2018
CHINA-UK WORKSHOP

CARBON RECYCLING
CONVERTING WASTE DERIVED GHG INTO CHEMICALS, FUELS AND ANIMAL FEED

June 3rd - June 5th, 2018, Beijing, China
2018年6月3日 — 6月5日， 中国　北京

Contact Information

Ms. Dandan Luo
Programme Manager
CAS-TWAS Centre of Excellence for Biotechnology
Institute of Microbiology, Chinese Academy of Sciences
No. 1 Beichen West Road, Chaoyang District, Beijing 100101
Tel: +86-10-64807351
Email: Luodd@im.ac.cn

Organizers

CAS-TWAS Centre of Excellence for Biotechnology (CoEBio)

Institute of Microbiology, Chinese Academy of Sciences (IMCAS)

中国科学院 - 发展中国家科学院生物技术卓越中心
CAS-TWAS Centre of Excellence for Biotechnology

GENERAL INFORMATION

Venue

Meeting Room E-301, Institute of Microbiology, Chinese Academy of Sciences
Address: No.1 Beichen West Road, Chaoyang District, Beijing 100101

Hotel Information

China National Convention Center Grand Hotel
Address: No.8 Beichen West Road, Chaoyang District, Beijing 100105
Tel: +86 10 8437 2008

Conference Secretariat

Dandan Luo

Email: luodd@im.ac.cn Tel: +86 10 64807351

Programme

Sunday, 3rd June 2018

09:00-21:00	**Registration**	**China National Convention Center Grand Hotel**

Monday, 4th June 2018

09:00-09:05	**Yin Li** - IM, CAS - Welcome Remarks	
Session I: Industry and C1		
Chair	**Yin Li** - IM, CAS	
09:05-09:15	**Nigel Minton** (SBRC Nottingham) – Introduction to the Workshop	
09:15-09:50	**Sean Simpson** (LanzaTech) – Commercial Exploitation of Acetogens	USA
09:50-10:25	**Stephen Poulston** (Johnson Matthey) – Biorenewables and Sustainibility	UK
10:25-11:00	**Photograph & Coffee Break**	
Session II: Tools and Capabilities		
Chair	**Nigel Minton** - University of Nottingham	
11:00-11:20	**Craig Woods** (SBRC Nottingham) – Gene Essentiality in C1 Chassis using Transposon-Directed Insertion Sequencing (TraDIS)	UK
11:20-11:40	**He Huang** (SIBS, CAS) – CRISPR-Cas9 Genome Editing and Site-specific Chromosomal Integration of Metabolic Pathways in C1 Chassis	China
11:40-12:00	**Robert Mansfield** (SBRC Nottingham) – Transforming the Untransformable	UK
12:00-12:20	**Ying Zhang** (SBRC Nottingham) – Gene Tool Development in Novel Methylotrophs	UK
12:20-13:30	**Lunch**	

Monday, 4th June 2018

Session III: Systems and Synthetic Biotechnology		
Chair	**Weihong Jiang** - SIBS, CAS / **Stephen Poulston** - Johnson Matthey	
13:30-13:50	**Philippe Soucaille** (INSA of Toulouse) – System Approaches: Lessons Learnt from Saccharolytic Clostridia	France
13:50-14.10	**Xinhui Xing** (Tsinghua University) – C1 Integrative Bioprocess: an Enabling Route to the Biorefinery of Low-grade Biomass for the Production of Biofuels and Biochemicals	China
14:10-14:30	**Rupert Norman** (SBRC Nottingham) – Optimizing the Production of Bulk Chemicals from Carbon Monoxide using a Genome-scale Model of *Clostridium autoethanogenum*	UK
14:30-14:50	**Min Jiang** (Nanjing Tech University) – Efficient Biobutanol Production from Organic Wastes by Unique Solventogenic Clostridium sp.	China
14:50-15:10	**Kati Kovács** (SBRC Nottingham) – Metabolic Engineering of *Cupriavidus necator* H16 for the Sustainable Production of C3 and C5 Monomers and Polymers	UK
15:10-15:30	**Ziyong Liu** (Qingdao Institute of Bioenergy and Bioprocess Technology, CAS) – Study on Ethanol Reassimilation in the Fermentation of *Clostridium ljungdahlii* with CO as the Source of Carbon and Energy	China
15:30-16:00	**Coffee Break**	
Chair	**Ying Zhang** - SBRC Nottingham / **Yangchun Yong** - Jiangsu University	
16:00-16:20	**Huifeng Jiang** (Tianjin Institute of Industrial Biotechnology, CAS) – A Synthetic Acetyl-Coenzyme A Pathway for One-carbon Assimilation	China
16:20-16:40	**Richard Dinsdale** (University of South Wales) – Optimising Mixed Culture Biomes for Utilising C1 Gases	UK
16:40-17:00	**Qiang Fei** (Xi'an Jiaotong University) – Opportunities and Challenges for Bioconversion of Methane into Bioproducts	China
17:00-17:20	**Zhen Cai** (Institute of Microbiology, CAS) – Synthetic Carbon Concentrating and Fixing Modules Enabled Heterotrophic Fixation of CO_2 in *E. coli*	China
18:30	**Welcome Dinner**	

Tuesday, 5th June 2018

Session IV: Anaerobic Biotechnology: Is There A Role for Biomethanation of CO_2 in Carbon Recycling?		
Chair	**Huifeng Jiang** – TIB, CAS / **Philippe Soucaille** – INSA of Toulouse	
09:00-09:20	**Li Xie** (Tongji University) – Biomethanation of H_2/CO_2/CO by Hydrogenotrophic Mixed Cultures under Thermophilic and Extreme-Thermophilic Conditions	China
09:20-09:40	**Yue Zhang** (University of Southampton) – Simultaneous Biogas Upgrading and Power-To-Gas within Anaerobic Digestion via Biomethanisation	UK
09:40-10:00	**Gang Luo** (Fudan University) – Biomethanation of H_2 and CO in Biogas Reactors Treating Organic Wastes	China
10:00-10:20	**James Chong** (University of York) – the Role of Metabolomics and Metagenomics in Elucidating Metabolic Pathways and Community Structure	UK
10:20-10:50	**Coffee Break**	
Chair	**Richard Dinsdale** - University of South Wales / **Xinhui Xing** - Tsinghua University	
10:50:11:10	**Wen Wang** (Beijing University of Chemical Technology) – Optimization of Biofuel Recovery from Food Waste with Anaerobic Digestate Pyrolysis and Syngas Biomethanation	China
11:10-11:30	**Mark Walker** (University of Waikato) –Modelling and Process Control of the *In Situ* Biomethanation of Hydrogen in Anaerobic Digesters	New Zealand
11:30-11:50	**Raymond Jianxiong Zeng** (Fujian Agriculture and Forestry University) – Organic Acids Production from H_2/CO_2 via Mixed Culture Fermentation	China
11:50-12:10	**Tim Patterson** (University of South Wales) – Life Cycle Implications for Biomethanation	UK
12:10-13.30	**Lunch**	

Tuesday, 5th June 2018

Session V: Novel Applications and Process Development		
Chair	**Sean Simpson** - LanzaTech / **Fengwu Bai** - Shanghai Jiao Tong University	
13:30-13:50	**James Millard** (SBRC Nottingham) – Engineered Microbial Factories For CO_2 Exploitation in An Integrated Waste Treatment Platform	UK
13:50-14:10	**Jingjing Xie** (Nanjing Tech University) – High Efficiency Microbial Electrosynthesis of Organics from Carbon Dioxide	China
14:10-14:30	**Changhao Bi** (Tianjin Institute of Industrial Biotechnology, CAS) – Development Progress of Syngas Utilizing Strains in the National High Technology Program of the Twelfth Five-year Project	China
14:30-14:50	**Claudio Avignone Rossa** (University of Surrey) – Microbial Electrosynthesis for the Capture and Transformation of CO_2 into Multicarbon Organic Compounds	UK
14:50-15:10	**Yangchun Yong** (Jiangsu University) – Manipulation of Bacterial Transmembrane Electron Transfer Towards Efficient Microbial Electrosynthesis	China
15:10-15:40	**Coffee Break**	
Chair	**Li Xie** - Tongji University / **James Chong** - University of York	
15:40-16:00	**Demao Li** (Tianjin Institute of Industrial Biotechnology, CAS) – Bioconversion of Low-Concentration Methane from Bio-Gas Projects for Biochemical Production	China
16:00-16:20	**Preben Krabben** (CPI) – Gas Fermentation at the Centre for Process Innovation	UK
16:20-16:40	**Zhiyong Huang** (Tianjin Institute of Industrial Biotechnology) – A Novel Trace Metals Model Promotes Solventogenesis Of *Clostridium Carboxidivorans* P7 And Enhances Higher Alcohol Production During Syngas Fermentation	China
16:40-17:00	**Yanming Wang** (SBRC Nottingham) – Continuous Alcohol Production from CO_2 by *Cupriavidusnecator*	UK
17:00-17:20	**Peng Hu** (Shanghai GTL Biotech Co., Ltd.) – The Commercialization of Gas Fermentation Platform for Biofuel and Bulk Chemicals Production	China
17:20-17:40	**Nigel Minton** - Closing Remarks	
18:30	**Dinner**	

Invited Speakers and Abstracts

Nigel Minton (SBRC-Nottingham, UK) - Speaker

Nigel has an international reputation for excellence in advanced molecular methods for the study and exploitation of microbial chassis. His research ranges from combating bacterial pathogens, through the development of novel cancer therapies to the sustainable production of chemicals and fuels from C1, C3 and C5/C6 feedstocks. He is the Director of a BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), one of six UK centres established during 2013/2014. SBRC Nottingham (http://sbrc-nottingham.ac.uk/) is focused on the sustainable production of platform chemicals from C1 gases, principally CO, CO_2 and CH_4 He is also Director of the BBSRC Network in Industrial Biotechnology and Bioenergy (NIBB) C1net.

Jacque Minton (SBRC-Nottingham, UK) - Workshop Co-ordinator

Jacque is Network Manager of C1net, one of the 13 Networks in Industrial Biotechnology & Bioenergy (NIBB) funded by the BBSRC. Their role is to create interaction between industry and academia and initiate collaborative research projects to encourage the growth of Industrial Biotechnology in the UK. C1net is dedicated to the development and scalable production of C1 gas fermentation for the whole IB community. It provides a cross-sector forum to foster and enhance collaboration between industry and academia with funding for conferences, workshops and Outreach and research. For more details and to join: www.c1net.co.uk.

Carbon Recycling: Converting Waste Derived GHG into Chemicals, Fuels and Animal Feed

NIGEL P. MINTON

BBSRC/EPSRC Synthetic Biology Research Centre (SBRC) School of Life Sciences, Centre for Biomolecular Sciences, University Park, University of Nottingham, Nottingham, NG7 2RD, UK

The continued use of fossil fuels is no longer tenable. A finite resource, their extraction, processing and exploitation results in environmental pollution and increased greenhouse gas (GHG) emissions. The challenge facing society is to identify sustainable and cleaner processes for chemical, fuel and food production, while at the same time reducing GHG emissions. Biological routes offer the most promising alternative where, to avoid conflict with the food chain, attention has largely focussed on using lignocellulosic biomass as the feedstock. However, its recalcitrance to deconstruction is making the development of economic processes extremely challenging.

One solution is to directly capture carbon before its incorporation into lignocellulose through the use of microbial chassis able to utilize single carbon (C1) gases (CO and CO_2) as a feedstock. Such gases are an abundant, low cost waste product from a wide range of industrial processes, including steel making, cement manufacture and power generation, as well as from the gasification or anaerobic digestion (AD) of renewable domestic or agricultural waste and residues. AD generates biogas, a mixture of C1 feedstocks CH_4 and CO_2. Together, CO, CO_2 and CH_4 are readily available UK and China-wide at low cost and in high volumes. Autotrophic, phototrophic and methanotrophic microbial chassis able to utilise these gases can be engineered to synthesise a broad array of requisite molecules in scalable biological processes.

Biomass-based MSW, for instance, may be converted to biogas (CH_4 and CO_2) through AD, or through gasification into Synthesis Gas (syngas), a mixture of CO, CO_2 and H_2. The CO and CO_2 may be used by autotrophic and phototrophic chassis as the carbon source for chemical/ fuel and single cell protein synthesis, whereas the CH_4 may be either used directly by methanotrophic chassis, or through its reformation into syngas, by autotrophic and phototrophic chassis.

Sean Simpson (LanzaTech, USA) - Speaker

Sean is a Co-founder and Chief Scientific Officer of LanzaTech, a global leader in gas fermentation, currently located in Chicago, USA. Under Dr. Simpson's leadership, the company has established a broad and unique patent portfolio covering all areas of gas fermentation, including fermentation processes and microbes, gaseous feedstock handling, and product and waste handling. LanzaTech is experienced in technology commercialization, with commercial units in China and Belgium under development. Dr. Simpson has over 20 publications and 130 patents. He has received a number of awards including the 2015 US Environmental Protection Agency (EPA) Presidential Green Chemistry Award.

Commercial Exploitation of Acetogens

SEAN SIMPSON

LanzaTech Inc. 8045 Lamon Ave, Skokie; IL. USA 60077

World energy demand is expected to increase by up to 40% by 2035. As concerns about the economic and social impact of climate change grow, the need for strategies to minimize the use fossil carbon resources in the production of fuel, chemical and nutritional products demanded by societies globally intensifies. Gas fermentation using C1 utilizing microorganisms allows for the sustainable production of fuels, chemicals and feed by recycling carbon from local, highly abundant, low-cost waste resources. The technology has been successfully demonstrated using a diverse range feedstocks that are composed of greenhouse gases carbon monoxide (CO), carbon dioxide (CO_2) or methane (CH_4), including waste gases from industrial sources (e.g., steel mills, processing plants or refineries), syngas generated from any biomass resource (e.g., unsorted and unrecyclable municipal solid waste, agricultural waste, or organic industrial waste) or biogas. Gas fermentation enables the production of low-carbon fuel, chemicals and feed products without the need to consume valuable agricultural commodities such as sugar or corn.

Synthetic biology techniques have been developed for certain gas fermentation model organisms to allow the production of a spectrum of valuable chemicals from gases. The commercial deployment of bacteria able to produce different chemical intermediates from gases paves the way for the operation of "product flexible" conversion facilities that are able switch between final products by deploying a different bacteria in their fermentation reactors according to changing market dynamics. In this way gas fermentation is a vital bridge in the effort to create value from waste streams and enable the perpetual capture of greenhouse carbon in valuable materials as part of an increasingly circular economic model.

Richard Dinsdale (University of South Wales, UK) - Speaker

Richard is Professor of Sustainable Environmental Systems in the Sustainable Environment Research Centre at the University of South Wales. His research activities are directed at optimizing mixed microbial cultures for the production of green chemicals from wastes including waste gases and low grade biomass resources. These products include energy vectors such as hydrogen and methane or electrons and other products such as volatile fatty acids and bioplastics. He has received funding from the UK research funders, EPSRC, BBSRC, NERC as well as EU funding. He has supervised over 15 PhD students to completion, has over 80 international peer reviewed journal papers and an H Factor of 30.

Optimising Mixed Culture Biomes for Utilising C1 Gases

RICHARD M DINSDALE

Sustainable Environment Research Centre, Faculty of Computing, Engineering and Science, University of South Wales, Treforest, CF37 1DL, UK

Mixed microbial cultures or microbial biomes play a critical role in many economically and environmentally important processes ranging from agricultural soils to industrial processes such as anaerobic digestion and the activated sludge process for the treatment of sewage. Mixed culture biomes offer a number of advantages over pure or single culture systems in that they are relatively cheap to source, do not require expensive sterilisation and can utilise diverse non-pure feedstocks for use as substrates. They also have disadvantages in that they are difficult to control and optimise to achieve optimum product yield and purity.

In the work at the University of South Wales, we have been working on using mixed culture microbiomes derived from anaerobic digesters to convert CO_2, CO and H_2 to either acetate or methane. Most of this work uses conventional gas fermentation technology but some of the work is using bioelectrochemical systems (BES) to convert CO_2 to formate/acetate. The gases, we are proposing to use using arise either from carbon intensive industries such as steel works or from biological processes such as anaerobic digestion or whisky production supplemented with energy from renewable electricity production.

We have a particular interest in acetate as it offers a number of advantages as a green platform chemical, it can be produced via a number of non-fossil fuel routes, it can be utilised a conventional chemical industry feedstock or used a as non-food based fermentation media for single cell protein, lipids, bioplastics, biosurfactants etc. and compared to many other chemical feedstock's its handling and distribution would be potentially easier and safer and have a lower global warming potential.

To improve the process monitoring and control of our processes we have been working on a number of process designs such as the "substrate shuttle" reactor concept, electro dialysis for product recovery and novel instruments for acetate measurement.

Stephen Poulston (Johnson Matthey, UK) - Speaker

Stephen is a research scientist based at the Johnson Matthey Technology Centre, a corporate research and development centre for Johnson Matthey (JM) based just outside Reading, UK. Following a PhD in chemistry at Cambridge University and a research position at Reading University he joined JM in 1998. He has worked on a wide range of heterogeneous catalyst and sorbent development projects including a number involving syn-gas conversion, methane activation and renewable fuels/chemicals from biomass and waste. Stephen is also he is a management board member of C1net.

Converting Waste to Fuels and Chemicals

STEPHEN POULSTON

Johnson Matthey Technology Centre, Blounts Court Road, Sonning Common, Reading, UK

Johnson Matthey is a global leader in sustainable technologies. More than half of JM's products have a direct environmental benefit, a figure that is set to increase as a key part of the company's growth strategy is to focus on emerging environmental opportunities. Johnson Matthey's Efficient Natural Resources (ENR) sector is one of four within JM group, and a key part of the company's strategy for future growth. The division is a global supplier of heterogeneous and homogeneous catalyst technologies for the production of bulk and fine chemicals including the production, purification and downstream conversion of syn-gas with applications including methanol synthesis and Fischer Tropsch catalysis. The Johnson Matthey Technology Centre based at Sonning Common in the UK is a central facility which acts as a focal point for the development of new technologies into emerging market applications. In this presentation, an overview of Johnson Matthey technologies and interests in the area of waste to fuels and chemicals will be presented.

Craig Woods (SBRC-Nottingham, UK) - Speaker

Craig studied at Imperial College London attaining a BSc in Biology before Craig completed his PhD at the BBSRC/EPSRC Synthetic Biology Centre, Nottingham. Craig's work focussed on the development of transposon mutagenesis strategies in the acetogenic bacterial species *Clostridium autoethanogenum*, and on the development of highly-robust gene transfer protocols for the same organism. His current work involves the implementation of Transposon-Directed Insertion Sequencing (TraDIS) in a variety of species of industrial interest.

Gene Essentiality in C1 chassis using Transposon-Directed Insertion Sequencing (TraDIS)

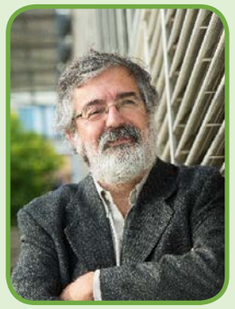
CRAIG WOODS, CHRISTOPHER HUMPHREYS, NIGEL P. MINTON

BBSRC/EPSRC Synthetic Biology Research Centre (SBRC) School of Life Sciences, Centre for Biomolecular Sciences, University Park, University of Nottingham, Nottingham, NG7 2RD, UK

Transposon Directed Insertion-site Sequencing (TraDIS) enables condition-specific determination of essential genes and a simultaneous estimation of fitness contribution for every gene in the genome. High-throughput functional genomics approaches such as this are required for gene discovery and characterisation to keep pace with increasingly powerful sequencing technologies.

TraDIS involves the creation of a large random transposon mutant library which is sequenced using the transposon integration site to prime a sequencing reaction into the adjacent interrupted gene. Genes essential for growth will be unrepresented or highly-under represented and will therefore represent candidate essential genes. A comparison of essential gene sets produced from different experimental conditions can reveal novel genes relevant to a given biological trait. TraDIS provides millions of reads and since the number of reads into a given gene corresponds to the prevalence of that transposon mutant in the pool, the relative abundance of mutants can be tracked and fitness contribution calculated through repeated periods of growth. The fitness contribution calculated from TraDIS have been demonstrated to correspond with those derived from one-on-one growth experiments.

Mutant libraries of Clostridium autoethanogenum, *Cupriavidus necator* and *Acetobacterium woodii* have been generated and the transposon insertion sites identified with TraDIS. These data have been used to predict the essential gene sets required for various physiological conditions including growth on CO and growth in the presence of limiting concentrations of 3-Hydroxypropionic acid.



Claudio Avignone-Rossa (University of Surrey, UK) - Speaker

Claudio is a Reader in Systems Microbiology at the University of Surrey, UK, with research interests in metabolic modelling and analysis and design of bioprocesses of interest for the pharmaceutical and biotechnology industries. His research has been supported by the Research Councils (BBSRC, EPSRC, EU) and by industry (Eli Lilly/Elanco, Green Biologics, GSK, Abbott, Avecia, etc). During the last 10 years, research in the Systems Microbiology group led by CAR has focused on the design and optimization of microbial processes employing Systems Biology approaches directed to bioproduct formation (antibiotics, proteins and other molecules of biological interest) and bioenergy production (biosolvents, biofuels and bioelectricity).

Microbial Electrosynthesis for the Capture and Transformation of CO_2 into Multicarbon Organic Compounds

CLAUDIO AVIGNONE ROSSA

Systems Microbiology Group, Department of Microbial Sciences,
University of Surrey, Guildford, GU2 7XH, UK

Microbial electrosynthesis (MES) or Electrofermentation (EF) can overcome some of the issues associated to conventional fermentation, such as stoichiometric and energy limitations, low yields, nutrient demands, etc. In MES, the extracellular redox potential is modified by supplying electrons through the cathode, therefore displacing the intracellular redox balance required to obtain the desired fermentation products. Therefore, it is possible to utilize compounds of low degree of reduction (e.g. CO_2) as carbon sources to synthesize reduced molecules by supplying exogenous electrons to specific microorganisms.

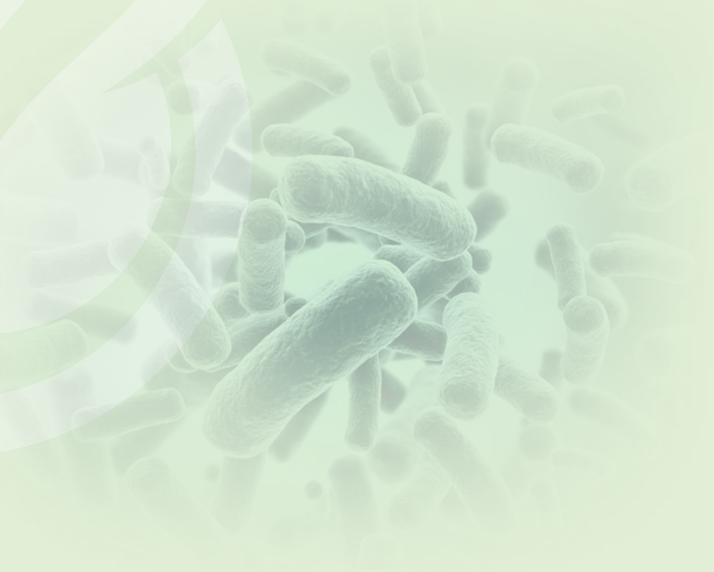
A limited number of microbial species are able to utilize C1 substrates for the synthesis of metabolic intermediates or precursors, notably photosynthetic microorganisms or species able to accumulate polyhydroxyalkanoates. However, despite their high efficiency for CO_2 capture, their metabolic repertoire is limited.

Several species are able to utilize external terminal electron acceptors. One such species is *Geobacter sulfurreducens*, a very well characterised electrogenic microbe able to utilize short chain fatty acids as C-sources and use an anode as final electron acceptor. When combined with the highly related species G. *metallireducens*, the reduction of CO_2 into a multicarbon compound is observed. Recently, the bioelectrochemical production of butyrate from CO_2 as a sole carbon source was demonstrated using a consortium of two Clostridium species, suggesting the potential application for biofuel production by MES.

Electrofermentation of CO_2 to produce valuable chemicals is a sustainable strategy that provides a viable alternative to current methods of chemical synthesis, contributing to the reduction of greenhouse gas emissions. The required electricity can be generated from renewable resources such as solar energy or wind power, and it should also be possible to utilize wastewater as the anodic feedstock. The net outcome of this process is the storage of energy in the covalent bonds of organic compounds synthesized from captured CO_2 with the reduction of pollutant levels in water.

Alan Burbidge (SBRC-Nottingham, UK) - Workshop Co-ordinator

Alan's undergraduate studies were in ecology. His PhD was in plant molecular biology and he subsequently spent 15 years in academic research at the University of Nottingham in plant molecular genetics. During this time he developed a strong interest in intellectual property. In 2003 he migrated into a role in university technology transfer specialising in life sciences licence deals and spin-out company formation. In 2014 he took on the role of managing the SBRC-Nottingham with an emphasis on IP and promoting collaboration with industry. Most recently he has been involved in developing strategy to create a sustainable environment for industrial biotechnology research to flourish within the university.



James Chong (University of York, UK) - Speaker

James is a molecular microbiologist. He has been at the University of York since 2004 and has recently redirected his research to focus on using molecular techniques to understand the dynamics of anaerobic microbial communities. James is currently a Royal Society Industry Fellow and works with Yorkshire Water to optimize resource recovery (particularly biogas) from the organic fraction of wastewater and sewage sludge.

The Role of Metabolomics and Metagenomics in Elucidating Metabolic Pathways and Community Structure

ANNA ALESSI & JAMES P.J. CHONG

Department of Biology, University of York, Wentworth Way, Heslington, York, YO10 5DD, UK

Anaerobic digestion (AD) relies on a complex community of interacting microbes in order to convert organic waste into biogas. Understanding how these communities change in response to different conditions and feedstocks, and what influences the reproducibility of these changes, is a potential route to improving solids destruction and increasing biogas output. Further extrapolation of this understanding could provide routes to changing the molecules produced by AD, ultimately resulting in higher-value products.

We are using a combination of untargeted metabolomics and metagenomics approaches to better understand the dynamics of the microbial communities underpinning the AD process. Here we examine some of the challenges resulting from the collection of large, complex datasets, and the factors that influence the reproducibility of community measurements. In particular we characterise how different methods of DNA extraction influence the apparent community reported by focusing on the characterisation of methanogenic species identified using these approaches and consider the potential of these measurements to inform approaches to “engineering” the microbial community.

Robert Mansfield (SBRC-Nottingham, UK) - Speaker

Rob is a genetic engineer of industrially relevant microbial systems. He has a longstanding interest in the development of tools for applied genetic engineering, including the design of new methodologies for establishing genetic transformation in recalcitrant organisms. Following his BSc in Biochemistry & Genetics at the University of Nottingham (2013), Rob completed a PhD in microbial genetic engineering within the SBRC (2018). He is continuing his research as a Research Fellow within SBRC, with a primary focus on the engineering of C1 fermenting microorganisms in pursuit of industrial applicable biocatalysts.

Transforming the Untransformable

ROBERT MANSFIELD

BBSRC/EPSRC Synthetic Biology Research Centre (SBRC) School of Life Sciences, Centre for Biomolecular Sciences, University Park, University of Nottingham, Nottingham, NG7 2RD, UK

Overcoming active restriction modification (RM) systems is essential for establishing basic gene-transfer in many microorganisms, and a valuable means of enhancing transformation efficiencies where basic transfer has already been achieved. This project centred around the development of an enhanced pipeline for establishing gene-transfer in recalcitrant microorganisms. The proposed transformation pipeline incorporates base-modification detection techniques with DNA-methylation mimicking concepts, for the production of tailored *E. coli* methylation donor strains. Lambda-red mediated recombineering has been employed to enable the rapid and stable creation of methylation donor systems, in a manner compatible with both conjugation- and electroporation-based transformation methodologies.

Philippe Soucaille (SBRC-Nottingham, UK) - Speaker

Philippe is Professor of Microbial Physiology at the National Institute for Applied Sciences (INSA of Toulouse, France) and Professor in Synthetic Biology at the University of Nottingham, UK. Until recently he was Chief Scientific Officer of Metabolic Explorer. He is an expert on the physiology and molecular genetics of bacteria and published more than 80 papers in this field. He and his group at INSA pioneered methods for the in vivo evolution of enzymes and metabolic pathways and successfully applied them to the production of several bulk chemicals. He is inventor or co-inventor on more than 200 patents. He has been a member of many national and international committees and served on the scientific advisory boards of several companies. He is associate editor of Biotechnology for Biofuels and board member of several journals.

Reviving the Weizmann Process for Commercial n-Butanol production

PHILIPPE SOUCAILLE[1,2,3] , NGOC-PHUONG-THAO NGUYEN[1],
CÉLINE RAYNAUD[2] & ISABELLE MEYNIAL-SALLES[1]

[1]Université de Toulouse, INSA, UPS, INP, LISBP, Toulouse, France.
[2]Metabolic Explorer, Biopôle Clermont-Limagne, Saint Beauzire, France.
[3]University of Nottingham, Nottingham, NG7 2RD UK.

The industrial production, by *Clostridium acetobutylicum*, of n-butanol for use as both a chemical and an alternative transportation fuel, is not currently economical due to low yield, titer and productivity. We used an advanced metabolic engineering approach to engineer C. acetobutylicum to produce n-butanol from glucose at a high yield: the deletion of genes encoding unwanted pathways was combined to the debottlenecking of the n-butanol pathway for maximizing both the yield of alcohol production and the n-butanol to ethanol ratio. We also designed a new continuous fermentation process using i) *in situ* extraction of alcohols by distillation under low pressure and ii) high cell density cultures to increase the titer, yield and productivity of n-butanol production to levels that have never been previously achieved in any organism. This process provides a means to produce n-butanol at performance levels that are now compatible with a commercial process.

Katalin Kovács (SBRC-Nottingham, UK) - Speaker

Katalin is a biotechnologist with a strong research interests in synthetic biology and biological (plant and bacterial) engineering. She is experienced in metabolic engineering of eukaryotic and prokaryotic organisms, focusing on the regulation of interacting and competing metabolic pathways and application of synthetic and systems biology tools for the sustainable production of chemicals and fuels. Current research activities include (1) creating and exploiting gas fermenting microbial chassis for the sustainable production of high value chemicals and bio-polymers, (2) microbial engineering for cellulosic substrate utilisation and added value chemical synthesis, and (3) plant chloroplast engineering.

Metabolic Engineering of *Cupriavidus necator* H16 for The Sustainable Production of C3 And C5 Monomers and Polymers

KATALIN KOVÁCS, ALEJANDRO SALINAS, CHRISTIAN GUDE,
CALLUM McGREGOR and NIGEL P. MINTON

BBSRC/EPSRC Synthetic Biology Research Centre (SBRC) School of Life Sciences, Centre for Biomolecular Sciences, University Park, University of Nottingham, Nottingham, NG7 2RD, UK

The facultatively chemolithoautotrophic bacterium *Cupriavidus necator* H16, is best known for its ability to store carbon in the form of poly (3-hydroxybutyrate) or PHB, a biodegradable and biocompatible natural polymer. When grown with organic substrates or H_2 and CO_2, under nutrient limiting, aerobic conditions it is able to accumulate large quantities of this polymer, up to 80% of its cell dry weight (CDW). This naturally synthesised bioplastic has relatively poor physical, thermal and mechanical properties (very brittle, highly crystalline, has a high melting temperature). Production of alternative homopolymers and copolymers with improved properties, and/or redirection of the carbon flux from PHB synthesis to the production of other high value monomers and polymers, makes this organism a great candidate for biological production of high value compounds.

Within the Synthetic Biology Research Centre in Nottingham (SBRC-Nottingham), we aim to metabolically engineer *Cupriavidus necator* H16 for the sustainable production of various biopolymers, in addition to C3 (ie. 3-hydroxipropinionic acid (3HP)) and C5 (5-aminovaleric acid (5-AV)) monomers and platform chemicals. One of our platform chemical target is 3-hydroxipropinionic acid or 3-HP, as it represents an important building block for the chemical industry and it can be utilized for the sustainable production of acrylic acid, 1,3-propanediol, methyl acrylate, acrylamide, ethyl 3-HP, malonic acid and potentially ethylene. It can also be polymerised to poly-3HP, a biopolymer with great physical and mechanical properties. In addition, 3-HP can potentially be biologically converted to higher chain, (such as C5) valued added monomers, which are of great industrial interest as the chemical synthesis of these compounds with two or more functionalized groups is not feasible.

To date we have successfully demonstrated the production of 3-HP via the beta-alanine intermediate by overexpression of a heterologous pathway and by re-directing the carbon flux from PHB to 3-HP. We have demonstrated that the 3-HP produced by the engineered strain can be incorporated into the natural produced polymer to form poly(3HP-3HB) co-polymer. Additionally, pathways for biological conversion of 3-HP to 5-AV were built and are currently being tested.

Preben Krabben (Centre for Process Innovation, UK) - Speaker

Preben is a Principle Scientist within the Industrial Biotechnology & Biorefining (IBB) platform of the Centre for Process Innovation (CPI). He has 25 years of experience in industrial biotechnology. His expertise covers microbial physiology, genomics, fermentation, feedstock processing, and metabolic modelling. He has worked 10 years on Clostridia and another 14 years on antibiotic fermentations. He has been working extensively in both academic and industrial settings, and he is a management board member of C1net.

Gas fermentation at the Centre for Process Innovation

PREBEN KRABBEN

The Centre for Process Innovation (CPI), Wilton Centre, Wilton, Redcar, Cleveland, TS10 4RF, UK

USA-based Calysta have partnered with CPI to demonstrate its state-of-the-art facility to manufacture sample quantities of FeedKind® protein, a proprietary, competitively priced new fish and animal feed ingredient targeted at replacing fishmeal. Produced using the world's only commercially validated gas fermentation process, FeedKind protein is a natural, traceable and safe non-animal source of protein. CPI integrated Calysta's novel loop reactor with the National Industrial Biotechnology Facilities (NIBF) to minimise both the capital required and time to complete the design and installation. CPI had not run this specific fermentation process prior to the start of this project; it was through CPI's know-how that the gap between laboratory batch and a scalable continuous demonstration process was successfully bridged.

The demonstration facility has shown positive results, achieving significant milestones within its first three months of operation. As of May 2017 the plant had successfully produced over four tonnes of dried material. This presentation will illustrate the development journey from laboratory to demonstration scale, highlighting the challenges faced in delivery of this process.

James Millard (SBRC-Nottingham, UK) - Speaker

James started work at the Clostridia Research Group (later the BBSRC/EPSRC Synthetic Biology Research Centre, Nottingham) in 2012 as a research technician developing synthetic biology tools for *Clostridium kluyveri*. His PhD (2013-2017) concerned the development of the acetogen Eubacterium limosum as a chassis for synthetic biology. This project involved the implementation of directed genetic techniques such as Allele-Coupled Exchange (ACE) and CRISPR, as well as random mutagenesis via a replicative Mariner transposon vector.

Engineered Microbial Factories for CO_2 Exploitation in An Integrated Waste Treatment Platform (ENGICOIN)

CHRISTOPHER J. MILLARD, JONATHAN P. BAKER, NIGEL P. MINTON

BBSRC/EPSRC Synthetic Biology Research Centre (SBRC) School of Life Sciences, Centre for Biomolecular Sciences, University Park, University of Nottingham, Nottingham, NG7 2RD, UK

CO_2 represents a potentially important raw material for the production of chemicals and fuels by microbial bio-catalysts. Large-scale exploitation of waste CO_2 for the production of chemicals and fuels would serve the dual purpose of mitigating CO_2 emissions and reducing the demand for fossil fuels.

The ENGICOIN project aims to produce an integrated set of microbial factories exploiting waste CO_2 and renewable H_2. The ENGICOIN consortium consists of twelve separate organisations, located in Italy, the Netherlands, Spain, Austria, Belgium, Sweden and the UK. Each microbial factory will be based on a separate organism, and will form an integrated bio-refinery. Because sources of waste CO_2 are widely distributed in geographic terms, the ENGICOIN project foresees the development of small-scale bio-refineries integrated into existing industrial plants and utilising their CO_2 and waste heat.

One of the organisms targeted for development by ENGICOIN is *Acetobacterium woodii*, an acetogenic bacterium which is capable of autotrophic growth on CO_2/H_2 and which primarily produces acetate. Employing genetic methods developed at the Synthetic Biology Research Centre (SBRC), an acetone production pathway will be implemented in *A. woodii*. Additionally, adaptive laboratory evolution (ALE) will be conducted to improve acetone tolerance in the modified strain. Gene transfer methods developed at the SBRC have enabled the generation of large transposon mutant libraries in *A. woodii*. Transposon-Directed Insertion Site Sequencing (TraDIS) will be used to identify genes essential for acetone tolerance, and to assess their relative contributions to fitness.

Rupert Norman (SBRC-Nottingham, UK) - Speaker

Rupert studied microbiology at the University of Nottingham and worked as an industrial placement student at Philips Research, Cambridge. His main academic interest is computational modelling of biochemical systems in bacteria. He is currently writing a PhD thesis about the construction and analysis of a genome scale model of *Clostridium autoethanogenum*.

Optimizing the Production of Bulk Chemicals from Carbon Monoxide Using a Genome-Scale Model of Clostridium autoethanogenum

RUPERT O. J. NORMAN

BBSRC/EPSRC Synthetic Biology Research Centre (SBRC) School of Life Sciences, Centre for Biomolecular Sciences, University Park, University of Nottingham, Nottingham, NG7 2RD, UK

Recent international directives promoting the reduced consumption of fossil fuels have warranted methods for effective carbon recycling. Subsequently, *Clostridium autoethanogenum* has attracted academic and industrial interest due to its ability to convert syngas components (CO, CO_2 & H_2) into valuable platform chemicals, including ethanol and 2,3-butanediol. Developing the metabolic conversions catalysed by *C. autoethanogenum* into an efficient bioprocess requires the accurate prediction of optimal metabolic steady states, which in turn necessitates the construction of a genome-scale model (GSM).

We have successfully constructed a predictive model, suitable for the integration of omics data sets and prediction of gene knock-out targets. Our model-simulated growth yields agree well with experimentally observed specific growth rates, while elementary modes analysis (EMA) confirms the availability of metabolic routes for acetate, ethanol, lactate and butanediol production. Elevated ethanol production is predicted to result from a reduction in pH levels. Similarly, we found that the switch from acetate to ethanol production occurs with increasing CO uptake rates under non-carbon limited conditions, finally leading to lactate and 2,3-butanediol production. Our results are consistent with trends observed in continuous cultures.

Our interdisciplinary approach for the construction, analysis and application of a genome-scale model provides insight into biological and biochemical principles which govern experimentally observed metabolic behaviour. Our results offer a rationale to aid the optimization of commodity chemical production from waste gases.

Tim Patterson (University of South Wales, UK) - Speaker

Tim is a Senior Lecturer and Researcher in Sustainability Analysis at the Sustainable Environment Research Centre, University of South Wales, UK. His research focus is the evaluation of novel biotechnology processes for the production of energy, fuels and chemicals and he has delivered collaborative research projects funded by the Welsh Government, Innovate UK, Biotechnology and Biological Sciences Research Council (BBSRC), the Department for Business, Energy and Industrial Strategy (BEIS) and EU Horizon 2020. Tim is part of a team at USW that has developed a novel mixed culture biomethanation process.

Environmental and Life Cycle Implications for Biomethanation

TIM PATTERSON

Sustainable Environment Research Centre, University of South Wales, CF37 1DL, UK

Using renewable electricity generated hydrogen and recovered industrial CO_2 has many potential advantages; reducing CO_2 emissions, large scale energy storage and production of low carbon CH_4. There are, however, technical and economic challenges that need to be considered. Availability and recovery of CO_2, electrolytic hydrogen production, the biomethanation stage and the end use of the produced CH_4 will all influence the economic viability and the environmental benefits and burdens of the system. These challenges should not halt the development and deployment of the process, but should influence the way in which industrial deployment evolves and inform an appropriate policy and financial framework that recognises the strategic value of such an integrated energy management approach.

Yanming Wang (SBRC-Nottingham, UK) - Speaker

Yanming has been a post-doctoral research associate at the Synthetic Biology Research Centre (SBRC), University of Nottingham since 2016. His current research interests and topics are gas fermentation process development and bioprocess integration. Yanming studied biotechnology at East China Normal University in Shanghai, and Lund University in Sweden. Before joining the SBRC, Yanming was an industrial biotechnology research scientist at VTT Technical Research Centre of Finland Ltd.

Continuous Alcohol Production from CO_2 by *Cupriavidus necator*

YANMING WANG[1], RAJESH BOMMAREDDY[1], NIGEL MINTON[1], ALEX CONRADIE[1,2]

[1]*BBSRC/EPSRC Synthetic Biology Research Centre (SBRC) School of Life Sciences, Centre for Biomolecular Sciences, University Park, University of Nottingham, Nottingham, NG7 2RD, UK*

[2]*Faculty of Engineering, University of Nottingham, Coasts Building, University Park, Nottingham, NG7 2RD, UK*

Biochemical routes of producing industrial alcohols from CO_2 would make the process more sustainable than the existing petro-chemical processes. *Cupriavidus necator*, capable of chemolithotrophic growth on CO_2, H_2 and air, was modified to host the isopropyl alcohol (isopropanol,IPA) and 2,3-butanediol (2,3-BDO) production pathways. A phosphorous limiting chemostat gas fermentation process was developed to characterise the IPA and 2,3-BDO production strains at lab scale safely. H_2 was introduced to the bioreactor through a separate sparger. The headspace O_2 concentration was monitored and controlled by a PID controller at the set point of 4% (below the O_2 percentage corresponding to the upper explosive limit of H_2). This improved the oxygen transfer rate, and allowed high cell density at steady states. The IPA concentration during the steady state was 1.2 g L^{-1}, which translates to the productivity of 0.02 g $L^{-1}h^{-1}$. 2,3-BDO concentration in the two different variants during the steady state was found to be 7 g L^{-1} with a productivity of 0.35 g $L^{-1}h^{-1}$. These are the highest productivities of IPA and 2,3-BDO reported to date in autotrophic cultures of *C.necator*.

Mark Walker (University of Waikato, New Zealand) - Speaker

Mark graduated with a MEng in mechanical engineering from the University of Durham in 2004 and went on to complete his PhD with the Bioenergy and Organic Resources Research Group (BORRG) at the University of Southampton in 2009, working on the application of mesh/membrane bioreactors to the anaerobic digestion (AD) of biodegradable municipal wastes. Following this he contributed to a variety research projects as a research fellow at the Universities of Southampton, Leeds and Sheffield, with his work continuing to focus on AD and biogas production from biomass and organic wastes. Recently Mark has been appointed as a lecturer in mechanical engineering at the University of Waikato in New Zealand.

Modelling and Process Control of the *In Situ* Biomethanation of Hydrogen in Anaerobic Digesters

MARK WALKER

Sustainable Engineering Research Group, School of Engineering, University of Waikato, New Zealand

Injection of hydrogen into anaerobic reactors can be used as a strategy to produce high quality biomethane directly from the anaerobic digestion (AD) process, a process called *in situ* biomethanation. Successful control of this process involves balancing the consumption of naturally produced carbon dioxide by hydrogenotrophic methanogens without exceeding the biochemical or mass transfer capacity of the AD system.

In this work Anaerobic Digestion Model No.1 (ADM1) was modified to allow simulation of the *in situ* biomethanation process. The model was calibrated and validated using experimental data and then used to explore process control options using gain scheduling with the objective of continuous production of high quality biomethane (>95%) by actuation of either the hydrogen injection rate and in some cases the biomass feeding rate.

The modelling work demonstrates that successful control of the biomethane quality, by variation of the hydrogen injection rate, can be achieved using gain scheduling with four scheduling parameters; the major components of biogas (CH_4, CO_2, H_2) and the pH of the digester. Depending on the desired control philosophy, the biomass feeding control can either be an open loop, limited once the consumption limit of hydrogen is reached, or controlled dynamically to match the hydrogen uptake demand.

Ying Zhang (SBRC-Nottingham, UK) - Speaker

Ying is a molecular microbiologist with strong interests in biological engineering. Her research applies the approaches of molecular biology, metabolic engineering, and synthetic biology to address problems in energy and human health, namely the design and creation of new biosynthetic pathways in microbial hosts for in vivo production of biofuels and platform chemicals from abundant crop feedstocks or wastes. Her work has been focused on the generation of novel clostridal strains with advanced properties such as enhanced biofuels production and selectivity. She is also interested in methane as a feedstock resource; using Methanotrophs (methane consuming bacteria) to add value.

Gene Tool Development in Novel Methylotrophs

YING ZHANG

BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), School of Life Sciences, University of Nottingham, Nottingham, NG7 2RD, UK

The rapid expansion of global methane production and capture, both in the form of natural gas and biogas from AD, has improved the accessibility of methane and consequently reduced the commodity price. This has generated increased interest in methane as a feedstock for novel, value-added processes, especially the biological conversion of CH_4 to fine and commodity chemicals. Methane-oxidizing bacteria, methanotrophs, that naturally consume methane as carbon and energy source have been identified and developed as an industrial workhorse to produce proteins, polymers, chemicals and fuels.

Strains currently used to answer the IB challenge of chemical commodity production are traditionally those already stored in strain collections and must be considerably modified to achieve their goals. Most research on methanotrophs has been on their ecology and some aspects of their primary metabolism, and while Syngas-utilising organisms have been characterised in more depth, it is timely to search for better strains.

We isolated new strains or species that can utilise CH_4 in the production of industrially-important chemicals, and developed an array of gene tools that will benefit widely in C1 research community.

Yue Zhang (University of Southampton, UK) - Speaker

Yue is a lecturer in Environmental Engineering at the University of Southampton. Her research is related to anaerobic biotechnology for both renewable energy production and organic waste management. Dr Zhang's work focuses on microbial aspects of the anaerobic digestion process in order to identify the practical benefits this could give in terms of engineering control of these systems, and the knowledge and understanding gained can be applied in both biofuel and bioproduct production. Another stream of her research is about biological wastewater treatment and pollution remediation.

Simultaneous Biogas Upgrading and Power-to-Gas within Anaerobic Digestion via Biomethanisation

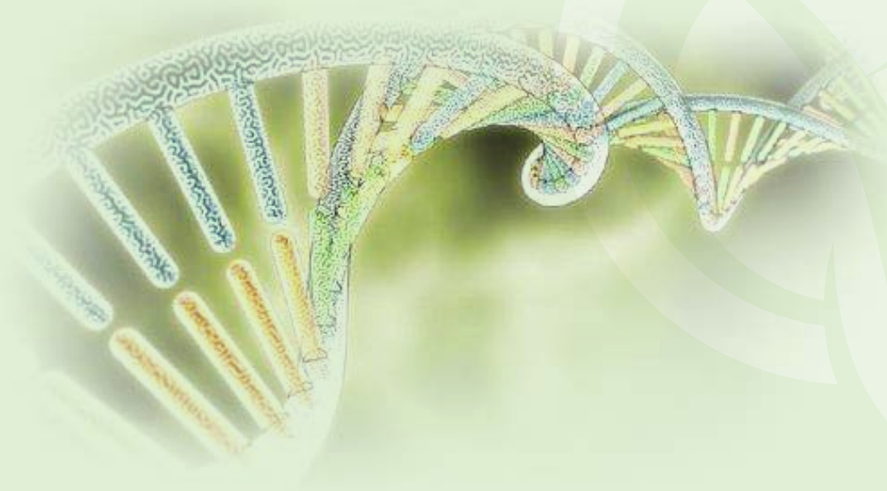
BING TAO, YUE ZHANG, SONIA HEAVEN, CHARLES BANKS

Water and Environmental Engineering Group, Faculty of Engineering and the Environment, University of Southampton, Southampton, SO17 1BJ, UK

Study on biomethanisation of CO_2 in conventional CSTR anaerobic digesters was conducted with the injection of external H_2. By utilising both internal and external CO_2, the methane production capacity of digesters was increased over 3 times of their original values from 0.288 to 0.920 $L_{CH4}\ g^{-1}\ COD_{org}$ at an organic loading rate of 3 g $COD_{org}\ L^{-1}\ day^{-1}$. pH of digesters was closely related to the partial pressure of residual CO_2 in the product gas and therefore maintaining a certain level of CO_2 partial pressure is a feasible pH control measure: a 3% of CO_2 partial pressure at headspace allowed pH stable at 8.2, when digesters were operated a total ammonia level of 700 mg N L^{-1} and VFA concentration less than 500 mg L^{-1}. The results indicate that this hybrid *in situ* / ex situ biomethanisation process has potential to be applied to anaerobic digestion plants with more than one digester: a single digester modified for biomethanisation can be fed with the biogas produced from the other conventional anaerobic digesters to increase the overall methane production.

Yin Li (Institute of Microbiology, CAS) - Speaker

Dr. Yin Li, a Principle Investigator at Institute of Microbiology, CAS.Yin Li obtained his Ph. D from Wuxi University of Light Industry in 2000. He then worked in the Netherlands and Ireland as a postdoctoral researcher, and was recruited by the Hundred Talents Program of CAS in 2006. Yin was selected as Young Affiliate of TWAS (The World Academy of Sciences for the advancement of science in developing countries). He is serving as the Director of CAS-TWAS Centre of Excellence for Biotechnology, Chair of the first executive committee of TWAS Young Affiliates Network(TYAN), Associate Editor of Chinese Journal of Biotechnology, member of the Editorial Board of *Microbiology, Microbial Cell Factories, Biotechnology Journal, Food Biosciences, and Industrial Biotechnology.* Yin is mainly interested in improving the performance of industrial microbes through molecular physiology and systems biotechnology. In recent years, his group is focused in engineering Escherichia coli and cyanobacteria strains to produce chemicals from glucose and CO_2, respectively. He has filed over 40 patents and published more than 100 papers in peer-review journals. He remains active collaboration with multinational enterprises including Shell, DSM, Nestle and a dozen of domestic enterprises.



He Huang (Shanghai Institutes for Biological Sciences (SIBS), CAS) - Speaker

Dr. He Huang, got her Ph.D. degree in 2015, is now working as an associate professor in Key Lab of Synthetic Biology, Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences (CAS). She has been long engaged in developing high-efficiency synthetic biology tools on the purpose of fast and accurate chromosomal editing. She has established efficient CRISPR-Cas9 genome editing tool in Streptomyces, *C.ljungdahlii* and *L.casei* respectively. Based on the effective tools, she devotes to improve the desired productive performance of various industrial and model microbes by metabolic pathway optimizing and rebuilding. Dr. He Huang has published research articles in *Applied and Environmental Microbiology, ACS Synthetic Biology, Acta Biochim Biophys Sin, BMC Genomics, Cell Research*, as the first author or co-first author.

CRIPSR-Cas9 Genome Editing and Site-specific Chromosomal integration of Metabolic Pathways in C1 Chassis

The real value of gas-fermenting clostridia resides on their potential of being developed into cell factories to produce various bulk chemicals and fuels. This process, however, is still being impeded by shortage of powerful genetic tools for precise and efficient chromosome manipulation. In the previous studies, we have developed the CRISPR-Cas9 system in *Clostridium ljungdahalii*, achieving a fast and effective gene deletion in 7 days. Based on this tool and the bacteriophage attachment/integration system from *Clostridium difficile*, we recently established a site-specific genome engineering technique in *C.ljungdahlii*, aimed at rapid and stable chromosomal integration of large DNA fragments. Using this system, one-step chromosomal integration of a butyrate-synthetic pathway (8.5 kbps) in *C. ljungdahlii* was achieved within 4 days, showing an integration efficiency of 100%. The resulting engineered strain yielded 0.923 g/L of butyric acid in fermenting simulated steel mill off-gas. Moreover, the engineered strain showed high stability in butyric acid production after sequential subculturing. This system has advantages over currently available genetic tools of clostridia in chromosomal integration of large gene cluster, and therefore, will play an important role in constructing gas-fermenting clostridial cell factories.

Xinhui Xing (Tsinghua University) - Speaker

Prof. Xin-Hui XING received his B.S. from South China University of Technology in 1985, and Ph.D. from Tokyo Institute of Technology in 1992. He had been Assistant Professor at Tokyo Institute of Technology from 1992 to 1998, and Associate Professor at Yokohama National University from 1998 to2001. He was selected as a full professor by the 100-Telent Scholar Program of Tsinghua University in 2000 and joined department of Chemical Engineering since then. He was appointed as the director of Institute of Biochemical Engineering in 2002. Currently he is the vice chairman of Department of Chemical Engineering. His research field covers biochemical engineering, evolution technology and instrumentation, high throughput technology, enzyme engineering, C1 integrative bioengineering, environmental biotechnology and bioenergy. He serves as the editor of Journal of Bioscience and Bioengineering, associate editor of Biochemical Engineering Journal, and editorial board of several domestic and international journals.

C1 Integrative Bioprocess: an Enabling Route to Biorefinery of Low-Grade Biomass for Production of Biofuels and Biochemicals

XIN-HUI XING[1,*] CHONG ZHANG[1], ZHIDAN LIU[2], JIANWEI HE[1], YUAN LU[1]

[1]MOE Key Laboratory for Industrial Biocatalysis, Department of Chemical Engineering, and Center for Synthetic and Systems Biology, Tsinghua University, Beijing, China
[2]College of Water Resources and Civil Engineering, China Agricultural University, Beijing, China

The future sustainable chemical and energy industry depends largely on renewable biomass resources for the production of chemicals, biopolymers and biofuels. Concerning the serious situation of worldwide food supply, “None-Food Biomass Strategy” is now becoming a big challenge for the development of biorefinery technology. Low-grade or waste biomass, which is the most abundantly and widely distributed biomass, especially for the agriculture-based countries like China, is a very important non-food biomass resource, while an emission source for environment pollution if not treated properly. However, the processing of such broadly distributing, low-grade and abundant biomass resource is obviously difficult for traditional chemical industry. Biorefinery of the low-grade biomass is a promising alternative, while reducing process costs and increasing carbon-conversion ratio are key issues for production of biofuels and biochemicals, which needs to develop integrative bioprocess technologies. In our study, we proposed an integrative bioprocess by co-production of H_2 and CH_4 from low grade biomass by two-stage anaerobic fermentation technology. This integrative bioprocess has the advantages of maximum energy recovery from biomass and high fermentation speed which is indispensable to large scale industrialization. Biohythane consisting of 0-20% (v/v) bioH_2 and 100-80% (v/v) bioCH_4 can be utilized as a new and green gas biofuel for transportation. BioCH_4 can be utilized as a sustainable and economic substrate for C1-utilizing microbes to produce various biochemicals and biofuels by developing the tools of metagenomics, system biology and synthetic biology. In this talk, design and optimization of metabolic pathways, strain improvement, microbial consortia and the C1 integrative bioprocess for refinery of low grade biomass and engineering of methanotrophic cell factory for utilization of BioCH_4 will be introduced.

Min Jiang (Nanjing Tech University) - Speaker

Prof. Min Jiang is the Dean of 2011 college and PhD supervisor of State Key Laboratory of Materials-Oriented Chemical Engineering and College of Biotechnology and Pharmaceutical Engineering in Nanjing Tech University. He is also appointed as the Deputy Secretary General of Jiangsu Biological Technology Associate, China and Coordinator for Cooperation in Biotechnology between Baden-Wurttemberg and Jiangsu. His research mainly focuses on bioprocessing engineering, synthetic engineering, lignocellulose degradation and bulk chemicals/fuels production. In recent five years, he has published more than 65 SCI papers in reputational journal including ACS *synthetic biology, Applied Environmental Microbiology, Biotechnology for Biofuels, Bioresource Technology* et al, 61 invention patens with 34 authorized, 2 English book chapters and 2 Chinese books. He also obtained The Second Prize of China Petroleum and Chemical Industry Federation in 2017, The Second Prize of Ministry of Education Technological Innovation in 2016, and The Second Prize of Jiangsu Science and Technology in 2015 et al.

Efficient Bioconversion of Lignocellulose into Biobutanol by Unique Solventogenic Clostridium Strains

MIN JIANG[a,b*] and FENGXUE XIN[a,b]

[a]State Key Laboratory of Materials-Oriented Chemical Engineering, College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Nanjing, 211816, P.R. China
[b]Jiangsu National Synergetic Innovation Center for Advanced Materials (SICAM), Nanjing Tech University, Nanjing, 211816, P.R. China

Butanol, a four-carbon primary alcohol is not only an important intermediate chemical, but also considered as a promising next-generation liquid fuel. However, the high cost of traditional feedstocks restricts the development of industrial acetone-butanol-ethanol (ABE) fermentation. A lignocellulosic biomass-based route has been one of the thorough solutions for sustainable development of ABE fermentation. Solventogenic *Clostridium* species is one of the few microorganisms able to convert xylose and other pentoses to desirable products (ethanol, butanol and acetone). But on glucose/xylose mixtures, xylose is often left over at the end of the fermentation due to the low xylose utilization efficiency. Another issue is the toxicity caused by inhibitors occurring in the hydrolyzed lignobiomass. Unless both glucose and xylose in the real lignocellulosic hydrolysate are completely utilized, the economics of converting lignocellulosic biomass into bio-based products are unfavorable. Moreover, direct butanol production from lignocellulose, known as consolidated bioprocessing (CBP) is widely recognized as the most attractive and potential strategy for converting cellulosic biomass to biofuel, since it offers outstanding potential for lower costs and higher efficiency. Therefore, unique solventogenic *Clostridium* strains with capabilities of efficient simultaneous utilization of glucose and xylose and even direct butanol production from lignocellulose through CBP are needed urgently.

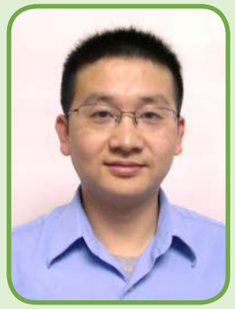
Ziyong Liu (Nanjing Tech University) - Speaker

Dr. Ziyong Liu got his PhD from Department of microbiology, Technical University of Munich, after he completed his master degree in Shandong University. Since 2016, he has been working as a post-doctoral research associate at the molecular microbiology engineering group, Qingdao institute of bioenergy and bioprocess of technology, CAS. Dr Liu's research interest is mainly on the metabolism and genetic engineering of anaerobic clostridia for biofuel production. His current work focuses on ethanol reassimilation in the fermentation of *Clostridium ljungdahlii*.

Study on Ethanol Reassimilation in the Fermentation of *Clostridium Ljungdahlii* with CO as the Source of Carbon and Energy

Ethanol production from syngas as a promising biofuel using Clostridium *ljungdahlii* has gained considerable attention in the recent past. However, acetate is the dominant product in the batch fermentation using CO as the source of carbon and energy. While ethanol titer is around 0.8 g/L and only one sixth of acetate titer under the same condition, low ethanol yield is the biggest challenge which prevents the commercialization of syngas fermentation into biofuels by microbial catalyst. Herein, C. *ljungdahlii* was able to produce around 5 g/L ethanol at 84 h in the late-exponential phase with 0.1 MPa at pH 6.0 in the batch fermentation with CO. Interestingly, ethanol started to be reassimilated in the stationary phase. To clarify this phenomenon, 13C marked ethanol and acetate metabolic experiment using resting cells, reducing equivalents analysis and comparative transcriptome between exponential and stationary phases were performed. The results showed that C. *ljungdahlii* produced amount of reducing equivalents in the exponential phase, accompanied with biomass accumulation. To balance the redox reactions, ethanol was produced in abundance. In the stationary phase, CO metabolism efficiency became lower and the biomass accumulation was ceased. At this moment, ethanol reassimilation was able to provide extra reducing equivalents to achieve the life circle of C. ljungdahlii. The clarification of ethanol reassimilation and biosynthesis metabolism paved the way to construct high ethanol yield genetic strain of C. *ljungdahlii* and highlight ethanol fermentation with syngas in industrial scale.

Huifeng Jiang (Tianjin Institute of Industrial Biotechnology, CAS) - Speaker



Huifeng Jiang received his Ph.D. (2008) from Kunming Institution of Zoology, Chinese Academy of Sciences, and was a postdoctoral fellow at Division of Nutritional Sciences, Cornell University between 2008 and 2012. He was a principle investigator in Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences (TIB-CAS) since 2012. Dr. Jiang's research focuses on understanding the origin and evolution of plant specialized metabolism at enzyme, pathway, and systems levels. Through synthetic biology and metabolic engineering approaches, he develops new platforms for producing high-value natural products in a sustainable manner. Dr. Jiang has won numerous awards in his career, including The Hundred Talents Program of the Chinese Academy of Sciences (2014), Tianjin Thousand Youth Talents Plan (2014) and Tianjin Innovative talent promotion program (2015). Until now he has published more than 30 papers in *PNAS, Genome Research, PLoS Genetics, Cell Research, Molecular Biology and Evolution, Biotechnology for Biofuels, Scientific Reports* and so on.

A Synthetic Pathway for Acetyl-Coenzyme A Biosynthesis

HUIFENG JIANG

Key Laboratory of Systems Microbial Biotechnology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China

Acetyl-CoA is a critical intermediate metabolite for all living life. It is also a key starting point for the biosynthesis of a variety of chemicals of industrial importance. In this study, we designed and constructed a novel synthetic acetyl-CoA pathway, designated as the SACA pathway. The key challenge is that the enzymes capable of catalyzing the reactions in the designed SACA pathway have not been found in nature. To address this challenge, two novel enzymes, glycolaldehyde synthase (GALS) and acetyl-phosphate synthase (ACPS), were de novo designed and characterized. The de novo designed enzymes enabled the SACA pathway to function in vitro and in vivo, converting formaldehyde into acetyl-CoA in only three steps. The SACA pathway is the shortest, ATP-independent, oxygen-insensitive pathway for acetyl-CoA biosynthesis from one-carbon compound. It is thermodynamically favorable, carbon-conserving, and most importantly, orthogonal to the cellular metabolism. Thus, the SACA pathway opens new possibility for cells to convert one-carbon compounds into acetyl-CoA, and consequently, into acetyl-CoA derived chemicals if the SACA pathway is incorporated with other cellular pathways. Moreover, as CO_2 can be relatively easily to be converted into one-carbon compound through electrochemistry, the SACA pathway also provides possibility to convert CO_2-derived one-carbon compound into useful chemicals in the future.

Qiang Fei (Xi'an Jiaotong University) - Speaker

Prof. Qiang Fei has been serving as a full professor of Xi'an Jiaotong University since 2016. From 2011-2016, he was working as a postdoc and staff engineer at MIT and the U.S. National Renewable Energy Laboratory (NREL) respectively. In 2007, he was selected in the"Thousand Young Talents Plan" of Shaanxi Province. Prof. Fei has been researching the construction and development of biocatalysts for biofuel production including bioethanol, biodiesel, isobutanol, and jet fuel. He has led and involved several projects funded ARPA-E、DOE and Shaanxi Province researching the biofuel production from recombinant microbes using sugars, natural gas, biogas as carbon sources. Prof. Fei has published more 30 peer-reviewed research papers and DOE milestone reports with a total citation of 480 times and H-index of 10. Currently, he is focusing on developing fermentation processes using biogas as substrates for the production of bio-based products and building techno-economic analysis (TEA) models for bioconversion of renewable carbon sources into value-added products

Enhanced Methane Biofixation for Biofuel Production by Recombinant Methanotrophic Bacteria

QIANG FEI

School of Chemical Engineering and Technology, Xi'an Jiaotong University, Xi'an, China.

The current crisis of global warming is primarily attributed to CO_2 production from excessive use of fossil fuels during recent decades, and has increased demand for renewable biofuels tremendously. Lipids are drawing considerable attention in relation to the production potential of biodiesel on the basis of their nontoxic, sustainable, and energy efficient proprieties. However, the high cost of microbial lipid produced by oleaginous microorganisms mainly stems from the high cost of glucose, which is estimated to be about 80% of the total medium cost. Therefore, considerable efforts have been directed toward minimizing the carbon source cost and finding new alternative carbon sources. Because of the relatively low price of biogas and increasing demands of liquid transportation fuels, attention has begun to turn to methantrophic bacteria for biofuel production. In this study, *Methylomicrobium buryatense* was investigated to achieve high lipid titer and productivity in high cell density cultivations (HCDC) using methane as the sole carbon source. The cell growth and lipid production from both strains were studied and compared in order to elucidate the influence of culture conditions on lipid production, gas uptake/evolution rate and glycogen accumulation in batch cultures of both strains. Finally, the fatty acid composition of membrane lipids was analyzed and characterized for diesel fuel production.

Zhen Cai (Institute of Microbiology, CAS) - Speaker

Zhen Cai has received her B.S. and Ph.D. degrees in Chemical Engineering from Tsinghua University in 2004 and 2009, respectively. She joined Institute of Microbiology in 2009 as an assistant professor and became an associate professor in 2014. Her previous research interests were enzyme and microbial engineering to improve their properties as well as robustness. Now her work mainly focuses on biological CO_2 fixation, including screening and engineering of the CO_2-fixing enzymes, and construction of the CO_2 fixation pathway. Dr. Cai has published tens of journal papers including Biotechnology for Biofuels, Scientific Reports, and Applied Microbiology and Biotechnology and applied one international patents and six Chinese patents. One patent has been licensed to a Chinese domestic company.

Synthetic Carbon Concentrating and Fixing Modules Enabled Heterotrophic Fixation of CO_2 in *E. Coli*

ZHEN CAI*, FUYU GONG, GUOXIA LIU, JUNLI ZHANG, XIAOYUN ZHAI, YIN LI*

CAS Key Laboratory of Microbial Physiological and Metabolic Engineering, State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China

Production of fuels from the abundant and wasteful CO_2 is a promising approach to reduce carbon emission and fossil fuels consumption. The slow growth rate of the naturally CO_2-assimilation autotrophs calls for investigation of the possibility of heterotrophic CO_2 fixation. However, it remains unclear how and how efficient that CO_2 can be incorporated into the central metabolic pathway of a heterotrophic microbe. In this study, synthetic carbon concentrating and fixing modules which mimic the cyanobacterial Calvin cycle and carbon concentrating mechanism were devised and incorporated into *E. coli.* Both relative quantification based on an isotropic assay and absolute quantification based on carbon balance have been developed to evaluate the CO_2 fixation rate. This strain was able to fix CO_2 at a rate of 40.2 mg CO_2 L^{-1} h^{-1}, which is comparable with that of fourteen autotrophic cyanobacteria and algae (11-147 mg CO_2 L^{-1} h^{-1}), demonstrating the potential of heterotrophic CO_2 fixation.

Li Xie (Tongji University) - Speaker

Li XIE currently works as full professor at the department of Environmental Engineering, Tongji University. Her main research interests focus on high strength wastewater treatment and municipal wastewater treatment, anaerobic reactor configuration and design, anaerobic processes optimization and development of sustainable solutions for wastewater management. Currently, she is involved mainly in projects related with biogas upgrading, hydrogenotrophic methanogenic process.

Biomethanation of $H_2/CO_2/CO$ by Hydrogenotrophic Mixed Cultures under Thermophilic and Extreme-Thermophilic Conditions

LI XIE

Department of Environmental Engineering, Tongji University, Shanghai 200092, China

Coke oven gas is considered as a potential hydrogen source for biogas bio-upgrading. However, the contained CO might impose toxicity to methanogens. In this study, the effect of CO on biomethanation performance and microbial community structure of mixed cultures was investigated under thermophilic (55°C) and extreme-thermophilic (70°C) conditions. The addition of 5% (v/v) CO did not inhibit hydrogenotrophic methanogenesis during semi-continuous operation, and 83-97% CO conversion to CH_4 was achieved. *Methanothermobacter thermoautotrophicus* was the dominant methanogen under both temperature conditions and the main functional archaea associated with CO biomethanation. Specific methanogenic activity test results showed that long-term 5% CO acclimation shortened the lag phase from 5 hrs to 1 hr at 55°C and 15 hrs to 3 hr at 70°C respectively. CO_2 was a preferred substrate over CO for biomethanation and CO consumption only started when CO_2 was completely depleted. The mixed cultures dominated with *M. thermoautotrophicus* showed a great potential in simultaneous hydrogenotrophic methanogenesis and CO biomethanation.

Gang Luo (Fudan University) - Speaker

Gang Luo is an associate professor in the department of Environmental Science and Engineering at Fudan University. He got PhD degree in Environmental Engineering from Tongji University in 2011 and then worked as postdoc in Technical University of Denmark until 2013. His research interests include anaerobic digestion, biomass to bioenergy, microbial ecology. He has developed several technologies including H_2 based biogas upgrading, syngas biomethanation together with organic wastes, and efficient H_2 production based on the inhibition of homoacetogens. His research received funding from NSFC, STCSM and MOST. He has published more than 50 ISI publications.

Biomethanation of H_2 and CO in Biogas Reactors Treating Organic Wastes

GANG LUO

Department of Environmental Science and Engineering, Fudan Uinversity, Shanghai,China

Syngas is produced by thermal gasification of both nonrenewable and renewable sources including biomass and coal, and it consists mainly of CO, CO_2, and H_2. H_2 can also be obtained by water electrolysis using excess electricity from wind mill. We developed efficient system for biomethanation of H_2 and CO in biogas reactors treating organic wastes. With H_2 itself, in-situ biogas upgrading could be achieved in biogas reactors. We found that the addition of H_2 resulted in increase of pH (from 8.0 to 8.3) due to the consumption of bicarbonate, which subsequently caused slight inhibition of methanogenesis, and also the methane content in the biogas was not very high due to the low solubility of H_2. Further study showed that co-digestion of cattle manure with whey and using hollow fiber membrane can solve the pH problem and increase the H_2 utilization rate. The maximum CH_4 content can be as high as 99% by the addition of H_2 without affecting the original biogas production. The biomethanation of CO in biogas reactors was also studied. It was shown that CO was inhibitory to methanogens, but not to bacteria. Anaerobic granular sludge (AGS) tolerated CO partial pressure as high as 0.5 atm without affecting its ability for organic degradation, which was higher than that of suspended sludge (<0.25 atm). Continuous experiments by upflow anaerobic sludge blanket (UASB) reactors showed AGS could efficiently convert synthetic wastewater and CO into methane by applying gas-recirculation to increase gas-liquid mass transfer.

Wen Wang (Tongji University) - Speaker

Wen Wang received her Ph.D. degree from department of Environmental Engineering, Tongji University. She went to Technical University of Denmark as visiting scientist during 2012 to 2013. After getting Ph.D, she has been working in School of Chemical Engineering, Beijing University of Chemical Technology as assistant professor since then. Her research is focusing on biomass to biofuels (biogas, biohydrogen, bio-acid) production. Dr. Wang has published about 30 academic papers, 1 book and has applied 11 China Patents. She has got more than 10 programs grants from National Science Foundation of China, Science and Technology Commission of Beijing Municipality, Fundamental Research Funds for the Central Universities etc.

Optimization of Biofuel Recovery from Food Waste with Anaerobic Digestate Pyrolysis and Syngas Biomethanation

WEN WANG and GUANGQING LIU

Biomass Energy and Environmental Engineering Research Center, Beijing University of Chemical Technology, Beijing 100029, China

A food waste integrated management system with anaerobic digestion, digestate pyrolysis and syngas biomethanation to optimize the biofuel recovery was investigated in this research under both mesophilic and thermophilic conditions.

Anaerobic digestion of food waste was evaluated first (phase I), and stable biogas productions were obtained in both two reactors. The methane production (MP) obtained under thermophilic condition (5404 mL/d) was about 20% higher than that under mesophilic condition (4520 mL/d).

The digestate from the two reactors were collected and used as the substrate of pyrolysis. 400°C and 700°C were selected as pyrolysis temperature by thermal gravimetric analysis. Results of digestate pyrolysis indicated that the yields of syngas and bio-oil increased with the increase of pyrolysis temperature, while the yield of char decreased. The yield of syngas was about 3 times higher at 700°C than that at 400°C, reached 103.87 mL/g under mesophilic condition and 159.85 mL/g under thermophilic condition, and the flammable component (H_2, CO and CH_4) contents were also 3 to 4 times higher at 700°C (57.0 vol% and 65.5 vol% under mesophilic and thermophilic conditions respectively) than those at 400°C.

The simulated syngas (H_2/CO: 5/4) was injected to the anaerobic reactors for further biomethanation evaluation. From phase II to V, the injected syngas volume increased from 500 to about 5000 mL/d, and the calculated conversion efficiency of H_2 and CO under mesophilic and thermophilic conditions were all over 97% in each phase indicating sufficient utilization of microorganisms. The MP in phase II to V increased 7- 33% compared with that in phase I under thermophilic condition. The MP in thermophilic reactor was about 13-25% higher than that in mesophilic during phase II to V. The calculated CH_4 yields (based on the stoichiometric equations) were lower than the experimental data, indicating that syngas was not only almost stoichiometrically converted to CH_4, but also the syngas enhanced the anaerobic degradation of FW. High-throughput sequencing analysis of 16S rRNA genes indicating that during syngas biomethanation, CO was converted into H_2 first and then into CH_4 under thermophilic condition, while acetate was the intermediate under mesophilic condition.

Raymond Jianxiong Zeng (Fujian Agriculture and Forestry University) - Speaker

Dr. Raymond Zeng obtained his PhD degree of Environmental Engineering from Advanced Water Management Centre, The University of Queensland in 2002. After 7 years academic experience in Denmark and Australia, in 2009 he joined University of Science and Technology of China as a professor of the Hundred Talent program, and relocated to Fujian Agriculture and Forestry University this year. His research area includes biological wastewater treatment and resource recovery, bioenergy production from wastes, bio-utilization of greenhouse gases, value-added chemicals from microalgae. Dr. Zeng has published more than 150 SCI paper with h index of 33.

Organic Acids Production from H_2/CO_2 via Mixed Culture Fermentation

RAYMOND JIANXIONG ZENG, FANG ZHANG

Department of Environmental Science and Engineering, Fudan Uinversity, Shanghai,China

Nowadays, complete biological conversion of organic wastes is still difficult, thus a significant amount of non-biodegradable materials remain in the effluent. Gasification is suggested as a good alternative for resource recovery. It converts waste into syngas of H_2, CO, and CO_2. Biological conversion of syngas into liquid chemicals, such as acetate, butyrate, caproate and caprylate, is an attractive concept. But, the low solubility of H_2 and CO in the water phase is the main challenge for their utilization. The hollow-fiber membrane biofilm reactor (HFMBR) is recently recognized as an elegant and attractive technology, in which gas permeates from inside of the membrane lumen and is directly consumed by biofilms naturally attached on the outer surface of the membrane without loss of gas through bubble formation. The high specific exchange surface area of HFMBR also promotes a high volumetric gas transfer rate, increases the product generation rate and reduces the investment cost.

On the other hand, to minimize operational costs and deal with variations in environmental and feedstock parameters, mixed culture fermentation (MCF) have been proposed as a promising approach to realize resource recovery and valuable biomaterials production, such as fatty acids. Thus, H_2 and CO_2 utilization by MCF in HFMBR were recently carried out in our lab, and the main outcomes were as follows:

In mesophilic MCF (35°C), a mixture of acetate (7.4 g/L), butyrate (1.8 g/L), caproate (0.98 g/L), and caprylate (0.42 g/L) was accumulated at pH 6.0, where *Clostridium spp.* (such as *C. ljungdahlii* and *C. kluyveri*) were the dominant bacteria. When pH was reduced to 4.0, the metabolite only consisted of acetate (12.5 g/L), and the dominant bacteria were identified as *C. ljungdahlii* and *C. drakei.*

- With temperature increasing to 55°C, *Thermoanaerobacterium* (66%) became the main bacterium, and acetate occupied more than 98.5% and 99.1% of total metabolites in batch and continuous modes of HFMBR, respectively.
- Low temperature favoured caproate production, for example, as temperature decreased to 25°C, acetate, ethanol, butyrate, and caproate were the main metabolites. Especially, the concentration of caproate reached 5.7 g/L in the batch mode of HFMBR.

Jingjing Xie (Nanjing Tech University) - Speaker

Dr. Xie, Jingjing, got Bachelor degree in Chemistry from department of Chemistry in Nanjing University and PhD degree in biochemistry from State University of New York at Albany. Then served as a postdoctoral fellow at the State University of New York and Cornell University before became professor at College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University. Research focuses on the construction of high efficient biocatalytic conversion systems.

Specific interests includes: Build up Microbial Electrosysthesis systems that utilize autotrophic microorganisms and sustainable energy to achieve efficient conversion of CO_2 to high value-added products; Design and build efficient biocatalysts based on enzyme engineering and synthetic biology to provide new technologies for green manufacturing of chemicals. Published over 60 papers with citations over 900.

High Efficiency Microbial Electrosynthesis of Organics from Carbon Dioxide

Since hydroelectric, wind and solar energy are extremely uneven at different times of the year or day, efficient energy storage is a prerequisite for the successful use of sustainable energy. Microbial electrosynthesis (MES) is a process in which microorganisms may use sustainable electrons derived from an electrode to reduce carbon dioxide to multicarbon, extracellular product. It is a potent strategy for storage of renewable electricity into value-added chemical commodities. A long-term operation of MES system was set up. Both novel 3D-cathode and self-assembled electroactive biofilm were studied to improve the production efficiency of acetate from CO2 by MES systems. Furthermore, butyrate was obtained through the processing regulation of the MES with mixed culture.

Changhao Bi (Tianjin Institute of Industrial Biotechnology, CAS) - Speaker

Changhao Bi currently works as a Professor of Tianjin Institute of Industrial Biotechnology CAS, Leader Scientist of Chines High Technology (863) research program from 2014. He Graduated from Nankai University for his Bachelor and Master study and obtained the Ph.D. in University of Florida in 2009, before worked in University of Delaware and Laurence Berkeley National Lab.

His main research area is Microbial Synthetic Biology and Metabolic Engineering, focusing on development and application of new methods and techniques, as well as explore of the frontiers of Synthetic Biology. He has published 22 SCI research article in Journals as *Metab Eng., ACS Synth., Microb. Cell Fact., ChemComm., .J. Bacteriol., AEM etc*, with about 200 citations.

Strain Development Progress of The National High Technology Project of The Twelfth Five

As a country with large resource consumption, China is facing severe situation of exhaustion of petroleum resource and increasing carbon emission. From 2015, The Key Technologies of Bio-transformation of Syngas project, funded by the National High Technology Funding, was carried out by several institutes of the Chinese Academy of Sciences collaborated with the Bao Steel company. This project is focused on basic and applicable researches of biosustainable syngas biotransformation technologies. We engineered syngas utilization bacterial strains, designed and built syngas fermenter, developed and optimized the fermentation process, and finally achieved efficient syngas biotransformation for important chemicals.

Strain development is the most fundamental part of the project. Genetic manipulation methods and especially CRISPR/Cas9 genome editing technology were established for C*lostridium ljungdahlii* for the first time, which enabled efficient manipulation of this bacterium. With this technique, competing pathways of ethanol were deleted, and heterologous four-carbon synthesis pathways were introduced, which caused production of and caused 0.32 g/L butanol, 1.9g/L butyrate and 0.8 g/L isopropanol production. CRISPR/Cas9 genome editing technology were also established for *Ralstonia eutropha* for the first time. In addition, the key restriction enzyme was identified, deletion of which enabled electroporation transformation of this bacterium. These key technologies enabled construction of efficient H_2 and CO_2 utilization of *R. eutropha* for production of PHB.

Yangchun Yong (Jiangsu University) - Speaker

Yang-Chun Yong, Ph.D., Professor. He received his Ph.D. degree from East China University of Science and Technology in 2009 under the supervision of Prof. Jian-Jiang Zhong. Before he joined in Jiangsu University in 2012, he worked as a research fellow at Nanyang Technological University, Singapore. His research interests mainly focused on bioremediation, biosensors, electrobiocommodities and solar fuels.

Manipulation Of Bacterial Transmembrane Electron Transfer Towards Efficient Microbial Electrosynthesis

YANG-CHUN YONG

Biofuels Institute, School of Safety and Environmental Engineering, Jiangsu University, China

Electroactive bacteria (EAB) are unique kinds of microorganisms that can transport intrancellular electrons to extracellular solid electrode or uptake electrons from the extracellular electrode or solid minerals. By utilizing this kind of bacteria, various interesting and promising bioelectrochemical systems have been developed. Among these systems, microbial electrosynthesis system has attracted much attention in recent years as it holds great promise to transform of CO_2 into value-added chemicals (electrobiocommodities or solar fuels) by using electricity or even solar energy as the reducing power [1, 2]. However, the efficiency of this microbial electrosynthesis system is still low due to the limitation of the bacterial transmembrane electron transfer.Thus, our lab focused on the manipulation of the transmebrane electron transfer process aimed to improve the electron transfer efficiency. By using the model EAB (*Shewanella oneidensis* MR-1) and a sensitive transmembrane electron transfer monitoring method, the electron uptaking properties (including the contribution of Mtr-electron transport chain and riboflavin) of this model EAB were detailed characterized (3, 4). Strinkingly, we found that phenazine can serve as a more efficient electron shuttle for Shewanella than riboflavin (5). Moreover, we developed series new electrodes and new electrode biofilm doping strategies for microbial electron uptaking, which greatly improved the bacterial transmembrane electron transfer efficiency (6-8).

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Demao Li (Tianjin Institute of Industrial Biotechnology, CAS) - Speaker

Demao Li is the associate professor of Biosystem engineering at Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin, China. Dr. Li received his Ph.D. degree from Institute of Oceanology, Chinese Academy of Sciences. He was a visiting scholar at the Department of Biological Systems Engineering, Washington State University in 2015-2016. His current research includes methane bioconversion and fermentation engineering. He is the author and co-author of 80 peer-reviewed original research papers and more than 15 issued patents.

Bioconversion of Low-Concentration Methane from Bio-Gas Projects for Biochemical Production

It is an indisputable fact that China's biogas projects are less profitable, which has greatly dampened the enthusiasm of investors. Treatment of high ammonia nitrogen wastewater from manure biogas projects is very difficult. Also, the main application of methane produced by biogas projects is the preparation of steam for combustion, heat generation, and gas for automobiles. In reality, there will not high demand for the heat from the biogas projects. At the same time, the excess power they send cannot enter the power grid, which results in a slow development of the domestic biogas industry. Therefore, bioconversion of methane to high-valued added chemicals, if the high ammonia nitrogen wastewater can be used will be better, will be urgently needed.

Thus, a methanotroph that can synthesize lycopene, C30 carotenoid and exopolysaccharides (EPS) with relative better performances from C1 substrates was isolated, and its performances were evaluated. The genome was sequenced and gene transfer operating system has been established. The ammonia inhibition has been removed through metabolism analysis. Then a closed loop for manure waste-methane and waste water-high value added biochemical will be formed which will be helpful for the whole industry.

Zhiyong Huang (Tianjin Institute of Industrial Biotechnology, CAS) - Speaker

Prof. Huang graduated from University of Tokyo, Japan, in 2000. From 2002 to 2008, he studied in the University of Missouri as a visitor fellowship and worked in the University of Georgia as research coordinator, respectively. In 2008, he was a Principle Investigator and assembled a group in Tianjin Institute of Industrial Biotechnology, CAS. Prof. Huang used to focus on Microbial ecology and environmental microbiology. And in recent years, his group did lots of research works on syngas fermentation using *Clostridia*, which was supported by Ministry of science and technology, and Chinese Academy of Sciences, respectively. Moreover, Prof. Huang was a Member of America Society of Microbiology; Professional Member of the Geological Society of America; Member of International Society of Microbial Ecology.

A Novel Trace Metals Model Promotes Solventogenesis of *Clostridium Carboxidivorans* P7 and Enhances Higher Alcohol Production During Syngas Fermentation

ZHIYONG HUANG, YIFAN HAN

Tianjin Institute of Industrial Biotechnology, CAS

The main constraints of biological treatment of volatile organic compounds are redundant residence time and low organic load. To overcome these problems, a xylene-degrading microbial consortium was obtained from municipal activated sludge by domestication. A trickling bio-filter was established with the consortium and operation factors were optimized. The removal efficiency (RE) was stable at more than 86%, and the maximum elimination capacity (EC) was 303.61g/m^3·h. Kinetic analysis of the xylene indicated efficient biological activity and gas liquid mass transfer efficiency. The main products from bio-degradation of xylene and CO_2 production were monitored during the experiments. Analysis of community structure showed that *Pseudomonas* sp. and *Sphingobium* sp. were the most dominant bacteria in the biotrickling filter, followed by *Burkholderia*sp., *Bacillus*sp., *Rhodococcus*sp., *Dokdonella*sp., *Novosphingobium*sp., *Pandoraea*sp., *Stenotrophomonas*sp., and *Comamonas* sp. The potential for bacteria synergistic effects were discussed.

Peng Hu (Shanghai GTL Biotech Co., Ltd.) - Speaker

Dr. Peng Hu received his Bachelor degree in Chemical Engineering from Huazhong University of Science and Technology (HUST) in China and Ph.D degree from Brigham Young University in the United States, respectively. From 2010 to 2014, Dr. Hu joined Metabolic Engineering and Bioinformatics Laboratory in Massachusetts Institute of Technology (MIT) as visiting student and Post-doctor associate. His research is focusing in developing biological solutions to solve challenges in health, environment and alternative energy. Especially, by applying the metabolic engineering and fermentation technology, Dr. Hu and his colleagues developed an integrated system that enabled the microorganisms to convert gas substrate (a mixture of CO_2, H_2, and CO) to liquid biofuels. During the past years, the research results of such gas-to-liquid (GTL) process have been reported in many top journals such as PNAS, Science, Nature, etc. In 2015, Dr. Hu co-founded the company GTL Biofuel Inc. in Boston and its Chinese affiliate Shanghai GTL Biotech Co. Ltd. As the CTO of the company, Dr. Hu leads a team of more than 15 scientists and engineers for the commercialization of the patented GTL technologies.

The Commercialization of Gas Fermentation Platform for Biofuel and Bulk Chemicals Production

In the quest for inexpensive feedstocks for the cost-effective production of liquid fuels, we have examined gaseous substrates that could be made available at low cost and sufficiently large scale for industrial fuel production. Here we introduce a new bioconversion scheme that effectively converts syngas, generated from gasification of coal, natural gas or biomass, into lipids that can be used for biodiesel production. We present an integrated conversion method comprising a two-stage system. In the first stage, an anaerobic bioreactor converts mixtures of gases of CO_2 and CO or H_2 to acetic acid using the anaerobic acetogen *Moorella thermoacetica*. The acetic acid product is fed as substrate to a second bioreactor, where it is converted aerobically into lipids by an engineered oleaginous yeast, *Yarrowia lipolytica*. We first describe the process carried out in each reactor and then present an integrated system that produces microbial oil using synthesis gas as input. The integrated continuous bench-scale reactor system produced C16-C18 triacylglycerides directly from synthesis gas, with an overall high efficiency and productivity. The presented integrated system demonstrates the feasibility of converting gaseous feedstocks to lipids for biodiesel production. Apre-commercial pilot based on this technologyin conversion of waste gases from steel mills to valuable liquid fuels and bulk chemicals is successfully running in Shanxi Province, China.

Introduction of the Institute of Microbiology, Chinese Academy of Sciences (IMCAS)

The Institute of Microbiology of the Chinese Academy of Sciences (IMCAS) is the largest microbiological research institution in China. It was founded on December 3, 1958, through the merger of the Institute of Applied Mycology and the Beijing Laboratories of Microbiology, both of which were affiliated to the Chinese Academy of Sciences (CAS). IMCAS was initially located in Zhongguancun, Haidian District, Beijing. In early 2007, the major part of the Institute was relocated to the CAS Life Science Park near the Olympic Village in Chaoyang District, Beijing. After over 50 years of development, it has become the nation's largest comprehensive research institution of microbiological science.

In the first 18 years from 1958 to 1976, IMCAS had eight research divisions covering the following disciplines: mycology, virology, physiology and biochemistry, biophysics, genetics, agricultural microbiology, industrial microbiology and geomicrobiology. During that period of time, great efforts were made to survey microbial resources and to study microbial metabolisms and genetic variations. A large number of microbial strains were found and improved for use in fermentation industries, agriculture and geological survey.

In 1978, in accordance with the strategic restructuring of CAS, IMCAS underwent institutional reorganization and set up eight divisions, i.e., divisions of fungal classification, bacterial classification, virology, microbial ecology, microbial metabolism, microbial enzymology, microbial genetics and microbial strain preservation. At the same time, new research support systems, including a fermentation pilot plant, a core facility, a radiation safety laboratory and an information center, were set up. In the following two decades, IMCAS made great strides in both basic and applied research, which were represented by a large number of scientific achievements of great significance to economic and social development.

In 1998, the CAS Knowledge Innovation Project was officially launched. Upon approval by CAS, IMCAS entered the Project on August 15, 2001, marking the beginning of a new phase in its history. With microbial resources, molecular microbiology and microbial biotechnology as three main areas of research, the Institute reshuffled its research divisions to establish a research center for microbial resources, a research center for molecular microbiology and a research center for microbial biotechnology. In 2004, IMCAS recognized microbial resources, industrial microbiology and pathogenic microbiology as its three areas of research, and set up nine research centers in the three areas. These include research centers for microbial resources, extremophiles, energy and industrial biotechnology, microbial metabolic engineering, environmental biotechnology, agricultural biotechnology, molecular virology and molecular immunology, and a joint research center for microbial genomics.

Since 2008, the Institute has endeavored to reorganize research and development activities into an innovative value chain with a biological resource center, a scientific research system and a technology transfer and transformation center as three interconnected units, and to carry out basic, strategic and prospective research in the areas of microbial resources, microbial biotechnology and pathogenic microbiology and immunology to meet national needs in industrial upgrading, agricultural development, human health, environmental protection, etc. At present, the scientific research system of IMCAS is comprised of five laboratories: State Key Laboratory of Microbial Resources, State Key Laboratory of Plant Genomics (jointly set up by IMCAS and the Institute of Genetics and Developmental Biology, CAS), State Key Laboratory of Mycology, CAS Key Laboratory of Pathogenic Microbiology and Immunology, and CAS Key Laboratory of Microbial Physiological and Metabolic Engineering.

IMCAS owns the largest fungal herbarium in Asia with nearly 500,000 specimens and the largest microbiological culture collection in China with more than 41,000 strains. In addition, it possesses a microbiological information center, a core facility, a Biosafety Level-3 laboratory and other supporting platforms. It also has a specialized library with more than 50,000 books/journals and an electronic library with more than 20,000 e-books and 9,000 e-journals in Chinese or English. Three national academic societies, i.e., Chinese Society of Microbiology (CSM), Chinese Mycology Society and Chinese Society of Biotechnology (CSBT), are currently affiliated to IMCAS. IMCAS publish the following academic journals in conjunction with these societies: Acta Microbiologica Sinica, Microbiology China, Mycosystem and Chinese Journal of Biological Engineering (in Chinese and English).

The Institute also attaches great importance to cooperation with international scientific community and industries. Since 2008, IMCAS implemented 50 international cooperative projects and 40 talent recruitment and international exchange projects. In 2010, IMCAS was elected to be the host institute of the World Data Center for Microorganisms. In 2012, IMCAS cooperated with Royal Holloway, University of London and launched Fungal Names, an international fungal names registration website and a Chinese fungal species database. In March 2013, the CAS-TWAS Biotechnology Center of Excellence was established at IMCAS. Each year, it organizes several international meetings in microbiology to further promote communication and exchanges in the international community.

IMCAS employs more than 480 staff members, of whom more than 300 are researchers including seven CAS academicians. There are 522 graduate students at IMCAS.

Through over five decades of unflagging efforts, IMCAS has developed into a comprehensive microbiological research institution with rich heritage, great strength and international reputation.

http://english.im.cas.cn/

Introduction of CAS-TWAS Centre of Excellence for Biotechnology (CoEBio)

CAS-TWAS Centre of Excellence for Biotechnology (CoEBio) was founded in 2013, it is an open and integrative platform aiming to enhance the biotechnology development in developing countries. CoEBio is supported by ChineseAcademy of Sciences (CAS) and the World Academy of Sciences (TWAS) for the advancement in developing countries, and affiliated at the Institute of Microbiology, CAS. CoEBio strives to improve and reinforce biotechnology education, scientific communication, project cooperation and strategic intelligence analysis with the purpose of providing biotechnological solutions to address the issues of resources, energy, population, health and environment issues within developing countries.

The main missions of CoEBio include:

1. Assisting developing countries in advancing the education of students and scholars, in the area of biotechnology.
2. Organizing biotechnology training courses, conferences, symposia, and workshops in developing countries.
3. Coordinating biotechnology related scientific research and development (R&D) projects for developing countries.
4. Strategic intelligence analysis for biotechnology development in developing countries.

http://www.cas-twas-coebio.org/

Introduction of CAS Key Laboratory of Microbial Physiological and Metabolic Engineering, IMCAS

CAS Key Laboratory of Microbial Physiological and Metabolic Engineering, affiliated to Institute of Microbiology, Chinese Academy of Sciences (IMCAS), was founded in April, 2013. The orientation of key laboratory is applied basic research in microbial biotechnology, focusing on industrial microbial physiology and regulation of metabolism, the reconstruction and optimization of biosynthesis pathway and physiological adaptation functionalities. Its objectives focus on the development of new technologies and new methods towards microbial physiological engineering and metabolic engineering, developing the next generation of strains and whole-cell catalysts with predominate industrial performance.

Our department now has 10 research groups and 46 researchers, including 1 CAS academician, 10 Professors, 10 associate Professors, 23 assistant Professors, also has 2 post-doctor associates and 64 graduate students.

The main research directions include:

1. Molecular genetics and efficient genetic manipulation systems
2. Molecular enzyme engineering and new biocatalytic process
3. Molecular physiology and advanced metabolic engineering.

http://www.im.cas.cn/jgsz/yjtx/gywswyswjsyjs/