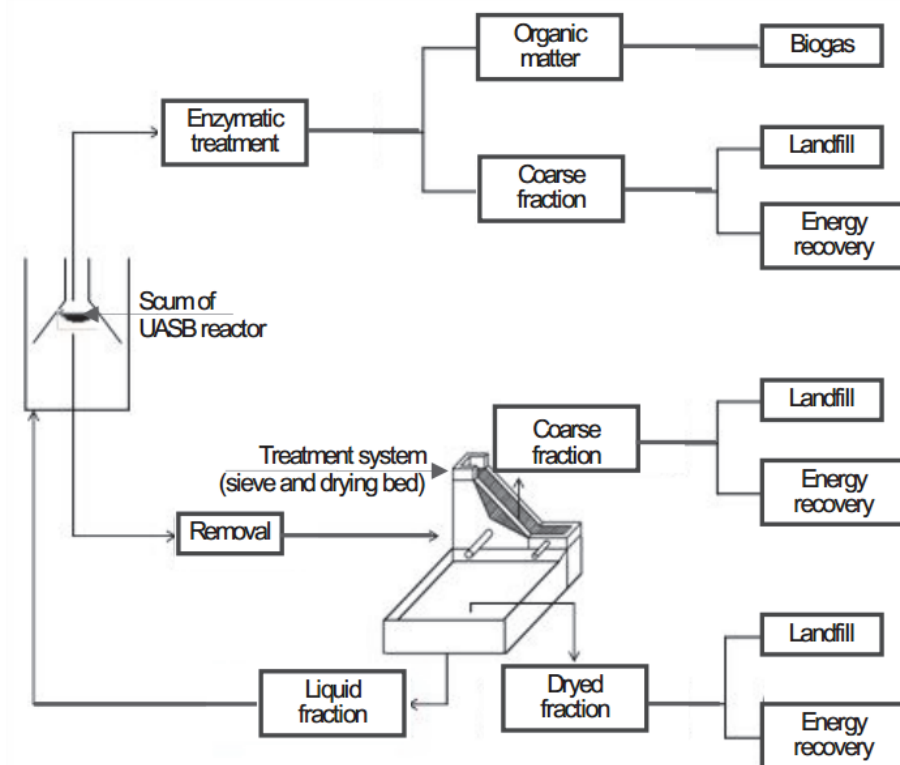


Fig. 12.7. Flow-sheet of some possibilities for scum management.

Another important aspect is the management of the scum removed from the reactors, since this material is very heterogenic and presents a high amount of coarse material originally present in the raw sewage, which was not retained in the screens and sieves of the preliminary treatment. The experience so far indicates some possibilities for the management of this material, as depicted in Fig. 12.7.

The use of static sieves and drying beds (Figs. 12.8 and 12.9) to allow the separation of the coarse material present in the scum was successfully tested at the Laboreaux STP in Brazil. The liquid fraction separated from the coarse material showed a very good infiltration rate in the drying bed, resulting in a virtually complete dewatering in less than three days. The dewatering period is a very important design parameter of the system, since large amounts of liquid are removed together with a very small coarse fraction of the scum.

4.6. Atmospheric methane emissions

Although biogas produced in UASB reactors treating domestic wastewater usually presents high methane contents, significant amounts of the gaseous products are not recovered (Hartley and Lant 2006; Noyola *et al.* 2006; Souza *et al.* 2011). With municipal wastewater as substrate, composition of the produced biogas is in the following intervals: 70–80% CH₄, 10–25% N₂ and just 5–10% CO₂ (Noyola *et al.* 1988;

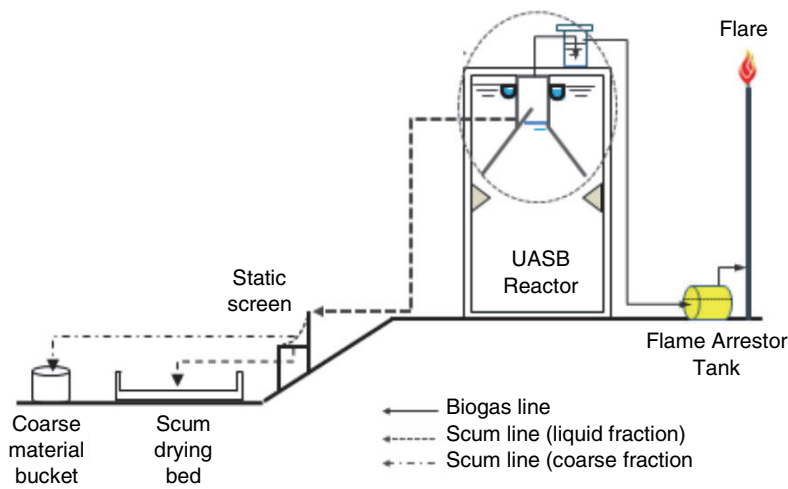


Fig. 12.8. Schematic flowsheet of the scum removal system tested at the Laboreaux STP.



Fig. 12.9. Pilot scum management system tested at the Laboreaux STP: (a) and (b) static sieve before and after scum application; (c) and (d) scum in the drying bed just after the removal operation and after the drying period (72 hours).

Souza *et al.* 2011; Souza *et al.* 2012; Lobato *et al.* 2013). The concentrations of the various gases are typical for sewage and can be ascribed to the high hydraulic flow and the relatively low temperature. The high N_2 content can be attributed to the solubilized N_2 in the influent, which inside the UASB, escapes from the liquid when the N_2 partial pressure drops to low levels. The low CO_2 content can be attributed to the high solubility of CO_2 and the high hydraulic flow. The recovered methane in the gaseous phase is well below the stoichiometric value of $0.35 \text{ Nm}^3 \cdot \text{kg}^{-1}$ COD removed, due to an important fraction that is dissolved in the effluent. In addition, a substantial fraction of suspended COD is non-methanized and leaves the reactor with the excess sludge. Under the general sewage conditions in Latin America, i.e. COD concentrations $<1000 \text{ mg} \cdot \text{L}^{-1}$ and temperatures around 20°C , the solubilized effluent CH_4 is between 30 and 41% (Souza *et al.* 2011) or also more than 50% of the produced amount (Noyola *et al.* 1988).

In general, high rates of methane losses occur at the exit hydraulic structures of the reactor, where the partial CH_4 pressure drops to zero, particularly under high turbulence conditions (Souza *et al.* 2012). In a recent study carried out by Souza *et al.* (2011), the authors found that although COD removal in the UASB reactor was considerably high (around 70%), only around 36% of the removed COD was recovered as biogas. In relation to the methane balance itself, less than 60% of the produced methane was effectively recovered as biogas in the gas chamber, while 36–40% of the methane left the reactor dissolved in the effluent. The remaining fraction (around 5%) was emitted as waste gas at the top part of the reactor in the settling zone. These losses not only represent loss of potential energy, but also contribute to the emission of greenhouse gases.

If methane losses are not taken into account, the theoretical estimation of biogas production for the purpose of energy recovery can go far above the field measurements. A model that allows for a better estimation of the methane losses, as well as the effective energy potential from UASB reactors treating domestic wastewater was developed by Lobato *et al.* (2012). The model considers the relevant COD conversion routes for energy potential estimation, allowing for the calculation of the effective COD fraction converted into methane, as well as the amount of methane dissolved in the final effluent or accounted as waste gas.

Figure 12.10 shows the COD mass balance obtained from the simulations performed by Lobato *et al.* (2012), in which the contribution of each portion of the mass balance of COD may be observed for the three simulations analyzed. In Fig. 12.10, the worst scenario considers systems operating with more diluted waste, higher sulfate concentrations, lower COD removal efficiencies, and higher rates of methane loss. The best scenario involves systems operating with more concentrated waste, lower sulfate concentrations, higher COD removal efficiencies, and lower rates of methane loss. The typical scenario considers intermediary values for the input data. In the worst case scenario, only 20% of the influent COD is recovered as methane in the gas chamber, while in the best case scenario it reached 39%. These results indicate that a significant fraction of the influent COD may not be recovered as biogas in the gas chamber, and the amount of methane available for energy generation will strongly depend on the amount of methane dissolved in the bulk liquid.

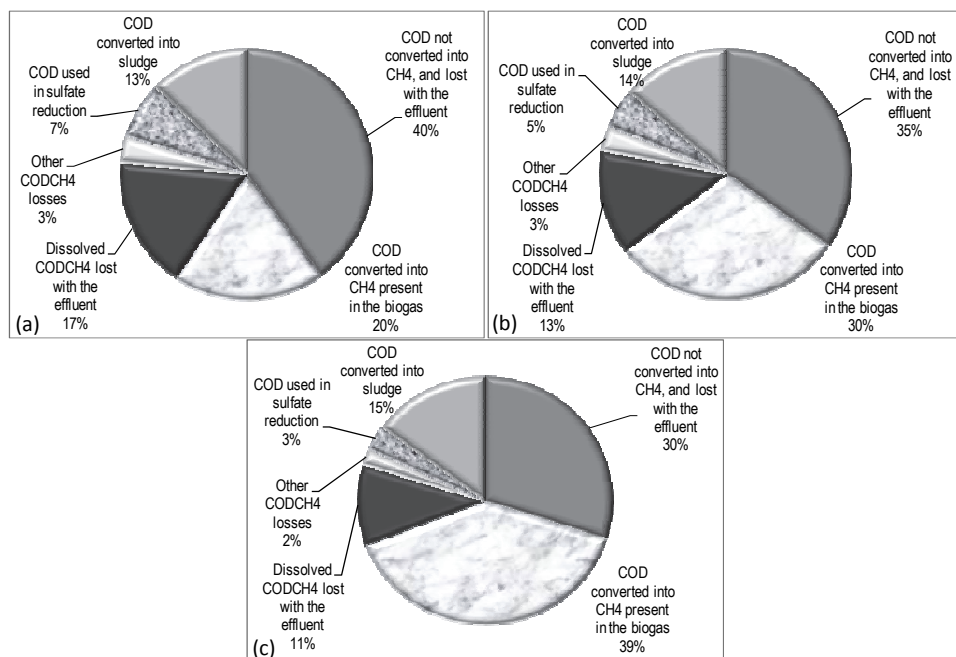


Fig. 12.10. Result of the simulations of COD mass balance in UASB reactors treating domestic waste, in relation to the influent COD for three scenarios: (a) worst; (b) typical; (c) best. Source: Lobato *et al.* (2012).

Based on the simulations performed by Lobato *et al.* (2012), the following unitary relationships were obtained for methane, biogas and energy production in UASB reactors treating typically domestic wastewater (Table 12.6).

Facing the lack of sanitation and treatment infrastructure in developing countries, the adoption of anaerobic treatment technologies for future sewage treatment plants would accomplish low capital investments and reduced operational costs, when compared to conventional full aerobic options. Whether the provision of adequate sanitation and treatment outweighs the potential emission of greenhouse gases coming from the UASB effluents is a debate that needs careful attention. Such a debate should include the actual quantitative contribution of these emissions relative to the global emissions from other sources worldwide.

Undoubtedly, the climate extremes, notably the scarcity of water due to prolonged drought periods, like Brazil has been suffering in densely populated areas in the last two years, will affect the composition of sewage, turning it more concentrated. In fact, that expected change would favor the anaerobic processes.

Table 12.6. Unitary relationships for the production of methane, biogas and energy in UASB reactors treating domestic wastewater.

Unitary relationship	Unit	Worst scenario			Typical scenario			Best scenario		
		Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean
Unitary methane yield	NLCH ₄ ·inhab.day ⁻¹	9.9	3.6	6.8	13.3	7.4	10.2	16.7	11.1	13.7
	NLCH ₄ ·m ⁻³ wastewater	81.7	16.7	42.2	103.7	34.8	64.2	134.6	51.8	81.3
	NLCH ₄ ·kg ⁻¹ COD _{removed}	154.1	66.0	113.4	185.8	124.2	158.3	219.1	173.9	196.0
Unitary biogas yield	NLbiogas·inhab.day ⁻¹	14.1	5.2	9.8	17.7	9.9	13.6	20.8	13.9	17.1
	NL biogas·m ⁻³ wastewater	116.7	23.8	60.3	138.3	46.4	85.6	168.3	64.8	101.6
	NLbiogas·kg ⁻¹ COD _{removed}	220.1	94.3	162.0	247.8	165.6	211.1	273.9	217.4	245.0
Unitary energy potential	MJ·m ⁻³ wastewater	2.9	0.6	1.5	3.7	1.2	2.3	4.8	1.9	2.9
	MJ·kg ⁻¹ COD _{removed}	5.5	2.4	4.1	6.7	4.5	5.7	7.9	6.2	7.0
	MJ·Nm ⁻³ biogas	25.1	25.1	25.1	26.9	26.9	26.9	28.7	28.7	28.7
	MJ·inhab.day ⁻¹	129.5	47.7	89.7	173.8	96.8	133.8	218.4	145.7	179.3

NLCH₄: Normal litre of CH₄; NLbiogas: Normal litre of biogas; MJ: Mega Joule

Source: Lobato *et al.* (2012)

5. Challenges

5.1. *Energy recovery from biogas*

Energy recovery from biogas is still in its early stages in Latin America. While many of the small STPs using UASB technology just vent the biogas, the majority of the larger STPs burn the biogas, in order to reduce odor and the emission of greenhouse gases (methane is the main constituent of biogas). In both situations, the biogas energy potential is simply wasted. However, the high calorific value of biogas generates great interest in exploiting this gas mixture in processes that require the use of energy, such as its use in sewage treatment plants, e.g., for post-treatment aeration systems. The use of biogas as a source of renewable energy could enable decentralized power generation and is in line with the concept of sustainable development.

5.1.1. *Constraints*

The main constraints related to energy (biogas) recovery from UASB reactors treating domestic wastewater are related to:

- high amounts of gaseous methane staying in solution in the bulk liquid and being washed out with the liquid effluent, resulting in high losses of energy potential. As discussed in Section 4.6, the measurements carried out in full-scale plants in Brazil accounted for around 36–40% of the produced methane leaving the reactor dissolved in the effluent. Other losses, via leaks and emissions via the surface of the settler compartment, can also occur.
- the existence of irregular connections and contributions of storm water to the sewerage system causing very high dilutions of the wastewater, resulting in sharp declines of the net biogas production during the rainy seasons.
- only a few reactors using proper flow-meters to measure the amount of biogas effectively produced, therefore posing serious difficulties in relying on the database available at the different treatment plants. Furthermore, the composition of the biogas is rarely evaluated and as a result, the power generation potential is unknown in most STPs.

5.1.2. *The necessary improvements*

The increasing number of UASB reactors in Latin America opens up a perspective for energy recovery in such systems, but there still exist a great deal of uncertainties regarding the seasonal variations of biogas production and the actual amount that can effectively be recovered for energy generation, as discussed earlier.

Besides the necessary improvements in the design and construction of UASB reactors, seeking the use of efficient devices for biogas collection (e.g., hermetic biogas chambers) and for dissolved methane recovery, there is also a strong need for a better understanding of the behavior of biogas production and recovery in sewage treatment plants. Brazil has moved forward in this direction, setting up a project in cooperation with GIZ (Germany) that aims at the monitoring of crucial parameters (including COD load

and biogas production) in ten full-scale plants in six different states of the confederation. The main result expected from this project is the establishment of ranges of biogas production per m^3 of treated sewage, for different plants' reality, and for regional and seasonal (dry and rainy season) conditions.

Other challenges that impair the use of biogas as an energy source are (Salomon and Lora 2009):

- the lack and high costs of the power generation technologies fuelled on biogas.
- biogas energy recovery systems for the small and very small scale are economically non-viable.
- difficulties to ensure the proper functioning of the biogas energy generation units in the long-term.
- low government investment in programs for the conversion of biogas to energy.
- economic viability.
- dependence on local conditions.
- storage and distribution of biogas.
- difficulties of small units to trade carbon credits.

Improved management and training should be the focus of a support program for small municipalities or operators in developing countries, regardless of the type of treatment process. Moreover, research and technology development efforts should be encouraged in order to provide small and reliable biogas burners and co-generation units to small anaerobic facilities, as well as simple means for capturing or degrading the dissolved methane in the effluent.

5.1.3. Possibilities

The energy content of biogas, with calorific value between $25.1\text{--}28.7 \text{ MJ}\cdot\text{Nm}^{-3}$ (considering methane concentrations between 70–80%), can be recovered for different applications such as: (i) direct use as fuel in boilers, furnaces and kilns to replace other types of fuels; (ii) generation of electricity for local use or sale to the utility power network; (iii) co-generation of electricity and heat; and (iv) alternative fuel aimed at injection into the natural gas line or use as vehicle fuel. In rural areas, the produced biogas can be used for cooking, lighting, refrigeration of food and water heating. In addition to these uses, the biogas generated in STPs can be used for drying and hygienization of sludge. The main alternatives for biogas management in the plant can be classified as follows (Fig. 12.11).

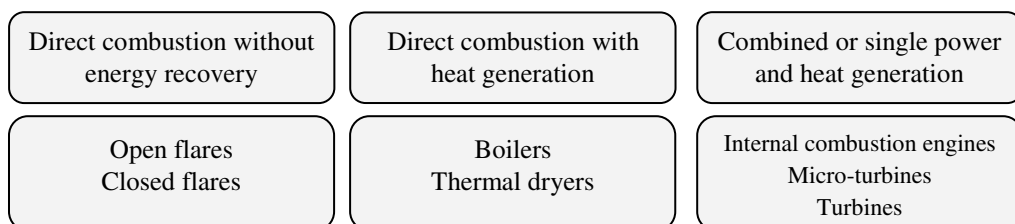


Fig. 12.11. Main alternatives for biogas management in STPs.

Because of the low rates of methane production and losses of dissolved methane in the effluent, the choice of co-generation of power and heat becomes challenging in small-scale systems. In this case, direct combustion with thermal energy recovery seems to be the simplest and highest cost/benefit alternative. If the heat is used for the purpose of sludge drying, an extra benefit will be the complete inactivation of helminth eggs, an important issue in developing countries. In the case of medium- and large-scale STPs, the co-generation of power and heat seem to be possible and feasible alternatives.

5.2. Energy recovery from sludge and scum

Energy sustainability is one of the main aspects to be addressed in the future of STPs, with particular emphasis on the use of sludge, the main by-product of the treatment process, as raw material for the production of energy. This might become a paradigm shift to the final destination of sludge, since landfills are the common final disposal option for this material in the region. Indeed, waste sludge should be evaluated as a source of energy after the dewatering stage. Regarding the scum, research should be pursued in order to characterize this material and determine its energy potential, while inserting the disposal of scum into operational routines of the plants.

Recent work by Rosa *et al.* (submitted) allowed for the characterization of the energy potential of the solid by-products of a sewage treatment plant consisting of UASB reactors and trickling filters (Laboreaux STP, Minas Gerais, Brazil). The results indicated that both the sludge dewatered in the filter press and the scum dewatered in the drying bed have significant low calorific values, in the order of 2.0 MJ·kg⁻¹, for these materials analyzed with moisture content of around 60%. Simulation studies carried out for this same STP (Rosa 2013) assessed the combined use of energy derived from the biogas and

Table 12.7. Main possibilities and benefits of using sludge and scum generated in STPs for energetic purposes.

Direct benefit	Indirect benefit	Type of benefit
Volume reduction of the material to be disposed in landfills	• Reduction of transportation costs	• Economical
	• Reduction of generation and emission of GHG due to the avoidance of the landfill	• Environmental
Source of thermal energy due to its combustion	• Potential use of heat in heating and hygienization processes	• Economical
	• Reduction of volume of the final residue	• Environmental
		• Economical
Electricity supply due to the use of steam and syngas generated in the sludge thermal processing	• Reduction in electricity costs at the STP or other units	• Economical
	• Renewable energy aggregation in the Latin American energy matrix	• Environmental
Sludge hygienization after thermal treatment	• Possibility of sludge use in agriculture, reducing the use of natural resources	• Environmental
	• Possibility of improvement of family agriculture	• Economical
		• Social

Source: Rosa (2013)

sludge generated in the plant for thermally drying the dewatered sludge. The results showed an impressive potential of drying and conversion of sludge into an energetic by-product, which could completely eliminate the generation of rejected material to be disposed off.

Although studies in this area are still in the early stages in Latin America, it is clear that there is a strong potential of using the solid by-products of STPs, after dewatering, to produce energy for local use, substantially reducing the transportation costs for final disposal in landfills. In summary, Table 12.7 presents the main beneficial aspects of energy recovery from sludge and scum generated in STPs.

5.3. Dissolved effluent CH_4 recovery

As discussed in previous sections, high amounts of gaseous methane (30–40% of the produced methane) are kept dissolved in the liquid effluent and therefore represent a matter of strong concern to the environment and potential energy losses. So far, only the methane recovered in the interior of the 3-phase separators of UASB reactors can be adequately managed (flared or used as an energy resource). Some alternatives to reduce the dissolved methane content in the effluent of anaerobic reactors have been proposed, such as micro-aeration using biogas (Hartley and Lant 2006) and degasifying membranes (Cookney *et al.* 2010), but none of them have yet proved to be fully viable or effective. Souza *et al.* (2012) indicated that significant reductions of methane and hydrogen sulfide dissolved in the final effluent can be attained simply by increasing the turbulence of the liquid. These gases can be emitted to a controlled atmosphere, therefore allowing for its recovery or treatment.

A recent study (unpublished results) carried out in a pilot-scale UASB reactor (Centre for Research and Training in Sanitation UFMG/COPASA, Belo Horizonte, Brazil) tested a dissipation chamber downstream the reactor to reduce the concentration of dissolved methane in the liquid effluent. For the best operation condition (free drop height of 1.10 m and 12 renews·h⁻¹), the median dissolved methane removal efficiency was 73%. This result indicates that dissolved methane can possibly be entirely released from the effluent in the case of more turbulent or long discharge pipelines and this calls for attention to the proper design of these discharge structures.

Other alternatives regarding CH_4 emission prevention are the use of membrane contactors, as discussed in Section 5.2 of Chapter 10.

5.4. Agricultural use of effluents

The application of sewage in the soil is based on two main aspects: the reuse of water, especially in (semi-) arid climate zones and the use of nutrients that can increase soil fertility. Additionally, the agricultural aptitude can be improved due to the organic matter supply.

The aforementioned aspects are associated with the reduction of effluent discharge into water bodies, reducing environmental impacts associated with dissolved oxygen depletion and eutrophication. In (sub) tropical countries like the Latin American ones, the application of effluent from anaerobic sewage treatment in agriculture is favored by the

temperature at which the soil is exposed, benefiting an intensive weathering activity, providing nutrients to the soil solution (Martelli 2011). However, this is a relatively recent practice in the region, lacking technical and scientific information, long-term assessments (Fonseca *et al.* 2007; Martelli 2011) and legal regulatory mechanisms.

The agricultural use of treated effluents gains remarkable importance due to the agricultural extension in countries like Brazil, mainly the family-based agriculture, which is responsible for 84% of the rural establishments in the country (data from the Brazilian Ministry of Agrarian Development 2013).

Sewage treatment in STPs based only on UASB reactors is not able to meet the WHO guidelines (WHO 2006) for reuse in agricultural systems. In this regard, Sousa *et al.* (2005) evaluated three post-treatment systems for a demo-scale UASB reactor (5 m³) — wetlands, rock beds and polishing ponds (5 in series) — with the goal to produce a final effluent for irrigation of cultures in semi-arid areas in northeast Brazil (a critical water shortage area). All post-treatment systems produced effluents without helminth eggs; meanwhile the UASB/polishing ponds was the only treatment scheme capable to produce an effluent for unrestricted irrigation (WHO 2006). However, the more advanced treated effluent contains insufficient macronutrients to the majority of regional crops cultivated in the semi-arid areas (Sousa *et al.* 2005).

It is worth noting the effort performed under the Brazilian Research Program on Basic Sanitation (PROSAB), which consolidated results from research that, among other objectives, aimed to develop technology related to the reuse of treated sewage. In this context, technologies were studied for the controlled use of wastewater for ferti-irrigation, hydroponics, aquaculture (fish farming and crustaceans' production) and livestock goats fed with effluent irrigated forage. The results are published in three book volumes: i) Sewage nutrients: utilization and removal (Nutrientes de esgoto sanitário: utilização e remoção); ii) Sewage treatment and utilization (Tratamento e utilização de esgotos sanitários); and iii) Treated sewage utilization in ferti-irrigation, hydroponics and aquaculture (Utilização de Esgotos Tratados em Fertilização, Hidroponia e Piscicultura). All of these books are available online (in Portuguese) on: <http://www.finep.gov.br/prosab/produtos.htm>.

6. Concluding Remarks

In the past 30 years, anaerobic high-rate treatment using UASB reactor technology has evolved from a pioneering academic exercise to an accepted alternative in sanitary engineering to address municipal wastewater. Particularly the feedback of the several pioneer full-scale plants was crucial to elucidate the limitations of the current design and managerial approaches and helped to improve the system, leading to standardized designs. This feedback also draws a research agenda for the various aspects that still need reconsideration. Factors like odor nuisance, scum formation and accurate hydraulic design are still being improved upon by researchers and field specialists. As such, the technology is under development and will remain so for the coming periods. Of interest is that the assets of anaerobic treatment are becoming boundary conditions for any environmental technology to be developed, such as no or little fossil fuel consumption,

plane and robust technology, and the recovery of resources. A concern of growing interest is the emission of the potent greenhouse gas CH_4 from anaerobic reactors. Indeed, in current full-scale UASB systems, large amounts of CH_4 are dissolved in the effluent and are emitted to the atmosphere when effluents are discharged. Current research emphasis is therefore directed to also recover the dissolved CH_4 , serving energy recovery needs, while preventing greenhouse gas emissions. Similar interests exist in developing cost-efficient technologies for the removal, or better, recovery of nutrients from anaerobic effluents.

Provided that non-controlled CH_4 emissions can be avoided, anaerobic reactor systems can potentially contribute to reduce fossil-based CO_2 emissions from the water sector to the atmosphere. In fact, the adoption of treatment systems with lower electricity needs, such as the anaerobic processes, may be a “low hanging fruit” policy measure, considering the new facilities that will be built in the Latin American countries, most of them lacking sufficient sewage treatment coverage (Noyola *et al.* submitted). As an example, the application of anaerobic plus aerobic processes in Mexico would result in a reduction of 4% of GHG emissions for the year 2030 (100% treatment coverage), together with lower investment and operational costs, if compared to a scenario with only aerobic processes. However, if methane is used for local electricity production in larger facilities, a 27% reduction would be accomplished (Noyola *et al.* submitted). In order to arrive at such achievements, all produced CH_4 , also the solubilized, must be captured, since the slightest CH_4 emission will offset the CO_2 emission reduction by avoiding fossil fuels for aeration.

Considering the introduction and further development of anaerobic sewage treatment in Latin America, the most important contributor to the success of UASB reactor systems is not the technology itself, but the acceptance in the engineering community, the governmental institutions, and the responsible agencies for the operation of the respective sewage treatment plants. Poor reactor performance can mostly be ascribed to poor operation and management, which pressurizes particularly the decentralized systems, where proper personnel and adequate understanding is not available. This immediately jeopardizes one of the striking advantages of UASB reactor systems: the possibility to install a compact small unit in small communities, not having the need to construct a large and very costly sewer network. Therefore, basic knowledge transfer and incorporation of this novel technology in the curricula of academic institutions is of prime importance to increase acceptance and to adopt the technology to local conditions. In this regard, Brazil indeed made big steps, with other Latin American countries steadily following.

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Chapter 13

Applications and the Development of Anaerobic Technology in China

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The Chinese government has been promoting anaerobic treatment technology, not only for pollution control, but also for bioenergy recovery. Millions of decentralized anaerobic digesters have been built using animal manure and crop stalk as feedstock to improve sanitary conditions and to produce biogas for heating and lighting for farmers and rural communities. Also, there are 22,570 medium- to large-sized anaerobic projects built for centralized animal manure treatment. In addition, over 2,000 medium to large anaerobic systems have been installed since the 2000s for the treatment of industrial wastewaters including alcohol, starch, pulp and paper using technologies such as CSTR, UASB, EGSB, IC, etc. Overall, more than 25,100 medium- to large-sized anaerobic systems are now in operation in rural China, producing 7.5×10^9 m³ of biogas annually. Based on Chinese government's 12th Five-Year Plan, biogas output will reach 21×10^9 m³ by 2020 from treatment of industrial wastewater, poultry wastes, municipal wastes and wastewater sludge; this amount will double by 2050. This chapter summarizes the development of anaerobic technology in China in the countryside as well as in industrial wastewater treatment. A number of case studies are presented.

1. Introduction

Even though Chinese farmers were familiar with anaerobic digestion much earlier, applications of modern anaerobic technology since then mid-1950s in China have experienced three stages of development (Fig. 13.1), i.e. pre-1990, 1990–2000, and post-2000. The first stage of early development originated in Wuchang city in the 1950s when a campaign was launched to exploit the multiple functions of biogas production, which simultaneously solved the problems of manure disposal and hygienic improvement of

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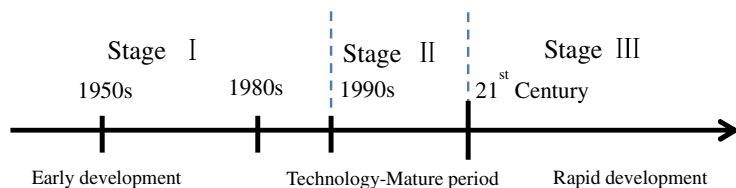


Fig. 13.1. Historical development of anaerobic treatment of agricultural wastes.

living conditions (Li and Ho 2006). A testing scheme was proposed using animal manure to produce biogas, which was the starting point of the first development stage. In the late 1950s, during the *Great Leap Forward* period, China set off a huge biogas movement. However, it failed at the end, due to the lack of technical support and practical regulation, resulting in the abandonment of over 100,000 biogas tanks (Xia 1988). Later, more than 7×10^6 new digesters were built during the late 1970s to early 1980s; unfortunately, half of these were soon abandoned owing to various technical barriers, such as gas and liquid leakage (Li and Ho 2006).

The second stage of development began in the 1990s. With more construction and operation experience, a series of national standards for biogas tanks were published. Some model eco-agricultural systems, integrating waste treatment, energy production and nutrient recycling, were promoted. These included the *Pig-Biogas-Fruit* system integrating pig breeding, biogas production and fruit tree planting in southern China (Chen 1997), and the *Integrated 4-Element* system combining pig breeding, biogas production and vegetable farming in greenhouses in northern China (Qi *et al.* 2005). Around the year 2000, there were 9.8×10^6 household rural digesters in operation in China. At the same time, the application of anaerobic processes for wastewater treatment also achieved remarkable development. Years of academic research led to the industrialization of high-rate anaerobic technology, as represented by the successful application of up-flow anaerobic sludge blanket (UASB) technology in China. Standards for UASB reactor design, including 3-phase separator and influent distribution systems, were gradually established. More than 300 UASB projects for industrial wastewater treatment plants were constructed during this stage, which provided considerable amounts of granular sludge to be used as seed for the new UASB systems in China. At the same time, an innovative hydrolysis–acidification pre-treatment process was developed and widely applied. This process significantly improved the overall performance of the wastewater treatment systems.

The third stage began after entering the 21st century. Application of anaerobic technology for agricultural waste treatment has increasingly attracted social and political attentions. According to the national standard (AQSIQ 2002), standard design for a household system is composed of three components, i.e. digester, household toilet and animal farm. A typical household biogas tank has a simple and practical structure of 6–15 m³, treating waste of 8–20 pigs or the equivalents of 1–2 cows or 150–200 chickens, capable of producing 0.8–2.0 m³ biogas daily. By 2008, there were over 31×10^6 anaerobic digesters installed in rural households producing 12.4×10^9 m³ of biogas annually. On the other hand, as the farms increased in size in recent years, many medium- to large-sized anaerobic systems have also been built in rural China for waste treatment

and for resource recycling. Such a practice is supported by the national policy to relieve environmental pollution pressure. According to the Minister of Agriculture (MOA 2006), anaerobic systems are classified into four categories based on the amount of biogas produced: small ($5\text{--}150\text{ m}^3\cdot\text{d}^{-1}$), medium ($150\text{--}500\text{ m}^3\cdot\text{d}^{-1}$), large ($500\text{--}5,000\text{ m}^3\cdot\text{d}^{-1}$) and super large (over $5,000\text{ m}^3\cdot\text{d}^{-1}$). Animal manure was the primary feedstock for anaerobic plants in the early period. However, with the increase of digesters and limited supply of animal manure, farmers have gradually used weeds and stalks of corn, wheat and rice as feedstock as well. As for industrial wastewater treatment, high-rate anaerobic process research and application were further developed. The expanded granular sludge bed (EGSB) process was successfully developed in the 2000s during the *10th 5-Year Plan*, resulting from the research and development effort on the design of reactor and flow pattern, as well as the understandings of reaction kinetics and characteristics of granular sludge. At present, there are over 2,000 large- and medium-sized industrial anaerobic treatment systems in China treating effluents from industries of distillery, brewery, sugar, starch, monosodium glutamate, beverages, and pulp and paper. Overall, more than 25,100 medium- to large-sized anaerobic systems are now in operation in China, producing $7.5\times 10^9\text{ m}^3$ of biogas annually (Shen 2011b). According to the Chinese government's *12th 5-Year Plan*, biogas output will reach $21\times 10^9\text{ m}^3$ by 2020 from the treatment of industrial wastewater, poultry wastes, municipal wastes and wastewater sludge; this amount will double by 2050 (Shen 2011b).

2. Applications of Anaerobic Technology in Industrial Wastewater Treatment

By the end of 2009, the discharge of industrial organic wastewater in China has reached $4.367\times 10^9\text{ m}^3$ (Shen 2011a), of which $1.757\times 10^9\text{ m}^3$ came from light industries, such as those producing alcohol, sugar, starch, monosodium glutamate, beverages, pulp and paper, and $2.610\times 10^9\text{ m}^3$ from other industries, such as pharmacy, slaughterhouse, petrifaction, natural rubber and furfural. If all treated by anaerobic processes, the overall biogas production would reach $28.083\times 10^9\text{ m}^3$, assuming 56% of methane content in biogas.

Table 13.1 shows the potential annual biogas production from industrial wastewaters from 2015 to 2050, and the corresponding target production set by the central government. The government's target for 2020 (Shen 2011b) is to treat $1.5\times 10^9\text{ m}^3$ of industrial wastewater and residue wastes by anaerobic processes, accounting for 23.7% of the total industrial discharge. It is estimated that there will be 5,000–6,000 anaerobic wastewater treatment systems in operation by then, producing $10\times 10^9\text{ m}^3$ of biogas (Shen 2011b). Top priority will be given to those from agricultural and food industries due to the advantages of centralized resources and high biogas production.

This section focuses on the discussion of the anaerobic treatment of wastewaters from alcohol, starch, and pulp and paper industries, which have been the major sectors using such technology in China. A 2009 survey estimated that there were nearly 2,000 anaerobic industrial wastewater treatment plants in China, over 60% of which were from these three industries (see Fig. 13.2); the rest came mostly from food processing, pharmacy, and others. Provinces and autonomous regions that have the highest biogas production are the following in descending order: Shandong, Jiangsu, Henan, Anhui,

Table 13.1. Biogas resources and target productions from industrial wastewater and residue (Shen 2011b).

Item	2009	2015	2020	2030	2050
Resources for biogas production ($\times 10^9 \text{ m}^3$)	28.083	35.000	42.125	51.000	63.750
Target biogas production ($\times 10^9 \text{ m}^3$)	5.000	7.500	10.000	17.850	22.300

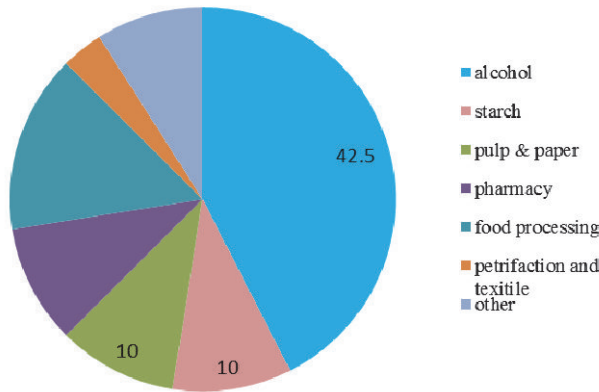


Fig. 13.2. The percentage of organic wastewater industries for biogas project.

Guangxi, Hebei, Sichuan, Guangdong, Zhejiang, Heilongjiang, Jilin and Inner Mongolia. Together, these accounted for over 70% of the total biogas production.

Anaerobic technology was firstly applied in wastewater treatment of the alcohol industry in China. In the very early stage, the traditional anaerobic contact digester (CSTR) was adopted. Later, with the development of anaerobic technology and investment in clean production processes, high-efficiency UASB technology started to be widely applied to alcohol, as well as to starch wastewaters. Subsequently, due to better control of granular sludge formation and the successful development of reactor design, material, 3-phase separator and inlet distribution system, EGSB technology was developed and applied rapidly. A team at Tsinghua University led by Kaijun Wang developed the suspended granular sludge bed (SGSB) process (Wang 2008), which had superior performance to EGSB on process efficiency and organic load (Zheng 2014). Treatment of pulp and paper wastewater in China initially used UASB and EGSB technologies with imported sludge granules. In 2002, Yanling He of Xian Jiaotong University developed a zero discharge process, and built the first plant using this technology at the Wanli recycled paper plant in paper-making wastewater treatment (He 2003).

2.1.1. Background

China has 38,000 alcohol production plants with an annual capacity of 7×10^6 tons, accounting for 47% of the world's production. It annually consumes 17.5×10^6 tons of grains, plus other raw materials such as corn, cassava and blackstrap. The majority of alcohol wastewater is derived from the distillation of the mature fermented mash. The residue after distillation is acidic, containing $50,000\text{--}70,000 \text{ mg-COD} \cdot \text{L}^{-1}$ and $10,000\text{--}40,000 \text{ mg-SS} \cdot \text{L}^{-1}$. Anaerobic contact processes were implemented in the early stage for the treatment of various alcohol wastewaters from fermentation with or without

decantation due to their robustness for the tolerance of suspended solids and to their simple start-up and operation. For example, the Second Distillery in Yantai, the Bo Xing Distillery and the Tai Chi Distillery all chose anaerobic contact processes, where COD loading ranged 4–5 kg-COD·m⁻³·d⁻¹, COD removal 75–85%, BOD removal 85–90% and biogas production rate 5 m³·m⁻³·d⁻¹.

Two kinds of pre-treatment processes for alcohol residues are mostly used: distiller's dried grains (DDGs) and DDG solubles. Of the two, the former pre-treatment is dominant in the market because production of the latter costs more in capital investment and operation, as well as energy consumption. For the treatment of alcohol wastewater from dried sweet potato and cassava, the UASB process, which is suitable for wastewater with a low concentration of suspended solids, was more often applied. Du *et al.* (1999) studied on the treatment of alcohol wastewater in Jingzhi Distillery at Shandong containing 10,000 mg-SS·L⁻¹ using a 2,700-m³ UASB reactor, and was able to remove 90% of COD, 89% of SS and 97% of BOD at loading rates ranging 7–12 kg-COD·m⁻³·d⁻¹ and HRT ranging 2–5 days with proper pre-treatment. Xu *et al.* (2006) achieved 85% COD removal from a wastewater-producing alcohol from cassava using UASB at 37 °C with loading rates up to 25 kg-COD·m⁻³·d⁻¹. Sun *et al.* (2010) achieved 95% of COD removal treating wastewater-producing alcohol from corn, using a 2-stage process combining solid–liquid separation and UASB at 35–37 °C with loading rates up to 8 kg-COD·m⁻³·d⁻¹. Compared to the anaerobic CSTR process in treating alcohol wastewater, UASB processes with DDG pre-treatment can not only recover solid residues in the wastewater for use as fodder or fertilizer, but are also cheaper in both capital investment and operation (Zhou 2001).

2.1.2. Case studies

(1) CSTR in Tian Guan Distillery

Tian Guan Distillery is a renowned enterprise in China with an annual capacity of producing 100,000 tons of alcohol from potato. It was also one of the earliest distilleries treating its wastewater (1,300–1,500 m³ daily) using anaerobic processes. The schematic flow diagram illustrated in Fig. 13.3 shows that wastewater, after DDG pre-treatment, is treated in two 5,000-m³ conventional anaerobic tanks in parallel with HRT of 10 d. The production of biogas is around 40,000 m³·d⁻¹.

The Tian Guan distillery plant upgrades its biogas by water-washing using a packed absorption column. After desulfurization and compression to 2.0 MPa, biogas is fed to the column bottom while water flows downward from the top. CO₂ is absorbed by water producing high-purity (>97%) methane before the removal of residual H₂S and water vapor. The methane produced is qualified by the national standard (GB/T18047-2000), and is further compressed to 25 MPa for use as vehicle fuel. The discharged water after washing firstly enters the desorption column with pressure reduced to 0.6 MPa, so that most dissolved methane is recovered. The total methane recovery is over 99%. The desorption column then regenerates the water by desorbing CO₂ with pressure further reduced to 2 kPa, and the regenerated water is recycled to the washing process. The key characteristics of the Tian Guan biogas upgrading plant are shown in Table 13.2.

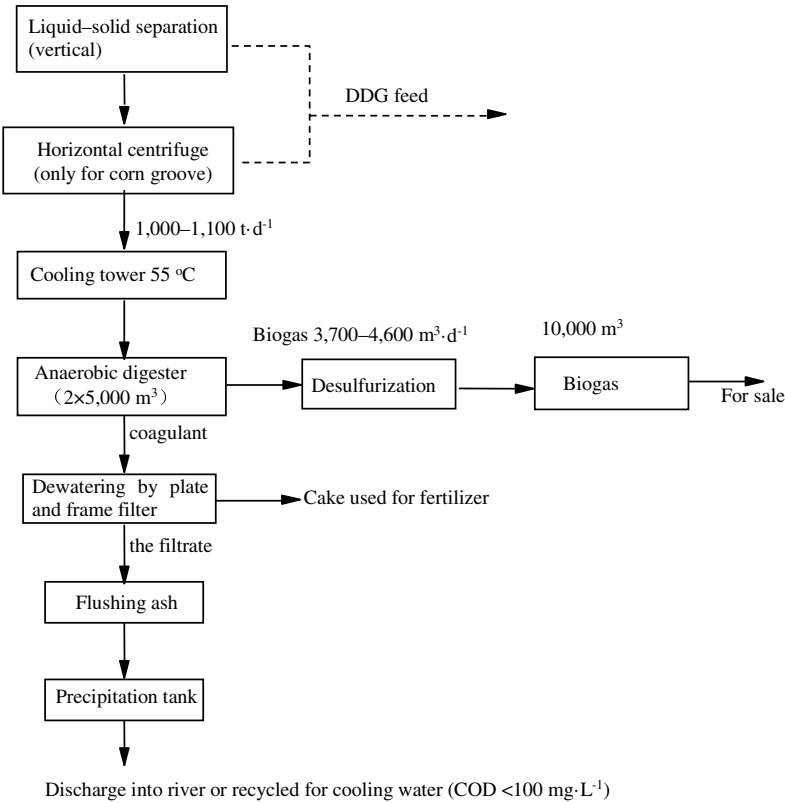


Fig. 13.3. Flowchart of the Tian Guan Distillery plant.

Table 13.2. Characteristics of the Tian Guan biogas upgrading plant.

	Composition (vol. %)	Temperature (°C)	Pressure
Raw biogas	CH ₄ 62%; CO ₂ 38%	30	3 kPa
Upgraded methane	CO ₂ ≤3%	30	25 MPa
Performance indicators			
Methane recovery	≥99%		
Water consumption	31 m ³ ·d ⁻¹		
Electricity cost	1,352 kwh·h ⁻¹		
Operation temperature	30 °C		
Adsorption pressure	2 MPa		
Desorption pressure level I	0.6 MPa		
Desorption pressure level II	2 kPa		
Standard applied	compressed natural gas as vehicle fuel (GB/T18047-2000)		

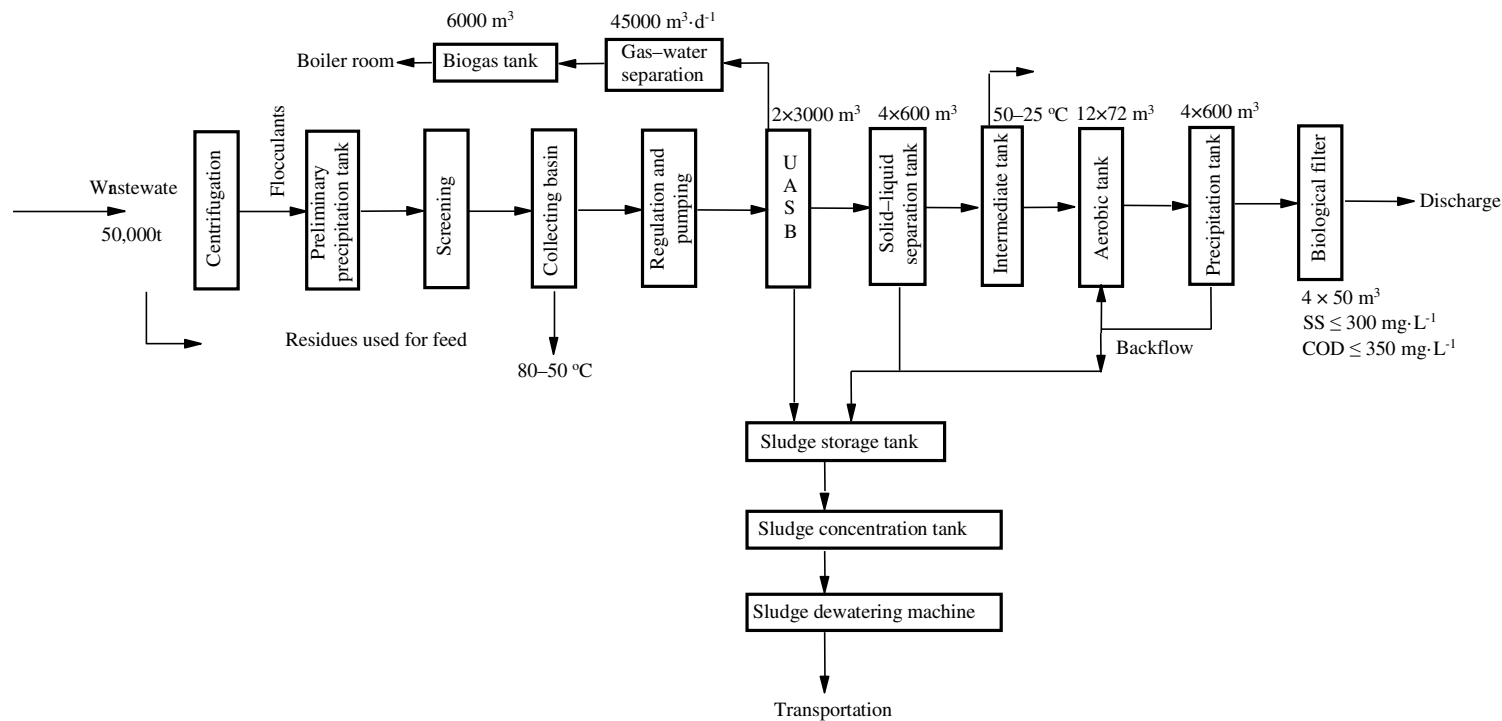


Fig. 13.4. The flowchart of the Fangting alcohol plant.

The setup in the Tian Guan distillery plant has achieved significant environmental benefits. Over 800,000 tons of alcohol wastewater are treated annually, removing 84% of COD, 90.8% of BOD₅ and 96.5% of SS. Over 33,000 tons of COD are converted to methane and are used to supply over 20,000 households as fuel. It reduces over 500 tons of SO₂ from exhausted gas and over 10,000 tons of solid residues.

(2) *UASB in Fangting Distillery in Xuzhou*

Fangting Distillery at Xuzhou is the largest distillery in Jiangsu Province. It has an annual capability of producing dried-potato-based 50,000 tons of ethanol and 25,000 tons of alcohol. Wang *et al.* (2014) designed a UASB system treating 2,400 m³·d⁻¹ of wastewater with pH 3–5 containing 30,000–50,000 mg-COD·L⁻¹, 15,000–30,000 mg-BOD·L⁻¹ and 30,000–40,000 mg-SS·L⁻¹.

As illustrated in Fig. 13.4, the alcohol wastewater first goes through a solid–liquid separation step, producing 400 m³ of solid residues a day. This pre-treated wastewater is then pumped into two 3,000-m³ UASB reactors in parallel. Under thermophilic conditions at 50–55 °C with three days of HRT, the process generates 45,000 m³ of biogas daily. Biogas produced is then stored in a 6,000-m³ float-cover gas storage tank for use as boiler fuel.

Table 13.3. Economic comparison between CSTR and UASB applied in alcohol wastewater digestion.

Process			UASB	CSTR		
Manufacturing plant			Fangting	Tian Guan		
Annual production of alcohol (t)			30,000	76,000		
biogas	output	(m ³ ·d ⁻¹)	6,000,000	12,000,000		
		(m ³ ·d ⁻¹)	20,000	40,000		
	usage	Household (m ³ ·d ⁻¹)	20,000	32,000		
		Alcohol plant (m ³ ·d ⁻¹)	/	8,000		
	annual income	(x10,000 yuan)	144	244.8	900	1,800
DDG	Recovery output (t·d ⁻¹)		/	80,000		
	annual income (x10,000 yuan)		350	460		
Annual discharge fee saving (x10,000 yuan)			216	280		
Starting time			1,996	1,985		
Wastewater	Quantity before separation (m ³ ·d ⁻¹)		2,400	1,300~1,500		
	Quantity after separation (m ³ ·d ⁻¹)		2,000	1,000~1,100		
	BOD (mg·L ⁻¹)		25,000			
	COD (mg·L ⁻¹)		50,000	30,000		
	SS (mg·L ⁻¹)		35,000	35,000		
	pH		5	5		
Days of operation a year (d)			300	300		
Annual operation (x10,000 yuan)	Power cost		158	99		
	Chemical cost		60	72		
	Staff wages		40	112		
	Depreciation and maintenance cost		202	86		
	Water cost			9		
	Management cost			53		
Total cost		258	439			

The UASB process with DDG pre-treatment and proper post-treatment removes 99.3% of COD and 99.4% of BOD from the wastewater, producing an effluent of pH 6.8, containing $330 \text{ mg-COD}\cdot\text{L}^{-1}$ and $123 \text{ mg-BOD}\cdot\text{L}^{-1}$. This is one of the largest UASB projects in treating alcohol wastewater in China, and is often served as a model system for demonstration.

2.1.3. Comparison of two aforementioned processes

Table 13.3 compares the key features between the Tian Guan CSTR-based system and the Fangting UASB-based system. Results show that both anaerobic processes are effective in COD removal, but the CSTR system produces an effluent that has difficulties in meeting the discharge limits. By contrast, the UASB process is superior in treatment efficiency, biogas production, effluent quality and costs. In general, the UASB process is recommended in China.

2.2. Starch-processing wastewater treatment

2.2.1. Background

China produces more than 10×10^6 tons of starch annually, second only to the U.S. in the world, of which 80% is produced from corn (Xu 2012), and most of the rest from potato and cassava. The rich acidic organic wastewater from starch processing mainly consists of soluble starch and protein. In recent years, due to the advancement of starch production technology and the demand for green production, the industry has consumed substantially lower volumes of water with rapidly increased practice of wastewater recycle. Typical wastewater characteristics are: $10,000\text{--}15,000 \text{ mg-COD}\cdot\text{L}^{-1}$, $1,000\text{--}3,000 \text{ mg-SS}\cdot\text{L}^{-1}$, $240\text{--}540 \text{ mg-TN}\cdot\text{L}^{-1}$, $15\text{--}130 \text{ mg-P}\cdot\text{L}^{-1}$ and pH 4.2–5.0 (Cai *et al.* 2007). The two main treatment processes used in China are UASB and EGSB.

2.2.2. Improvement of the UASB system

Since 2000, the UASB system has been developed very rapidly in China, accounting for over 50% of the anaerobic projects for industrial wastewater treatment with over 1,000 installations. Li *et al.* (1996) showed that UASBs were feasible to treat starch-rich wastewater from a flour plant. Zhang *et al.* (2002) also showed that a UASB reactor could treat starch wastewater at a loading rate of $10 \text{ kg-COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ with over 90% of COD removal. Similar results were demonstrated by Guan and Zheng (2004), who showed that the UASB system could remove 92% of COD from starch wastewater consistently at the loading rate of $11 \text{ kg-COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. In the year of 2004 alone, over 100 UASB systems were installed in China.

The key features of UASB systems include the 3-phase separator, influent distribution system and structure material (Wang, 2001). In the past decade, the modular 3-phase separators made of engineering plastic were designed and manufactured in China. Both rectangular- and round-shaped modular separators were developed, depending on the UASB reactor shapes. These separators can be easily assembled on site. Figure 13.5

shows an example of rectangular modular 3-phase separator and UASB for a starch processing plant at Xinxu of Tengzhou in Shandong Province.

The influent distribution into the UASB reactor is crucial to the effective operation of the process. The key criteria are to ensure even wastewater distribution and to provide effective mixing. In the early stage, most influent distribution for UASB reactors used dendroid perforated pipes (Fig. 13.6). The pipes, having 10–20-mm holes, were installed at 45°-angles with a distance of 1–2 m apart from one another, each serving an area of 1–4 m². In practice, some holes would be blocked unpredictably due to incompletely pre-treatment. Therefore, a one-hole-one-pipe distribution system was implemented, and has been widely used in the starch-processing wastewater treatment projects in China.

Reinforced concrete was widely used for the construction of UASB in early years. It was soon found to have some disadvantages, including the long construction time, large land requirement and poorly controlled construction quality. Some projects used steel but it was costly and had corrosion problems. In recent years, the construction technique of Lipp was introduced from Germany. Lipp silo may be fabricated and installed on site, like reinforced concrete, but achieves better air tightness. It has become the preferred choice in China, because of the short construction time, low cost and good quality, especially in starch-processing wastewater treatment plants, like the one in Zhucheng (Fig. 13.7), Binzhou, Linyi, and many other cities.

It is well known that granular sludge is the key factor to achieve high efficiency for UASB reactors. In the 1990s in China, however, well-cultured sludge granules were not available. They had to either be imported from abroad or inoculated from flocculent sludge from anaerobic digesters. Start-up using flocculent sludge would take at least 3–6 months, and the loading rate had to be kept low at below 5 kg-COD·m⁻³·d⁻¹, even for readily biodegradable wastewaters. Granular sludge was soon developed in well-operated UASB reactors treating starch wastewater using an operating strategy developed by Wang of Tsinghua University (Wang 2007). The strategy is as follows: the reactor first treats wastewater of low concentration at high hydraulic loading; effluent recirculates to dilute the incoming wastewater and to reduce the demand of alkalinity; the organic loading then increases by rapidly increasing the hydraulic loading. Following such a strategy, substantial improvement was reached after the loading rate reached 2 kg-COD·m⁻³·d⁻¹.

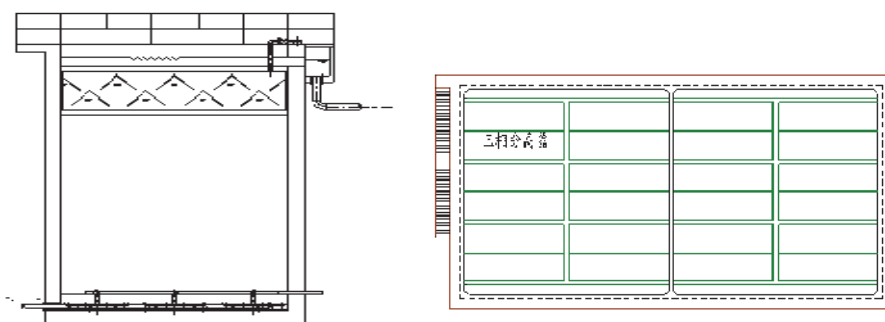


Fig. 13.5. The rectangular modular 3-phase separator and rectangular UASB installed at Xinxu starch processing wastewater treatment plant.

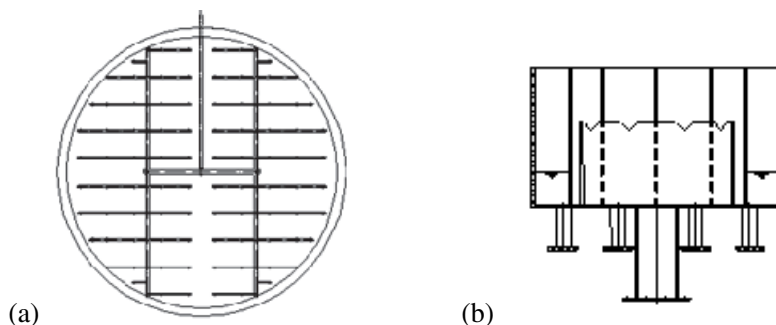


Fig. 13.6. Influent distribution into the UASB reactor: (a) perforated pipes distribution and (b) one-hole-one-pipe distribution.



Fig. 13.7. Applications of the Lipp technology in the Zhucheng Starch Processing Plant ($4000 \text{ m}^3 \cdot \text{d}^{-1}$) and in the Zhucheng Starch Joint-Stock Company ($6000 \text{ m}^3 \cdot \text{d}^{-1}$), both in Shandong Province.

The Zhucheng Starch Joint Stock Company is a medium-sized plant producing 80,000 tons of corn starch annually. In the reconstruction project of its wastewater treatment, a UASB reactor was added. The UASB had a diameter of 21 m, height of 6.7 m and an effective volume of $2,300 \text{ m}^3$. The startup strategy for granular sludge was adopted in this reactor. Figure 13.8 illustrates the changes in loading, COD concentrations of influent and effluent, and pH during the operation.

During the start-up stage, 138 tons of dewatered anaerobic digester sludge (25% total solids) from a municipal wastewater treatment plant was used to seed the UASB reactor after being diluted with starch wastewater to $15 \text{ g} \cdot \text{L}^{-1}$. The sludge was inoculated by recycling and the sludge concentration was reduced from $4,000 \text{ mg} \cdot \text{L}^{-1}$ to $1,500 \text{ mg} \cdot \text{L}^{-1}$ after three days. The loading was initially at $0.5 \text{ kg} \cdot \text{COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$, and it was increased gradually stepwise by $0.5 \text{ kg} \cdot \text{COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ for each increment. In order to form sludge granules, the influent was diluted from the original $20,000 \text{ mg} \cdot \text{COD} \cdot \text{L}^{-1}$ to $3,000\text{--}4,000 \text{ mg} \cdot \text{COD} \cdot \text{L}^{-1}$ by recirculating the effluent, and the pH was adjusted by the addition of lime water. The loading reached $5 \text{ kg} \cdot \text{COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ after 40 days and the sludge granules began to appear with diameters below 0.2 mm. The increase of loading rate resulted in the increase of effluent pH and the washout of flocculent sludge. The addition of lime water was gradually decreased and stopped at the end. Granular sludge began to form and grow to the size of 1–2 mm (a few even reached 3 mm or more). The sludge bed began to form and had the height of about 1.0 m.

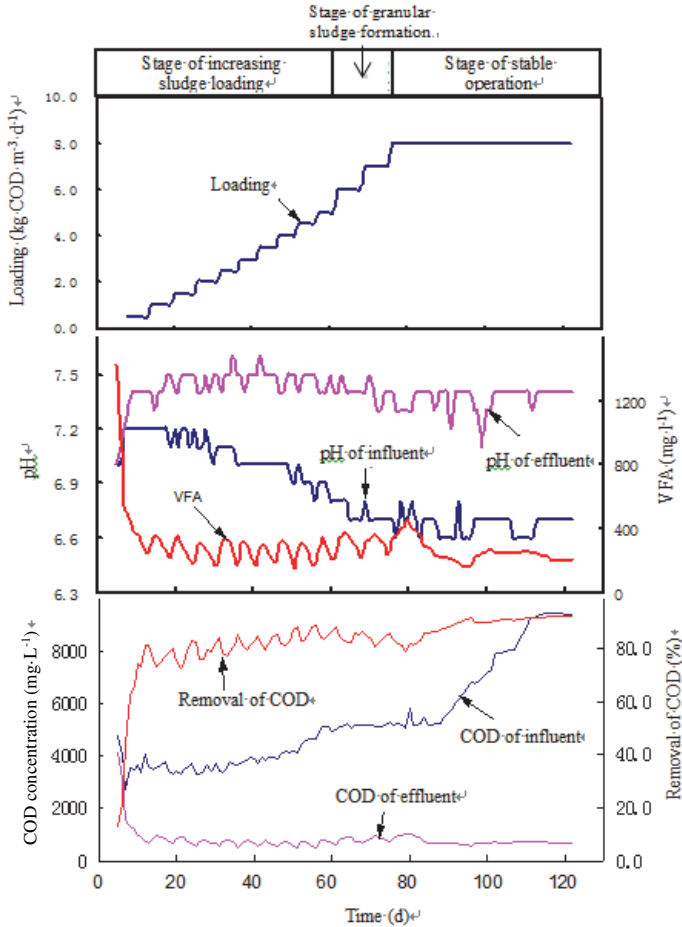


Fig. 13.8. Key parameters during the start-up for the development of granular sludge.

During the stage of granular sludge formation, loading rate further increased from $5 \text{ kg-COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ stepwise with increment of $1.0 \text{ kg-COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$. The granular sludge grew and reached 3–5 mm, while the height of granular sludge bed reached 2.0–2.5 m. When the loading reached the final level of $8 \text{ kg-COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ with stable operation, the granular sludge was considered to be successfully cultivated. From then on, the influent COD was increased and effluent recirculation decreased. The whole cultivation process of granular sludge took about three months. The granular sludge appeared smooth and neat, with abundant microorganisms at the surface. The interior of sludge granules were dense without noticeable pores, and the microbial distribution was sparse with noticeable microbial autolysis (Fig. 13.9).

2.2.3. Application and promotion of UASB

In addition to the development of the 3-phase separator, influent distribution system and new construction material, the rapid promotion of UASB in China may also be attributed to the establishment of bases for the supply of granular sludge. About $2,000 \text{ m}^3$ of

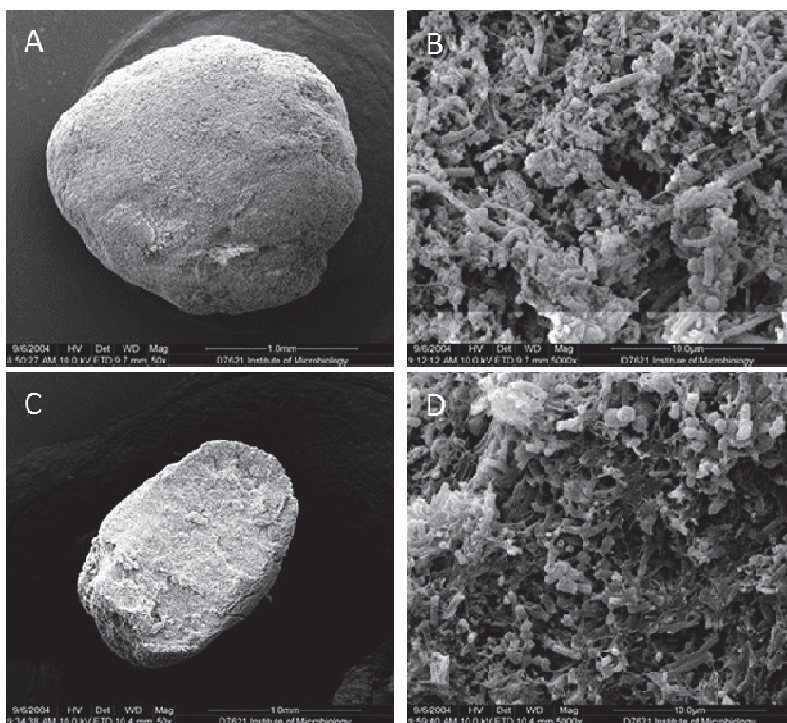


Fig. 13.9. The SEM of (a) appearance of granular sludge x50, (b) surface of granular sludge x5000, (c) cross section of granular sludge x50 and (d) interior of granular sludge x5000.

granular sludge may be produced yearly from a reactor treating $6,000 \text{ m}^3 \cdot \text{d}^{-1}$ of wastewater containing $10,000 \text{ mg} \cdot \text{COD} \cdot \text{L}^{-1}$. According to the scheme of granular sludge cultivation and the distribution of starch producers in China, a large number of granular sludge production bases with an annual yield of $5,000 \text{ m}^3$ have been established in China, including many in Shandong, Hebei, Liaoning Provinces in the north, Sichuan and Yunnan Provinces in the west, and Guangdong Province in the south. The establishment of granular sludge supply bases created conditions for the successful start-up of many UASB reactors. These bases produce sufficient granular sludge to meet the primary need for the home market, and help to promote the anaerobic processes in wastewater treatment in China.

Figure 13.10 illustrates the distribution of major UASB systems in China, most of which treat agricultural industrial wastewater containing high levels of organic pollutants with good biodegradability. The figure shows that UASB systems in China have been installed in clusters surrounding these established bases of granular sludge supply.

2.3. Development and application of EGSB

2.3.1. The application of EGSB in industrial wastewater treatment

Near the end of the 1990s, the third generation of anaerobic bioreactors, EGSB, had been developed in China (Hu 2003). The Shandong Shifang was successful in developing



Fig. 13.10. Application of UASB in China.

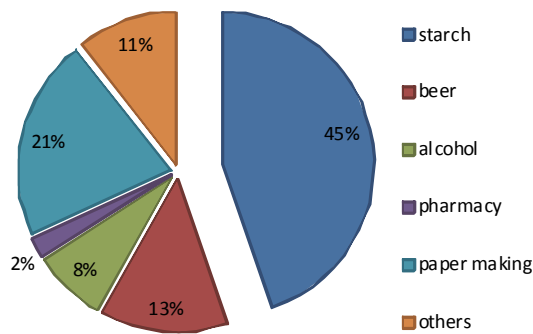


Fig. 13.11. Industrial sectors using EGSB (based on a survey of 136 plants).

EGSB with a series of demonstration projects. The EGSB technology has gradually become mature and widely used in China, as illustrated in Fig. 13.11, based on a survey of 136 plants, mostly installed by Paques and Shandong Shifang. It shows that the main applications are still related to those of agricultural, food and beverage industries. About 66% of the 136 installations were treating wastewaters from starch, brewery and distillery industries, whereas pharmaceutical, pulp and paper accounted for another 23%.

2.3.2. Problems resulted from fluidization in EGSB reactor

An early laboratory EGSB reactor was tested using granular sludge taken from a UASB reactor treating starch wastewater as seed sludge. The sludge concentration was 65.2 g-VSS·L⁻¹ with a COD removal activity of 1.6 g-COD·g-VSS⁻¹·d⁻¹. The organic loading

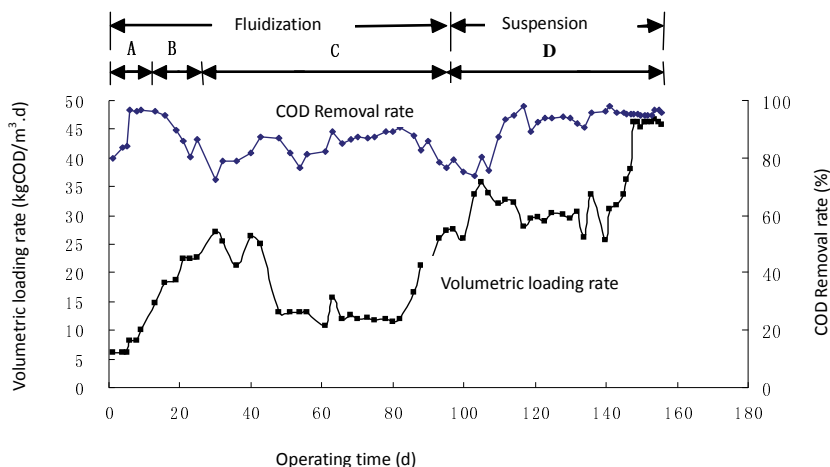


Fig. 13.12. The volumetric loading rate and COD removal in EGSB reactor.

rate (OLR) was $6 \text{ kg-COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ during start-up, and was increased within 24 days to $23 \text{ kg-COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$. The COD removal efficiency ranged 86–96% and stable reactor performance was obtained, as illustrated in Fig. 13.12.

After operating at the OLR of $23 \text{ kg-COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ for three days, the OLR was further increased to $27 \text{ kg-COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$. A complete fluidization of sludge bed was observed in the reactor. By cutting recirculation, the up-flow velocity was reduced from $4.0 \text{ m} \cdot \text{h}^{-1}$ to $3.0 \text{ m} \cdot \text{h}^{-1}$, while fluidization was still maintained. Although fluidization may improve mass transfer, the intense collision and friction also result in the disintegration of sludge granules and the increase of sludge washout. After running for two weeks, about half of the granular sludge was lost. On day 44, the OLR was decreased to $13 \text{ kg-COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$, and kept at that level for nearly 50 days. After the gradual restoration of reactor performance, the OLR was increased to $26 \text{ kg-COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ on day 92 with an up-flow velocity of over $3 \text{ m} \cdot \text{h}^{-1}$. This resulted in fluidization again. The fluidization phenomenon appeared several times at the OLR of $24 \sim 26 \text{ kg-COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ with up-flow velocity over $3 \text{ m} \cdot \text{h}^{-1}$. This seriously hindered the stable and reliable operation of the expended granular sludge reactor.

As can be seen from the above example, the expansion of the granular sludge bed increased with the increasing up-flow velocity of gas and/or water. On one hand, this improves treatment efficiency, whereas on the other hand, the excessive bed expansion affects the stability and the washout of the granular sludge. Controlling the degree of sludge bed expansion is crucial to the reactor performance.

2.3.3. The concept of the anaerobic suspended granular sludge bed reactor (SGSB)

Figure 13.13 (Chen 1985) illustrates the flow patterns in fluidized bed that have been well studied by chemical engineers. Before reaching the theoretical critical flow velocity (U_{mf}), it already begins to fluidize or move at the flow rate of U_{bf} ($< U_{mf}$). Therefore, U_{bf} is the actual velocity at which the particles start to move freely. The particles are completely fluidized until the flow velocity achieves U_{tf} ($> U_{mf}$). The velocity ranging

between U_{bf} and U_{tf} is a transition from the fixed bed to the fluidized bed; the reactor operated in this stage is called as expanded bed reactor. The fixed bed and fluidized bed can be easily controlled, whereas the transition state is more sensitive to the up-flow velocity and thus more difficult to control and operate as such.

However, Wang *et al.* (2007) found that fluidization of biosolid particles, which are soft and non-uniform in particle size and density, behaved differently from those shown in chemical engineering literature. He found that there were actually two different states during the transition, i.e. expanded state ($U_{bf} \sim U_{mf}$) and suspended state ($U_{mf} \sim U_{tf}$), as illustrated in Fig. 13.13.

At the beginning of the transition state, with the flow velocity increasing, the void rate increases and the corresponding expansion of the sludge bed appears. However, the majority of sludge particles move within a certain limited space inside the bed, and the relative positions of particles remain unchanged. This is defined as the expansion state ($U_{bf} \sim U_{mf}$), and the bioreactor running under this state is called the expanded bed reactor. With the further increase of flow rate, the particles are in motion and their relative positions change dramatically (Fig. 13.14). Under this state, the particles are suspended

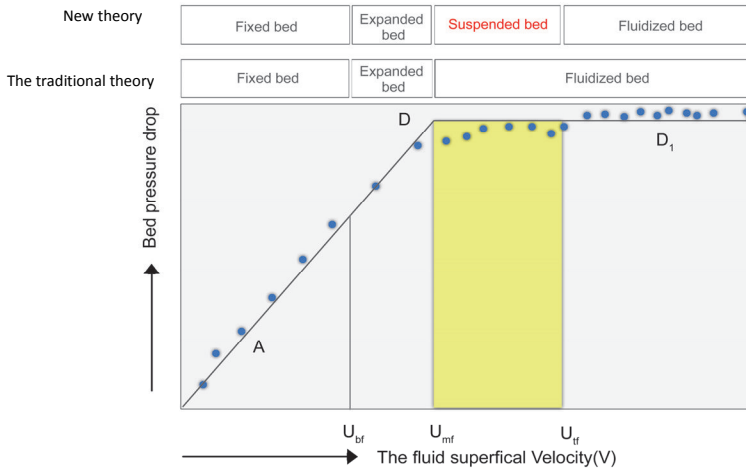


Fig. 13.13. The actual critical state of fluidization.

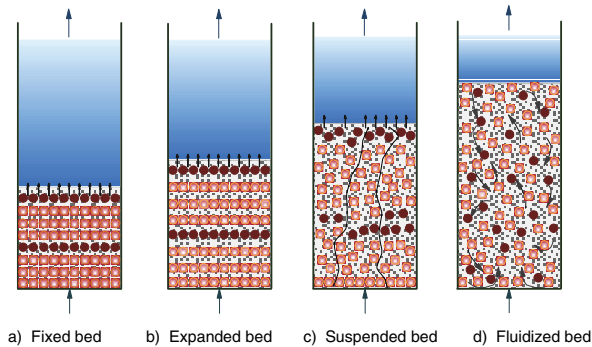


Fig. 13.14. The four states of sludge bed.

($U_{mf} \sim U_{tf}$); likewise, the bioreactor running under the state is called the suspended bed reactor. Therefore, according to the expansion rate and flow velocity, Wang *et al.* (2007) classified the up-flow anaerobic bioreactor into four types: fixed bed (bed expansion <5%, and up-flow velocity low), expanded bed (5–20% and <4.0 m·h⁻¹), suspended bed expansion (20–70% and <4.0 m·h⁻¹), and fluidized bed (70–100% and >8.0 m·h⁻¹).

2.3.4. Full-scale SGSB reactor performance

A full-scale SGSB experiment was conducted using an EGSB reactor with a diameter of 5 m, height of 15 m and a working volume of 275 m³. The operation of the reactor was divided into five phases: (A) start-up with increasing loading rate, (B) high loading rate, (C) ultra-high loading rate, (D) second startup after a shutdown for overhaul, and (E) a repeated ultra-high loading rate phase. The operation lasted for more than one year, and results are summarized in Fig. 13.15, Fig. 13.16 and Table 13.4 (Fang 2005; Wang *et al.* 2007).

As shown in Table 13.4, Fig. 13.15 and Fig. 13.16, the reactor was started with 8.0 kg-COD·m⁻³·d⁻¹. After one week, the COD removal efficiency reached above 80%. During the next 26 days, the loading rates gradually increased from 9 kg-COD·m⁻³·d⁻¹ to 25 kg-COD·m⁻³·d⁻¹, ending the start-up phase. The loading rate was then further increased 32 kg-COD·m⁻³·d⁻¹ for 138 days, at the end of which, the amount of sludge accumulated reached 19 tons with a maximum methanogenic activity of 1.5 g-COD-CH₄·g-VSS⁻¹·d⁻¹. The next 28 days were operated at an ultra-high load phase with an average loading rate of 40.7 kg-COD·m⁻³·d⁻¹ and the maximum loading rate being 50 kg-COD·m⁻³·d⁻¹.

Table 13.4. Operational conditions in a suspended bed reactor.

Operation stage	Start-up	High load	Ultra-high load	Second start-up	Ultra-high load
Operation time (d)	44	153	28	14	91
OLR(kg-COD·m ⁻³ ·d ⁻¹)	8–25	30.1(average)	40.2(average)	10–34	38.3(average)
COD removal (%)	84.1	88.3	89.6	89.7	90.1
Sludge in reactor (ton)	2.1–6.7	6.7–19	19	13.2–15.3	15.3

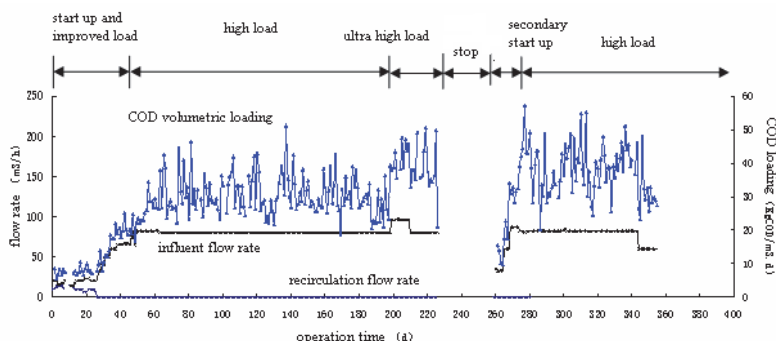


Fig. 13.15. The influent flow rate, recirculation flow rate and COD loading rate of a SGSB reactor.

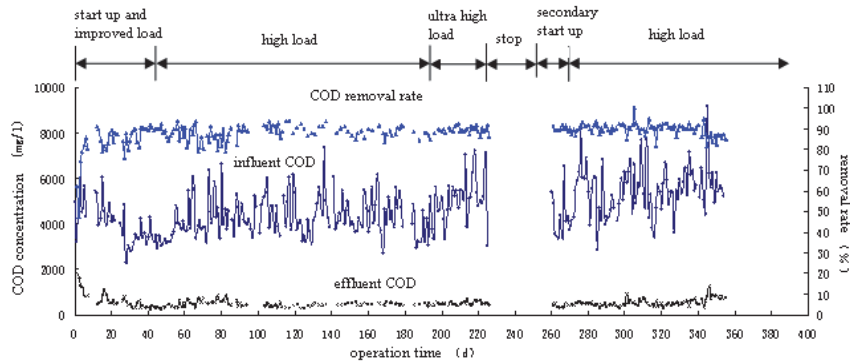


Fig. 13.16. COD removal efficiency of SGSB reactor.

Table 13.5. Performance comparison between suspended bed reactors and other types of anaerobic reactors.

Reactor (wastewater)	Load rate ($\text{kg-COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$)	Hydraulic load ($\text{m}\cdot\text{h}^{-1}$)	Gas load ($\text{m}\cdot\text{h}^{-1}$)	COD removal (%)	Bed expansion (%)	
Suspended bed (starch wastewater)	38.3	4.5	8.5	90.1%	30–70%	suspended bed
IC (brewery wastewater, The Netherlands)	24	---	---	80%	>100%	full fluidization by recirculation
IC (brewery wastewater, The Netherlands)	19.2	7.5	2.7	60%	>100%	full fluidization by recirculation
EGSB(corn starch wastewater, USA)	20.8	2.8	3.4	87%	>70%	actual fluidized bed
EGSB (bread yeast, Germany)	40	8.0	4.0	90%	>100%	actual fluidized bed

After the second start-up, the reactor was again operated at ultra-high load rates ranging $20\text{--}55\text{ kg-COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$, with an average of $38\text{ kg-COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. During which, the COD removal averaged 90.1%, and the effluent contained $170\text{ mg}\cdot\text{L}^{-1}$ of VFA and $1,402\text{ mg}\cdot\text{L}^{-1}$ of alkalinity. The operation performance of the suspended bed reactor was compared with other types of reactors in Table 13.5. Results show that the anaerobic suspended bed reactor has obvious advantages in full-scale operations, as it is operated at a reduced recirculation flow rate, ensuring that the reactor has good mixing without disrupting the stable structure of sludge granules.

3. Agricultural Waste Treatment

3.1. Poultry manure treatment

3.1.1. History and applications

In the 1980s, anaerobic plants treating cow manure had been successfully built at the Feng Huanshan cow farm in Chengdu and the animal husbandry farm affiliated with

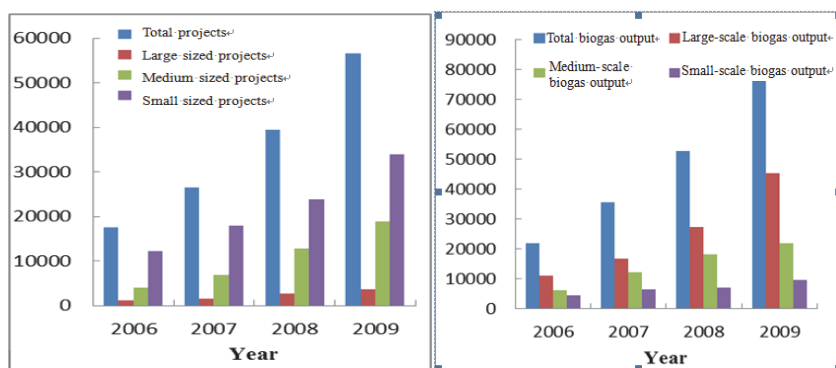


Fig. 13.17. Number of anaerobic treatments for agricultural wastes and biogas production (2006–2009).

Zhejiang Agricultural University in Hangzhou. The former used the underground plug-flow reactor, and the latter used the underground hydraulic digesters; both were operated at ambient temperature (Zhou *et al.* 1990). The efficiencies of these treatment plants were low, even with a HRT of about 40 days, producing biogas at $0.13\text{--}0.3\text{ m}^3\cdot\text{m}^{-3}\cdot\text{d}^{-1}$. In the 1990s, with the development of large-scale livestock and poultry farms, water pollution originating from these breeding sites became increasingly severe. During that time, the main purpose of building anaerobic plants was to treat wastewater with little attention paid to biogas production. For example, a swine wastewater treatment plant built in 1990 by Shenzhen Agriculture and Animal Husbandry Company used an up-flow blanket filter (UBF) process with influent COD of about $9,500\text{ mg}\cdot\text{L}^{-1}$. Operated at ambient temperatures ($16\text{--}33\text{ }^{\circ}\text{C}$), it reached a biogas production rate of $0.8\text{--}1.3\text{ m}^3\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ (Xu *et al.* 1991). The wastewater treatment plants of Hangzhou Dengta built in 2000 applied a process combining UASB and SBR (sequencing batch reactor) to treat $3,000\text{ m}^3\cdot\text{d}^{-1}$ of swine wastewater (Deng *et al.* 2007). These types of treatment plants were also used in northern China. For example, swine wastewater treatment plants in Masanjia Pig Farm in Liaoning built in 1994 used the UASB process to treat wastewater containing $4,000\text{--}5,000\text{ mg-COD}\cdot\text{L}^{-1}$ at ambient temperatures ($9\text{--}13\text{ }^{\circ}\text{C}$). It achieved a volumetric biogas production rate of $0.2\text{--}0.3\text{ m}^3\cdot\text{m}^{-3}\cdot\text{d}^{-1}$.

After 2000, the Chinese government has provided financial support to promote the development of anaerobic treatment plants. As a result of such financial incentives, the application of anaerobic technology has been rapidly applied for poultry manure treatment (Fig. 13.17). In 2012, according to one estimate, 80,703 anaerobic plants of various scales were established for livestock and poultry manures, producing about $1.67\times 10^9\text{ m}^3$ of biogas. The power-generating capacity reached 135.74 MW, and a total of 344.46 GWh of electricity was generated (MOA 2012).

3.1.2. Technology development

Nearly all kinds of anaerobic digestion processes have been investigated in China for the treatment of animal wastewater (Li *et al.* 2010), including CSTR, plug-flow anaerobic

reactor, UASB, up-flow solids reactor (USR), anaerobic contact digester (AC), anaerobic sequential batch reactor, anaerobic baffled reactor (ABR), UBF, internal circulation reactor (IC), EGSB, etc. Anaerobic digestion of the feedstock with high suspended solids, such as cow or chicken manures, mainly used CSTR, USR, and plug-flow anaerobic digesters. Anaerobic digestion of the feedstock with low suspended solids and low COD, such as swine and dairy wastewaters, mainly used UASB, AC, UBF and IC reactors. The presence of SS in reactors can also inhibit the formation of granulated sludge. These factors restrict the microorganism retention in the reactor. Xu *et al.* (1991) carried out a comparison study on the anaerobic treatment of screened pig farm wastewater using UBF, UASB and ABR reactors. The results consistently showed that UBF reactors performed better than UASB, which was slightly better than ABR. At 10 °C and a loading rate of 1.9–2.3 g-COD·L⁻¹·d⁻¹, the average gas production rate was 0.32–0.51 m³·m⁻³·d⁻¹, and the COD removal efficiency was 82.2–91.0%. At 15 °C and 2.5–2.6 g-COD·L⁻¹·d⁻¹, the gas production rate was 0.57–0.59 m³·m⁻³·d⁻¹; and at 25 °C and 5.5–5.7 g-COD·L⁻¹·d⁻¹, 1.93–2.01 m³·m⁻³·d⁻¹.

In China, the average biogas production rate is 0.212 m³·m⁻³·d⁻¹ for small anaerobic digesters, 0.274 m³·m⁻³·d⁻¹ for medium-sized digesters, 0.523 m³·m⁻³·d⁻¹ for large ones, and 0.827 m³·m⁻³·d⁻¹ for the super large (DEST, MOA). In general, the larger the digester, the more efficient the operation. That is because the small- and medium-sized digesters often use the traditional, less efficient processes, such as underground hydraulic digester and plug-flow reactor, whereas the larger ones use the advanced processes, such as CSTR, USR, and UBF.

Anaerobic digestion processes applied in China vary substantially depending on locations. For example, Xu *et al.* (2010) surveyed 172 anaerobic treatment plants in Fujian Province treating wastewater from large-scale pig farms, and found 34.57% of them used the ABR process, 42.59% used the underground plug-flow reactor, 12.35% used the CSTR, and 4.32% used the UASB and UBF (Fig. 13.18). However, Tang *et al.* (2012) surveyed 38 large- and medium-sized anaerobic plants treating manure and slurry of swine, cow and chicken in Beijing. He found 21% of these plants used CSTR, and the rest used USR.

3.1.3. Case study

(1) Minhe

Minhe Animal Husbandry is the first Chinese poultry company listed in the public stock market and it has installed the largest anaerobic treatment systems in Asia treating chicken manure and producing electricity from biogas (Fig. 13.19). The plant, which is located near Penglai of Shandong Province, processes 360 ton·d⁻¹ of manure with 20% of TS produced by 600,000 breeding hens and 3,000,000 broiler chickens from 23 farms. The plant consists of eight CSTR digesters divided into two parallel trains. The CSTR digesters operate at mesophilic conditions, the temperatures of which are kept by using the waste heat recovered from the process and from electricity generators. The biogas produced is desulfurized using biological filters reducing the H₂S content to below 200

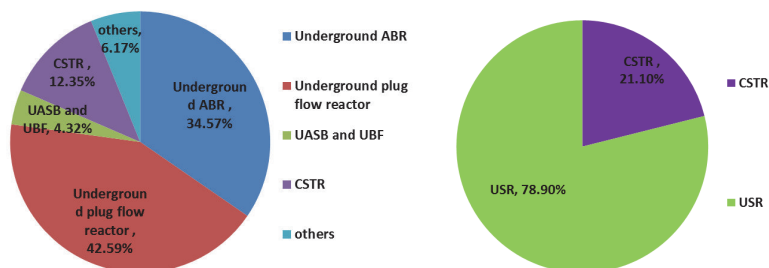


Fig. 13.18. Anaerobic reactors used for agricultural wastes in Fujian Province and in Beijing.

ppm. After dewatering and purification, the biogas is stored in double membrane tanks before being used for power generation. Most waste heat is used for heating the digesters, and the rest for heating the chicken farms and office rooms. The digestate is used as organic fertilizer for neighboring farms growing grape, apple and corn.

The Minhe plant has been in operation since October of 2008. It has treated $360 \text{ ton} \cdot \text{d}^{-1}$ of chicken wastes producing $30,000 \text{ m}^3 \cdot \text{d}^{-1}$ of biogas, which is used to produce 60,000 kWh of electricity. It has earned credits for reducing the emission of 80,000 tons of CO_2 equivalent annually, and was the first of such plants in China to qualify in the United Nation's Clean Development Mechanism (CDM) program. The electricity produced has been sold to the national grid. Unlike many government-funded anaerobic treatment plants, which often lack economic incentive to optimize the plant operation, Minhe as a private enterprise has strong motivation to improve the process efficiency and has become the pioneer of bioenergy production in China.

Minhe's anaerobic treatment process may be divided into three steps (Fig. 13.20): pre-digestion, primary digestion and post-digestion. The pre-digestion step can be split into three parts: manure collection, mixing and sand removal. Manure and wastewater collected from the chicken farms are first mixed vigorously to break up lumps and to release sand particles. The mixture is then conveyed to two $1,600\text{-m}^3$ separation tanks, in which sand particles are removed from the bottom and feathers skimmed from the top. Slurry in the middle section of the tank, after adjusting to a TS level of 6–8.9%, is then used to feed the anaerobic digesters.

Each of the eight anaerobic tanks is $3,300 \text{ m}^3$ in size with an active volume of $3,000 \text{ m}^3$ for digestion. The tanks are operated in two trains in parallel. Each train has four tanks, three being primary digester and one secondary. The retention time is about 30 days in the primary digester and ten days in the secondary digester.

Each day, $300\text{--}400 \text{ m}^3$ of digestate is transferred from the two secondary tanks into a $2,200\text{-m}^3$ post-fermentation tank for 4–5 days of retention before being finally fed to a $50,000\text{-m}^3$ holding pond. The nutrient-rich digestate in the pond is sold to neighboring fruit farms and vineyards as organic fertilizer. The biogas produced in all anaerobic tanks is stored in a $2,000\text{-m}^3$ holder. After desulfurization by biofilters, dewatering and purification, the biogas with 97% methane is used for heating and for electricity generation.



Fig. 13.19. Minhe anaerobic plant treating chicken manure.

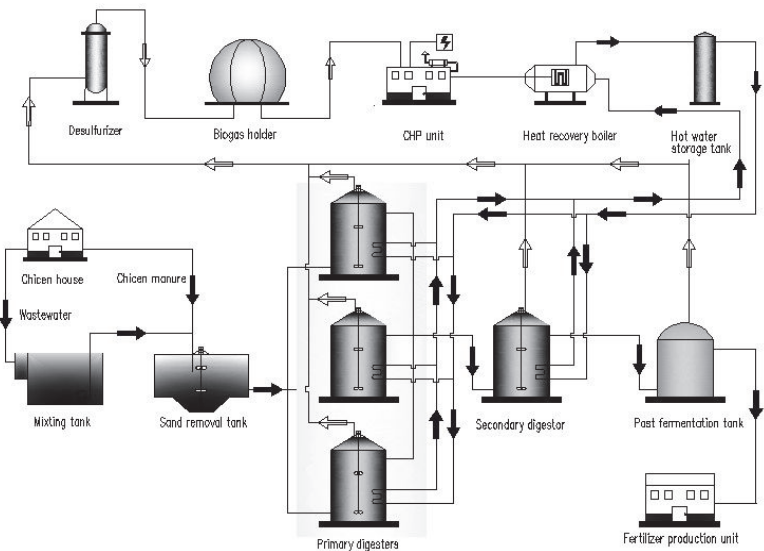


Fig. 13.20. Flow diagram of Minhe anaerobic treatment plant.

Table 13.6. Key process parameters for the biogas plant during the first half-year of 2012.

Items	Unit	Parameters
Annual loaded manure (wet weight)	ton	231,143
Daily loaded material (wet weight)	ton	628±65
Total solids (TS)	%	6~8.9
Loading rate (wet weight)	kg m ⁻¹ d ⁻¹	34.9±3.6
Loading rate (as TS)	kg m ⁻¹ d ⁻¹	3.1±0.5
HRT (primary)	day	30
HRT (secondary)	day	10
Daily biogas production	x10,000 m ³	2.97±0.6

Due to its very positive impact on the environment and its overwhelming success in operation, Minhe is expanding its capacity by adding 12 new digesters with a total additional volume of 36,000 m³. The new additions are expected to be completed by October 2014. With the expanded digesters, the plant will have a new capacity of treating 700 tons of chicken wastes each day, producing 70,000 m³ of biogas daily. A pilot study is being conducted with the financial support from Germany Federal Ministry for Economic Cooperation and Development to upgrade the biogas to natural gas quality for better market values. Minhe is also in collaboration with a team from Lund University (Sweden) for the codigestion feasibility study using local energy crops as co-substrates of chicken manure.

(2) Dengta Pig Farm in Hangzhou

Dengta Pig Farm at Hangzhou has 120,000 pigs, producing 2,500 – 3,000 m³·d⁻¹ of wastewater. The wastewater treatment flow diagram is illustrated in Fig. 13.21. Characteristics of wastewater and those of effluent from each step of treatment are summarized in Table 13.7. Grids in wastewater are first removed in a settling chamber before entering the collection tank. After screening, the wastewater is pumped to an equalization tank mixed with supernatant from the sludge gravity thickener and belt press. Solids retained by the screen are sent to a fertilizer factory for composting (Deng *et al.* 2007).

After equalization, wastewater is treated in UASB reactors with effluent recirculation. The biogas produced is used as fuel for cooking or boiler heating after desulfurization and dewatering. The effluent of the anaerobic digester is discharged into an anaerobic settling tank. The mixed liquid of digested wastewater and raw wastewater are treated using aerobic SBRs, which are operated in three 8-h cycles. Excess sludge is thickened in a tank before dewatered by belt press after dosing with cationic polyacrylamide (Deng *et al.* 2007).

After five weeks of start-up, the process removed 80% of COD producing a stable effluent with <1,000 mg·COD·L⁻¹. Since most of the dry excrement was transferred directly to a fertilizer factory, while a part of the raw wastewater was directly added to the digested wastewater to adjust the ratio of carbon to nitrogen, the amount of wastewater was largely decreased. The actual inflow rate to the UASB ranged 1,200–2,200 m³·d⁻¹. This corresponded to a HRT of 3–5 days and an OLR of 2.0–5.0 kg·COD·m⁻³·d⁻¹. During the winter, the temperature of the anaerobic digester was kept only at 10–15 °C, resulting to the lowering of COD removal efficiency from 85% to 60–75%.

Although the wastewater COD fluctuated greatly (weekly average ranging from 4,000 to 15,000 mg·L⁻¹), effluent COD was 750–1,500 mg·L⁻¹. Biogas production ranged 5,000–7,000 m³·d⁻¹ in the summer, and 2,500–3,500 m³·d⁻¹ during the winter (Deng *et al.* 2007). The SBR reactors removed more than 80% of residual COD and over 98% of NH₄⁺-N, producing an effluent containing 250–350 mg·COD·L⁻¹ and <15 mg·NH₄⁺-N·L⁻¹.

Results in Table 13.7 show the process removes 96.7% COD, 99.7% BOD₅, 98.4% NH₄⁺-N, 96.8% TN and 98% SS (Deng *et al.* 2007).

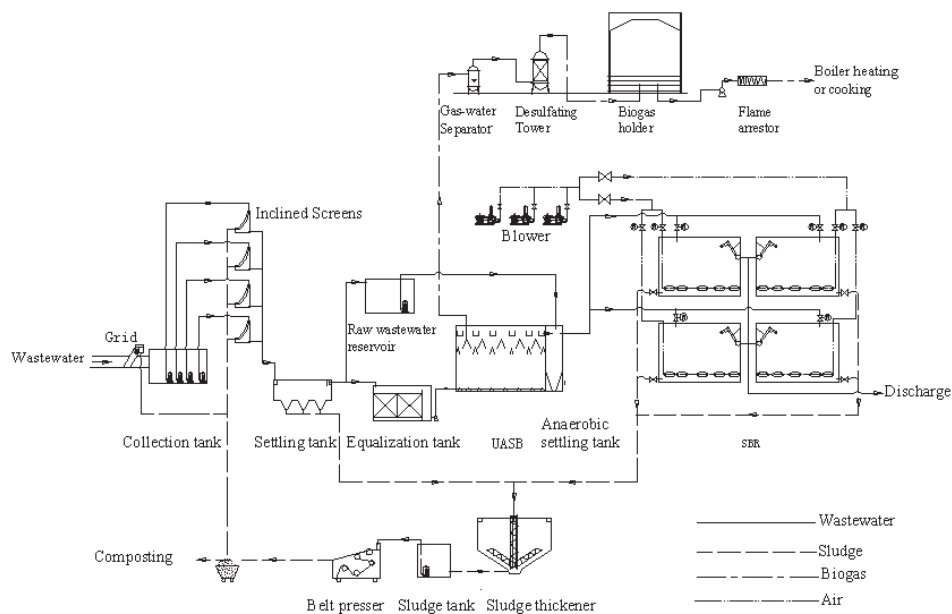


Fig. 13.21. Process flow chart of swine wastewater treatment plant in the Hangzhou Dengta general livestock farm.

Table 13.7. Total removal of pollutants of swine wastewater by wastewater treatment plants (unit: $\text{mg}\cdot\text{L}^{-1}$).

Unit	Influent		Effluent of equalization tank		Effluent of UASB		Effluent of SBR	
Item	Average	n	Average	n	Average	n	Average	n
COD	9,194±5,455	20	6,283±1817	311	1,150±390	311	302±50	210
BOD ₅	5,295±523	12	3,912±374	12	223±78.3	12	15.6±6.2	7
NH ₄ ⁺ -N	637±162	20	639±175	311	609±148	311	10.2±10.9	210
TN	1,326±672	12	1,085±467	12	753±231	12	41.9±14.2	7
SS	7,306±5,552	12	2,351±699	12	735±318	12	90±28	7

3.2. Crop stalks treatment

3.2.1. History and application in China

Anaerobic treatment of crop stalks in rural China began in the 1970s, during which about 6 million digesters were built at the beginning, mostly using animal manure as feedstock. With the increased number of digesters, farmers began to use weeds, stalks of corn, wheat and rice as feed as well. More recently, starting in the early 2000s, the focus was on the digestion of crop stalks in medium- and large-sized reactors. China in 2010 produced about 840×10^6 tons of crop stalks, of which 700×10^6 tons could potentially be collected for processing, including 210×10^6 tons of rice stalk, 154×10^6 tons of wheat stalk and 273×10^6 tons of corn stalk. About 70% of the total stalks was made use of in 2010 and this number is expected to increase to 80% by 2015.

Table 13.8. Anaerobic treatment of crop stalks in China.

Year	Projects number	Projects in operation	Household served ($\times 10^3$)
2009	178	159	11.8
2010	273	246	40.6
2011	341	294	56.9
2012	409	334	71.5
2013	434	372	78.3

The development of anaerobic treatment of crop stalks from 2009 to 2013 in China is summarized in Table 13.8. Some large biogas projects with annual output of $3\text{--}15 \times 10^6 \text{ m}^3$ were constructed by private funding at Dezhou in Shandong, Chifeng in Inner Mongolia, and Tangshan in Hebei. The biogas produced is used for heating and for vehicles after purification.

3.2.2. Technology development

In the early 2000s, the anaerobic treatment of crop stalks in China mostly used the CSTR process. Pre-treatment processes, both chemical and biological, to improve the stalk's biodegradability were investigated by the Beijing University of Chemical Technology and China Agricultural University. Through enhanced mixing, both heat transfer and mass transfer between the stalks and the inoculum were improved, resulting in better anaerobic treatment efficiency. They also studied new processes, such as full hybrid wet anaerobic digestion, membrane-covered dry fermentation and integrated 2-phase anaerobic digestion. Many other institutes have also conducted studies in this area, two of the prominent ones being the Chinese Academy of Agricultural Engineering and the Biogas Institute under the Ministry of Agriculture.

3.2.3. Case study

(1) Full hybrid wet anaerobic digestion

At Dezhou of Shandong Province, a fully hybrid wet anaerobic digestion system was built to treat crop stalks producing biogas for a centralized gas supply project, using the process developed by the Beijing University of Chemical Technology. As shown in Fig. 13.22, the process includes kneading, chemical pre-treatment, anaerobic digestion, biogas purification, biogas storage, and pipeline transportation. It processes 185 tons of crop stalk a year. The process consists of a 720-m^3 anaerobic digester (half-underground built by concrete) and a 50-m^3 pressurized tank (Fig. 13.23). It was designed to produce $300 \text{ m}^3 \cdot \text{d}^{-1}$ of biogas for the consumption of 300 nearby households; the actual biogas production was however about half the amount. The liquid slurry is partly used in stalk pre-treatment and partly provided to neighboring fruit farmers as fertilizer free of charge.

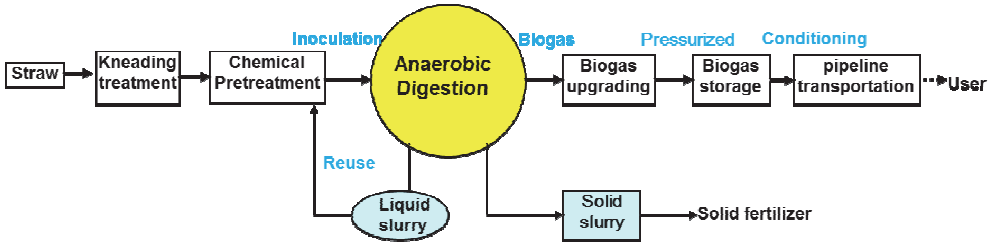


Fig. 13.22. Process flow chart of full hybrid wet anaerobic digestion technology.



Fig. 13.23. Pre-treatment tank (left) and half-underground concrete fermentor (right).

(2) Membrane covered dry fermentation process

The Chinese Academy of Agricultural Engineering has successfully developed a process in full-scale operation by combining membrane-covered dry anaerobic digestion with aerobic composting. Figure 13.24 illustrates the flow diagram of the process. Membrane cover is used (Fig. 13.25) so as to facilitate the feed and discharge of materials. After anaerobic digestion, the residues are quickly fermented and dried by mechanical devices or by sunlight to achieve dry composting. Medium- and small-sized treatment systems often encountered the problems of feedstock shortage, low biogas production and difficulties in slurry consumption. To overcome these problems, this new process uses the loop inoculation technology for stalks through the modes of liquid - solid combination and 2-phase integration by recycling the fermentation liquid on the basis of vertical digesters, which expands the raw materials for biogas and solves the problems of slurry generation. It has changed from the traditional ways of waste stalk treatment, which mostly use SBR fermentation.

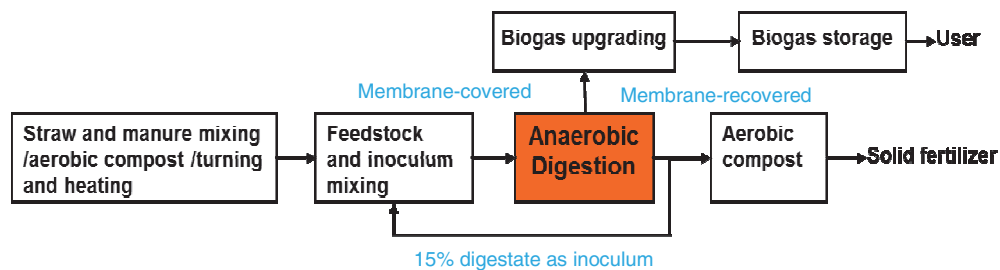


Fig. 13.24. Process flow chart of membrane-covered dry fermentation process.



Fig. 13.25. Membrane-covered trough bioreactor.

4. Development of Hydrolysis–Acidification Based Anaerobic Wastewater Pre-Treatment Technology and Its Applications

4.1. Low-strength domestic wastewater (sewage)

4.1.1. Development of hydrolysis process

Various anaerobic processes have been widely studied and applied for sewage treatment since the 1980s, particularly in the tropical and subtropical regions, such as Brazil, Thailand and India (McCarty 2001; Matsuo *et al.* 2001; Lettinga *et al.* 1983; Genung *et al.* 1979). In China, research on UASB treatment of sewage began in 1983 (Liu *et al.* 1984). Results were not encouraging, as the process required long HRT and removed only 30–60% of COD, especially at low temperatures. Aerobic post-treatment was thus

required to meet the requirements of the discharge regulations; this would further increase the total HRT and investment cost. The long HRT was mainly ascribed to the slow and sensitive methanogenesis stage. Wang *et al.* (1988) proposed a simplified anaerobic sewage treatment process, in which sewage without primary settling was hydrolyzed and partially acidified under anaerobic conditions without reaching the methanogenesis stage (this process is hereafter referred to as the “hydrolysis process”).

The hydrolysis process requires much shorter HRT (i.e. 2–3 h) and less stringent operational conditions, as compared to the conventional anaerobic process (Table 13.9). Consequently, hydrolytic reactors no longer need 3-phase separators, stirrers, sealing and heat insulation, making them much easier in scaling up and in operation. As described in Table 13.9, the facultative microorganisms are dominant in the hydrolytic reactors, which are effective at converting non-soluble, complex and even recalcitrant organic compounds into small soluble organic molecules to improve the biodegradability of sewage for high-efficiency aerobic post-treatment, which may require an additional 2.5 h. Nearly 100 sewage treatment plants in China have now used this hydrolysis process with aerobic post-treatment since the 1990s. Full-scale data have shown that this new process reduces the capital investment and operational cost, including energy consumption, by about 30%.

4.1.2. *Performance of the hydrolytic reactor*

(1) Enhanced removal of COD, BOD₅ and SS

Table 13.10 compares the performances of the pilot hydrolytic reactor (treating 1,800 m³·d⁻¹ of sewage without primary settling) and the primary settling tank of the conventional activated sludge at the Gaobeidian sewage treatment (Beijing). As shown in Table 13.10, the hydrolytic reactor performed much better than the primary settling tank, particularly in SS removal (up to about 80%). Moreover, the hydrolytic reactor was shown to be much more resistant to the influent fluctuations and thus could replace the primary settling tank as an effective pre-treatment method.

(2) Resiliency towards organic shock loads

As demonstrated in Fig. 13.26, the COD removal efficiency increased with the sewage COD concentration. When organic loads were increased from 1.95 to 8.8 kg·m⁻³·d⁻¹, only a slight increase in the effluent COD (ranging 207–316 mg·L⁻¹) was observed, indicating that the hydrolytic reactor has high resiliency towards the organic shock loads.

(3) Sludge reduction

Figure 13.27 shows the 1-year overall mass balance of COD and SS in the pilot hydrolytic reactor. Results show that the process removed ~82% of sewage SS, almost half of which are small particles that could not be removed by the conventional primary settling tank. Moreover, the process hydrolyzed up to 53.5% of the sewage SS and reduced 30% of sludge production as compared to the combined system of the primary settling tank and the anaerobic sludge digester.

Table 13.9. Overview of the differences between the hydrolysis and anaerobic digestion processes.

Items	Hydrolysis	Acidogenic phase in 2-phase AD	Single-phase AD
Eh (mV)	0	-100--300	< -300
pH	6.5--7.5	6.0--6.5	6.8--7.2
Temperature	uncontrolled	controlled	controlled
Dominant microorganisms	facultative microorganisms	facultative and anaerobic microorganisms	anaerobic microorganisms
Methane production	very tiny amount	small amount	huge amount
End products	low-strength organic acids	high-strength organic acids, small amounts of CH ₄ /CO ₂	CH ₄ /CO ₂

Table 13.10. Performance of the hydrolytic reactor vs. the primary settling tank.

Parameters	Hydrolytic reactor			Primary settling tank		
HRT (h)	2.5	3.0	3.5	1.67	2.22	3.33
COD removal (%)	43.0	41.3	40.6	—	—	—
BOD ₅ removal (%)	29.8	33.1	28.1	18	12	17
SS removal (%)	82.6	74.8	79	42	40	47

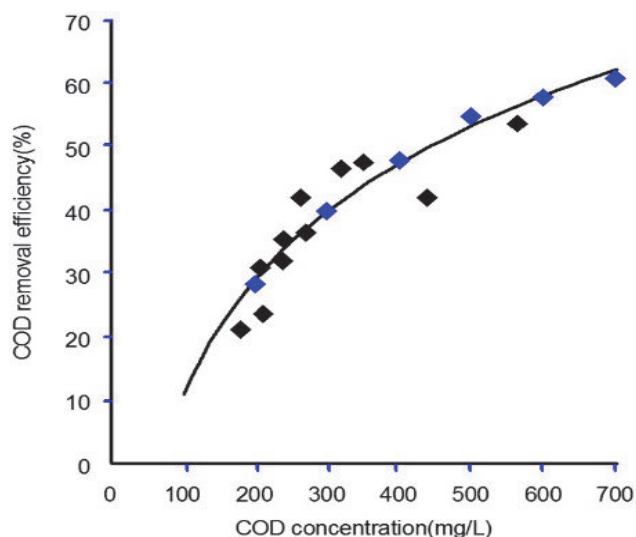


Fig. 13.26. Effect of influent COD concentration on COD removal efficiency.

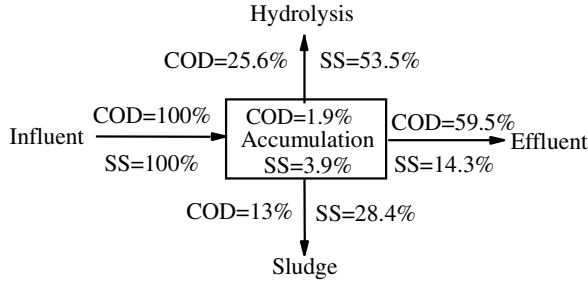


Fig. 13.27. The mass balance of COD and SS in the pilot-scale hydrolytic reactor.

4.1.3. Operational and engineering aspects of the hydrolytic reactor

(1) Start-up

When the SS of raw sewage is less than $100 \text{ mg} \cdot \text{L}^{-1}$, sludge from the anaerobic digester ($5\text{--}10 \text{ g} \cdot \text{L}^{-1}$) could be used as the inoculum for the start-up of the hydrolysis reactor. Due to the low COD concentration and high buffer capacity of sewage, the hydrolytic reactor can be operated at full load immediately after inoculation with no consideration of acidification. At the initial stage, the SS and turbidity of the effluent are quite high and large amounts of methanogens are washed out. After 10–15 days, the effluent becomes clear and $\sim 40\%$ COD removal could be reached, indicating that the mature facultative microorganisms for hydrolysis and acidification are gradually formed. The start-up is expected to be completed when COD removal, sludge concentration and activity become stable. If the SS of raw sewage is higher than $100 \text{ mg} \cdot \text{L}^{-1}$, the start-up of the hydrolysis reactor can be performed directly without inoculation.

(2) Influent distribution system

The uniform influent distribution is of great importance for the stable operation of the hydrolytic reactor. The branched pipeline system (Fig. 13.28) was widely adopted in the early stage of development, such as those used at the Gaobeidian, Miyun and Changji plants. In branched pipeline systems, the pipeline is symmetrically arranged. The outlets of each branched pipe situate in the middle of their respective service areas, about 20 cm from the bottom of the reactor. The sewage from the outlets is subsequently distributed by the reflecting cones, located right below the outlets. The branched pipeline system can basically achieve a balanced distribution of influent if correctly installed.

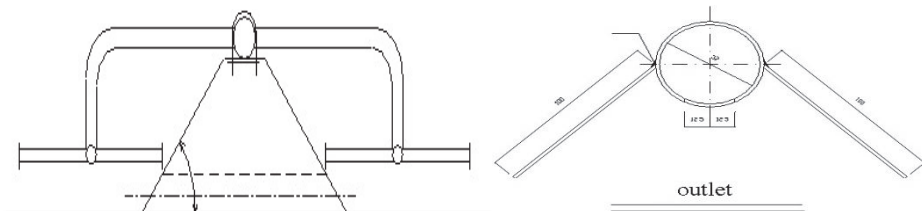


Fig. 13.28. The branched pipeline systems for influent distribution.

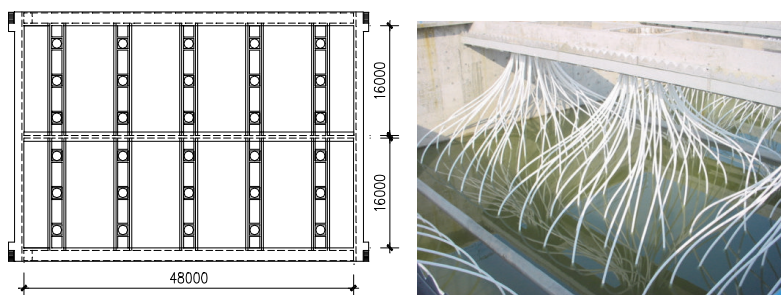


Fig. 13.29. The EIP-SOP distribution systems used in the hydrolytic reactors of Miyun sewage plant (second-stage, $30,000 \text{ m}^3 \cdot \text{d}^{-1}$).

More recently, a strategy has been recommended for the influent distribution system for UASB reactors; that is, each inlet pipe with a single outlet point (EIP-SOP). This has been applied in several sewage plants, such as Miyun (second-stage, $30,000 \text{ m}^3 \cdot \text{d}^{-1}$) and Aksu ($60,000 \text{ m}^3 \cdot \text{d}^{-1}$). This strategy can not only ensure the uniform influent distribution but also facilitate the scale-up of the hydrolytic reactors. The EIP-SOP distribution, system used in the hydrolytic reactors of Miyun sewage plant (second-stage, $30,000 \text{ m}^3 \cdot \text{d}^{-1}$) is illustrated in Fig. 13.29.

(3) Two additional crucial factors: Up-flow velocity and sludge withdrawal

The relationship between the reactor height (H) and up-flow velocity (v) is shown as below:

$$V = Q/A = V/(HRT \cdot A) = H/HRT \quad (13.1)$$

where Q represents the flow rate and A represents the cross-section area of the reactor. The reactor height is directly related with the up-flow velocity of the influent. The increase of up-flow velocity helps to enhance the contact between the influent organics and the sludge. However, overly high up-flow velocity leads to the washout of the sludge. Therefore, there is an optimal range of up-flow velocity and reactor height. And in most cases, a height of 4–6 m and an up-flow velocity of $0.5\text{--}1.8 \text{ m} \cdot \text{h}^{-1}$ are adopted.

In general, the performance of the hydrolytic reactor can be improved with the increase of sludge concentration. However, when the sludge blanket height exceeds a certain value, sludge washout will take place and the effluent quality will start to deteriorate. Thus, excess sludge should be discharged. Meanwhile, it is important to discharge the low-activity sludge while retaining the high-activity sludge. Experience has shown that the following are crucial to the sludge withdrawal:

- height of the supernatant liquid layer should be maintained at 0.5–1.5 m;
- sludge discharge can be performed regularly, namely once or twice a day;
- sludge level detector is required to determine the sludge discharge time;
- excess sludge from the top half of the sludge blanket should be removed;
- multiple sludge discharge points should be set in the vertical direction;
- occasional sludge discharge from the lower level of the reactor is required;

- at SRT >15 days, 25% of sludge hydrolysed in winter, and 50 % in summer; and
- the design of the sludge withdrawal system should consider the worst scenario.

4.1.4. Case Study

In the past three decades, the hydrolysis process has been widely used in treating sewage in various regions of China, as shown in Table 13.11. Figure 13.30 shows a typical flow diagram of the sewage treatment employing the hydrolysis process.

Performance of the Chengbei sewage treatment plant at Wuxi is chosen as a case study to be discussed as follows: In this plant, 100,000 m³·d⁻¹ of sewage is first hydrolyzed followed by aerobic post-treatment using an oxidation ditch process. There are four hydrolytic tanks (56 x 15 x 5 m) in use with an average HRT of 4 h. The sludge retained in the hydrolytic tanks could reach 980 m³·d⁻¹ (1.5% TS). In order to ensure the balanced influent distribution in the large hydrolytic tanks, the EIP-SOP distribution system was used. The monthly average performance of the hydrolytic tanks is demonstrated in Fig. 13.31. More than 50% of the COD and SS could be removed in the hydrolytic tanks most of the time, even in the winter seasons.

4.2. High-strength industrial wastewater

4.2.1. Concept of separating acidogenic and methanogenic processes

Before the 1970s, the single-stage anaerobic treatment was the most widely applied process for treating high-strength wastewaters. Since the microorganisms that are

Table 13.11. STPs with the hydrolysis process (partial data).

Number	Year	Capacity (t·d ⁻¹)	Main processes	Provinces or municipalities
01	1992	15,000	Hydrolysis-Activated Sludge	Beijing
02	1989	30,000	Hydrolysis-Oxidation Ditch	Henan
03	1989	30,000	Hydrolysis-Activated Sludge	Xinjiang
04	1997	100,000	Hydrolysis-Activated Sludge	Hubei
05	1998	6,0000	Hydrolysis-AICS	Xinjiang
06	1995	60,000	Hydrolysis-Land Treatment	Shandong
07	1991	10,000	Hydrolysis-Oxidation Pond	Shenzhen
08	1998	10,000	Hydrolysis-Activated Sludge	Xinjiang
09	2002	30,000	Hydrolysis-Modified UNITANK	Xinjiang
10	2002	100,000	Hydrolysis-Modified UNITANK	Sichuan
11	2002	50,000	Hydrolysis- Modified SBR	Jilin
12	2003	80,000	Hydrolysis-(SBR)AICS	Xinjiang
13	2004	40,000	Hydrolysis-(SBR)AICS	Zhejiang
14	2006	100,000	Hydrolysis-Oxidation Ditch	Jiangsu
15	2007	50,000	Hydrolysis-(SBR)AICS	Jilin
16	2008	30,000	Hydrolysis-A/O	Heilongjiang
17	2009	30,000	Hydrolysis-(SBR)AICS	Jilin
18	2009	100,000	Hydrolysis-A/O-Advanced Treatment	Hebei
19	2010	10,000	Hydrolysis-SBR	Xinjiang

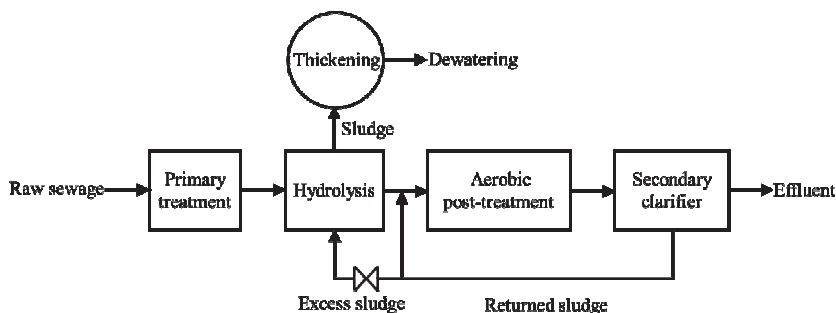


Fig. 13.30. Typical flow diagram of the sewage treatment with the hydrolysis process.

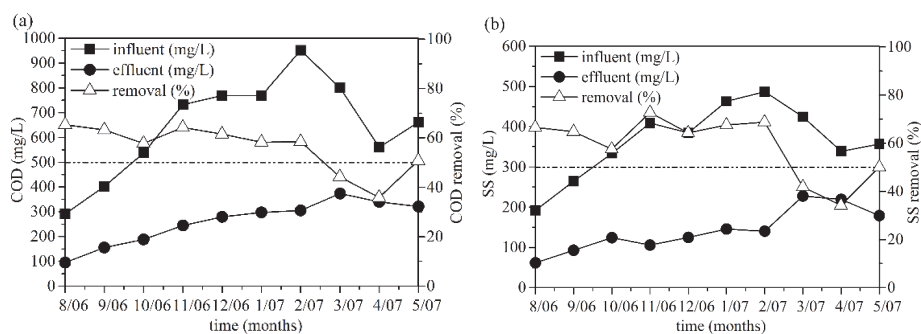


Fig. 13.31. The monthly averages of (a) COD and (b) SS of the hydrolytic tanks (2006–08 to 2007–05) at Wuxi Chengbei sewage treatment plant.

responsible for acidification and methanogenesis have distinct preferences for environmental conditions, it is very hard to maintain a balance in a single reactor in which both the acidifiers and methanogens can be prosperous. When such a balance is not achieved, the activity of methanogenesis is suppressed due to the so-called “rancidity”. This phenomenon might jeopardize the stability of the entire treatment operation. The concept of separating the two bio-reactions has been proposed by Pohland and Ghosh (1971). In the 2-stage anaerobic system, the acidogenic phase and methanogenic phase are operated separately by adjusting physicochemical parameters (such as using selective inhibitors, oxidation–reduction potential or pH) and/or hydraulic parameters (such as HRT). By carefully choosing the operational parameters for the two individual phases, optimal conditions for the two types of microorganisms can be achieved, and as a consequence, the treatment capacity and operation stability can be enhanced.

Acidogenesis plays a key role in improving the biodegradability of recalcitrant pollutants in high-strength industrial wastewaters, and consequently, the stability of the methanogenic process downstream. In the 1990s, many studies were conducted on the microbiology, biochemistry, ecology, and operation strategy related to the acidogenic process. Ren *et al.* (2005) demonstrated that: (1) pH and redox potential are two critical

factors, and (2) limiting ecological factors have great impact on the acidifiers and their population dynamics. By revealing how these limiting ecological factors work, feasible control strategies to further improve the treatment capacity could be developed.

4.2.2. *Development of integrated processes*

Based on the concept of separating individual biodegradation processes, four integrated processes were developed for the treatment of wastewater from chemical industries, such as printing and dyeing, pharmaceutical and fermentation: (1) 2-phase anaerobic–aerobic process, (2) hydrolysis–acidification–oxidation process, (3) a process for the simultaneous removal of carbon, nitrogen and sulfur coupling with bio-sulfur recovery, and (4) internal cycling–hydrolysis–oxidation process. Aside from these, 12 patented instruments were developed to facilitate the operation of these processes. These processes are reliable by controlling the bio-community, hydraulic pattern, and physiological–ecological parameters. For 20 years, they have been widely applied in China with more than 20 demonstration projects. Data from full-scale operations show a 30–40% increase in acidification efficiency of recalcitrant chemicals, 20–30% increase in biodegradability, 20–40% increase in organic loading rate for the methanogenic reactor downstream, and 10–20% increase in the overall process capacity (Ren *et al.* 2005). The operational cost for each m³ of wastewater treated was lowered by 2.0 yuan. Two prestigious national prizes were awarded in 2004 and 2010 for the recognition of these achievements.

4.2.3. *Case Study*

(1) Two-phase anaerobic–aerobic process for pharmaceutical and chemical wastewaters

Wastewaters from the pharmaceutical industry, producing both traditional Chinese drugs as well as modern Western drugs, and chemical industry are often with intense color and concentrated with recalcitrant pollutants. A 2-phase anaerobic–aerobic integrated process was developed with the physiological ecology of acidifiers in mind, in reactor design and in the control of hydrological condition operations. Figures 13.32 and 13.33 illustrates two schemes of this process, which have been proven to be highly cost effective for the treatment of various wastewaters with COD ranging 4,000–20,000 mg·L⁻¹. The development has been awarded with a series of patents in reactor design and operation (ZL98240801.3, ZL00206243.7, ZL96251960.X, and ZL992132525).

The plant of Harbin No. 2 Traditional Chinese Medicine produced 1,500 m³·d⁻¹ of wastewater with 19,000 mg-COD·L⁻¹, 4,000 mg-BOD·L⁻¹ and 450 mg-SS·L⁻¹. Using this 2-phase process, the treated effluent contained 150 mg-COD·L⁻¹, 60 mg-BOD·L⁻¹ and 70 mg-SS·L⁻¹. The operational cost was 0.9 yuan for each m³ of treated wastewater or 0.09 yuan for each gram of COD removed. Comparing to traditional wastewater treatment processes, this process saved 3.6x10⁶ yuan in capital cost and 164x10³ yuan in annual operation.

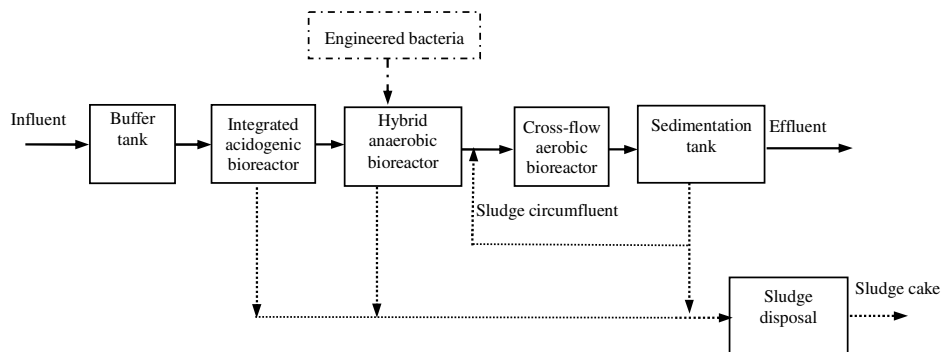


Fig. 13.32. Two-phase anaerobic-aerobic process (Scheme 1).

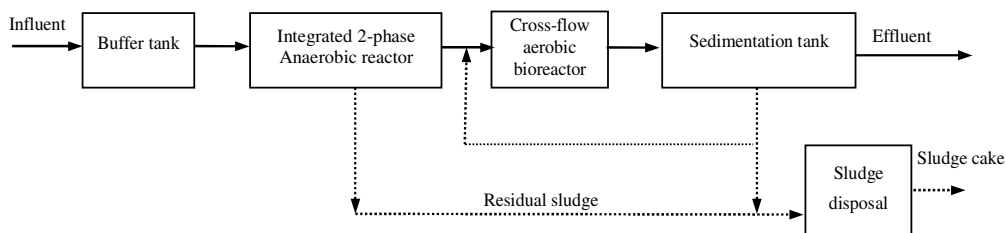


Fig. 13.33. Two-phase anaerobic-aerobic process (Scheme 2).

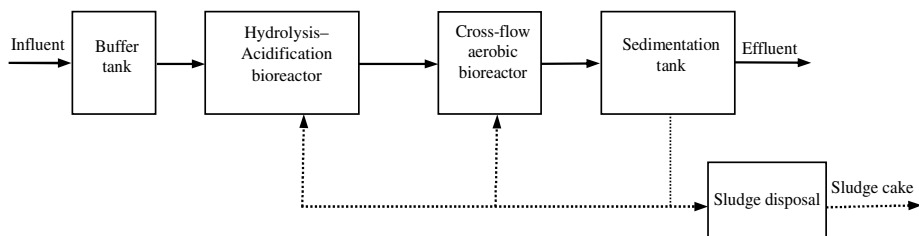


Fig. 13.34. Hydrolysis-acidification-oxidation process.

(2) Hydrolysis-acidification-oxidation process for printing and dyeing wastewater

Figure 13.34 illustrates the schematic flow diagram of the hydrolysis-acidification-oxidation process, which has been applied to the treatment of printing and dyeing wastewater. The process was used at the Siling dyeing plant in Jiangsu Province treating $4,000 \text{ m}^3 \cdot \text{d}^{-1}$ of wastewater with $3,000 \text{ mg-COD} \cdot \text{L}^{-1}$, $700 \text{ mg-BOD} \cdot \text{L}^{-1}$, and a chromacity of $4,000\text{--}12,000 \text{ mg} \cdot \text{L}^{-1}$. The treated effluent has been substantially improved to the quality of $<100 \text{ mg-COD} \cdot \text{L}^{-1}$, $<60 \text{ mg-BOD} \cdot \text{L}^{-1}$, and a chromacity of $<180 \text{ mg} \cdot \text{L}^{-1}$ with an operational cost of 1.0 yuan per m^3 of treated wastewater. Comparing with the traditional processes, this process saved the 2.96×10^6 yuan in capital cost and 4.38×10^6 yuan in annual operation.

The keys to the success of this process are two folds: (1) It consolidates the hydrolysis–acidification bioreactor, which can be operated under high loading rates and has the features of optimized flow patterns, easy to operate and low HRT; the removing of ammonium enhances the biodegradability by as much as 30%; and (2) it incorporates the design of cross-flow aerobic reactors with a specially designed packing material and changes the flow pattern to enhance oxygen transfer; the process is operated at low HRT and it reduces the sludge production.

(3) A novel process for pharmaceutical wastewaters

Figure 13.35 illustrates the flow diagram of a novel process, which was developed for the simultaneous removal of carbon, nitrogen and sulfur coupling with bio-sulfur recovery for wastewater with high levels of nitrogen and sulfate (Yuan *et al.* 2014). It uses a 2-phase anaerobic process, effectively reducing sulfate at the acidification step and recovering the elemental sulfur at the subsequent denitrification step (Chen *et al.* 2009). The process was used at the Huabei Pharmaceutical treating 20,000 m³·d⁻¹ of wastewater with 12,000–15,000 mg-COD·L⁻¹, 4,800–6,000 mg-BOD·L⁻¹, 1,000 mg-SS·L⁻¹, 2,300 mg-TN·L⁻¹, and 5,000–6,000 mg-SO₄²⁻·L⁻¹. The treated effluent has been substantially improved to the quality of <100 mg-COD·L⁻¹, <50 mg-BOD·L⁻¹, <100 mg-SS·L⁻¹, <100 mg-TN·L⁻¹, and <50 mg-SO₄²⁻·L⁻¹. The cost for treating each m³ of wastewater is 1.5 yuan per m³.

The key successful features of this process are: (1) high resiliency towards the shock loading and the toxicity of sulfurous compounds; (2) effective recovery of elemental sulfur by using a microaerobic regulation method (Xu *et al.* 2014); (3) over 90% of sulfur recovery by dosing coagulants to enhance sedimentation; and (4) effective removal of ammonium by the use of aerobic contact pond.

(4) Internal cycling–hydrolysis–oxidation process for brewery wastewater treatment

Figure 13.36 illustrates the flow diagram of an internal cycling–hydrolysis–oxidation process at the Huarun Brewery of Anhui Province treating 5,500 m³·d⁻¹ of brewery wastewater with 4,000 mg-COD·L⁻¹, 1,000 mg-BOD·L⁻¹ and 1,000 mg-SS·L⁻¹. The

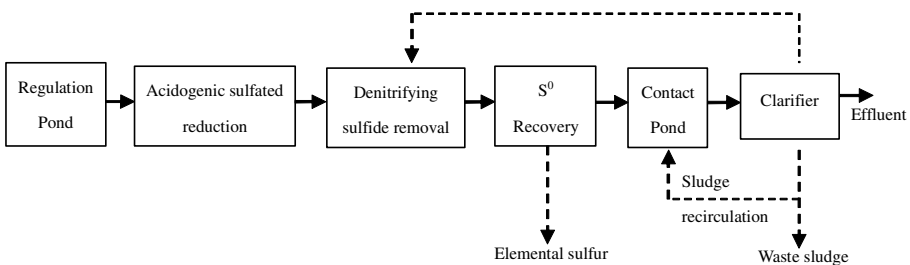


Fig. 13.35. A novel process for wastewater with high levels of nitrogen and sulfur.

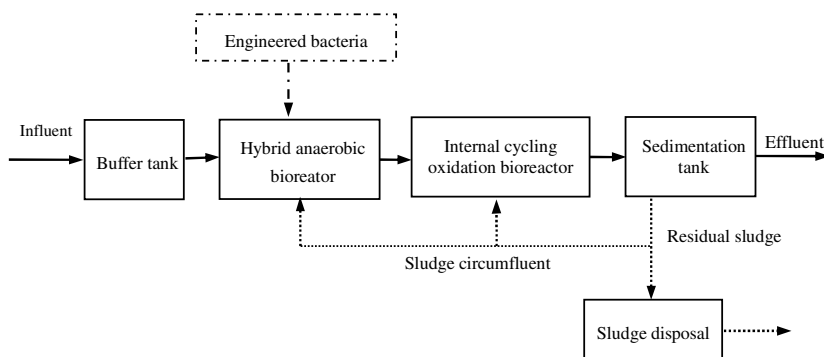


Fig. 13.36. The internal cycling–hydroxylation–oxidation process for wastewater from the breweries.

treated effluent produced is of the quality of $100 \text{ mg-COD}\cdot\text{L}^{-1}$, $40 \text{ mg-BOD}\cdot\text{L}^{-1}$, and $50 \text{ mg-SS}\cdot\text{L}^{-1}$. The operational cost is 0.6 yuan per m^3 of treated wastewater. Comparing with the traditional processes, this process saved the 0.22×10^6 yuan annual in operation. A few key features are summarized as follows: (1) start-up was shortened to 15 days by using good quality seed sludge with engineered bacteria; (2) hydrolysis and acidification, which take place in the internal cycling–hydrolysis–oxidation reactor, were controlled at pH 5.5–6.5 and $0.2\sim 0.5 \text{ mg}\cdot\text{L}^{-1}$ of DO; (3) aerobic stage was operated at $1.5\sim 3 \text{ mg}\cdot\text{L}^{-1}$ of DO and the return sludge ratio was kept at 50–80%; and (4) waste sludge was substantially reduced.

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II. Challenges Towards Sustainability

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Chapter 14

Development of Anaerobic Digestion of Animal Waste: From Laboratory, Research and Commercial Farms to a Value-Added New Product

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A systematic study on thermophilic anaerobic digestion of poultry waste was carried out at North Carolina State University over the last 35 years. The concept and experiments were initiated in the laboratory and technical feasibility was proven by a pilot-scale digester on the university farm and subsequently full-scale in China. Multiple benefits of the digester system in animal and crop productions, waste conversion, biogas production, nutrient recycle, farm sanitation and CO₂ reduction were demonstrated. The possibility of financial gain was projected for an integrated system with an anaerobic digester at its core. Also, interestingly, a feather-degrading bacterium was isolated from the digester and, subsequently, a bacterial keratinase was purified. This enzyme was found useful in processing feather meal, degrading prion protein — which is the putative cause of bovine spongiform encephalopathy or commonly known as mad cow disease — and improving protein digestibility in animal feeds. As a feed enzyme, keratinase is now produced at industrial scale and marketed globally. A total of 12 patents were generated from this series of studies from waste management and energy production to enzyme technology. In the foreseeable future, new biotechnologies, including genomics, proteomics and bioinformatics, are able to probe into the complex bio-process for better understanding and improving biogas production. An integrated system, or *Holistic Farming*, is proposed to facilitate not only waste management and energy production, but also agricultural and environmental sustainability. This system could drive a new model of agriculture for the 21st Century.

1. Introduction

1.1. Early history

Biogas has been around in natural environments where biomass decays and decomposes by the action of microbes in the absence of air to produce the gas mixture of CH₄ and CO₂. It was first discovered in swamp areas, and this bio-process is known as anaerobic digestion. It became the basis of an Indian government outreach program to provide villagers with cooking fuel from the digestion of farm manure (Tietjen 1975). This process was also used for lighting in the UK. Starting in the mid-20th century, systems

were developed in the UK and Germany for the treatment of sewage sludge. Several sewage plants ran vehicles powered by biogas.

1.2. Biogas from animal farms in China

Biogas technology developed independently in China. In the early 1900s, an engineer from Taiwan named Luo Guo-Rui (羅國瑞) invented the first water-pressured digester to treat night soil and provide biogas for lighting and cooking for household use. In 1930, he started a company in Shanghai based on his patented digester and biogas light (Shi 2010). In 1957, the Chinese government launched a policy of developing new sources of energy in rural areas with the small “biogaser” (5–10 m³), similar to Mr. Luo’s water-pressured design for each household with 5–10 pigs. Since then, the small digesters have been widely used and promoted with government subsidies. In recent years, the total number of household digesters was estimated at 3.5×10^7 , providing 12.4×10^9 m³ biogas per year as a form of energy to reduce wood and coal burning in rural China (Li 2011).

As the Chinese economy grew rapidly from the 1980s onward, many large farms were installed in China, and, as a result, the total number of medium- (100–500 m³) and large- (>500 m³) sized digesters was increased and estimated to be 1,000 in 2011 (Li 2011). For example, this author assisted in the design and operation of the first thermophilic anaerobic digester (TAnD) in China in 1992 to process five tons of chicken manure each day (see below). In recent years, the Chinese have built many large biogas plants. The largest plant is on a poultry farm in Shandong Province. With 7 million chickens on the farm, the digester system processes approximately 700 tons of manure into 70,000 m³ of biogas per day. Half the amount of the biogas is converted into electricity and the other half is purified and compressed to power vehicles (Sun, XM, the owner, personal communication, 2014). In addition to the large biogas plants, 35 million small household-type digesters are being extensively used in rural China. For both waste management and energy production, the Chinese will continue to develop their biogas technology and commercialization of large and small digesters (Shih and Chen 2009). Industrialization of anaerobic digestion has been actively discussed in China (Jiang *et al.* 2011; Li 2011).

1.3. Biogas in Germany

Germany is the leading country in global development of anaerobic digestion, in terms of technology, growth rate and commercialization. The number of large digesters grew rapidly from 139 in 1992 to near 8,000 in 2013 (Nagele 2013). Most biogas plants are installed for electric power generation with a total capacity of about 3,530 MW. Being short on natural resources, Germany is dependent on the import of petroleum and natural gas. In 2011, the German government announced plans to eliminate nuclear energy by 2022, which provides 15% of its current national energy need. Instead, an aggressive plan calls for complete energy self-sufficiency by 2050 through the development of renewable energies. Biogas, one of the options, is predicted to replace 30% of natural gas by 2030. Many different kinds of biomass and organic wastes are digested or codigested for biogas production. Biogas has earned a nickname, “Cinderella Energy”, transforming itself from a by-product of waste treatment into a clean and renewable energy. In addition to

Germany, many other countries in Europe, including the UK, the Netherlands, Denmark and Sweden also have active biogas programs.

1.4. Biogas in the U.S.

Ethanol has been targeted as the biofuel for development in the U.S. during the last 10–20 years. However, the interest in the use of methane is on the rise, regardless of whether it is derived from natural shale gas or biogas from anaerobic digestion. The discovery of the abundant natural reserve of shale gas in North America will inevitably change the energy landscape in the future for the U.S. and, potentially, for the world (The Economist 2012). On the other hand, because of the benefits of biogas in its renewability, waste reduction, generation of carbon credits, co-production of organic fertilizer, and the relative ease in production, purification and utilization, it is gathering more interest and attention in the U.S. In 2014, the U.S. Department of Agriculture, US Environmental Protection and the U.S. Department of Energy jointly published a document, entitled *Biogas Opportunities Roadmap*, calling on voluntary actions to reduce methane emissions and increase energy independence. They projected that the integration of the multiple processes and value capture of the co-products would make biogas energy financially viable.

2. Thermophilic Anaerobic Digestion (TAnD)

2.1. Laboratory studies

In the early 1980s, a systematic study of TAnD of poultry manure was initiated at North Carolina State University (NCSU). What followed was a series of new discoveries. Multiple lab-scale digesters were first set up to carry out experiments to determine the biological potential of biogas production from poultry manure (Fig. 14.1). Three major parameters, namely temperature, concentration and retention time, were studied to determine the operational optima for the highest biogas production rate. It was found that thermophilic temperatures (50–60 °C) supported high biogas rates (Huang and Shih 1981; Huang *et al.* 1982). Relatively higher concentration of chicken manure and shorter

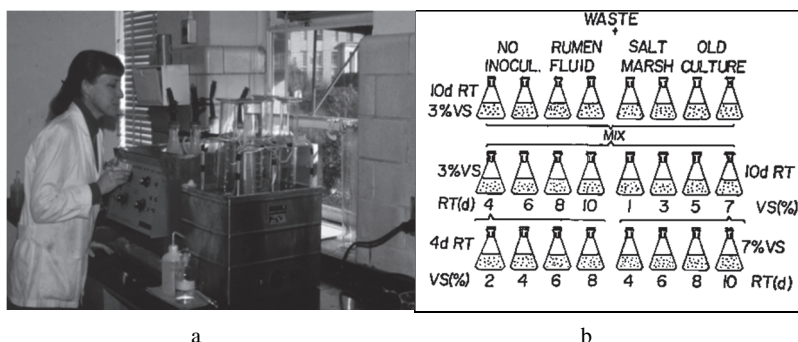


Fig. 14.1. Laboratory anaerobic digesters. a: The set-up of laboratory digesters. b: The experimental design.

Table 14.1. Biogas potential from thermophilic and mesophilic anaerobic digestion of chicken manure.

	Mesophilic	Thermophilic
Temperature, °C	35	50
Volume, liter	1.0	1.0
pH	7.5–8.0	7.5–8.0
RT, days	10	5
Influent, % VS	6	6
Effluent, % VS	2.8	3.3
Gas Rate, $v \cdot v^{-1} \cdot \text{day}^{-1}$		
Biogas	2.5	4.5
CH ₄	1.5	3.0
Gas yield, L·kg-VS ⁻¹		
Biogas	420	400
CH ₄	250	250
Energy, cal		
Input	2,000	7,000
Output	13,500	27,000
Net	11,500	20,000
Net/output, %	85	74

Note: Energy input is for heating 15 °C influent to 35 or 50 °C; output, 9,000 cal·L-CH₄⁻¹.

RT: retention time. VS: volatile solids.

retention time could be used for long-term stable operation. From the laboratory digesters, the overall efficiency at thermophilic temperatures was found to be higher than that at mesophilic temperature (30–40 °C) and much higher than that at ambient temperatures. The average net energy gains were 74% from TAnD and 85% from the mesophilic. The performances at two different temperatures are summarized in Table 14.1.

2.2. Pilot-scale digester

To confirm the laboratory results, a simple pilot plug-flow type digester was designed, constructed and operated on the NCSU research farm. It was attached to a hen house with 4,000 laying hens generating 400 kg manure daily (Fig. 14.2). After three years of operation, the performance of the pilot digester confirmed the laboratory data and proved the concept and technical feasibility of TAnD (Steinsberger and Shih 1984). With the NCSU digester system, it further demonstrated the multiple benefits of the system. The benefits included the biogas heat for drying the digestate and brooding baby chicks (Jiang *et al.* 1987), the dry digestate residue as feed supplement for nutrients recovery (Steinsberger *et al.* 1987), and the digester effluent for aquaculture (Shih 1987). An integrated system called “Holistic Farming” was proposed in concept (Shih 1985; Shih 1987). In addition, TAnD was found to be effective at destroying and controlling pathogens that may exist in animal excreta (Shih 1988; Lee and Shih 1988).

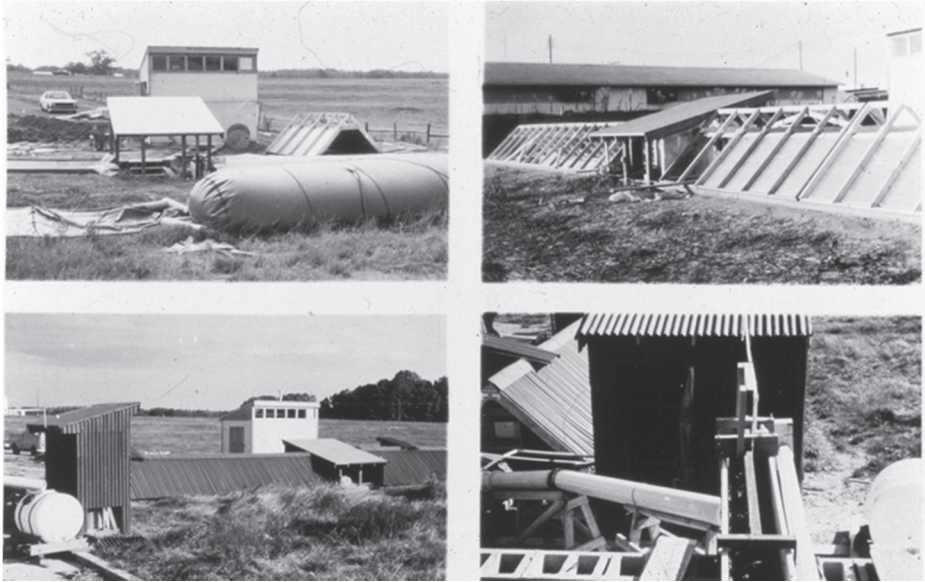


Fig. 14.2. Construction of NCSU pilot-scale TAnD for poultry waste from 4,000 laying hens.



Fig. 14.3. Beijing Liu-min-ying TAnD for 50,000 laying hens. (Designed and supervised by the author).

2.3. Farm operations

Subsequently, TAnDs with the similar plug-flow design were constructed and operated in Asia, one in Taiwan for 2,000 hogs and one in Beijing for 50,000 laying hens, both designed and supervised by the author. The TAnDs were compact (100 m^3), that processed approximately 5 tons of manure each day ($\text{RT}=10$ days). In Beijing, it produced biogas for cooking and heating for a village of 200 households and co-produced organic fertilizer as a major source of income (Fig. 14.3). This project has been in operation for more than 20 years and is still running, initially supported by a grant from the United Nations Development Program (Shen and Zhao 1995; Shih 2012). The success

of this project has made a positive impact on the development of biogas in China. Many large biogas plants have been constructed and commercialized in recent years in China. Although most of them are operated at mesophilic temperatures (30–40 °C), the concept of Holistic Farming has been well adapted in China. The author's pioneering work was honorably cited in the cover story of Genetic Engineering and Biotechnology News published in 2011 (GEN News 2011).

3. Keratinase

3.1. *The discovery*

During daily operation of the TAnD at the NCSU research farm, an interesting observation was made that resulted in a serendipitous discovery. The initial observation was made that shed feathers mixed in the poultry manure disappeared in the digestion process. Following three years of methodology development, experimental design and a painstaking research, the bio-degradation of feathers was finally proven by the isolation of a feather-degrading bacterium, *Bacillus licheniformis*. It was named strain PWD-1 as the first isolate from a poultry waste digester (Fig. 14.4). This bacterium can grow in a medium with feathers as the sole source of organic substrate, because it secretes a keratinase enzyme capable of hydrolyzing feather keratin (Williams and Shih 1989; Williams *et al.* 1990).

The keratinase was subsequently purified from the culture medium. It was characterized as a heat-stable protease, consisting of a single monomeric protein with a molecular weight of 33 kDa, digesting a wide range of substrates, including casein, elastin, collagen and keratin (Lin *et al.* 1992). The encoding gene, *ker A*, was isolated, sequenced and over-expressed by genetic manipulations (Lin *et al.* 1995; Lin *et al.* 1997; Wang *et al.* 2004). Large-scale production of this enzyme was accomplished first in a 150-L pilot fermenter in the lab (Wang and Shih 1999) and then in 50-kL industrial fermentation tanks for commercial production.

3.2. *Applications of keratinase*

Early in 1991, the PWD-1 culture with active keratinase was used to incubate chopped chicken feathers to produce a partially hydrolyzed “feather-lysate”, which was subsequently tested for partial replacement of soybean meal in a test feed. The result indicated that feather-lysate indeed had the digestibility or nutritional value close to soybean meal, the major protein source for agricultural animals (Williams *et al.* 1991). This was the first study to indicate the potential of keratinase for converting feathers into a more digestible feather meal since raw, unprocessed feathers are poorly digestible. The follow-up studies and commercial applications helped realize this potential.

Independently, the author and his research team began to test the keratinase as a feed additive in chicken feed. A small amount of crude keratinase was supplemented to the control feed to grow young chicks. Surprisingly, the feed supplemented with the enzyme significantly improved chick growth. Further studies on the NCSU research farm (Odetallah *et al.* 2005) and on commercial farms (Wang *et al.* 2006) confirmed the initial

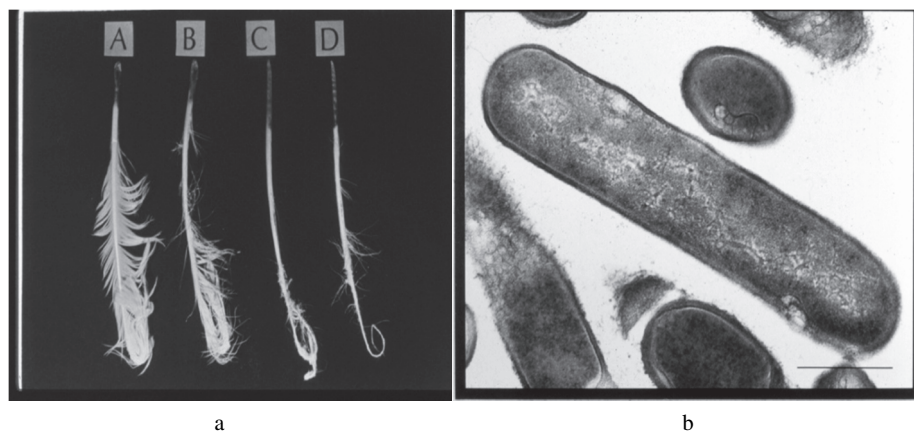


Fig. 14.4. (a) Bio-degradation of feathers in the culture of *B. licheniformis* PWD-1, and (b) Electron microscopy of PWD-1 bacterium.

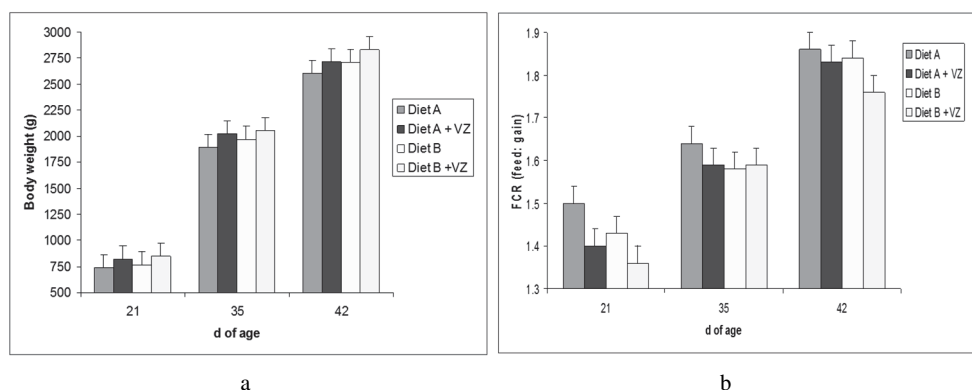


Fig. 14.5. Research farm test (1,000 growing chicks each treatment) of the effect of the keratinase (VZ) additive to the low-protein (Diet A) and high-protein (Diet B) diets. a: Body weight. b: Feed conversion rate (FCR).

discovery, which was conducted in laboratory cages (Odetallah *et al.* 2003). One of the representative results is illustrated in Fig. 14.5. More research concluded that the growth promoting effect is due to the enhanced protein digestibility in the gastrointestinal tract and, consequently, improved nutrition and growth of the animal (Wang *et al.* 2008). It was estimated that supplementation of this enzyme could help spare up to 2% dietary protein in feed. Given the fact that the global annual production of poultry and swine feeds is approximately 500 million tons, the saving of global feed cost by keratinase and other similar enzymes are proportionally quite large. In commercial development, a biotechnology company, namely BioResource International, was established to produce and market the enzyme worldwide (www.briworldwide.com).

In addition to the applications in animal feed and nutrition, the keratinase was tested for enzymatic degradation of prion protein. Prion protein, the putative cause of bovine spongiform encephalopathy (BSE) or mad cow disease, is notoriously resistant to

common proteases and regular disinfection methods, because of its unique protein structure and mis-folding into a tightly packed polymer (Prusiner *et al.* 1998). It was noted by the author that feather keratin has some structural similarity to prion protein. This author, therefore, collaborated with his colleague in the Netherlands to conduct a test to determine if enzymatic degradation of prion protein was possible. Interestingly, a series of tests confirmed the discovery of the first prion degradation by an enzyme, the PWD-1 keratinase (Langeveld *et al.* 2003). However, because more proteases using modified processes were found to be equally effective and the experiments with afflicted animals are highly risky and prohibitory expensive, the prion work was discontinued in the author's laboratory except for some *in vitro* studies (Chen *et al.* 2005).

4. Future Development

4.1. Microbiology and metagenomics

Anaerobic digestion produces biogas as a renewable energy on the one hand and protects the environment by waste management on the other. As the energy crisis and climate change are of global concern, the process of anaerobic digestion and the energy use of biogas are becoming increasingly important. Many scientists and engineers across their disciplines are turning their interest to the microbiology, biochemistry and biotechnology, in order to understand better the bio-process and to improve the technology biologically in the long run.

In anaerobic digestion, roles of different groups of microorganisms and degradation pathways are known in general terms (Whitman *et al.* 2006; Wilkie 2008; Cheng 2010). Some of them, notably the archaeal methanogens, have been isolated and identified. However, the majority of microorganisms in the digester are uncultivable and remain unidentified. The digester consists of a large variety of interactive microorganisms, enzymes and genetic functions. Little is known about their specific roles in specific processes (Hung *et al.* 2011; Saini *et al.* 2011). The microbial ecosystem is so complex that the digester has been regarded as a "black box" for a long time. The majority of the unexplored portion of microbial diversity has been referred to as "microbial dark matter" (Rinke *et al.* 2013). Lack of understanding of the bio-processes may result in low activity, malfunction or even failure of a digester in operation. To improve the technology in the long run, the black box must be unlocked and the dark matter characterized.

Using the tools of high-throughput genome sequencing, two genomic methods can be used to study the microbial complexity and functional diversity of anaerobic digestion. One is through cultivation-independent molecular surveys based on conserved marker genes, chiefly small subunit ribosomal RNA (SSU rRNA) (Rajendhran and Gunasekaran 2011). The other is through shot-gun sequencing of DNA (metagenomics) from the environment (Handelsman 2004; Gilbert and Dupont 2011). With these methods, many laboratories have explored microbial populations and their functional roles in anaerobic digestion (Krakat *et al.* 2010; Zhang 2010; Supaphol *et al.* 2011; Jaenicke *et al.* 2011). The first metagenomic study of the pig waste digester was recently published (Liu *et al.*

2015). This has demonstrated the feasibility of genomic study of the dynamics of microbial community in animal waste digesters.

4.2. Proteomics and metabolomics

The anaerobic digester is a unique, man-made, functional microbial ecosystem. More than 500 different bacterial species are believed to participate in the anaerobic digestion process (Whitman *et al.* 2006; Wilkie 2008; Cheng 2010). The microbial communities in the digester are diverse and dynamic. Varieties of microorganisms, enzymes and genes co-exist functionally, working syntrophically to accomplish the digestion process. How to control the biological process or metabolic pathway has always been the big puzzle for scientists in the field. However, with the current tools of proteomics and metabolomics in combination of metagenomics, we may be able to offer the answer to the question.

Biological variations, such as microbial population, enzyme activity, metabolic intermediates and gene expression, are believed to depend on the operational conditions of a digester and the different metabolic states or stage of the digestion process. Modern HPLC-MS for proteins (Thingholm *et al.* 2006; Sugiyama *et al.* 2007) and GC-MS for metabolic intermediates (Fancy and Rumpel 2008; Kanani *et al.* 2008) can be used to analyze the digester mixture at different performances under various operational conditions. If some key enzymes are related to biogas production, they can be identified and the key metabolic pathways mapped. Metabolic intermediates, such as short-chain fatty acids, monosaccharides and glycolysis intermediates, can be analyzed to confirm the pathway. The goal is to identify the key enzymes and key steps in the digestion process. Integration of meta-genomics and proteomics are believed to be able to delineate the metabolic control and genetic regulation of the overall process. Eventually, the bio-process may be manipulated and biologically improved, along with hardware improvement by engineering.

4.3. Enzyme discovery and gene mining

A significant fringe benefit of genomic and proteomic studies is the potential of discovery of novel enzymes and isolation of their genes from the anaerobic digester, a highly diverse and resource-rich microbiological ecosystem. The newly isolated enzyme and gene system may be cloned in a different expression systems, such as *Escherichia coli*, *Bacillus*, or *Pichia*, for production purposes. Furthermore, the enzyme produced may have an entirely different functional role for bio-industrial applications. The discovery and diversified applications of keratinase from a feather-degrading *Bacillus licheniformis* PWD-1 (Shih 2012) as described above is a perfect example. Beyond the production of biogas energy, an anaerobic digester is potentially a novel and rich source of value-added new products.

4.4. Codigestion

Codigestion is another area of new development. Anaerobic digestion has very diverse feedstock that includes all kinds of organic biomass, including animal waste, agricultural

residues, sewer sludge, food wastes and industrial wastes. Agricultural residues such as corn stalks, wheat straw, rice straw and other lignocellulose-rich bio-wastes generated in large volumes can be used as substrates. Some of them, such as corn stalks, have been identified as energy crops for biogas production (Bouallagui *et al.* 2009; Kacprzak *et al.* 2009; Xia *et al.* 2012). However, the mixture of agricultural residues with animal manure greatly enhances the biogas production by several folds over that from either of them alone. This is because lignocellulosic wastes have a very low nitrogen content (C/N ratio: 60–80), while it is high in animal manure (C/N: 5–10). The mixture of the two different kinds of feedstock at a C/N ratio 25–35 supported better anaerobic digestion and more biogas production. Combining animal manure with agricultural biomass for codigestion is a promising direction for future development. In other words, anaerobic digestion is expected to convert both animal and agricultural wastes generated on farm into biogas energy. The digestate or residue from the digester can be processed into fertilizer and returned to the farmland for nutrient recycling.

5. Future Development

In summary, the systematic studies of thermophilic anaerobic digestion of poultry waste were carried out from the laboratory to the farm, and successfully practiced in China. The discovery of keratinase from the digester triggered another set of studies from microbiology and biochemistry, to enzyme technology. In application, keratinase as a co-product of the system set the stage for a start-up biotechnology company, now a global business. The entire work has been reviewed at different stages (Shih 1987, 1993, 2012; Shih and Wang 2006, 2008) and the endeavor has resulted in 12 U.S. and international patents.

The development of the anaerobic digestion of poultry waste is a case study demonstrating the systematic approach. From this case study, an important lesson was learned. The organic compositions of animal manure from different species are different. The microbial populations from the animal excreta and from the seed culture of a digester also vary. The results of early studies suggested that the same systematic approach is useful or even necessary. When a different type of animal waste from a different species is used, it is important to have a well-designed laboratory study to determine its operational optimum for a high biogas rate. For codigestion with different plant materials, the same approach is recommended to reach the optimal operation.

Another important lesson was learned: basic study and application research is a 2-way street. It was the laboratory study that optimized the operational conditions and found the higher biogas rate at thermophilic temperatures. The farm operation of the digester helped make the discovery of keratinase. Basic and application research of keratinase gave rise to a new biotechnology company and introduced a new enzyme product to the world. In the future, new developments in genomics and proteomics will help open up the “black box” of the biology of anaerobic digestion. The acquired knowledge will eventually improve the bio-process for higher biogas production.

In the agricultural production of crops and animals, the multiple benefits of anaerobic digestion in waste conversion, biogas production, nutrient recycling, farm sanitation

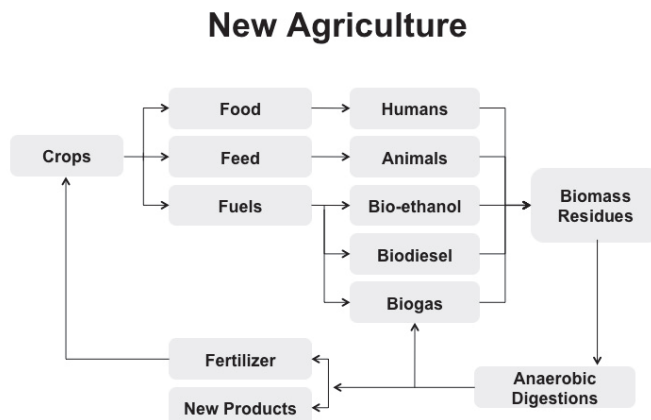


Fig. 14.6. New agriculture. The agricultural products are food, feed and bio-fuels. All excreta and residues will be processed by anaerobic digestion to produce fertilizer for crops, biogas for energy and new bio-products of high value.

and CO₂ reduction have been studied and demonstrated. When all sectors are integrated with an anaerobic digester at its core, the financial gain for the total system is feasible. In addition to waste management and energy production, the system will facilitate the sustainability of both agriculture and environment. The integrated system, or Holistic Farming (Fig. 14.6), has the potential to be the new agriculture of the 21st Century.

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Chapter 15

Role of Anaerobic Digestion in Increasing the Energy Efficiency and Energy Output of Sugar Cane Distilleries

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The chemical energy in sugar cane is roughly equally divided into three fractions: (1) juice, the liquid part; (2) bagasse, the fibrous part of stalks; and (3) straw, the leaves and tops of the cane. So far, only the juice has productive use as a raw material for sugar and alcohol production. In this chapter, it is shown that anaerobic digestion can be applied to generate energy from sub-products, reducing the environmental impact of distilleries. Fermentation of cane juice leads to the generation of wastewater called vinasse, which is spread on the cane fields, using its nutrients. If vinasse is submitted to anaerobic digestion before using the nutrients, the produced biogas can generate electric energy at a rate of $45 \text{ kWh} \cdot \text{t}_c^{-1}$ for a conversion efficiency of 40%. Presently, bagasse is burnt at sugar mills and distilleries for the generation of electric energy with boilers and steam turbines producing a $100 \text{ kWh} \cdot \text{t}_c^{-1}$ at the maximum conversion efficiency of 16%. However, the application of anaerobic digestion to bagasse may increase the energy production potential to $150 \text{ kWh} \cdot \text{t}_c^{-1}$. Another $150 \text{ kWh} \cdot \text{t}_c^{-1}$ can be added to the distillery's energy output from cane straw that generally remains on the fields when cane is harvested by anaerobic digestion as well. Currently, the production rate of sugar cane in Brazil is about 720 million $\text{t}_c \cdot \text{y}^{-1}$ with a production potential electric energy of $124 \text{ TWh} \cdot \text{y}^{-1}$ from vinasse and bagasse, compared to a consumption of about $500 \text{ TWh} \cdot \text{y}^{-1}$. Hence, this represents 25% of the national consumption.

1. Introduction

Sugar cane is planted on a very large scale in Brazil, for both sugar and ethanol production. The current production of about 720 million tons per year is roughly evenly divided to generate the two products. From the produced ethanol, about 5% is used for beverage production (cachaça), 15% is exported and 80% is used as automotive fuel,

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either as a 20–25% dehydrated additive to gasoline or as hydrated (azeotropic) ethanol (UNICA 2010). Worldwide annual ethanol production for fuel is about $89 \times 10^6 \text{ m}^3$ (see Table 15.1), with the U.S. producing more than half of the total from corn and Brazil more than a quarter from cane.

To evaluate the suitability of biomass as a basis for ethanol production, three factors are of great importance: (1) the yield per unit area of agricultural land occupied for biomass production; (2) the costs of conversion of the biomass into ethanol; and (3) the amount of fossil energy consumption required per unit of produced ethanol. Although conversion of cellulose to ethanol has been demonstrated to be technically feasible, the costs are still too high for large-scale applications. The remaining options for ethanol production are starch with corn and cereals as important sources and sugars with cane, sorghum and beets as the main cultures. Sugar cane is a very suitable plant for ethanol production in the sense that a high yield is possible, the conversion of sucrose to ethanol is relatively simple and little fossil energy is required for the production. In Brazil, the average production rate is of the order of $80 \text{ t} \cdot \text{ha}^{-1} \cdot \text{y}^{-1}$ of cane and some $7,000 \text{ L} \cdot \text{ha}^{-1} \cdot \text{y}^{-1}$ of ethanol, much higher than the yield of corn (some $3,600 \text{ L} \cdot \text{ha}^{-1} \cdot \text{y}^{-1}$). The low demand for fossil energy is due to the composition of cane when it is harvested. Sugar cane on the field is composed of three fractions: (1) juice, the liquid part of the plant; (2) bagasse, the fibrous part; and (3) straw, the leaves and tops. Each of these three parts have about 1/3 of the stored chemical energy of the plant (Seabra 2008), but in practice, during the harvest, the straw remains unused on the field and is either burnt or is slowly decomposed if harvesting is done mechanically. The cane stalks that arrive at the industrial plant (sugar mill or distillery), after being washed, are submitted to a separation process of the juice and bagasse by milling and extraction with water. Thus, an easily combustible energy source becomes available in the form of finely cut chips of bagasse and is used as an energy source for both electric energy and steam in the industrial operations, which therefore are largely independent of external energy.

Figure 15.1 shows the flow sheet of ethanol production from cane juice in so-called autonomous distilleries. In sugar factories, the juice is partially transformed into sugar by crystallization. The remaining molasses, a dense syrup of dissolved sucrose, is also used for the production of ethanol at attached distilleries.

Table 15.1. Ethanol production in several regions
(<http://ethanolrfa.org/pages/World-Fuel-Ethanol-Production>).

Region	$10^6 \text{ m}^3 \cdot \text{y}^{-1}$	Fraction	Energy crop
USA	50.5	0.568	corn
Brazil	23.8	0.267	cane
Europe	5.2	0.059	beet, cereal
China	2.6	0.030	several
India	2.1	0.023	cane
Canada	2.0	0.022	corn
Rest	2.0	0.031	

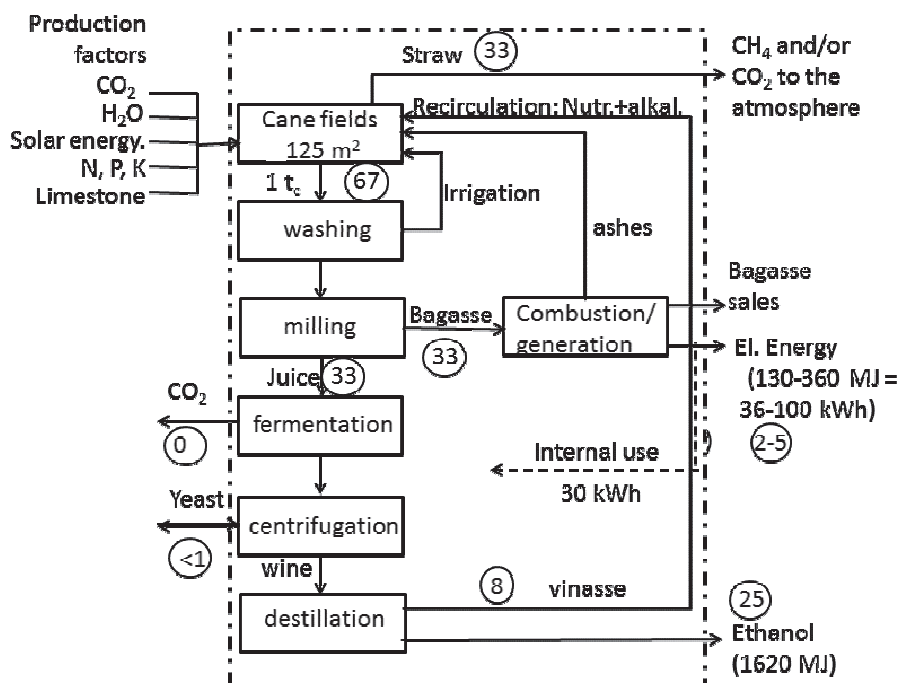


Fig. 15.1. Flow sheet of a traditional ethanol distillery with 1 ton of sugar cane as raw material and self-sufficient in electric energy. Encircled numbers indicate the chemical energy content (% of total).

However, even if sugar cane is a biomass with favorable characteristics for ethanol production, the current production process such as depicted in Fig. 15.1 is unsatisfactory both from the economic and the environmental view point. The figure shows that only about 25% of the energy in sugar cane is actually converted into ethanol, whereas 75% is discarded or grossly misused. In this chapter, we discuss that there is a potential to increase the energy output of ethanol distilleries, boosting the profitability and reducing the environmental impact of the agricultural and industrial processes. Overall mass balances show that there exist excellent opportunities to apply the anaerobic digestion process, both for the production of methane as an energy source and as a treatment option for waste water and residuals, in agreement with the recommendations of Pabón-Pereira *et al.* (2011, 2013).

2. Mass and Energy Flows in Ethanol Production

Per ton of raw sugarcane on the field, about 160 kg of sucrose is produced, whereas the mass of bagasse and straw is 135 kg each, mostly composed of carbohydrates. To evaluate the energy contained in cane, it is convenient to transform these masses into COD equivalents or MJ. For sucrose, this is 1.07 kg-COD·kg⁻¹ sucrose, whereas for bagasse and straw, the COD equivalent was experimentally determined as 1.26 kg-COD·kg⁻¹ of dry weight (Van Haandel 2005). Hence, in a ton of sugar cane, there is a mass of 170 kg COD in each of the three fractions: juice, bagasse and straw. Van

Haandel and Lettinga (1994) have shown that independent of the nature of organic material upon oxidation, the released chemical energy is about $13.7 \text{ MJ} \cdot \text{kg}^{-1} \text{COD}$. Hence, the chemical energy in cane is $3 \times 13.7 \times 170 = 6,900 \text{ MJ}$ per ton of cane (t_c), divided in three equal parts of 2,300 MJ for each of the three fractions.

Figure 15.1 shows the main flows of energy in ethanol distilleries. During fermentation, about half of the sucrose mass is converted into CO_2 , not containing any chemical energy. Currently, this CO_2 is released to the atmosphere without any use. During fermentation, there is also some production of yeast, but the related energy is less than 1%. In addition, during the fermentation process, a little energy is lost as heat, but this is also an insignificant fraction. The obtained ethanol after distillation represents about 25% of the energy, whereas 8% is discharged with vinasse. On the other hand, bagasse is burnt to produce the electric energy for the industrial operations, which is about 30 kWh or 108 MJ (electric) per ton of cane (t_c). The bagasse required for energy production depends strongly on the efficiency of the power generation, which in turn, depends on the steam pressure. In practice, electric conversion efficiencies range between 6–16% of the chemical energy of 2,300 MJ in bagasse, depending on the applied pressure which ranges from 20 Bar to a maximum of 80 Bar. Thus, the required bagasse fraction to be burnt for energy generation ranges from 78% (20 Bar unit) to 30% (80 Bar unit). The excess bagasse is sold to be used in agriculture (cattle feed) or industry (as a source for cellulose). Since the value of bagasse is low and the costs of a top-of-the-line boiler is very high, most distilleries prefer units with low or medium steam pressure, so that a considerable fraction of the bagasse is burnt at the distillery to satisfy the demand for industrial operations. It is concluded that energy production at ethanol distilleries currently occurs with a conversion efficiency of only 25% of the available chemically stored energy, with ethanol as the only relevant product. Sub-products like vinasse, bagasse, straw, yeast and CO_2 are hardly regarded and may have a small or even a negative value.

3. Rational Use of Materials at Ethanol Distilleries

From the flow sheet in Fig. 15.1, it can be seen that there are several possibilities to improve the rational use of materials in the production process of ethanol:

- The organic material in vinasse can be used in an anaerobic digester to produce methane that can generate electricity.
- Anaerobic digestion can also be applied for the production of methane from bagasse, yielding a much higher energy production.
- The straw can be harvested and submitted to the same treatment as bagasse, and
- The dissolved carbon dioxide liberated at the fermenters of cane juice can be used in ponds to produce algae that can be used for biodiesel production

As a result, the output of useful products at the distilleries would no longer be limited to ethanol, but include significant amounts of other forms of energy: electric energy and biodiesel. These aspects will be discussed in the following sections.

3.1. Rational use of vinasse

As shown in Fig. 15.1, after milling, the sugar cane juice is mixed with yeast, which performs the conversion of sucrose to ethanol, releasing carbon dioxide, which escapes to the atmosphere. At the end of the fermentation, the suspension that consists of yeast, ethanol and unconverted organic material in water is centrifuged to separate the yeast. The resulting liquid “wine” with 7–9% of ethanol is distilled, thus separating the product ethanol (azeotropic mixture of 96%) and the wastewater. The proportion between ethanol and waste water is of the order of 1:12 to 1:15 depending on the operational conditions. About 90 L or 72 kg of ethanol are produced per ton of cane. The produced waste water is called stillage, distillery slop or vinasse and has a COD content of about $500 \text{ kg}\cdot\text{m}^{-3}$ of ethanol. The COD of 1 m^3 of ethanol at 96% purity can be calculated as 1605 kg COD. Therefore, a fraction of $500/(500+1,605) = 24\%$ of the chemically energy in the “wine” is not converted to ethanol, but is lost in the vinasse. The organic material of vinasse is predominantly soluble and biodegradable, so also this energy fraction can be recovered. Table 15.1 shows the typical composition of settled vinasse.

Current practice in Brazil is to use raw vinasse for ferti-irrigation, so that the nutrients are recycled. By using the vinasse, the alkalinity demand of the cane fields for pH correction is reduced, probably due to removal of the volatile fatty acids abundant in raw vinasse by biological oxidation or methanization in the soil. The application of vinasse is limited to $600 \text{ m}^3\cdot\text{ha}^{-1}\cdot\text{y}^{-1}$ to avoid salinization by potassium. Since vinasse is applied once per year, this is equivalent to a load of $2 \text{ kg}\cdot\text{COD}\cdot\text{m}^{-2}$. The use of raw vinasse may lead to serious environmental problems. When vinasse is spread on the fields, it penetrates into the soil. Considering the applied sludge load of $2 \text{ kg}\cdot\text{COD}\cdot\text{m}^{-2}$ very likely, there will be insufficient oxygen to oxidize the COD. Therefore, part of the organic material may decompose anaerobically producing the potent greenhouse gas CH_4 . On the other hand, under these conditions, methanotrophs will thrive in the upper layer of the soil and convert part of the methane, but undoubtedly, some of the produced CH_4 will escape to the atmosphere. Such a release could have a very significant environmental impact, since CH_4 has 21 times of a stronger impact than CO_2 as a greenhouse gas.

The best way to avoid the undesirable release of methane from the cane field, is to remove the biodegradable material from vinasse by applying anaerobic digestion in a controlled manner to the raw vinasse. The resulting biogas can then be captured and easily converted into electric energy in special generators equipped with explosion motors, having an electric conversion efficiency of up to 40%. High-rate anaerobic systems, such as the up-flow anaerobic sludge blanket (UASB) reactor are available to produce the biogas from the organic material (van Lier 2008). The feasibility of using the UASB reactor for the digestion of vinasse has been demonstrated by many authors. Recently, van Haandel *et al.* (2013) showed at pilot-scale, that a digestion rate of more than $40 \text{ kg}\cdot\text{COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ can be maintained, whereas at full-scale, plants' rates of more than $20 \text{ kg}\cdot\text{COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ have been observed (Weiland *et al.* 2009). Knowing that anaerobic digestion leads to the generation of $\frac{1}{4}$ of a kg CH_4 for each kg digested COD and that 1 kg CH_4 in an efficient generator produces about 5 kWh of electric energy, a digestion rate of $20 \text{ kg}\cdot\text{COD}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ leads to the production of $5 \text{ kg}\cdot\text{CH}_4\cdot\text{m}^{-3}\cdot\text{d}^{-1}$, which

can be transformed into $25\text{kWh}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$, or a power generation potential of $1\text{ kW}\cdot\text{m}^{-3}$; see also van Lier *et al.* (2008). At this rate, energy production is a very profitable operation unless the energy price is extremely low. Even when anaerobic digestion is carried out at high rate, the conversion efficiency for vinasse digestion remains very high, in the range of 80–95% because of the easily biodegradable nature of the organic material. As shown above, the production potential of methane from vinasse is at least is $100\text{ kg}\cdot\text{CH}_4\cdot\text{m}^{-3}$ ethanol. The electric-energy-producing potential of the methane derived from vinasse digestion is $500\text{ kWh}\cdot\text{m}^{-3}$ ethanol, which is about 1.5 times the energy required for industrial operations. Therefore, it can be concluded that the energy production potential by applying anaerobic digestion of vinasse wastewater amply exceeds the industrial energy demand of the distillery.

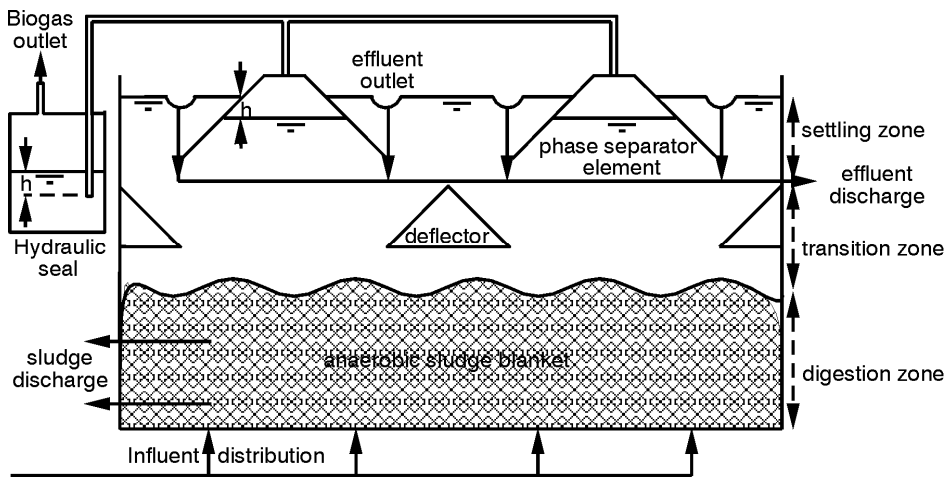


Fig. 15.2. Schematic representation of a UASB reactor (Van Haandel and Lettinga 1994).

Table 15.2. Main characteristics of vinasse from autonomous ethanol distilleries after simple settling.

Parameter	Per m ³ of ethanol	Per ton of cane	Per m ³ of vinasse (eth/vinasse = 1/15)
Vinasse (m ³)	15	1.4	1
Organic material (kg COD)	500	45	33
Volatile fatty acids (kg VFA)	100	9	6.6
Suspended solids (kg STS)	75	7	5
Nitrogen (kg TKN)	9	0.8	0.7
Phosphorus (kg P)	6	0.5	0.4
Potassium (kg K)	15	1.4	1
Sulfate (kg S)	5	0.5	0.3
pH	4	4	4
Temperature (°C)	80	80	80

In order to have a high conversion rate in any biological treatment system, there are basically two conditions that must be met: (1) intense contact between the entering organic material and the microbial mass in the system must be assured and (2) a large mass of microorganisms must be maintained in the system. It can be seen in Fig. 15.2 that both conditions are fulfilled in UASB reactors; the incoming flow is distributed over the bottom of the reactor and follows an upward path until it is discharged at the top passing through a layer of sludge. The large mass of microorganisms is maintained due to the presence of an internal settler at the top section of the reactor (van Lier *et al.* 2008).

Even though the organic material in vinasse is an excellent substrate of anaerobic digestion, there are some constraints related to raw vinasse digestion, which must be considered before the process can be applied. These constraints are:

- (1) Ethanol production is a seasonal activity with periods of activity of six months per year. Even if it is possible to keep the activity of the methanogenic sludge in the UASB reactor during periods between harvests, thus maintaining the treatment capacity, from an economic viewpoint, it is undesirable to have the treatment unit standing idle for six months per year. Also, if the electric energy is sold, year-around production must be guaranteed to obtain the maximum price. It is not possible to keep untreated vinasse for a long time: after a month in a reservoir, the organic material and hence the electric energy production potential is reduced by 30–40%, potentially emitting potent greenhouse gases to the atmosphere. The problem is less pressing in attached distilleries as these produce molasses to continue distillation after the harvest period. A solution for autonomous distilleries could be to produce organic material by hydrolysis of part of the bagasse as can be seen in the next section. This hydrolysis during periods between harvests would guarantee a continuous supply of soluble organic material to be introduced in the anaerobic digester, generating energy for commercialization.
- (2) Raw vinasse has a very high temperature (80–90 °C) and a high suspended solids concentration (5 g·L⁻¹). The suspended solids in vinasse are mostly yeast cells that are not separated in the centrifugation unit. To minimize the suspended solids load to the UASB reactor, a settling phase of the vinasse for 12–24 hours can be included prior to anaerobic digestion. Such a storage period not only reduces the suspended solids content of the vinasse, but it also lowers the temperature due to convective heat dissipation. High loadings of suspended solids may affect the operational performance of UASB, since these solids are mostly composed of protein-rich material that can give rise to the formation of scum on top of the liquid surface and in the phase separator elements. Considering the high digestibility of the material, the storage period should be limited to maximally one day to avoid the loss of organic material. The settled solids can be used as cattle feed or organic fertilizer. Van Haandel *et al.* (2013) showed that high rates of digestion (up to 40 kg-COD·m⁻³·d⁻¹) are possible both at the thermophilic and mesophilic optimal temperatures (55 and 35 °C, respectively). Mesophilic digestion is commonly applied and is considered to have a high operational stability. Thermophilic treatment requires a more cautious start-up procedure, since thermophilic seed material is not available whereas the required operational procedures differ (van Lier 1996).

- (3) Raw vinasse contains sulfate, although in Brazil, in a modest concentration of about $300 \text{ mg-S}\cdot\text{L}^{-1}$. In the anaerobic reactor, the sulfate is reduced to sulfide by sulfate-reducing bacteria, a process that is not desirable, but unavoidable. The stoichiometric relationship between oxidation of COD by sulfate and of anaerobic digestion is such that for the reduction of 1 mol of sulfate (32 g S) to sulfide, 64 g of COD must be oxidized, which prevents the formation of $64/4 = 16 \text{ g}$ of methane. Hence, the reduction of $300 \text{ mg-S}\cdot\text{L}^{-1}$ of sulfate oxidizes $600 \text{ mg}\cdot\text{L}^{-1}$ of COD, which is only 2% of the available COD concentration in vinasse ($\sim 33 \text{ g}\cdot\text{L}^{-1}$), so that the loss of methane production is insignificant. The main problem associated with sulfur is related to biogas usage, since due to the high biogas/effluent ratio (like 10:1), most of the reduced sulfur will be in the gas phase as hydrogen sulfide. To avoid hydrogen sulfide gas is transforming into corrosive sulfuric acid, when the biogas is used in a generator, its presence must be avoided. Several methods can be applied, as reviewed by Lens and Hulshoff Pol (2000).
- (4) The high concentration of organic material in vinasse requires a high alkalinity to maintain a neutral pH in the reactor, necessary for proper performance. The neutral pH may be established by reducing the acidity or by increasing the alkalinity. The acidity may be reduced by mixing the effluent with the influent and recirculating the mixture. In this case, the acid influent will transform the bicarbonate of the effluent into carbonic acid, which will subsequently desorb as CO_2 from the mixture, taking acidity with it. Consequently, the pH in the reactor will increase. Recirculation of the effluent has several advantages:
- a. By recirculation, the up-flow velocity of the liquid is increased and as a result, the sludge bed is expanded and the contact between sludge and organic material improves;
 - b. The increased mixing prevents stratification of COD concentrations in the reactor and thus prevents local pH drops;
 - c. Due to recirculation, the COD concentration of the mixture is reduced and this in itself causes a reduction of the required alkalinity for maintaining a neutral pH;
 - d. The mechanism of removing carbon dioxide from the reactor by desorption results in a reduction of the acidity in the reactor, which in itself leads to a lower alkalinity for maintaining a neutral pH;
 - e. The removal of CO_2 from the recycled effluent automatically leads to a lower CO_2 and higher methane fraction in the biogas, which increases its calorific values, being an advantage when it is used for electric energy generation; and
 - f. Part of the bisulfide in the effluent will be transformed into hydrogen sulfide due to mixture with the acid influent. The hydrogen sulfide may then be removed from the mixture due to desorption in the same way as CO_2 is removed.

Even when recirculation is applied and the alkalinity demand is reduced, the composition of the vinasse is such that some alkalinity addition is required to maintain a neutral pH and stable operational conditions. For alkalinity addition, the following agents can be considered:

- a. Lime may be added, but even though this is a cheap alkaline material, there are limitations to its use. Firstly, the increase in calcium ions may induce precipitation

- of calcium carbonate on the sludge granules, decreasing their methanogenic activity. Secondly, lime has a fraction of silica and other inorganic solids that will accumulate in the reactor, also leading to a decrease in methane production;
- b. It may be considered to substitute lime for soda (Na_2CO_3) or caustic soda (NaOH). Indeed, these materials do not have the problems of lime, but their price is high and application requires caution; and
 - c. Urea can also be used as an alkaline agent as it readily decomposes in the UASB reactor, forming ammonium and carbon dioxide. It is more expensive than lime, but much easier to handle. It must be considered that urea is used anyway to supply nitrogen on the cane fields, so that in fact its addition to the reactor serves two purposes: alkaline agent and fertilization. Thus, in reality, the urea can be added at marginal cost to the reactor.

3.2. Rational use of bagasse and straw

3.2.1. Electric energy production by combustion of bagasse

Traditionally, bagasse is used for the generation of electric energy for sugar mills and ethanol distilleries. The generation unit consists of two parts: (1) a boiler in which steam is generated and (2) a steam turbine in which the pressure of the steam is used to move the turbine and produce electric energy. In this case, the efficiency of electric energy generation depends on the steam pressure that can be obtained, but it is much lower than the one obtained by generators with explosion motors that use liquid or gaseous fuels. In most distilleries, the steam pressure is 20–40 Bar and the efficiency of chemical energy conversion into electric energy is only 6–10%. Recently, high pressure boilers have been introduced with a steam pressure of 80 Bar and a conversion efficiency of around 16%. It was shown above that the fraction of bagasse to be burnt varies between 30–78% depending on the conversion efficiency. The wide-spread practice of producing electric energy by the combustion of bagasse is questionable, as much larger energy quantities may be generated by other methods, which may even have a lower production cost. This is due to the fact that solid fuel has a much lower conversion efficiency (maximum of 16%) than the one that can be obtained in generators with an explosion motor, using liquid or gaseous fuel. Biogas generators, for example, can routinely generate electric energy with conversion efficiency up to 40%. Thus, even if the chemical energy in bagasse is about four times higher than that in vinasse (33 against 8% of the total cane energy), the maximum electric energy that can be obtained by burning bagasse (100 kWh) is not much more than double the energy from the biogas obtained from vinasse digestion (45 kWh).

3.2.2. Energy production by conversion of bagasse to ethanol

Many research centers and engineering firms are developing technology to convert lignocellulosic material, such as bagasse, into ethanol. Bagasse is essentially composed of cellulose, hemicellulose and lignin in COD proportions of approximately 38:37:25. The aim of most processes to produce ethanol is to first destroy the lignocellulosic bagasse fibers in a water phase by thermal, chemical, physical or biological methods. The

cellulosic and hemi-cellulosic material is then accessible for hydrolytic enzymatic attack. Cellulose produces glucose that is converted into ethanol. The conversion of hemicellulose into ethanol is also possible (Ahring *et al.* 1996), but the process is much more complex due to the nature of the formed intermediate products, and much less suited to be fermented than glucose. The lignin is separated from the liquid phase and used as a fuel or material for industrial processes. The technical feasibility of ethanol production has already widely been demonstrated, but it is not yet possible to produce the fuel economically from lignocellulosic materials, even when these have a low or negative value.

As soon as part of the bagasse can be converted into ethanol on a sound economic basis, anaerobic digestion could be applied to produce biogas from the fraction of cellulose and hemicellulose that are not converted into ethanol. In that case, only the lignin fraction would not be used to produce ethanol or methane, but it could be a useful product by itself for several industrial and agricultural applications (Harkin 1969). Anaerobic digestion of hydrolyzed cellulose and hemicellulose would be relatively easy compared to direct anaerobic digestion of bagasse (Wang *et al.* 2015).

3.2.3. *Electric energy production by anaerobic digestion of bagasse*

Since ethanol production from bagasse is not yet an economically feasible process, the possibility of using anaerobic digestion plus methane combustion as an alternative to bagasse combustion for the production of electric energy was investigated. The reason for this investigation was that even in efficient boilers, the electric conversion efficiency is low (16%). Though the fibrous bagasse is a poorly biodegradable material, it was expected that a higher efficiency of electric energy production could be obtained by anaerobic digestion.

To investigate the potential for bagasse digestion, a pilot-scale project was carried out at the laboratory of the University of Campina Grande. The main objective was to establish if, and under which conditions, it is possible to convert a fraction of the cellulose and hemicelluloses (accounting for some 75% of the total COD of bagasse) into methane. The potential of useful energy production by anaerobic digestion is high: a maximum of $0.75 \times 2,300 = 1,725$ MJ or 480 kWh theoretical value of chemical energy would be available for conversion into methane. Assuming again a 40% efficiency of the generator, the electric energy production potential would then be $0.4 \times 480 = 190$ kWh \cdot t $^{-1}$, which is almost double the maximum value for direct bagasse combustion under optimal conditions.

The investigation considered many variables of the process, which are briefly mentioned here:

- (1) Reactor configuration. The best results were obtained with a 2-step reactor system: a completely mixed hydrolysis reactor, daily fed with bagasse (50% humidity) and water to maintain the solids concentration at the desired level, followed by a separated UASB reactor in which the hydrolyzed material from bagasse was digested (Fig. 15.4). In the hydrolytic reactor, the pH was low (in the range of 4–5), but the hydrolytic and acidogenic bacteria worked well at a low pH and produced the substrate for the UASB reactor.

- (2) Type of bagasse. Two types of bagasse were tested: (a) raw bagasse as it leaves an autonomous distillery, having a humidity of 50%, and (b) steam-exploded bagasse (steam application at 205 °C for 8 minutes). Steam explosion is a widely applied method to improve the biodegradability of bagasse when it is used as cattle feed. By applying steam at a high pressure and temperature in a closed reactor, water is introduced in the pores of the bagasse structure and when the pressure is suddenly released, the water starts to boil vigorously thereby destroying (exploding) part of the fibres. Figure 15.5 shows the visual difference of raw and steam-exploded bagasse.
- (3) Operation. Several hydrolytic reactors with volumes of 20 L were operated in batch fed mode at a constant sludge age, R_s ; once per day, a fraction $1/R_s$ was removed and appropriate amounts of new bagasse, together with the required volumes of water to maintain the desired solids concentration in the reactor, were then fed and thoroughly mixed with the contents. After filtration of the withdrawn hydrolyzed solids, part of the (diluted) filtrate was used to feed the UASB reactor. No attempt was made to recover the microorganisms from the hydrolyzed slurry.
- (4) Operational conditions. Each hydrolysis reactor was operated under a particular set of operational conditions, so that the influence of these on the hydrolysis and digestion performance could be tested and thus, the optimal operational conditions could be selected. (a) The hydrolysis reactors were operated at the optimal temperatures for mesophilic and thermophilic digestion, 35 and 54 °C, respectively; (b) the solids concentration in the reactors was varied between 10 and 17%; and (c) the solids retention time was varied between $R_s = 15$ and 25 d. Also, different types of enzymes to increase the hydrolysis rate and/or efficiency were used: rumen from goats and industrial enzymes. In addition, a cocktail of micronutrients was added to some hydrolytic reactors. All hydrolytic reactors were inoculated with anaerobic sludge obtained from a UASB reactor treating vinasse.
- (5) Anaerobic reactors. The UASB reactors were fed continuously with the diluted effluent of the hydrolysis reactor, maintaining an upward velocity $0.25 \text{ m}\cdot\text{h}^{-1}$. The UASB reactor was operated at ambient temperature (25 °C) and no intentional sludge withdrawal was applied, so that the sludge production was equal to the solids particles that were discharged in the final effluent. The applied organic loading rate was $10\text{--}20 \text{ g}\cdot\text{COD}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ and granular sludge from a vinasse treatment unit was used as inoculum.

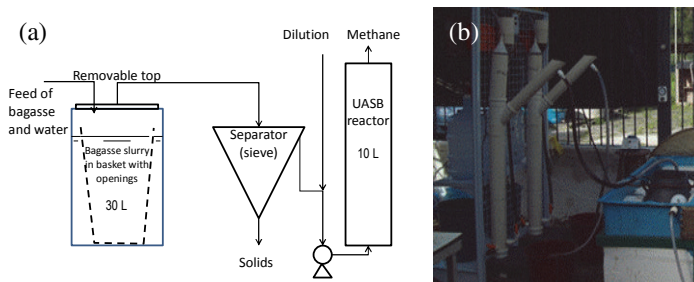


Fig. 15.4. (a) Experimental set up for a system with hydrolysis + digestion units for bagasse digestion; and (b) hydrolysis + UASB reactors (only one hydrolytic reactor shown).



Fig. 15.5. Photos of raw (right) and exploded bagasse (left).

The experimental results obtained under different operational conditions were compared and led to the following conclusions:

- (1) Thermal pre-treatment had an effect on the hydrolysis efficiency as measured by the decrease in suspended solids in the hydrolyzed slurry and COD increase in the liquid phase.
- (2) The addition of enzymes had a moderately strong effect during the first week after the additions started for all tested enzymes. However, after a few weeks, the slurry without enzyme addition gradually improved its performance, apparently being able to produce its own enzymes. After this period, the application of the industrial enzymes led to a marginally improved hydrolytic efficiency. Therefore, enzyme additions were eventually abandoned, as the high cost did not warrant the slightly improved result. Enzymatic pre-treatment of bagasse before hydrolysis was tested and also found to be efficient.
- (3) By contrast, the addition of micronutrients had a considerable effect on the hydrolytic efficiency.
- (4) The acid effluent from the hydrolytic reactor could be fed without alkalinity addition to the UASB reactor. Apparently, the concomitant alkalinity generation during methanogenesis was sufficient to increase the pH to a neutral value and thus, maintain stability in the reactor.
- (5) The hydrolytic efficiency at 54 °C was higher than at 35 °C for all operational conditions, but the difference was not high: a maximum removal efficiency of 70% at 54 °C against 62% at 35 °C for a sludge age of $R_s = 20$ d.
- (6) The applied sludge age in the solids reactor had a significant impact, especially in the range from $R_s = 15$ to 20 d, where the removal efficiency of solids increased from 52–66 % at 35 °C. A further increase to $R_s = 25$ d led to an increase to 70%.
- (7) The fraction of suspended solids in the reactor had a strong impact on the reactor performance. The higher the allowed solids concentration, the higher the solids loading and the volumetric hydrolysis rates, but the hydrolysis efficiency tended to decrease with increased solids concentration. The reason for this might be attributed

to the formation of inhibitory (intermediate) compounds during the hydrolysis process (e.g., Fernandes *et al.* 2015) or that the thicker slurry at high solids concentrations posed mass transfer limitations in the reactor. The highest volumetric rate of hydrolyzed material production at a sludge age of 20 days was obtained at a suspended solids fraction of 15%.

- (8) The UASB reactors were inoculated with granular sludge of high activity UASB reactors, i.e., the specific methanogenic activity SMA was $>1.2 \text{ g-COD} \cdot \text{gVSS}^{-1} \cdot \text{d}^{-1}$ and the UASB reactor was operated at specific volumetric loading rates varying between 10 and $20 \text{ kg-COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$. The hydrolysed effluent was diluted in such a manner that the upward velocity of the liquid in the digester zone was 0.25 to be in $0.5 \text{ m} \cdot \text{h}^{-1}$.
- (9) The UASB reactors had an average COD removal efficiency of 74%, which means that of the organic material in the hydrolyzed effluent, 74% was transformed into methane. This was the average of the COD reduction in the reactor, which agreed with the amount of COD of the produced methane. Thus, the maximum overall bagasse to methane conversion was of the order of $0.74 \times 0.70 = 52\%$. The relatively low efficiency of anaerobic digestion could possibly be attributed to the presence of humic matter in the hydrolytic effluent as recently shown elsewhere (Fernandes *et al.* 2015), but this could not be confirmed experimentally
- (10) The UASB reactors were inoculated with granular sludge obtained from reactors treating vinasse. The UASB sludge remained granular but the activity slowly deteriorated from a very high initial value ($1.2 \text{ g-COD} \cdot \text{gVSS}^{-1} \cdot \text{d}^{-1}$) to about half of this value ($0.5 \text{ g-COD} \cdot \text{VSS}^{-1} \cdot \text{d}^{-1}$). Possibly the reduction of the activity can be attributed to the absorption of inert solid particles that were in the influent or the presence of toxic humic matter introduced with the influent or generated during digestion.

The results of the investigation show the possibility of transforming 52% of the chemical energy of bagasse into methane. It is important to point out that the maximum theoretical conversion efficiency of bagasse to methane is equal to the fraction of cellulose and hemicelluloses, which is about 75%, as lignin cannot be methanized. Thus, the maximum experimental conversion of 52% means that a fraction 69% of the theoretical maximum conversion was actually obtained. Again, admitting a conversion efficiency of methane into electric energy, the efficiency would be $0.40 \times 0.52 = 20\%$ or $0.2 \times 600 = 120 \text{ kWh} \cdot \text{t}_c^{-1}$. It is therefore concluded that it is feasible to produce 20% more electric energy from bagasse by anaerobic digestion than by combustion with the best available equipment. It must be considered that under these conditions, 30% of the bagasse solids are not hydrolyzed and thus still available for combustion. This can yield 30% of $100 = 30 \text{ kWh} \cdot \text{t}_c^{-1}$ in a high-pressure boiler. Hence, by applying hydrolysis plus anaerobic digestion plus combustion of the unhydrolyzed part, the maximum energy production would be $120 + 30 = 150 \text{ kWh} \cdot \text{t}_c^{-1}$, as opposed to $100 \text{ kWh} \cdot \text{t}_c^{-1}$ for direct combustion. Thus, an increase of 50% is achievable. It is important to stress that in the calculations, it is assumed that combustion occurs at its maximum efficiency of $100 \text{ kWh} \cdot \text{t}_c^{-1}$, but in practice, these boilers are rarely employed due to their high cost and complex operation. Thus, in practice, the increase would be much greater than 50%.

If the digestion of bagasse can be transformed into a profitable operation, it can also be considered to change the harvesting process of cane and bring the straw together with the cane to the distillery to be treated in the same way as bagasse. This could lead to an additional production of 120 kWh from anaerobic straw digestion and 30 kWh from combustion of residual organic material. A similar approach is now being established in the Netherlands, where remainders of sugar beets are collected from the field for digestion and subsequent energy generation (Corré and Langeveld 2008).

In Table 15.3, the input and output energy flows relative to 1 t of cane at ethanol distilleries are shown for five different scenarios: (1) actual normal operating conditions; (2) maximum energy production from bagasse combustion; (3) anaerobic digestion of vinasse and bagasse (v+b); (4) anaerobic digestion of vinasse, bagasse and straw (v+b+s); and (5) theoretical maximum energy production from sub-products. To make a direct comparison easier, the chemical energy of juice, bagasse, straw and ethanol have all been converted to kWh (1 kWh = 3.6 MJ). The table shows the actual conversion of chemical energy in cane into useful products can be increased from 27–44%, an increase of 62%.

The experimental data can be used to estimate the required volume of the hydrolytic and the UASB reactor for energy production from bagasse. This is shown here for a sludge age of 20 d, a hydrolysis efficiency of 68% and a solids concentration of 15% in the hydrolysis reactor. If it is assumed that for exploded bagasse and for hydrolyzed bagasse 1 g-TSS = 1.26 g-COD, the COD of 1 t \cdot d $^{-1}$ or 135 kg \cdot d $^{-1}$ (dry weight) is 170 kg \cdot d $^{-1}$, of which 68% or 115 kg is in the liquid phase and 55 kg are solids. The humidity of these solids is 85%, so that the effluent volume is 55/0,15 = 365 L \cdot d $^{-1}$. The reactor volume of the hydrolysis and methanogenesis reactors can now be estimated as follows (see also Fig. 15.6):

Table 15.3. Energy flows in kWh \cdot t $^{-1}$ at distilleries under different conditions: (1) traditional, (2) top of the line boiler, (3) applying anaerobic digestion of vinasse and bagasse, (4) applying AD of vinasse, bagasse and straw and (5) maximum theoretically achievable energy production.

Process	Input	Output				
		1	2	3	4	5
		Tradi- tional	Maxim. combust.	AD (v+b)	AD (v+b+s)	Maximum theory
Juice	600					
Ethanol		450	450	450	450	450
Vinasse				45	45	45
Bagasse	600					
Fermentation				120	120	180
Combustion		30	100	30	30	
Straw	600					
Fermentation					120	180
Combustion					30	
Total	1,800	480	550	645	795	855
Conv. Effic.		0.27	0.31	0.36	0.44	0.48

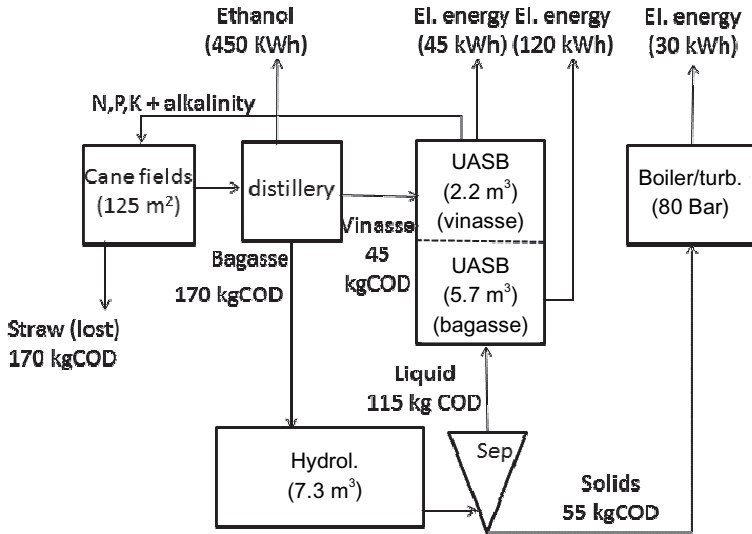


Fig. 15.6. Reactor configuration and operational conditions for anaerobic bagasse digestion relative to a mass flux of $1 \text{ t} \cdot \text{d}^{-1}$.

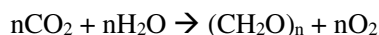
- (1) $1 \text{ t} \cdot \text{d}^{-1}$ of cane has a dry bagasse mass of $135 \text{ kg} \cdot \text{d}^{-1}$ or 170 kg COD . If in the reactor, there is hydrolysis of 68%, the effluent has still $0.32 \times 170 = 55 \text{ kg} \cdot \text{d}^{-1}$ of solids.
- (2) Since the solids fraction in the reactor and in the effluent is kept at 15%, the total daily discharged volume is $55/0.15 = 365 \text{ kg} \cdot \text{d}^{-1}$, with $365 \times 0.85 = 311 \text{ L}$ water.
- (3) The effluent slurry of $365 \text{ L} \cdot \text{d}^{-1}$ of the hydrolysis reactor represents 5% of the reactor volume (the retention time in the complete mix reactor is $R_s = 20 \text{ d}$), so that the hydrolysis reactor has a volume of $365/0.05 = 7,300 \text{ L} \cdot \text{t}^{-1}$ or $7.3 \text{ m}^3 \cdot \text{t}^{-1}$. The COD of bagasse is $170 \text{ kg} \cdot \text{t}^{-1}$ so that the organic loading rate is $170/7.3 = 23 \text{ kg} \cdot \text{COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$.
- (4) The hydrolyzed COD is 68% of the bagasse COD i.e., $0.68 \times 170 = 115 \text{ kg} \cdot \text{COD} \cdot \text{t}^{-1}$. The COD of the liquid fraction is $115/311 = 370 \text{ kg} \cdot \text{m}^{-3}$. If an upward velocity of $0.25 \text{ m} \cdot \text{h}^{-1}$ is to be maintained in the subsequent UASB reactor, the required flow would be $0.25 \text{ m}^3 \cdot \text{h}^{-1}$ or $6 \text{ m}^3 \cdot \text{d}^{-1}$. Thus, a considerable dilution of $6/0.311 = 19$ times would be required. This water could be the effluent from the UASB reactor or fresh water if the effluent has toxic materials, or detoxified water if inhibition is caused by humic matter. Detoxification of humic matter can be achieved by applying bivalent cations like calcium to the reactor broth (Azman *et al.* 2015).
- (5) If an organic loading rate of $20 \text{ kg COD} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$ is admitted for UASB reactor, the required volume would be $115/20 = 5.7 \text{ m}^3 \cdot \text{t}^{-1}$.
- (6) If a height of 5.7 m is admitted for the UASB reactor (a normal value in practice), the area of the reactor would be $1 \text{ m}^2 \cdot \text{t}^{-1}$.

- (7) The unhydrolyzed solids could be submitted to the same milling process as the one used for separation of juice from bagasse, thus producing solids with 50% humidity, which can be burnt directly with 16% efficiency in a top-quality boiler.
- (8) In practice, the UASB reactors for vinasse and for bagasse would probably be the same in which a mixture of both vinasse and hydrolyzed bagasse could be digested. Thus, the vinasse could be processed during the cane harvest, avoiding loss of organic material.
- (9) The production rate of electric energy would have to be spread out over the year, so that during the harvest, the distillery consumption would be guaranteed by its own generation and the excess would be produced continuously over the whole year.

An aspect that stands out in the experimental investigation and in Fig. 15.6 is that the required reactor volumes of the hydrolytic and the UASB reactor are large and hence will have considerable construction costs, despite the fact that the hydrolytic reactor is a simple flow-through, probably earthen reactor. To reduce the hydrolytic reactor size, it is necessary to increase the hydrolysis rate. This may be achieved by a number of actions: (1) better pre-treatment of the bagasse, increasing its biodegradability by mechanical, chemical, physical or thermal methods (Hendriks and Zeeman 2009), (2) increase the activity of the mass of microorganisms, which may include the introduction of specific enzymes or enzymatic pre-treatment (Wang *et al.* 2015) and (3) improve the operational conditions with actions such as more intense agitation, recycling of microorganisms, etc. It is concluded that even though a very large improvement of the useful energy production from bagasse by applying anaerobic digestion has been demonstrated, there are still important improvements to be made in order to obtain a stable process with improved digestion efficiency while operating at a higher rate, thereby reducing the investment and operational costs.

3.3. Rational use of CO₂

The net result of CO₂ in relation with ethanol distilleries is zero in the sense that the CO₂ production resulting from the different processes is equal to CO₂ synthesized to produce the required sugar cane. For ease of calculation, the organic material in 1 t of cane is equated to 3x160 kg carbohydrate ((CH₂O)_n), for which a stoichiometric mass of 44/30x480 = 705 kg CO₂ is required conforming the reaction equation:



It can be seen in Fig. 15.7 that the mass of CO₂ in the fermentation process and burning the methane generated in the anaerobic digestion of bagasse and vinasse (a total of 305 kg-CO₂-t_c⁻¹) can be obtained in relatively pure form. While this CO₂ has no chemical energy, it can nevertheless be used to generate energy in the form of biodiesel. It is well known that algae are the most productive biomass for the generation of biodiesel (Sheehan *et al.* 1998; Cristi 2007; Borowitzka 2013). Because the cells grow in aqueous suspension, where they have more efficient access to water, CO₂ and dissolved nutrients, microalgae are capable of producing large amounts of biomass and usable oil in high rate algal ponds. This oil can then be turned into biodiesel. Important growth factors

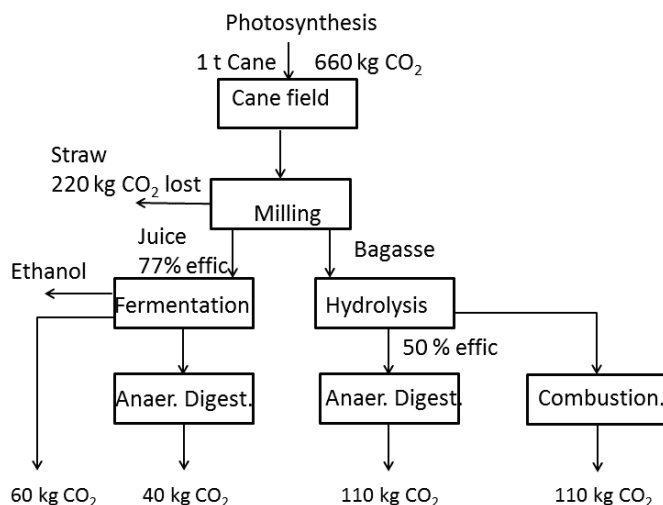


Fig. 15.7. CO₂ masses in the different flows at ethanol distilleries (per ton of sugar cane).

for algae are the presence of nutrients (especially nitrogen, phosphorus and potassium) and the availability of CO₂. Nutrients are abundantly available in vinasse and if a pond is used for vinasse discharge after anaerobic digestion, the CO₂ generated in different operations at the distillery could be used to increase the concentration in the pond to or near the saturation value. This would have an important effect on the rate of algae production (Schenk *et al.* 2008). In practice, the production of algae is about 0.5 t dry weight per t CO₂ with a maximum oil fraction of 40–60%. Therefore, theoretically it is possible to produce $0.5 \times 0.4 = 0.2$ t of oil per t CO₂. The 305 kg of CO₂ released per t_c at distilleries could therefore in theory yield about 60 kg of oils from which biodiesel could be produced.

Although reports vary widely on the rate of biodiesel production from algae, it is generally agreed that the required area for algae is much lower than for plants. Atabani *et al.* (2012) estimated that the yield of oil from algae varies from 60–140 m³·ha⁻¹·y⁻¹, depending on lipid content and operational conditions, which is 10–23 times as high as the next highest yielding crop, palm oil, at 6 m³·ha⁻¹·y⁻¹. For the lower value of 60 m³·ha⁻¹·y⁻¹, the area required for the production of 60 L of oil, expected to be feasible with the nutrients and CO₂ from 1 t_c, the required area of the algae production would be 10 m², much less than the 125 m² of cane field for the production of 1 t_c.

However, in order to transform the suspension of algae in water into biodiesel, there are three difficult downstream process operations to be carried out: (1) separation of the algae from the water, (2) separation of the oil from the algae and (3) transformation of the oil into biodiesel. Harvesting is mostly done by physical–chemical methods (flotation, flocculation and filtration). The oil is obtained from the algae by extraction. Algal oil is converted into biodiesel through a transesterification process; oil extracted from the algae is mixed with alcohol and an acid or a base to produce the fatty acid methyl esters that make up the biodiesel. An excess of methanol is used to force the reaction to favor the right side of the equation. The excess methanol is later recovered and reused (Demirbas

and Demirbas 2011). The algae mass after extraction of the oil can be used as forage for animal feed, or it may be digested together with bagasse. Depending on its composition, diesel oil has a COD value of about $2.5 \text{ g-COD} \cdot \text{g}^{-1} \text{ oil}$, and a density of about $0.9 \text{ g} \cdot \text{L}^{-1}$, so that 60 L represents 54 kg with a COD of 135 kg and an energy content of $1,690 \text{ MJ} \cdot \text{t}^{-1}$, about the same as that of ethanol ($1,620 \text{ MJ} \cdot \text{t}^{-1}$).

It is concluded that even if there are currently no distilleries that are producing biodiesel from the CO_2 and nutrients they release, there is nevertheless a very important production potential of this material. In magnitude, both the energetic and monetary value biodiesel from CO_2 might even exceed that of ethanol, today the only product of importance at ethanol distilleries.

4. Discussion

In the preceding sections, it has been shown that it is technically feasible to increase the energy efficiency and output of ethanol distilleries and at the same time, widen its portfolio. Instead of producing only ethanol, the distilleries can be large producers of electric energy by applying anaerobic digestion of organic material in vinasse and bagasse and eventually also in straw. In addition, energy can be produced from algae-based biodiesel production, to be generated from carbon dioxide and nutrients that are released at the distilleries and the nutrients in vinasse. The economic feasibility of generating these additional products will be determined by optimized downstream processing and environmental aspects.

Regarding environmental constraints, it must be noted that the application of controlled anaerobic digestion of vinasse prevents any methane emission from uncontrolled anaerobic decomposition from raw vinasse when it is applied to the cane fields. It is known that the impact of methane gas as a cause of the greenhouse effect is 21 times greater than CO_2 . It has been shown that 100 kg CH_4 (equivalent to 2.1 t- CO_2) can be generated per m^3 of ethanol produced. This may be compared with the CO_2 release by combustion of gasoline. Knowing that 1 m^3 of ethanol (800 kg) is equivalent to 700 L of gasoline (500 kg), and assuming that gasoline is predominantly octane (C_8H_{18}), it can be calculated that the CO_2 release upon gasoline production would be 1.5 t per 700 L of gasoline. Hence, if equivalent amounts of ethanol and gasoline are used, the release of methane from the associated vinasse has a worse GHG effect than the CO_2 generation due to gasoline combustion. Based on assumptions that the methane generation potential by raw vinasse in the soil is actually realized, the balance of greenhouse gas emissions is negative for ethanol fuel, even if it is considered that the use of ethanol is neutral, having “pre-paid” the CO_2 released upon combustion due to photosynthetic CO_2 consumption during cane production. The methane emission by vinasse digestion in the soil would undermine the idea of sustainability, which is the central pillar and indeed the rationale whereupon the production of “green” energy rests.

Another important environmental aspect is that by substituting fuel used at thermoelectric power plants by methane generated at distilleries, there would be no net CO_2 emission, since the CO_2 released by combustion would have been “pre-paid” when the organic material transformed into methane was grown on the cane fields.

The rational use of sub-products for electric energy generation leads to clean production at sugar and ethanol plants and their environmental impact is strongly reduced as can be seen from Fig. 15.2. There are five production factors for cane growing: (1) solar energy, (2) carbon dioxide, (3) water, (4) nutrients (NPK) and (5) alkalinity for pH correction of the soil. Solar energy is required to maintain an adequate temperature and for photosynthesis, the basic process of energy conversion. CO_2 is available in the air albeit at reduced concentrations for maximum photosynthesis. The three other factors are directly affected by rational use of sub-products: water becomes available for the cane when recirculation is applied and the nutrients and alkalinity in the recycled water minimize the need for fertilizer and alkaline material. It has been estimated that recirculation of nutrients may reduce the demand for new fertilizer by 70%, whereas the reduction of limestone or other alkaline material depends on the solid condition (Van Haandel 1994). Thus, from the five basic production factors of cane production, three are drastically affected. Basically, the distilleries have the potential to become producers of clean energy, in which solar energy is transformed into liquid fuels for automotive energy, ethanol and biodiesel (if CO_2 is used) and electric energy with use of a minimum amount of materials and a relatively small footprint.

The economic feasibility of introducing new methods to stimulate a more efficient use of the sub-products generated at ethanol distilleries of sugar cane is somewhat uncertain, because several factors are of importance and the cost-benefit ratios of most of these factors have not yet been established or are not in the hand of the energy producers. Having established the technical feasibility of electric energy generation by anaerobic digestion of vinasse and bagasse, the economic feasibility of electric energy production is set by two factors: the production costs and the price of electric energy. In Brazil, the government sets the price of energy and this may be a reason why entrepreneurs are not eager to dedicate capital to these activities and incentives by the government are too little and too few.

It is most likely that even when anaerobic bagasse digestion starts to be applied, it would take many years until the optimal conditions for maximum profit and minimum environmental damage would be established at full scale. Comparatively, at the beginning of the Brazilian ethanol program some 40 years ago, the production was 70 L of ethanol per ton of cane and the cane production was $65 \text{ t} \cdot \text{ha}^{-1} \cdot \text{y}^{-1}$ on average. Today, these figures are $90 \text{ L} \cdot \text{t}^{-1}$ and $80 \text{ t} \cdot \text{ha}^{-1}$, respectively, so that ethanol production has risen from $4.5\text{--}7.2 \text{ m}^3 \cdot \text{ha}^{-1} \cdot \text{y}^{-1}$, due to a series of improvements in both agricultural and industrial production methods. It is to be expected that a similar pattern would develop if bagasse digestion and production of biodiesel from CO_2 and nutrients were adopted. In the case of biodiesel production via algae from nutrients and CO_2 little more than principles have been established and optimization of full-scale production processes are still in a distant future.

The extra energy production due to a more rational use of sub-products from sugar mills and distilleries is a considerable fraction of the electric energy demand in Brazil, which today stands at about $500 \text{ TWh} \cdot \text{y}^{-1}$ (EPE 2013), of which about 80% is hydropower. By applying anaerobic digestion, the generation potential from vinasse is $45 \text{ kWh} \cdot \text{t}^{-1}$ and $150 \text{ kWh} \cdot \text{t}^{-1}$ from bagasse (Table 15.2). The current cane production rate

in Brazil is about $360 \times 10^6 \text{ t} \cdot \text{y}^{-1}$ for production of each sugar and ethanol. Thus, the annual production potential from sub-products is calculated at 54 TWh from cane for sugar and 70 TWh from ethanol production. This production could cover $124/500 = 25\%$ of the total electric energy demand in Brazil. If an effort is made to recover the cane straw, the energy production can be increased with an amount equivalent to the energy from bagasse: 108 TWh. Thus, the energy production potential would be 232 TWh or 46% of the energy demand. This is a very large fraction of the demand and it would make other alternatives like thermo and nuclear power plants unnecessary for the foreseeable future. The energy from cane sub-products has the advantage that production is diffuse (almost all states in Brazil produce cane), so that expensive transmission lines are reduced. Another important advantage of the production of electric energy from sub-products is that the knowledge for production is already available or can be locally developed, so that there is no need to buy expensive foreign technology, as is the case with most other alternatives.

On the other hand, when realizing the cost-effective potential of anaerobic digestion to reclaim almost all of the chemically stored energy in sugar cane by-products, it makes sense to consider the digestion of the entire sugar cane plant, without producing ethanol. If the production of an energy carrier is the goal of the industry, then the production process could be simplified drastically if anaerobic digestion were applied to the entire crop. The maximum recoverable energy would then be $2300 \times 0.8 + 2,300 \times 0.5 + 2,300 \times 0.5 = 4120 \text{ MJ}$ or $4120/6900 = 60\%$ conversion efficiency, assuming a 80% methanization of the COD in juice and 50% for COD in bagasse and straw, which has been shown to be feasible in this chapter. With the development of more efficient processes and techniques, the efficiency could be even higher. The advantage of methane as a product is that it is already very widely used as an energy source for electric energy generation and as an automotive fuel. The produced CH_4 can be subsequently upgraded to pure methane and pressurized to an automotive fuel by means of various *ex situ* or *in situ* upgrading techniques.

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Chapter 16

With AnWT and AnDi Systems towards a more Sustainable Society

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AnDi and AnWT processes, properly integrated with their supplementary biological processes and physical–chemical treatment systems, provide optimal routes for achieving sustainability in the domains of environmental protection, renewable energy and food production, which then in turn might represent effective crowbars to implement the drastic changes recommended by the Brundtland Committee for realizing a more sustainable society.

1. Evolving Awareness of Importance of the *Sustainability* Notion

Prior to the 1960s/1970s, the sustainability notion was not a real issue in the domain of Environmental Protection (EP), neither in society nor in general. However, this changed gradually, as a result of the development of innovative high-rate Environmental Anaerobic Technologies (EATs) in the 1960s, and in the Netherlands, sustainable EP (EP_{sus}) even became a major issue in the mid-1970s following the successful implementation of the UASB process for industrial wastewater treatment. The definite eye-opener undoubtedly was the publication of the final report of the Brundtland Committee (1987), *Our Common Future* with its clear analyses and recommendations for drastic changes (see Table 16.1). Although they are still very relevant and in fact more urgent than in 1987, it looks as if the Brundtland report is being overruled by several prestigious world-conferences and the publication of numerous reports and studies, all meant to elucidate the ‘sustainability’ notion.

Nevertheless, starting from 1987, sustainability became a hot topic, although for many people likely too hot; they got fed up, presumably confused with all these studies and conferences, and undoubtedly because they were faced with numerous narrow-minded, highly tunneled interpretations, frequently guided by self-interest, *viz.* for practically all separate sectors in society, including that of EP. Despite that, fortunately in the EP world, there exists common sense about the need to develop appropriate — resource saving and/or recovering — technologies, systems/concepts and to get these properly implemented and applied.

Table 16.1. Some statements/recommendations in *Our Common Future* by Brundtland (World Commission on Environment and Developments 1987).

Humanity has the ability to make development sustainable – to ensure that it meets the needs of the present - without compromising the ability of future generations to meet their own needs.

Poverty is not only evil in itself, but sustainable development requires meeting the basic needs of all and extending to all the opportunity to fulfill their aspirations for a better life. A world in which poverty is endemic will always be prone to ecological and other catastrophes.

Sustainable development can only be pursued if population size and growth are in harmony with the changing potential of the ecosystem.

People are the ultimate resource. Improvements in education, health, and nutrition allow them a better use of resources they command, to stretch them further.

The highly subsidized, incentive driven agriculture of industrialized market economies generates surpluses that depress prices and erode the viability of the often neglected agriculture of developing countries.

Renewable energy offers the world potentially huge primary energy sources, sustainable in perpetuity and available in one form or another to every nation on Earth.

2. Sustainability Prospects of the Natural Biological Mineralization & Synthesis (NBM&S) Treatment Concepts

AnDi and AnWT methods comprise the *Natural Biological Mineralization (NBM)* part of natural life cycles; they elicit their mineralization capabilities from the broad class of archae bacteria (methanogens, acetogens, sulfate-reducing bacteria). The NBM-based technologies/concepts lead to valorization of dead organic matter, viz. biogas, hydrogen, maybe electricity, organic soil conditioners, while they improve the conditions for nutrient recovery for reuse and for valorization, viz. by applying aerobic and/or photo-synthetic treatment processes, i.e., the *Natural Biological Synthesis (NBS)* part of life cycles. Micro-aerobic ($A_{e_{micr}}$) organisms/processes can be regarded as the bridge between these ultimate systems. Thanks to timely starvation of all 'mortals' and the effective mineralization of the dead organic matter left, the conditions for the conception of 'improved' next generations from existing and from newly evolving species remain optimal — and we are dealing with a balanced clean environment.

With these features, the NBM and NBS parts of the NBM&S life cycle can be considered as crucial instruments to achieve a sustainable way of protecting our life environment, of producing green energy and food. This is particularly the case when they are combined with appropriate (generally already available) *physical-chemical (PC)* methods, because practically all relevant sustainability criteria then can be met, viz.

- i) Resource preservation, recovery and/or valorization, e.g., by closing water/substance cycles, focusing on on-site applications,
- ii) Application of plain and low cost technologies, e.g., widely applicable, non-laborious, long life time

- iii) Common interest directed, e.g., by achieving a high extent of self-sufficiency, citizen participation, open and democratic decision-making.

Despite the NBM&S event originating from early stages of life, awareness of its existence emerged only around the 19th–20th century switch. But from then onwards, microbiologists, ecologists and biochemists succeeded to disclose its basic fundamentals, while engineers developed technologies to benefit from its features. Ultimately, in the 1960s/1970s, biotechnologists developed innovative high-rate AnWT systems in joint efforts with microbiologists, and soon, also the required supplementary systems for post-treatment and resource recovery.

3. The Potentials of AnWT/Andi Systems for the Domains of EP, Renewable Energy and Food

Workers in the EAT field are convinced that AnWT and AnDi systems offer big prospects to enforce sustainable developments in the EP domain, and they enable the realization of substantial savings in energy demands of conventional EP tackles and production of energy as well. They therefore offer also big potentials for the domain of *renewable energy* (En_{ren}) and adjacent fields like that of *food production* ($FoPr$).

3.1. The EP domain

The development of high-rate Anaerobic Waste(water) Technologies (AnWTs) in the second half of the last century represented an important first step towards sustainability in the EP domain, i.e., for the efficient removal of bio-degradable organic pollutants, the production of energy and mineralized organic matter (soil conditioner), for the conversion of N and P compounds in ammonia and phosphate and for reducing metal ions. Regarding the situation of the application and further implementation of high-rate AnWT and supplementary NMB&S-PC methods in the industrial sector, we can conclude that in many countries, the discussion about the need/sense simply ceased, AnWT systems got their position of combined primary–secondary treatment method and the supplementary systems gradually also found their way. Nevertheless, a number of industrialized countries, e.g., U.S., Russia and France, are far behind in that respect; in these countries, well established AeWT-directed consultants and/or decision-makers likely succeed finding means to maintain their powerful position to implement their outdated systems and to obstruct the implementation of modern high-rate AnWT. I'll not discuss the state of art of AnWT-technology for industrial wastewater and sewage (pre-)treatment as it is the issue of several other chapters in this book.

3.2. The En_{ren} -domain

In this domain, application of AnDi/AnWT treatment systems particularly offer potentials in 'biomass energy-conversion', viz. the production of green energy carriers from energy crops like sugar cane. Nevertheless, we see that so far, the involved industries focus almost exclusively on the production of liquid fuel, i.e., ethanol. It looks relevant to elaborate this 'case' in the light of the sustainability notion, because this 'liquid fuel'

route, in essence, cannot compete with the 'gaseous fuel' route, i.e., the production of methane using one of the presently available AnDi/AnWT technologies. Nevertheless, it happens.

In present practice of alcohol production from sugar cane in Brazil (see Chapter 15), only 25.5% of the sugar cane energy content is converted into ethanol, 5.3% lands in the vinasse (the complex high-strength wastewater), 33.3% in the bagasse fraction, more than half of which is used for energy supply of the distillery, while 33.3% (straw fraction) is burnt on the field before cutting. So far, very few alcohol companies implemented modern high-rate AnWT as a treatment method for the vinasse, despite the feasibility of e.g., the UASB system that already had been demonstrated in Brazil in the 1980s. As the biodegradability of vinasse amounts to roughly 80%, application of AnWT would improve the net energy production from cane with at least 4% and set an end to nowadays' practices of spreading the untreated highly polluted vinasse over the cane fields. But application of AnDi processes offers more perspectives for distillery companies, i.e., it would enable them to produce a significant amount of methane from i) the bagasse and ii) the straw fraction, *viz.* at least 46% of the cane energy content, using an estimated biodegradability of 65% for both these fractions (this can be higher depending on e.g., the operational condition of the AnDi process; see Chapter 15). Consequently, in addition to 25.5% alcohol-energy, the total net energy production of a distillery could increase by roughly 50%, making up a total of about 75% net energy conversion, while the non-biodegradable residues can find applications as soil conditioners and/or (after drying) as energy sources for meeting the power demands of the distillery. And still, all the nutrients for fertilization of the fields are left in the liquid effluent of the AnDi process; moreover by implementing AnWT processes for vinasse treatment in the distillery plant an end is set to spreading practices of vinase-biodegradable matter over the fields, and consequently to the release of methane and carbon dioxide due its anaerobic conversion in the soil.

Considering these figures, it is clear that the sustainability of the present biomass energy conversion event can be improved significantly. Rather than focusing on the production of alcohol it would be better to prefer the production of methane, and not only for reason of its higher energy conversion efficiency and environmental protection features, but especially also because the AnDi technology is much simpler than that of the alcohol production, while it enables a more decentralized application (reducing transport) and could encourage the development of local cultivation of food crops and others crops as well. The latter, in turn, will lead to the improvement of biodiversity in nature, restoration of landscapes and — last but not least — to a significantly improved livelihood for residents. The '*sugar cane energy crop*' case is an illustration; unfortunately, there exist many other examples revealing similar forms of wasting of energy crops, including various other types of cultivated biomass. In terms of sustainability, it is a dramatic situation.

3.3. The FoPr domain

In traditional farming, it has been common practice for millennia to collect crop, animal and human residues in order to return them to the fields for fertilization and soil conditioning purposes. However, until recent times this happened without incorporating AnDi systems for producing biogas and improving the fertilizing value of these residues, e.g., making nutrients better available and releasing these residues from their obnoxious odor 'generation capacity'. The knowledge of these systems simply was not available until the first half of the last century; as a matter of fact, real awareness of the enormous potentials of the application of the AnDi technology for the agricultural, horticultural and animal husbandry sector evolved during the eighties: only since the last three decades has it increasingly led to sophisticated highly integrated industrialized practices and to modern on-site food production tackles as well, all these systems are based on water and substance closed loop principles. These offer enormous potentials for future food production; virtually nothing is lost and the products are of high quality.

Unfortunately, the developments in modern 'industrialized animal breeding practices', e.g., in the Netherlands, are much less positive, although also here, AnWT/AnDi systems have found wide-scale applications. However, as these systems are merely effective in removing biodegradable organic matter, and animal breeding branches often are based on excessive import of cheap fodder, closed-loop practices are impossible, because there simply is no land available for the enormous amounts of nutrients produced.

As a matter of fact, the focus of research on the development of the UASB system in the Netherlands during the 1970s was particularly on finding application in the agro-food industry, which was often highly polluting. Generally, just a part of the cultivated crop is used, leaving the remainder as waste(water). The first successful application of the UASB system concerned treatment of sugar beet wastewater, followed by several others. Likely the most difficult case in the Netherlands was the potato starch industry. It comprised one of the heaviest polluting industries in the country until late in the 1980s. The production process of this industrial branch focused exclusively on the starch fraction of the potato, leaving the highly polluting juice fraction as 'waste' for the environment. It found its way untreated to the canals, leading to tremendous nuisance problems for residents and serious damage to the environment. Despite comprehensive long-term experiments the potato starch industry was unable to develop processes for making marketable products from the proteins present in the juice fraction. Ultimately, in joint efforts with research institutes and universities and thanks to substantial governmental financial support, the involved potato starch company succeeded to implement the UASB process. This not merely resolved the pollution problems with organic fraction of the wastewater, but made the industry also greatly self-sufficient in its power demands. However, it was just the first step; still a lot of effort needed to be done to find solutions for the removal, recovery and/or direct valorization of the high concentrations of ammonia, phosphate and potassium contained in the effluent.

4. The Integrating Role of AnWT/AnDi Systems in Sectors of EP, En_{ren} and FoPr

The above discussions indicate that AnWT/AnDi technologies deserve the position of primary treatment/conversion system rather than AeWT systems. As a matter of fact, Nature provides a clear additional argument for that; it shows that the supply of free oxygen to e.g., fresh plant juices leads to the formation (via condensation reactions) of soluble non-biodegradable polymers (*viz.* humic acids) from originally well biodegradable compounds, i.e., especially from highly reduced compounds, such as those present in potato juice. Consequently, it leads to lower biogas production.

Overlooking the developments concerning the implementation of AnWT/AnDi systems in the various sectors, the question arises: *‘Why is it so difficult to choose for real sustainability?’* It is a complex social-economic matter, amongst other short-term interests of established institutes, companies and politicians dominating decision-making. The main reason likely can be found in the fact that present practice is economically sufficiently profitable for the distillery companies; apparently, the liquid fuel prices are high enough and the production/labor cost sufficiently low, while there doesn’t exist yet an interesting market for gaseous fuel, nor for electricity (generated from methane). Common residents cannot afford any luxury such as electric equipment, governments are unable or unwilling to choose for modern forms of electric transport, and things look to be controlled by industries and companies that benefit automobile and air transport. Some of these liquid fuel directed entities attempt to enforce liquid fuel production by stimulating the development of (bio)chemical-physical technologies providing a higher extent and rate of bio-conversion of the non- or the poorly biodegradable fraction of energy crops or non-food agro-residues. It looks as though they are not aware that these residues are essential for maintaining a high soil quality, like they are not for the application of the AnDi energy conversion route.

5. Resource Recovery and Valorization-Directed NBM&S (Post-)Treatment Systems

As mentioned above, AnWT/AnDi systems need to be integrated with supplementary NMB&S treatment steps for the removal and recovery of nutrients and/or with photo synthesis based NBS methods for nutrient valorization. Users of AnWT-systems, i.e., including the millions of residents applying septic tanks, are aware of the frequently obnoxious, *viz.* seriously stinking and turbid, characteristics of the effluents of these systems. It is a clear warning, i.e. *‘Don’t drink this water, it is still a risk for human health and the environmental quality!’* On the other hand, it is slightly misleading; the risks for environmental damage are much less dramatic than it looks to be at first sight, at least for domestic types of wastewaters. The absolute amount of obnoxious (generated) polluting compounds in the anaerobic effluent, *viz.* immensely stinking and highly toxic volatile reduced S-compounds, is generally very low. Regarding the latter aspect, relatively simple complementary (post)-treatment steps suffice for their removal, because the ‘oxygen demand’ for eliminating them is relatively low and the biological oxidation process proceeds quite fast. And since the precursors of these compounds present in the domestic wastewater are removed almost completely in a well performing AnWT (pre-)

treatment step, the ‘stinking nuisance problem’ has been definitely eliminated. As a matter of fact, Nature clearly demonstrates how to tackle the problem; many common people noticed this natural happening of ‘self-purification’ in slightly polluted surface water, for instance prevailing at the effluent discharge point of a well performing septic tank. In essence, we are dealing here with the Ae_{micr} conversion phenomenon: an aerobic biological process proceeding at very low dissolved oxygen concentrations. In the case of septic effluents, just relatively small amounts of oxygen suffice in order to achieve a superb result, *viz.* absence of mal-odor and a clear water phase a few meters ahead of the discharge point. The Ae_{micr} event apparently constitutes the ‘bridge’ between putrefaction and a clean aerobic environment (e.g., clean water).

5.1. The potentials of the Ae_{micr} conversion phenomenon

Visual observations no doubt were always of crucial importance for innovative technological developments. So in the 1980s, we observed manifesting the peculiar formation of elementary sulfur near the top of the settler of a UASB pilot plant. It made us aware that the Ae_{micr} treatment principle might offer very promising potentials for applications in practice as a first post-treatment step of anaerobic effluents, particularly including industrial effluents containing a high concentration of reduced S-compounds. Nature dropped it on our plate, more or less pushed us to initiate research on the development of proper (high-rate) Ae_{micr} treatment technologies. We commenced investigations focusing on the conversion of sulfide and volatile organic sulfur compounds into elementary sulfur, and attempted to improve the understanding of the involved microbiological, biochemical and physical–chemical fundamentals. The objective was to develop a high rate Ae_{micr} technology for that purpose, which in essence implies an effective manipulation of the first step in the oxidative part of the biological S-cycle (S_{biol} -cycle) by restricting/controlling the supply of oxygen. It turned out to be quite feasible and resulted in a high-rate S_{bio} – Ae_{micr} system. This innovative process already found ample full-scale application, *i.e.*, for treating anaerobic effluents, raw wastewaters, biogas, natural gas and polluted air. It became a major commercial activity of Paques B. V. Interestingly, the importance of the system is not just sulfide removal, but also the production of elementary bio-sulfur, because it has peculiar features, *i.e.*, it acts as fungicide, can be used as fertilizer, represents an attractive raw material in the chemical industry, and it offers interesting potentials for removing and recovering heavy metals from contaminated (e.g., Zn) soil and/or wastewaters, such as acid mine drainage water and specific, e.g., Cu-, Zn-, Co-contaminated industrial effluents. For latter applications the S_{bio} process needs to be integrated with both the reductive and the oxidative part of the S_{biol} cycle, but complete oxidation in this case. It comprises an extended S_{biol} cycle based treatment system, and already found full-scale application in the Netherlands for remediation Zn-polluted soil.

An additional fascinating Ae_{micr} -application comprises the removal of colloidal matter from anaerobic effluents and/or raw wastewaters by a kind of growth, entrapment, sorption and flocculation mechanism; it is still an important issue of research, *viz.*

Potentials of Ae_{micro} Treatment

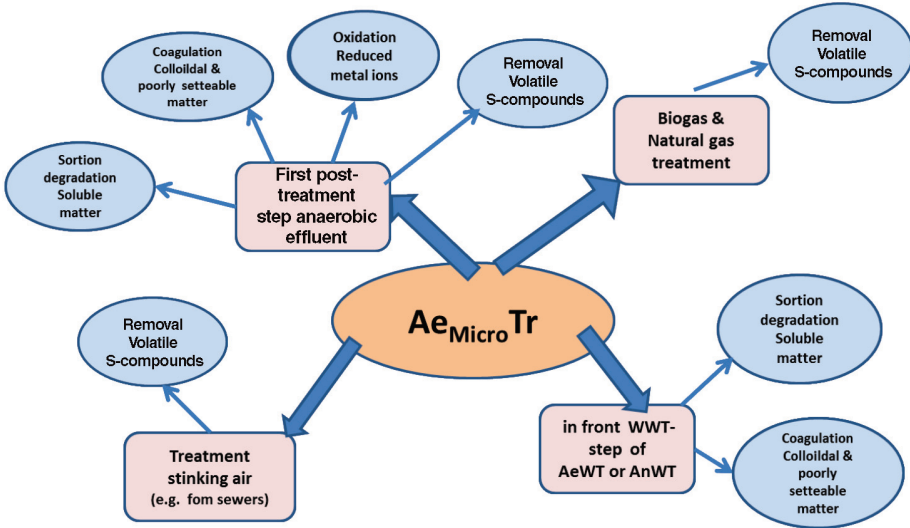


Fig. 16.1. The potentials of the micro-aerobic treatment concepts.

comprising investigations directed to assess the proper operational conditions and to develop appropriate reactor systems. Systems like rotating bio-disk, activated sludge, attached film systems and expanded Ae_{micro} granular sludge reactors look feasible. Likely, the door is open for numerous innovations. As a matter of fact, this specific feature of the Ae_{micro} process is exactly what is needed to make high-rate AnWT sewage (pre-)treatment systems more attractive, because so far, these suffer from a relatively poor efficiency of removing colloidal matter. We may expect the development of various types of integrated Ae_{micro}AnWT systems in the near future, e.g., comprising the use of the Ae_{micro} step either in up-front pre-treatment and/or in post-treatment position. The ‘up-front’ application of Ae_{micro}WT looks like it is not really new; likely it already found application as high-rate A-step of the so-called A/B-process developed in Germany in the 1970s, although it was not recognized as an Ae_{micro}WT system that time.

5.2. Application of AeWT systems

AeWT systems have important functions in the NBM&S treatment concept as the polishing step for removing remaining pollutants and pathogens, for nitrification and possibly even as a valorization method for the production of alginate, and by integrating them with photosynthetic NBS systems, for the production of algae, duckweed and/or water hyacinth.

6. Prospects of Integrated NBM&S-PC Tackles

The above discussions indicate that we may expect numerous interesting innovations, e.g., various types of integrated NBM&S systems in all kinds of scales and settings. They will include specific versions of high-rate AnWT reactor, such as systems enabling the production of electricity instead of methane/hydrogen. Moreover, most of these inventive concepts will apply innovative Ae_{micro}WT and AeWT systems based on the use of a specific bacterial cultures, e.g., algae, fungi. We may expect the development of systems capable degrading recalcitrant and/or toxic compounds, and systems applicable under extreme environmental operational conditions, such as very high pressure(s), high temperature(s) and/or low pH. Some of them are already underway.

6.1. *Physical–chemical (PC) processes for resource recovery and valorization*

The systems based on the NBM&S concept can and, in fact, often need to be supplemented with appropriate PC methods in order to attain the sustainability goals. As a matter of fact, various PC events already occur (naturally) in NBM&S-treatment systems, *viz.* induced by the bio-degradation processes taking place. This particularly happens in anaerobic treatment. These bio-conversions lead to drastic changes in the reaction medium, e.g., in pH, redox potential and composition; these induce precipitations of insoluble salts, like metal-sulfides (including those of trace elements), metal-hydroxides, metal-carbonates and metal-phosphates, e.g., magnesium–ammonium–phosphate (struvite) and calcium–phosphate. These events happen particularly in high(er)-strength waste(water)s, which once again points to the enormous (additional) benefit of the ‘*dilution prevention prerequisite*’. Obeying Nature will be granted, we’ll achieve substantial higher overall treatment efficiencies and a significantly easier nutrient recovery.

Supplementary to these naturally occurring PC events, we can incorporate a wide range of ‘man-made’ PC processes. Many of them provide an efficient removal and recovery of nutrients and metals, e.g., systems like stripping–absorption and chemical precipitation and application of specific ion exchange methods for the removal/recovery of (heavy) metals, phosphate, potassium and ammonia. A perfect option for the removal and recovery of ammonia and phosphate can be found in pairing a ‘one or two step stripping/absorption process’ with the Anammox process as a final step. And likely various others of these types of integrated NBM&S-PC optimizations will emerge, though it depends on numerous factors; in fact, each situation is unique and has its own optimal sustainable solutions, and many of them will be ‘holistic’ and innovative.

7. How to Achieve the Optimal Implementation of NBM&S/PC Tackles in Domains of EP and Renewable Energy

Despite their great potentials, we see that the implementation of NBM&S/PC systems in many countries is quite a laborious process. It is far more than just developing the appropriate reactor systems and assessing the optimal operational conditions and

sequence of systems in the treatment. Actually the major problem, the cases mentioned above illustrate this, is to develop sufficient awareness, skill and confidence (for all involved stakeholders) concerning the big potentials of the modern tackles. The question often is how to use the by-products efficiently and profitably, especially when the required skill is not available. But a major difficulty often is that innovative systems need to compete with widely applied (and accepted) systems with the relied established commerce, policy and decision-makers.

Casting back over the developments in the EP sector, we observe that in many countries, public authorities in the Public Sanitation sector frequently remained obstinately reluctant with regard to the substitution of conventional AeWT systems as a secondary sewage treatment method for high-rate AnWT, e.g., the UASB system, despite all accompanying benefits. Unfortunately, the 'superiority matter' is a rather complex affair; we always face the restriction that its optimal operational conditions need to meet specific criteria in order to benefit maximally from its application. Looking to present wastewater practice, the latter often is not sufficiently the case; for instance the pollution strength of huge amounts wastewaters generated worldwide (e.g., domestic sewage) is too low for optimal application of AnWT. As a matter of fact, it implies that nowadays waste(water) collection and transport practices are far from they needs to become regarding above mentioned Pollution Problem Prevention (P3) consideration. But nowadays it is difficult to release sanitation practice from its widely embraced comfort utility, the flushing toilet, which in essence, lies on the basis of the prevailing 'dilution malaise'. This malaise 'obstructs' the proper functioning of conventional septic tanks, forces authorities to make immense investments in sewerage systems, and is detrimental for the optimal application of modern high-rate AnWT systems and supplementary resource recover methods, especially at lower ambient temperature conditions. What can be done? Efforts can be, and occasionally are being, made to reduce water consumption of flushing toilets and/or by applying vacuum toilet and transport systems. Fortunately, in the industrial pollution control sector successful efforts increasingly are being made to limit '*Waste-dilution practices*'. However, as mentioned above, in the modern industrialized animal breeding sector, we see the opposite happening, i.e., the generation of huge amounts of highly polluted liquid manure requiring expensive treatment. It is far from sustainable, finds its cause in the excessive expansion of this industry due to the import of enormous amounts of cheap fodder from oversea. We need to return to practices resembling traditional forms of animal husbandry. And similar things apply for human residues. These natural residues need to remain undiluted as much as possible in order to enable their appropriate application in agriculture.

As elucidated, we see similar adverse developments in liquid fuel production from cultivated biomass in the En_{ren} sector. On the other hand in this sector, we fortunately see very promising developments in the solar energy field.

So with respect to the question of whether the door has opened sufficiently for achieving sustainability in the domains of EP, En_{ren} and FoPr, there are reasons for moderate optimism. Regarding the successes already obtained in the development and implementation of integrated recourse recovery and valorization directed NMB&S-PC systems, in the prevention of waste dilution and to some extent in the optimization of

transport, substantial progress has been made towards sustainability. Serious attempts are made and are underway to close water and substance loops. A very important feature of these developments is that they are applicable at almost any scale and location, and often completely independent of any complex infrastructure. It is obvious that they offer spectacular potentials, including local food production practices. Awareness undoubtedly will grow, also in the biomass part of the En_{ren} sector. The achievements attained and the growing awareness will act as a crowbar to get rid of outdated conventional tackles and established self-interested structures. We'll see more bottom-up actions, but things need time to become mature before they become generally accepted, even when they greatly comply with the sustainability criteria. It looks unavoidable that established conglomerates of policy-/decision-makers, businessmen and academics in society stick on their own expertise and commercial interests; they therefore generally are unable to make the proper choices for common interest in society. Nevertheless, we may hope that appropriate policy- and decision-making increasingly will come off so that progress will be made towards a more sustainable society.

8. On the Route towards a Sustainable Society

Considering how things develop(ed) in human history, it looks justified to conclude that mankind is on the route to a sustainable society (SOS_{sus}); it always was our mission and that will not change. And we succeeded in making progress, improved our understanding of what is happening and increasingly also why it is happening. We therefore don't want to escape from acting accordingly, i.e., elucidating the question '*What is SOC_{sus} and what is it for*'? The appeal of the Brundtland Committee to mankind in essence is to create the decent conditions in society enabling citizens to find '*Happiness, Harmony, Liberty and Justice*'. It implies the moral dedication to *eliminate society from its defects*, particularly those of *inequality and injustice*, and of structures, institutions, actions which are merely *self-interest directed*. Brundtland recognized that human behavior is strongly related to the quality of society; it is in line with the ideas underlying Thomas Moore's *Utopia*, and also with the inspiring vision of philosopher, Jesuit priest and paleontologist *Teilhard de Chardin*, who stated that mankind arrived at a critical moment in the process of *transition to a higher stage in evolution*. *We have our future in our own hands*, we should start using all available knowledge, insights, culture, marvelous tools, etc. to work towards the wellbeing of everybody. And mankind continues developing ever-advancing instruments at breakneck speed; we therefore can designate '*the optimal further development of the human species*' as an ultimate objective of SOC_{sus} . The latter implies that we need to focus on the following three main sustainability aspects:

- Conservation of fossil and natural resources, i.e., by closing water and substance loops in all sectors in society.
- Social security, viz. issues like problem prevention, social equity, liberty, justice and robust production and supply of basic needs,
- Human resource development by study and higher education ('*Bildung*').

Since the appearance of our species, life's evolutionary development became affected ever more by our involvement; new creations have become less incidental and also less directed towards physical perfection(s), but instead became more spiritual and reflecting of nature, and increasingly sprung from our enormous human creative capabilities and longings, such as to behold, to experience, to feel, or what so ever, the essence of the unintelligible conceptions: '*Justice, Fraternity, Liberty, Equality, Beauty, Dignity, Happiness, Democracy,*' etc.

From the abundance of examples in nature and from our own history as well, we can know that the quality of life in *cooperative types of communities* is superior compared to that in communities controlled by *self-interest and noxious types of competition*, as these have destructive effects. Although 'our' disgusting behavior generally is attributed to our individual human weaknesses, I think the reason lies particularly in the precarious absence of *social security and justice* in societies. Unfortunately, it looks as if we are incapable and/or unwilling to release our societies from those types of ugly malaises; because it is an immensely difficult and risky task, it needs the realization of the drastic changes as recommended by Brundtland. Unfortunately, very few people have confidence in the promises of such drastic changes and the vast majority of (world) leaders support that skepticism. And these leaders have an argument, *viz.* that the enormous achievements in technology, prosperity, public health, freedom, etc. in the industrialized world mainly can be attributed to the very competition-directed incentives of the '*free market*' economical system; the communistic regimes failed dramatically due to lack of competition. But the 'free market' ideology has a high price; it clashes head-on with the merits of a sustainable society (SOC_{sus}), and therefore, irrevocably will come to an end. We need a *transition of our society to a higher stage*, irrespective of the concomitant high risks. And as it happens, contemporary generations have the privilege of witnessing a *human-made transition*; it manifests in numerous incredible man-made creations, emerging throughout practically all domains of society, e.g., in the EP domain, and it likely will continue with ever-increasing tempo. These encouraging developments undoubtedly will have a very positive impact, i.e., will lead to a more balanced society, gradually excelling in a high degree of self-sufficiency at regional, community and family level, in substantially better livelihood, enabling citizens to develop their aspirations.

Like always before in our history, the coming generations will obtain even better tools than their ancestors to experience and to demonstrate the superiority of a society based on *cooperative actions* and on *sustainable production/treatment technologies/concepts*: in brief, a society in which Brundtland's recommendations for drastic changes have been greatly implemented, consequently starting to resemble the Utopia described by Thomas Moore five centuries ago. Although Moore's Utopia is never attainable, it is incomparably more appealing than our present heavily *competition*-based society with its desperate *survival fights*. Unfortunately, hardly any of the political parties have the courage to take the leap forward towards a SOC_{sus}, to admit that we need to get rid of excesses of the prevailing 'free market' ideology; they witness that citizens increasingly understand that our still widely embraced ideology is inherently unable to cope with the 'employment' problem. Leaders, private and public, desperately beg scientists and

research institutes for job-creating innovations; they all beg for an époque of new economical growth in their region/country, despite of being aware that the types of growth they have in mind are generally non-sustainable. Practically none of the ruling entities see any prospects in things like sharing meaningful employment, providing all citizens with a kind of basis income, creation of significant employment in education and in developing human talents, and obviously not at all in skipping types of meaningless employment. It is designated as far too much '*Utopia*'; our leaders prefer to advertise the enormous prospects and challenges of the 'free market' ideology, although they know they are unrealistic dreams, that they push a dead-end uncontrollable system, where the benefits are for very few at the top and the price needs to be paid by the vast majority. However, it will become increasingly difficult to mislead citizens; people want open and honest information from public and private authorities, more 'real democracy', and they want our marvelous achievements to primarily serve things of common interest. Fortunately, we see that occasionally valiant, democratic and visionary leaders stand up and really attempt to make progress in attaining the required transitions. Consequently, there are reasons for some optimism.

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Index

- 16S rRNA, 33
- 16S rRNA gene, 74
- 2,5-dihydroxybenzoate, 34
- 2-hydroxybenzoate, 34
- 341F/806R, 80
- 3-dimensional configuration, 4
- 3-hydroxybenzoate, 34
- 3-phenyl-propionate, 34
- 454 pyrosequencing, 84
- 4-carboxybenzaldehyde, 32
- 4-hydroxybenzoate, 34

- ABR: anaerobic baffled reactor, 316
- acetaldehyde, 249
- acetate, 13, 14, 20, 32
- acetates, 244, 255, 257
- acetic acid, 246, 249, 255
- acetoclastic, 34
- acetoclastic genera, 4
- acetoclastic pathway, 4, 93
- acetogenesis, 92
- acetogenic bacteria, 244
- acetotrophic, 93
- acid products, 111
- acid-base equilibrium, 113
- acidogenesis, 92, 249, 329
- acidogenic phase, 325, 329
- Actinobacteria*, 85
- activated carbon, 172
- activated sludge, 211, 229, 236
- activated sludge process, 249
- ADM1, 145
- adsorption, 219, 221, 230, 235
- advective-diffusive, 135
- AEM: anion exchange membranes, 174
- aerobic, 381, 382
- aerobic biofilter, 258, 259
- aerobic biological treatment, 264
- Aerobic post-treatment, 323
- AeWT, 382
- AF: anaerobic filter, 33, 244
- AFB: anaerobic fluidized bed, 244
- AFBR: anaerobic fluidized bed reactor, 211, 213
- agricultural, 290
- agricultural biomass, 348
- agricultural production, 348
- agricultural residues, 348
- agricultural use of effluents, 289
- agricultural wastes, 348
- agriculture, 349
- air-cathodes, 172
- alcohol, 299
- alcohol industry, 300
- alcohol/aldehyde dehydrogenase (*adhE*), 94
- algae production, 369
- Alkali products, 111
- ambient temperature, 198
- ammonia, 109, 111, 113–119, 234
- ammonia bicarbonate, 113
- ammonia inhibition, 117–119
- ammonia-nitrogen, 198
- anaerobic, 375, 381
- anaerobic ammonium oxidizing (anammox), 95
- anaerobic bagasse digestion, 367, 371
- anaerobic benzene degradation, 91
- anaerobic bioreactors, 84, 89, 97
- anaerobic biotechnology, 243, 245, 248, 249
- anaerobic contact, 33
- anaerobic conversion process, 267
- anaerobic digester, 74, 87

- anaerobic digestion, 3–5, 13, 75, 83, 86, 88–90, 92, 93, 95–97, 109–111, 113, 118, 120, 122, 127, 277, 353, 355–360
- Anaerobic Dynamics Membrane Bioreactor (AnDMBR), 83
- anaerobic effluents, 276, 278, 279, 291
- anaerobic filter, 248
- anaerobic methane oxidation, 89, 95
- anaerobic methanotrophic (ANME) archaea, 91
- anaerobic methen oxidation (AOM), 91
- anaerobic process, 245, 249
- anaerobic processes, 273, 291, 323
- anaerobic sewage treatment, 263, 273, 289, 291
- anaerobic technology, 73, 75, 78, 83, 264, 272
- anaerobic treatment, 212
- anaerobic wastewater pre-treatment, 323
- anaerobic-aerobic systems, 263
- anammox, 95, 234, 275
- Anammox: Anaerobic ammonia oxidation, 49–64
- AnDi, 375
- animal excreta, 348
- animal waste, 347
- AnMBR: anaerobic membrane bioreactor, 135, 191–194, 196, 197, 202–206
- anode, 170
- antibiotic resistance, 96
- antibiotic resistance genes, 96
- antibiotics, 245, 248
- AnWT, 375
- application, 73
- application research, 348
- aquaculture, 342
- archaea, 19, 246
- archaeal methanogens, 346
- Archaea*-specific primer sets, 80
- aromatic carboxylic acid compounds, 32
- ASM1, 145
- autogenerative, 6
- automotive fuel, 353, 372
- axenic culture, 5
- B. licheniformis* PWD-1, 347
- Bacillus licheniformis*, 344
- bacteria, 19
- bacterial pathogens, 97
- Bacteria-specific primer set*, 80
- Bacteroidetes*, 41, 85–87, 92, 96
- bagasse, 353–356, 359, 361–368
- bagasse combustion, 362
- bagasse digestion, 362
- basic study, 348
- bed expansion, 214
- bed stratification, 217, 218
- benzoate, 17, 26, 32, 249, 255, 257
- benzoic acid, 255
- bicarbonate alkalinity, 111–113
- big wastewater treatment plant, 267
- binning, 97
- bio-augmentation, 5
- biochemistry, 136, 348
- biocompatibility, 170
- biodegradability, 324
- biodegradation, 73, 74, 90, 92, 94
- bio-degradation of feathers, 344
- biodiesel production, 356
- bio-economy, 9
- bio-electrochemical communication, 4
- bioenergy, 73, 83, 97
- biofilm, 211, 214, 236
- biofuel, 341
- biofuel production, 73
- biogas, 6, 109–112, 114–117, 119, 122, 126, 127, 129, 339, 380
- biogas energy potential, 286
- biogas in China, 344
- biogas in Germany, 340
- biogas management, 287
- biogas opportunities roadmap, 341
- Biogas production, 226
- biogas-sparging, 219
- biohydrogen, 83, 88, 196
- bioimaging techniques, 91
- bioinformatics analysis, 73
- biological desulfurization, 126, 127
- biological S-cycle, 381
- biological variations, 347
- biology of anaerobic digestion, 348
- biomarker, 75, 78
- biomass, 110, 113, 119, 128
- biomethanation, 5

- bioreactors, 243–249, 252, 254, 256–258,
260, 261
- biorefinery, 6
- BioResource International, 345
- biosolids, 211, 212, 223–225, 231, 236
- bio-sulfur, 381
- bio-sulfur recovery, 330
- black box, 346
- BMP: biochemical methane potential, 145
- BOD removal efficiency, 263
- BOD₅, 217, 223, 225, 236
- boiler, 202, 203
- branched pipeline system, 326
- Brazil, 378
- brewery, 245
- brewery wastewater, 332
- Brundtland Committee, 375
- BSE: bovine spongiform encephalopathy,
345
- BSM2, 156
- bulk VSS concentration, 226, 229
- bulk wasting, 226
- butyrate, 14, 15
- C/N ratio, 113, 348
- C/N: Carbon/nitrogen ratio, 49, 58
- Ca. Cloacamonas acidaminovorans*, 41
- cake layer, 219
- Caldiserica*, 41
- Candidatus Cloacamonas*, 90
- cane juice, 353
- cane production factors, 370, 371
- cane straw, 353, 372
- CANON: completely autotrophic nitrogen
removal over nitrite, 63
- capacitance, 170
- CAPEX, 264
- carbohydrates, 244
- carbon credit, 341
- carbon dioxide, 360
- catalyst layer, 172
- cathodes, 171
- CCA: cloth cathode assembly, 177
- CDM: Clean Development Mechanism, 317
- CEA: cloth electrode assembly, 177
- cellulose, 83, 84, 87–90, 93, 94, 96, 245,
246
- CEM: cation exchange membranes, 174
- centralized sewage treatment, 271
- ceramic membranes, 219
- CFD, 150
- characterization, 133
- chemical cleaning, 218
- chemical energy, 7
- China, 340, 344
- chlorinated solvents, 32
- Chloroflexi*, 85, 89
- chlorophenols, 247
- Cinderella Energy, 340
- clean energy production, 371
- cleaning, 193, 194
- climate change, 6, 8, 9
- Clostridiales*, 88
- Clostridium*, 88
- Clostridium ultenense*, 17
- CO₂, 227
- coagulants, 235
- co-culture, 34
- COD removal, 215, 225, 231
- COD removal efficiency, 283
- COD: chemical oxygen demand, 61
- COD: chemical oxygen demand removal,
177, 182
- co-digestion, 113, 133, 319, 347, 348
- coffee grounds, 114, 195
- cogeneration of power and heat, 288
- colloidal, 381
- commercial development, 345
- commercial production, 344
- compact anaerobic process, 276
- comparative metagenomics, 97
- competition, 125, 126
- composite samples, 225
- composition of settled vinasse, 357
- composition of the biogas, 286
- conductivity, 170
- confidence, 146
- confocal scanning laser microscopy, 33
- Continuously Stirring Tank Reactor
(CSTR), 83
- control pathogens, 342
- control the biological process, 347
- conversion efficiency, 353, 356–358, 361,
362, 365, 372
- conversion of bagasse to ethanol, 361
- co-products, 341

- Cost-effective pathogen removal, 276
- cost-effectiveness, 263, 278
- coulombic efficiency, 168
- crop stalks, 320
- CSLM-FISH: fluorescence *in situ* hybridization, 33
- current collector, 173
- current limitations and constraints, 272

- DAF: downflow anaerobic filter, 244, 245
- Dairy processing wastewater, 202
- dark matter, 346
- DDG solubles, 301
- DDG: distiller's dried grains, 301
- de novo* assembly, 97
- DEAMOX: Denitrifying ammonia oxidation, 63
- decentralized approach, 269
- degradation pathways, 346
- Deltaproteobacteria*, 91
- demonstration projects, 310
- denitrification, 165
- design optimization, 232
- Desulfomonile*, 40
- Desulfovibrio*, 91
- desulfurization, 317
- developing countries, 269, 277, 278, 284, 287, 288
- diffusion layer, 172
- digestate, 6, 348
- digital sequencing, 82
- direct anaerobic digestion, 362
- direct combustion, 278, 288
- direct electron transfer, 169
- discharge structures, 278, 289
- dissociation equilibrium, 109
- dissolved effluent methane recovery, 286
- dissolved methane, 211, 227, 231, 232, 234, 235, 237
- dissolved sulfide, 124, 125
- dissolved sulfide and methane, 278
- distillery, 353, 354, 378, 380
- distributed parameter, 148
- DNA virus, 96
- DNA: Deoxyribonucleic acid, 60
- DNA: extraction, 78
- DO: dissolve oxygen, 61, 63, 64
- DO: dissolved oxygen, 217
- domestic wastewater, 323
- domestic wastewater treatment, 264
- Double-chamber MFC, 174
- downflow MFC, 175
- down-flow stationary fixed film, 33
- dry composting, 322
- dual-index sequencing, 77

- each inlet pipe with a single outlet point (EIP-SOP), 327
- EAT: environmental anaerobic technologies, 375
- eco-agricultural systems, 298
- ecosystems, 74
- effluent concentration, 265
- effluent quality standards, 267
- EGSB: expanded granular sludge bed, 299
- EGSB: expanded granular sludge blanket, 33
- electrode potential, 165
- electromotive force, 166
- electron, 13
- electron transfer, 141
- elemental sulfur, 127
- elemental transformation, 110
- energy balance, 235
- energy balance and footprint, 230
- energy consumption, 221, 230
- energy conversion efficiency, 353
- energy efficiency, 168
- energy in cane, 356
- energy potential, 283, 286, 288
- energy production, 231
- energy recovery from biogas, 286
- energy recovery from sludge, 288, 289
- energy rich biogas, 263
- energy-neutral, 211
- environment, 339, 346, 349
- environmental health, 269
- environmental impacts, 289
- enzymatic degradation of prion protein, 345
- Enzymatic pre-treatment, 364
- enzyme, 346, 347
- enzyme discovery, 347
- enzyme technology, 348
- EP: environmental protection, 375
- ethanol, 6, 14, 21, 356, 366, 378
- ethanol distilleries, 355, 356, 358, 361, 366, 368, 370, 371

- ethanol production, 355
- ethanol production from bagasse, 362
- ethanol stillage, 201
- ethylene glycol, 249, 257, 258
- Euryarchaeota*, 85
- excess sludge withdrawal, 269, 280
- exergonic, 15
- exoelectrogen, 168, 169
- Expanded Granule Sludge Bed, 83
- expanded-bed, 312
- extensions, 148
- external cross-flow membrane filtration
 - module, 192
- external submerged, 192
- external tubular membrane module, 192
- extracellular electron transport, 168, 169

- facultative microorganisms, 324–326
- farm operation, 348
- fats, 244
- feather meal, 339, 344
- feather-degradation, 347
- feather-lysate, 344
- feed additive, 344
- feed cost, 345
- feed enzyme, 339
- feed supplement, 342
- feedstock, 347
- fermentation, 143, 246
- fertilizer, 9
- filtration, 192
- filtration flux, 195
- fine chemical, 245
- Firmicutes*, 85
- FISH: Fluorescence in-situ Hybridization,
 - 60
- fixed-bed, 313
- flat-sheet, 219
- flat-sheet membrane, 193, 198, 200, 202
- fluidization, 314
- fluidized bed, 314
- fluidized media, 215, 218, 219, 220, 221, 236
- fluidized-bed membrane bioreactor, 194
- flux, 194, 198, 219–221, 223, 225, 228, 231,
 - 232, 236
- food, 376–379, 385
- food processing, 245, 247, 248, 252
- food waste, 194, 196, 197, 348

- footprint, 211, 214
- FoPr*: food production, 377
- formate, 13, 22
- fossil fuel consumption, 290
- fouling, 211, 212
- Free Market*, 386
- full hybrid wet anaerobic digestion, 321
- full-scale, 191, 200, 205, 206
- full-scale municipal anaerobic sewage, 264
- full-scale plants, 264, 280, 286, 287, 290
- full-scale submerged AnMBR, 201
- full-scale UASB reactors, 280
- function, 74, 76, 78, 83, 92, 94, 95, 97

- GAC: granular activated carbon, 194, 211,
 - 213, 214, 219–221, 224, 229, 230, 232, 236, 247
- gas collector, 246
- gas composition, 226
- gas flow, 149
- gas sparging, 212, 218
- GC-MS for metabolic intermediates, 347
- gene expression, 83, 93, 95
- gene mining, 347
- gene, *ker A*, 344
- genes, 347
- genetic functions, 346
- genetic regulation, 347
- genomics, 347
- Geobacter*, 4, 95, 169
- Germany, 340, 341
- GHG emissions, 291
- glycosyl hydrolase (GH), 94
- granular sludge, 33, 298
- Great Leap Forward*, 298
- greenhouse gas, 228, 232, 237
- growth promoting effect, 345
- GS Junior System, 75

- H₂, 33
- H₂-utilizing methanogen, 15
- headspace, 126–128
- heat stable protease, 344
- Henry's law, 115
- heterotroph, 14
- high hydraulic loading, 306
- high through-put genome sequencing, 346
- high-rate, 203

- high-rate anaerobic (pre-)treatment, 263
- high-rate treatment, 290
- High-strength industrial wastewater, 328
- high-throughput, 73, 74, 76
- high-throughput sequencing (HTS), 74
- HiSeq platforms, 75
- Holistic Farming*, 339, 342, 344, 349
- hollow-fiber, 219
- hollow-fiber membrane module, 198
- homo-acetogenesis, 89
- horizontal gene transfer, 79
- household biogas tank, 298
- household digesters, 340
- household rural digester, 298
- HPLC-MS for proteins, 347
- HRT: hydraulic retention time, 63, 182, 211, 214, 215, 218, 221, 223, 224, 229, 231, 232, 236, 263, 268, 301
- hybrid wet anaerobic digestion, 321
- hydraulic design, 290
- hydraulic flow, 275, 283
- hydraulic loading, 246
- hydraulics, 134
- hydrodynamics, 133
- hydrogen, 13, 136, 244, 248
- hydrogen sulfide, 122, 123, 127, 234, 235
- hydrogen yield, 197
- hydrogenotaxis, 5
- hydrogenotrophic methanogen, 34
- hydrogenotrophic methanogenesis pathways, 93
- hydrogen-producing AnMBR, 196, 197
- hydrogen-producing bacteria, 89
- hydrogentrophic, 93
- hydrolysis, 92, 137, 212, 244
- hydrolysis process, 323
- hydrolysis reactor, 362
- Hydrolysis-(SBR)AICS, 328
- hydrolysis-acidification, 330, 331, 332
- hydrolysis-acidification pre-treatment process, 298
- hydrolysis-acidification-oxidation, 330, 331
- Hydrolysis-Activated Sludge, 328
- hydrolytic reactor, 324, 326, 327
- hydrolytic tank, 328, 329
- hydrolytic/cellulolytic biofilm, 92
- hydroquinone, 34
- IC: internal circulation reactor, 316
- IC50: The half maximal inhibitory concentration, 58
- Illumina, 75–78, 80
- Illumina sequencing, 34
- inclined plate, 246
- Indirect electron transfer, 169
- industrial, 200
- industrial organic wastewater, 299
- industrial wastes, 348
- industrial wastewater, 245, 254, 260, 261, 297, 298
- industrial wastewaters, 191
- industries, 299
- influent distribution system, 305, 308, 326, 327
- inhibition, 117–120, 126, 138
- injection, 127
- input characterization, 133
- insoluble organic polymers, 244
- integrated processes, 330
- integrated two-phase anaerobic digestion process, 321
- interactions, 74, 91, 92, 97
- internal circulation, 33
- internal cycling-hydrolysis-oxidation process, 330, 332
- internal resistance, 167
- interspecies electron transfer, 17, 22, 95
- interspecies H₂ transfer, 20
- intragenomic heterogeneity, 79
- investment costs, 271
- ion activity, 139
- Ion exchange*, 234
- Ion Torrent, 75, 77, 97
- ionization equilibrium, 124
- IPT: Isotope pairing technique, 60
- isophthalate, 34
- Jacobian, 152, 153
- keratinase, 339, 344, 345, 347, 348
- laboratory digester, 341, 342
- large digesters, 340
- leach bed, 135
- library construction, 82

- life technologies, 75
- lignin, 5
- limestone, 371
- lipids, 203
- Lipp, 306, 307
- low-strength wastewater, 214, 215
- Luo Guo-Rui, 340

- mad cow disease, 339, 345
- management and training, 287
- management of liquid, 272
- manure, 339–342, 344, 348
- mass balance, 135
- mass transfer, 129
- MBfR: membrane biofilm reactor, 63
- MBR: membrane bioreactor, 191
- MCA: membrane cathode assembly, 176
- mcrA* gene, 90
- mechanistic models, 144
- medium size treatment plants, 265
- Membrane, 173
- membrane bioreactors, 212
- membrane covered dry fermentation process*, 322
- membrane fouling, 193, 194, 206
- membrane module, 195, 202
- mesophilic, 84–88, 91, 94, 112, 116, 119, 359
- mesophilic anaerobic digestion, 120, 342
- mesophilic vinasse digestion, 359, 363
- metabolic, 347
- metabolic control, 347
- metabolic pathway, 92, 93
- metabolism, 3, 5
- metabolomics, 347
- metagenome, 74, 77
- metagenomic approaches, 83
- metagenomics, 16, 26, 73, 346, 347
- metaproteome, 74, 83, 97
- metatranscriptome, 74, 77
- metatranscriptomics, 16, 41
- methane, 6, 13, 22, 140
- methane emissions, 281
- Methanobacteriaceae*, 36
- Methanobacterium*, 36, 85
- Methanobacterium formicicum*, 22
- Methanobrevibacter*, 40
- Methanococcus*, 4
- Methanoculleus*, 36, 85
- methanogen, 15
- methanogenesis, 14, 92, 117, 120, 126, 244, 245, 249, 324, 329
- methanogenic archaea (methanogens), 33
- methanogenic consortia, 5
- methanogenic ecosystem, 14
- methanogenic phase, 329
- methanogens, 78, 79, 85–88, 90, 248, 257, 258
- Methanolinea*, 36
- Methanomassiliicoccus*, 36
- Methanomicrobiales*, 86
- methanophenazine, 4
- Methanosaeta*, 4, 36, 85, 246
- Methanosaetaceae*, 217
- Methanosarcina*, 4, 246
- Methanospirillum*, 34
- Methanospirillum hungatei*, 16
- Methanothermobacter*, 21, 22
- methyl tert-butyl ether, 5
- Micro-aerobic ($A_{e_{micr}}$), 376
- microbial community, 347
- microbial dark matter, 346
- microbial diversity, 346
- microbial fingerprinting, 80
- microbial fuel cell, 164
- microbial nanowires, 169
- microbial reactions, 3
- microbiology, 73, 346, 348
- microbiome, 3, 4
- microfiltration, 193
- microorganisms, 346, 347
- micro-pollutants, 236
- MinION™ of Oxford Nanopore, 76
- MiSeq, 75
- mis-folding, 346
- modelling, 133
- modelling objective, 134
- modern anaerobic technology, 297
- Modern high-rate UASB reactors, 263
- modular three-phase separators, 305
- moisture, 123, 127, 128
- molasses, 354, 359
- molecular NH_3 , 117–119
- molecular techniques, 73, 74, 84, 86, 89, 91
- MPA: methane producing archaea, 123, 125
- MSG: Monosodium glutamate, 62

- municipal sewage treatment, 264
- municipal wastewater, 191, 197, 198
- nanofiltration, 193
- naphthenic acid, 32
- NBM&S: Natural Biological Mineralization & Synthesis, 376
- NBS: *Natural Biological Synthesis*, 376
- NCSU research farm, 342
- new agriculture, 349
- next-generation sequencing (NGS), 74
- NGS: Next generation sequencing, 60
- nitrate, 137
- nitrogen, 198
- nitrophenols, 247
- non-specific amplification, 82
- novel genes, 93
- novel microorganisms, 89
- NRBF: nitrogen removal biofilter, 63
- NRR: nitrogen removal rate, 64
- nucleotide barcodes, 77
- nutrient recycling, 348
- nutrient removal, 211, 230, 234, 235, 263, 273
- nutrients, 128, 353, 357, 368, 369–371
- OCV: open circuit voltage, 166
- odor nuisance, 276, 290
- odor treatment, 278
- odorous emissions, 277, 278
- OLAND : oxygen limited autotrophic nitrification denitrification, 64
- OLR: organic loading rate, 215, 217, 223
- OMZ: oxygen minimum zone, 60
- one-hole-one-pipe distribution, 306, 307
- operating strategy, 306
- operational conditions, 357, 360
- operational constraints, 273, 279
- operational costs, 263, 278, 284, 291
- operational optima, 341
- operational optimum, 348
- operational parameters, 86
- operational routines, 279, 280, 288
- OPEX, 264
- optimal implementation, 383
- organic biomass, 347
- organic fertilizer, 343
- organic loading, 246, 247, 254, 306, 310, 330
- organic matter and nutrients, 269
- organic shock loads, 324
- organic solid wastes, 194, 195
- ORL: organic loading rate, 182
- ORP: oxidation-reduction potential, 55
- ORR: oxygen reduction reaction, 166
- our common future*, 375, 376
- overpotential, 166
- oversaturation, 232
- Oxford Nanopore, 76
- PacBio RS of Pacific Biosciences, 76
- packing density of the membrane, 221
- packing ratio of the fluidized media, 222
- paired-end sequencing, 77
- parallel electrical connection, 183
- Parameter estimation, 152
- parameters, 138
- para*-toluate (*p*-Tol), 32
- patents, 348
- pathogen removal, 276
- pathogens, 75, 83, 96, 97
- PCR bias, 81
- PCR: Polymerase chain reaction, 60, 79
- PCR-cloning, 84, 87
- p*-cresol, 34
- Pelobacter venetianus*, 17
- Pelotomaculum*, 34
- Pelotomaculum isophthalicum*, 17
- Pelotomaculum terephthalicum*, 18
- Pelotomaculum thermopropionicum*, 22
- Pelotomaculum, Sulfurovum*, 91
- permeate, 195, 203, 205
- petrochemical, 8, 243–245, 247
- PGM of Ion Torrent, 75
- pH, 138, 227, 305, 325
- pH buffer, 111, 113, 117
- pharmaceutical and chemical wastewaters, 330
- phenol, 18, 26, 34
- phenolic inhibition, 247
- phenolic resin, 243, 245
- phosphate, 234
- phosphorous, 148
- phosphorus, 198
- phosphorus removal, 275
- photo-electron industries, 245
- phthalate, 34
- phylogenetic biomarker, 78
- physicochemical, 136

- physiological ecology, 330
physiological-ecological parameters, 330
pig waste digester, 346
Pig-Biogas-Fruit system, 298
pilot digester, 342
pilot-scale, 200, 203
plug-flow, 135
Plug-flow Anaerobic Digester, 83
plug-flow type digester, 342
PMF: proton motive force, 53
pollution control, 73
Pollution Problem Prevention (P3), 384
polycyclic aromatic hydrocarbons, 31
polyethylene terephthalate, 32
polyvinylidene difluoride, 219
POME: palm oil mill effluent, 203–206
population equivalent, 263, 265
pore size, 193, 198, 219
post-treatment, 232, 234, 235
post-treatment flowsheets, 264, 269, 270, 275, 276, 278, 280, 290
potential loss, 166
potential of biogas production, 341
poultry manure, 314, 315, 341
poultry waste, 348
poultry waste digester, 344
power and current densities, 167
precipitation, 139
primer design, 80
printing and dyeing wastewater, 331
prion protein, 345
probe design, 80
process engineering design criteria, 268
propanol, 17, 21
propionate, 14, 21, 22
protein, 75, 93–95
protein digestibility, 345
protein structure, 346
protein-rich substrates, 122
proteins, 244, 247
Proteobacteria, 85
proteomics, 347
proton-reducing H₂-producing, 14, 15
psychrophilic, 215
PTA wastewater, 255, 256
PTA: purified terephthalic acid, 32
p-toluic acid, 249, 255
public health, 269
pulp and paper wastewater, 300
purification, 317, 321
PWD-1 keratinase, 346
pyrosequencing, 34

qualified operators, 279

reactor configuration, 362
reactor volumes, 368
recirculation, 182, 360
recovery, 117, 119, 120
recycled, 357
redox potential, 329
reductionism, 3
regulation, 347
relaxation, 225, 228
renewable energy, 346, 375
resource preservation, 376
resource recovery, 291, 377, 380, 383
resource recycle, 9
RET: reversed electron transport, 53
retention time, 341, 342
reuse, 8
reverse osmosis, 193
ribotype microdiversity, 79
RNA extraction, 78
Roche, 75–77
rRNA: Ribosomal ribonucleic acid, 60

S. aromaticivorans, 34
SAA: Specific Anammox activity, 59
SAF-MBR: staged anaerobic fluidized
 membrane bioreactor, 223, 225, 229–232,
 234, 236
salinization, 357
sanitary systems, 271
sanitation, 8, 264, 267, 271, 272, 276, 279,
 284, 289, 290
SBR: sequencing batch reactor, 61, 315
scanning electron microscope, 34
scouring, 220, 221
scum removal, 268, 279, 280, 282
secondary treatment methods, 276
seed culture, 348
Self nutrient supplement reactor, 128
self-agitated, 128, 129
sensitivity, 152
separators, 173

- sequencing strategies, 75
- sequencing-by-synthesis, 76
- series electrical connection, 183
- sewage, 6, 7, 9, 133, 323, 324, 326, 327
- sewage influent flow, 269
- sewage treatment, 267, 272, 279, 280, 284, 291
- sewer network, 277, 291
- sewer sludge, 348
- sewer systems, 271, 272
- SGSB: suspended granular sludge bed, 300
- shale gas, 341
- SHARON: Single-Reactor high-activity
 - ammonium removal over nitrite, 61, 63
- shot-gun sequencing of DNA (metagenomics), 346
- simultaneous removal of ammonia and nitrate, 275
- simultaneous removal of carbon, nitrogen and sulfur, 330, 332
- single AFMBR, 231
- single cell, 82, 97
- single molecule real time sequencing, 76
- single-cell genomics, 41
- Single-chamber MFC, 176
- size distribution of STPs, 266
- sludge, 114, 119, 128, 129
- sludge digester, 135
- sludge drying, 288
- Sludge hygienization, 288
- sludge reduction, 324
- sludge retention time, 328
- sludge withdrawal, 327
- small STPs, 265, 267, 286
- small subunit ribosomal RNA (SSU rRNA), 346
- Smithella*, 91
- Smithella propionica*, 17
- SNAD: simultaneous partial nitrification,
 - anaerobic ammonium oxidation and denitrification, 61
- social security, 385
- solar energy, 371
- solid and gaseous by-products, 272
- solid byproducts, 288, 289
- SOLiD System, 75
- solid waste, 151
- solid-liquid separation, 191, 192
- solubilization, 133
- soybean meal, 344
- sparging, 194
- speciation, 138
- specific growth rate, 246
- Sphingobacteria*, 86
- Spirochaetes*, 41
- Spirochetes, 85
- Sporotomaculum syntrophicum*, 17
- SRAO: Sulfate reduction and ammonium oxidation, 55, 56, 64
- SRT: Solid retention time, 57, 61
- SS, 328, 329
- stacked MFC, 178
- starch, 245, 299
- starch processing wastewater, 305, 306
- start-up, 178
- start-up biotechnology company, 348
- state of the art, 263
- steam explosion, 363
- Stoichiometry, 109
- STP: sewage treatment plant, 263, 266,
 - 276–278, 286, 288, 290, 291
- Stripping tower*, 233
- structure, 75, 77, 83, 86–88, 91, 95, 97
- submerged, 192, 200, 202
- submerged AnMBR, 194, 198
- submerged flat-sheet membrane module, 192
- substrate, 180
- substrate to biomass concentration ratio, 202
- substrate-oxidizing heterotrophs, 14
- sugar cane, 353, 377, 378
- sulfate, 122, 123, 126, 137
- sulfate reducing bacteria (SRB), 120
- sulfate reduction, 225, 228, 231
- sulfide, 109
- sulfur, 148
- sulfurous compounds, 332
- suspended granular sludge bed (SGSB)
 - process, 300
- suspended-bed, 313
- suspended-bed expansion, 313
- sustainability, 7, 267, 272, 278, 288, 349, 375
- sustainable society, 375, 385, 386
- Synergistetes*, 85
- synthesis, 110
- synthetic polyester fiber, 245
- syntroph, 13, 15
- Syntrophaceticus schinkii*, 17

- syntrophic, 244, 246, 252, 261
- syntrophic acetate oxidation, 89
- syntrophic association, 13, 16, 74, 90, 91
- syntrophic bacteria, 129
- syntrophic metabolizers, 92
- syntrophic substrate-oxidizing bacteria (syntrophs), 33
- syntrophisms, 144
- Syntrophobacter*, 36, 91
- Syntrophobacter fumaroxidans*, 22
- Syntrophobacter wolinii*, 16
- Syntrophomonas wolfei*, 16, 22
- Syntrophomonas zehnderi*, 5
- Syntrophorhabdus*, 34
- Syntrophorhabdus aromaticivorans*, 17, 91
- Syntrophospora bryantii*, 17
- Syntrophothermus lipocalidus*, 16
- syntrophs, 90
- syntrophus*, 34, 91
- Syntrophus buswellii*, 16, 17
- Syntrophus gentianae*, 17
- syntrophy, 13, 141
- systematic studies, 348

- TA: terephthalate, 32
- TAnD: thermophilic anaerobic digestion, 340, 341, 348
- tank, 135
- TEEM: thermodynamic electron equivalents model, 56, 57
- temperature, 341, 342, 348
- Tepidanaerobacter acetatoxydans*, 17
- Tepidanaerobacter syntrophicus*, 17
- terephthalate, 5, 26
- terephthalate, TA, 243, 249, 256
- terephthalate-degrading, 94
- terephthalic acid, 31
- the Netherlands, 375, 379, 381
- theoretical critical flow velocity, 311
- Thermacetogenium*, 36
- Thermacetogenium phaeum*, 17, 22
- thermautotrophicus*, 21, 22
- Thermoanaerobacter*, 88
- Thermoanaerobacteriales*, 88
- Thermoanaerobacterium*, 88
- thermodynamics, 144
- thermophilic, 86–88, 93–95, 112, 118, 119, 123, 129, 246
- thermophilic AnMBR, 195, 203
- thermophilic cellulose methanization, 95
- thermophilic vinasse digestion, 359, 363
- Thermotogae*, 94
- three-phase separator, 246, 249, 300, 306, 308, 324
- tiedjei*, 40
- TMP: trans-membrane pressure, 220, 225, 228
- toxic, 117, 123
- transmission electron microscope, 34
- treatment of domestic sewage, 264
- treatment process scheme of the STPs, 267
- trickling filter, 245
- tubular membrane, 193, 200
- two-phase anaerobic-aerobic integrated process, 330
- two-phase anaerobic-aerobic process, 330, 331
- two-stage anaerobic system, 329
- two-stage fermentation, 246
- two-stage treatment, 203

- UASB, 19, 147, 192, 200, 202
- UASB reactors, 263–265, 267–269, 272, 273, 275–281, 283, 284, 286, 288–290
- UASB systems, 291
- UASB: Up-flow anaerobic sludge bed, 63
- UASB: upflow anaerobic sludge blanket, 33, 125, 243, 263, 298, 357
- UASB-process, 375
- UBF: upflow blanket filter, 315
- ultrafiltration, 193
- uncertainty, 134
- underground hydraulic digesters, 315
- underground plug-flow, 315
- underground plug-flow reactor, 315, 316
- United Nations Development Program, 343
- Upflow Anaerobic Sludge Bed (UASB), 83
- upflow MFC, 175
- upflow velocity, 214, 311, 312, 327
- USR : upflow solids reactor, 316
- U-tube, 128

- valorization, 376
- venturi, 233
- VFA, 111, 112, 119, 126
- VFA: volatile fatty acid, 217, 225
- vinasse, 357, 378
- vinasse digestion, 358, 361

- volatile fatty acids, 247
- volatile organic compounds, 31
- VSS destruction, 226

- waste gas management, 273
- waste management, 346
- Waste-dilution*, 384
- wastes, 73, 76, 83–88, 90, 95
- wastewater, 73, 74, 76, 83, 86, 87, 92, 96
- wastewater treatment, 163
- wastewater treatment coverage, 264
- wastewater type, 180
- water, 353–355, 357, 361, 363, 367, 369, 371

- water and wastewater treatment, 96
- water pressured digester, 340
- winery, 245, 248, 249
- WWTP: wastewater treatment plants, 52, 59

- X_c, 147
- xenobiotics, 5

- yeast, 356
- yeast fermentation, 245

- zero discharge process, 300
- anaerobic fluidized bed membrane bioreactor, 218