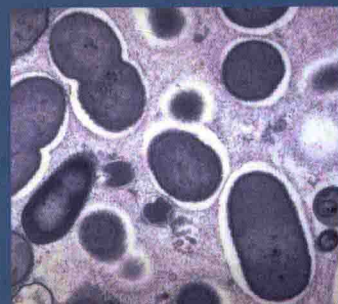
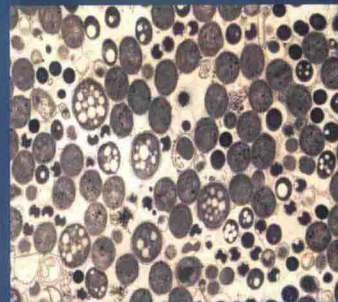




# Anaerobic Biotechnology

Environmental  
Protection and  
Resource Recovery

Herbert H. P. Fang  
Tong Zhang



Imperial College Press

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## Preface

Anaerobic biotechnology plays a significant role for the two issues crucial to our society in the 21<sup>st</sup> 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 21<sup>st</sup> 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.

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\* 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-intensive 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  $\text{NH}_3$  and  $\text{H}_2\text{S}$  (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 21<sup>st</sup> 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

Herbert H. P. Fang  
Tong Zhang  
*Environmental Biotechnology Laboratory*  
*Department of Civil Engineering*  
*The University of Hong Kong*

## 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).

## 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 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).



**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

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**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.



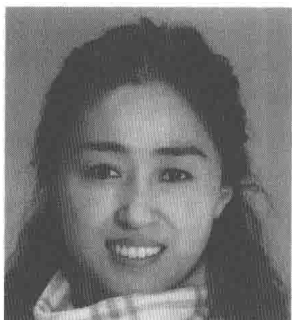
**Adalberto Noyola** is an environmental engineer with a PhD in wastewater treatment engineering (INSA Toulouse, France). He is a senior researcher and Head of the Institute of Engineering at the National Autonomous University of Mexico (UNAM). He has worked on anaerobic treatment of organic wastes, 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.



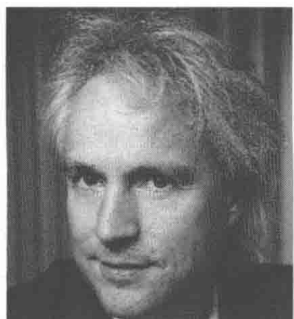
**Kaijun Wang** has been the Professor of School of Environment at Tsinghua University since 2008. He got his doctoral degree from the Environmental Technology Department of the Wageningen Agricultural University (the Netherlands) and served as the Chief Engineer of Beijing Municipal Environmental Protection 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).

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