C1net

Conference 2

25-26 July 2016
Nottingham, UK

Programme and Abstracts

C1net Management Board

Nigel Minton (PI)	SBRC Nottingham, UK
David Fell (CoI)	Oxford Brookes University, UK
Ian Fotheringham	Ingenza Ltd, UK
Michelle Gradley	BioSyntha Technology Ltd, UK
Edward Green	Chain Biotech Ltd, UK
Arild Johannessen	Calysta, UK
Preben Krabben	Green Biologics, UK
Robin Mitra	Centre for Process Innovation Ltd, UK
Sean Simpson	LanzaTech, USA
Bob Tooze	Sasol UK Ltd, UK
Phillip Wright	University of Newcastle, UK
Jacque Minton	SBRC Nottingham, UK

Conference Secretariat:

Jacque Minton
Centre for Biomolecular Sciences,
Clifton Boulevard,
University Park,
Nottingham,
NG7 2RD

Tel: 0115 846 6287
email: jacqueline.minton@nottingham.ac.uk

With the acknowledged help of Tom Bailey, Janice Sablitzky and Louise Dynes

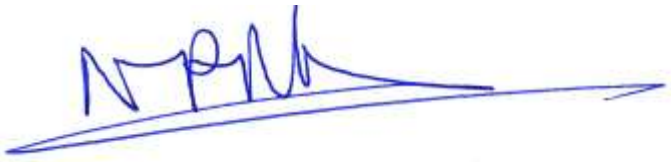
WELCOME

Current energy and chemical needs are largely met by the extraction and processing of the fossil fuels oil, gas and coal. Such resources are limited and their use causes environmental pollution and greenhouse gas (GHG) emissions. The challenge facing humankind is, therefore, to identify new, sustainable and cleaner processes for chemical and energy generation.

C1net champions the use gas fermenting microbes that are able to grow on C1 gases, such as CO, CO_2 and CH_4, that may be derived from non-food sources such as waste gases from industry as well as 'synthesis gas' (CO & H_2) produced from domestic and agricultural wastes. This enables low carbon fuels and chemicals to be produced in any industrialized geography without consumption of valuable food or land resources.

Since its initiation in March 2014, C1net has created a cross-disciplinary community of academics and industrialists working together to achieve the networks goals. Much progress has been made: Membership currently stands at 309 with members from Europe, India, USA and Russia, we have 259 followers on Twitter and a total of 8 POC awards of £50,000 have been made.

We, the management board, are all passionate in our belief that the manufacture of chemicals and fuels from C1 gases using microbial fermentation chassis has a significant role to play both from a commercial and societal perspective. It is, therefore, with much pleasure that we welcome you to the second conference of the BBSRC NIBB, C1net.



Nigel P Minton
On behalf of the C1net management board

GENERAL INFORMATION

CONFERENCE VENUE AND ACCOMMODATION

East Midlands Conference Centre and Orchard Hotel,
University Park Campus, The University of Nottingham, Nottingham, NG7 2RJ

ORAL PRESENTATIONS

Oral presentations will be in the Banqueting Suite Hall B. The length of oral presentations is scheduled 15 tor 30 min (check programme), within that presenters should allow 5 min for discussion. All presentations should be prepared in a form of MS Power Point slide show and stored on USB sticks or CD/DVD. The use of a personal computer or Mac is not possible. This can be done anytime, by handing it to the duty IT technician, but at least 2-3 hours before your session or the evening before for early morning presentations.

ELEVATOR PITCHES

Elevator pitches will be in the Banqueting Suite Hall B. They should no longer than 3 minutes with 2 PowerPoint slides (or soap box pitch if you prefer). All pitches were submitted to the conference organiser in advance, before 11 July 2016 and will be pre-loaded for you.

POSTER PRESENTATIONS

Poster presentations will be in the Banqueting Suite Hall A. The maximum recommended poster size is A0 portrait (90 cm × 120 cm). Velcro tabs will be provided. The presenting author should stand by his/her poster for the whole length of the session.

DATA PROTECTION

Presenters please let your audience know if they should not tweet or record your work.

SOCIAL EVENTS

Welcome BBQ, Sunday, 24 July 2016, 19:00, Orchard Hotel, University Park
Conference Dinner, Monday, 25 July 2016, 20:00, Banqueting Suite Hall B, East Midlands Conference Centre, University Park

TAXIS

DG Taxis 0115 9 607607 or ask the hotel.

PROGRAMME

MONDAY 25th July 2016		
SESSION 1 - Chair: David Fell (Oxford Brookes University, UK) – **Hall B - East Midlands Conference Centre**		
09.00 – 09.05	**Jacque/Nigel Minton** *SBRC-Nottingham, UK*	Welcome
09.05 – 09:35	**Rolf Thauer** *Max Plank Institute, Germany*	How Acetogens Form Ethanol when Growing on Syngas
09.35 – 10.05	**Sean Simpson** *LanzaTech Inc, USA*	Recycling Carbon: Gas fermentation Enables Recycling of Waste to Valuable Commodities
10:05 – 10:20	**Florence Annan** *SBRC-Nottingham, UK*	Using Synthetic Biology to fix the Pantothenate Pathway in two Gas Fermenting Species of Clostridia
10:.20 – 10:35	**Phillip Wright** *Newcastle University, UK*	Quantitative Proteomic Profiling of *Clostridium autoethanogenum* Strains (POC FUNDED)
10:35 - 10:50	**Peter Rowe** *SBRC-Nottingham, UK*	Developing a Modularized Toolset for CRISPR-Based Technologies in Acetogens
10:50 - 11:15	**Coffee/Tea Break**	**Hall A - East Midlands Conference Centre**
Session 2 - Chair: Arild Johannessen (Calysta, UK) - **Hall B - East Midlands Conference Centre**		
11:15 – 11:45	**Josh Silverman** *Calysta, USA*	Methane biotechnology: a C1 platform for production of value-added feed and chemicals
11:45 – 12:15	**Colin Murrell** *University of East Anglia, UK*	Catalytic and Metabolic Versatility in Methanotrophs
12:15 – 12:30	**Tom Smith** *Sheffield Hallam University, UK*	Bioremediation of Inorganic Pollutants by Methane-Oxidising Bacteria
12.30 – 12:45	**David Fell** *Oxford Brookes University, UK*	Metabolic Modelling to Support Synthetic Biology in C1net Organisms (POC FUNDED)
12:45 – 13:00	**Ying Zhang** *SBRC-Nottingham, UK*	Industrially-Driven Discovery of C1-utilising Microorganisms (POC FUNDED)
13:00 – 14:30	**Lunch**	**Hall A - East Midlands Conference Centre**
Session 3 - Chair: Sean Simpson (Lanzatech, USA) - **Hall B - East Midlands Conference Centre**		
14:30 – 15:00	**Miriam Rosenbaum** *RWTH Aachen University, Germany*	Characterizing and Advancing *Clostridium ljungdahlii* for Bioelectrochemical Applications
15:00 – 15:15	**Jasbir Singh** *Hel Ltd, UK*	Practical Approach to Flammability Hazard Problems in Gas Fermentation Research
15:15 – 15:30	**Valentina Mangiafridda** *Centre for Process Innovation, UK*	C1 Gas Fermentation Lab in CPI: Scale-Down Flexibility Integrated With Safety Operations
15:30 – 16:00	**Richard Dinsdale** *University of South Wales, UK*	In-situ Multiphase Compartmentalised Substrate Shuttle Bioreactor for Protein and Platform Chemicals (POC FUNDED)
16:00 – 16:15	**Coffee/Tea Break**	**Hall A - East Midlands Conference Centre**
16:15 - 17:15	**PITCHES**	**Hall B - East Midlands Conference Centre** 15 x 3 minute pitches
17:15 – 19:00	**POSTERS/ Refreshments**	**Hall A - East Midlands Conference Centre** (Concurrent MB meeting - 18:00 – 19:30 - Suite 4 EMCC)
20:00	**Dinner**	**Hall B - East Midlands Conference Centre**

TUESDAY 26th July 2016		
Session 4 - Chair: William Gabrielli (Sasol UK Ltd) - **Hall B - East Midlands Conference Centre**		
09.00 – 09:30	**Christian Kennes** *University of La Coruña, Spain*	Bioconversion of CO-rich Syngas/Waste Gas to Higher Alcohols by *Clostridium* in Bioreactors
09.30 – 10:00	**Linsey Garcia-Gonzalez** *VITO NV, Belgium*	Valorization of CO2-rich Off-gases to Monomers and Polymers Through Biotechnological Process
10:00 – 10:15	**Mahendra Raut** *University of Sheffield, UK*	Optimizing low cost C1/C2 compound production and fermentation from biomass solid waste (POC FUNDED)
10:15– 10:30	**Reuben Carr** *Ingenza, UK*	Analysis of Sustainable Feedstock Options and Biotechnology Readiness for Developing Industrial Scale Fermentation to Produce Chemicals
10:30 – 10:45	**William Zimmerman** *University of Sheffield*	Microbubble Intensification of Bioprocessing: Bioreactor Acceleration and Downstream Separations
10:45 – 11:15	**Coffee/Tea Break**	**Hall A - East Midlands Conference Centre**
Session 5 - Chair: Edward Green (Chain Biotech, UK) - **Hall B - East Midlands Conference Centre**		
11:15 – 11:45	**Peter Dürre** *University of Um, Germany*	Autotrophic Acetogens: Genomics, Physiology, and Biotechnological Applications
11:45 – 12:15	**Stéphane Guillouet** *INSA de Toulouse, France*	Metabolic pathway engineering in *Cupriavidus necator* as platform for biofuel and chemicals production from CO_2
12:15 – 12:30	**Samantha Bryan** *SBRC-Nottingham, UK*	Engineering Improved Ethylene Production in *Cupriavidus necator*
12:30 – 12:45	**Katalin Kovacs** *SBRC-Nottingham, UK*	3-HP Production in *Cupriavidus necator*
12:45– 13:00	**Noah Mesfin** *Oxford Brookes University, UK*	Modelling Central Carbon Metabolism of *Acetobacterium woodii* DSM1030 Using a Genome-Scale Metabolic Model
13:00 – 14.30	**Lunch**	**Hall A - East Midlands Conference Centre**
Session 6 - Chair: Nigel Minton (SBRC-Nottingham, UK) - **Hall B - East Midlands Conference Centre**		
14:30 – 15.00	**Arren Bar-Even** *Max Planck Institute, Germany*	The Formate Bio-Economy Concept: Addressing Humanity's Grand Challenges
15:00 - 15:15	**Florian Oswald** *Karlsruhe Institute for Technology, Germany*	Upgrading the Toolbox For Fermentation of (Crude) Syngas: From Syngas to Malic Acid
15:15 – 15:30	**Bart Pander** *SBRC-Nottingham, UK*	Functional Carbonic Anhydrase of *Clostridium autoethanogenum*
15:30 – 15:45	**David Hodgson** *Durham University, UK*	Carbamate Trapping—A New Tool for Understanding Protein-CO2 Interactions
15:45 – 16:00	**Verena Kriechbaumer** *Oxford Brookes University, UK*	PMMO In Plants
16:00 – 16:10	**Nigel Minton** *SBRC-Nottingham, UK*	Wrap up and Next Steps
16:10 - 17:00	**Refreshments/ Depart**	**Hall A - East Midlands Conference Centre**

POC PRESENTATIONS

David Fell - Oral
POC-2-kierzek-C1net
Metabolic modelling to support synthetic biology in C1net organisms.
PI-Andrzej M. Kierzek, University of Surrey
6 months
£50K

Mahendra Raut - Oral
POC-3-wright-C1net
A proteomic approach to optimizing gas fermentation in industrially relevant acetogens
PI-Phillip Wright, University of Sheffield
6 months
£50K

Ying Zhang - Oral
POC-4-zhang-C1net
Industrially-driven discovery of C1-utilising microorganisms
PI- Ying Zhang, SBRC, Nottingham
12 months
£50K

Richard Dinsdale - Oral
POC-5-dinsdale-C1net
In-situ Multiphase Compartmentalised Substrate Shuttle Bioreactor for Protein and Platform Chemicals
PI-Richard Dinsdale, University of South Wales
6 months
£50K

Frank Sargent - Poster
POC-8-sargent-C1net
A Synthetic Approach to bioconversion of carbon dioxide to formic acid
PI-Frank Sargent, University of Dundee
6 months
£50K

Phillip Wright- Oral
POC-11-wright-C1net
Optimizing low cost C1/C2 compound production and fermentation from biomass solid waste
PI-Phillip Wright, University of Newcastle
6 months
£50K

ELEVATOR PITCHES (order of presentations)

1. Gary Burgess, Bio Dynamic Ltd, UK
Technical Director - AD and Biomethane Plant Engineering
gary.burgess@biodynamicuk.com

2. Alex Conradie, The University of Nottingham, UK
Professor - C1 continuous fermentation, Metabolic engineering, Process Control
alex.conradie@gmail.com

3. Andrew Crombie, University of East Anglia, UK
PDRA - Molecular Biology, Genetics of Methane Oxidising Bacteria
a.crombie@uea.ac.uk

4. Frederik De Bruyn, Bio Base Europe Pilot Plant, Belgium
Business Developer - Scale-up fermentation
Frederik.de.bruyn@bbeu.org

5. Marco Gianesella, Pera Technology, UK
Senior Energy Technologist - Energy, Environmental and Process Engineering
m.gianesella@peratechnology.com

6. Bernhard Guentner, Syngip BV, The Netherlands
CEO - Strain development, Metabolic Engineering, Gas fermentation
info@syngip.com

7. Gary Hayes, Hgen Cleantech, UK
Chairman - Methanation of Hydrogen
info@hgencleantech.com

8. Rob Howarth, The University of Nottingham, UK
Business Engagement Executive - Business Development
rob.howarth@nottingham.ac.uk

9. Naglis Malys, SBRC Nottingham, UK
Senior Research Fellow - Biochemistry, Systems and Synthetic Biology
Naglis.Malys@nottingham.ac.uk

10. Jon McKechnie, The University of Nottingham, UK
Lecturer - Techno-economic analysis, biochemical production of fuels and chemicals
jon.mckechnie@nottingham.ac.uk

11. David Ortega, SBRC Nottingham, UK
PhD student – Anaerobic organisms utilising C1 gases
stxdo4@nottingham.ac.uk

12. Mahendra Raut, University of Sheffield, UK
Postdoctoral Researcher - Microbiology, Proteomics, Metabolomics
m.raut@sheffield.ac.uk

13. Alba Serna Maza, University of Southampton, UK
Research fellow - Anaerobic digestion, Membrane bioreactors, Biogas upgrading
asm3g10@soton.ac.uk

14. Thomas Smith, Sheffield Hallam University, UK
Professor - Methanotrophs , Enzymology, Biotechnology
t.j.smith@shu.ac.uk

15. William Zimmerman, University of Sheffield, UK
Professor - Fluid and microbubble dynamics, Bioreactor acceleration and downstream separations
w.zimmerman@shef.ac.uk

6. Bernhard Guentner, Syngip BV, The Netherlands
CEO - Strain development, Metabolic Engineering, Gas fermentation
info@syngip.com

POSTERS (order of presentations)

1. Swathi Alagesan, SBRC Nottingham, UK
Construction and Evaluation of a RBS Library for *Cupriavidus necator* H16

2. Saul Alonso Tuero & Valentina Mangiafridda, Centre for Process Innovation
C1 Gas Fermentation Lab in CPI: Scale-Down Flexibility Integrated With Safety Operations

3. Christian Arenas, SBRC Nottingham, UK
Engineering the Production of 3-Hydroxypropionic Acid Via Malonyl-CoA Pathway in *Cupriavidus necator* H16

4. Ronja Breitkopf, SBRC Nottingham, UK
Succinate Fermentation in *Clostridium autoethanogenum*

5. Frederik De Bruyn, Bio Base Europe Pilot Plant, Belgium
A key role for shared pilot facility to deploy gas fermentation

6. Muhammad Ehsaan, SBRC Nottingham, UK
Modular Vectors For *Cupriavidus necator* H16 and Generation of Restriction Negative Strain

7. Mohamed El-Esawi Universities of Tanta, Egypt and Nottingham, UK
Biotechnological Applications of Microorganisms Growing on C1 Compounds

8. Marco Garavaglia, SBRC Nottingham, UK
Identification of Conditionally Essential Genes in *Cupriavidus necator* H16 Using TraDIS

9. Satbir Gill, Ingenza, UK
Novel Industrial Exploitation of Methanotrophic Pathways

10. Anne Henstra, SBRC Nottingham, UK
GASCHEM: Optimising C1 gas fermentation by the acetogen *Clostridium autoethanogenum*

11. Rahul Kapoore, The University of Sheffield, UK
Photoautotrophic Carbon Uptake by Microalgae for Biotransformations

12. Naglis Malys, SBRC Nottingham, UK
Assembly and quantitative evaluation of genetic elements for controlling gene expression in *Cupriavidus necator*: combinatorial engineering for chemical production

13. Noah Mesfin, Oxford Brookes University, UK
Modelling Central Carbon Metabolism of *Acetobacterium woodii* DSM1030 Using a Genome-Scale Metabolic Model

14. James Murray, Imperial College London, UK
Isolating Novel Carboxydotrophs

15. Rupert Norman, SBRC Nottingham, UK
Constructing a Genome Scale Metabolic Model of *Clostridium autoethanogenum* for Metabolic Engineering

16. Rajan Patel, SBRC Nottingham, UK
Engineering Synthetic RNA Devices to Expediate the Evolution of Metabolite Producing Microorganisms

17. Nicole Pearcy, SBRC Nottingham, UK
Identification of Target Knock Outs in *Cupriavidus necator* using Elementary Modes Analysis

18. Dhivya Puri, Fiberight, UK
Clean Gas fermentation Feedstocks from MSW

19. Frank Sargent, University of Dundee, UK
A Synthetic Approach to Bioconversion of Carbon Dioxide to Formic Acid

20. Frederik Walter, SBRC Nottingham, UK
Production of β-alanine as a precursor of 3-hydroxypropionate in *Cupriavidus necator* H16

21. Alex Wichlacz, SBRC Nottingham, UK
Shifting the balance: Metabolic pathway analysis and inhibitor design in *Clostridium autoethanogenum*

22. Craig Woods, SBRC Nottingham, UK
Transposon Mutagenesis of *C. autoethanogenum*

23. Louise Dynes, SBRC Nottingham, UK
C1net Outreach Activities

INVITED SPEAKERS

Professor Rudolf Thauer

Max Planck Institute, Germany

Professor Thauer is a biologist and a retired professor of microbiology and heads the Emeritus group at the Max Planck Institute for Terrestrial Microbiology in Marburg. Professor Thauer taught in the faculty of Biology at the Philipps University in Marburg (Germany) for about 15 years and is known primarily for his work on the biochemistry of methanogens.

He received the Gottfried Wilhelm Leibniz Prize by the Deutsche Forschungsgemeinschaft in 1986, among numerous other honours including honorary doctorates from ETH Zurich, University of Waterloo and the University of Freiberg. In 1991 he became founding director of the Max Planck Institute for Terrestrial Microbiology in Marburg.

Dr Sean Simpson

LanzaTech, USA

Dr. Simpson is Co-founder and CEO of LanzaTech, a global leader in gas fermentation that has grown from a team of 3 in 2005 to a global company with entities in the US, UK, NZ, China, and India. Under Dr. Simpson's leadership, the company has established a broad and unique patent portfolio covering all areas of gas fermentation, including fermentation processes & microbes, gaseous feedstock handling, & product and waste handling. LanzaTech is experienced in technology commercialization, with pre-commercial demonstration plants operating in China and Taiwan and commercial plants in development. LanzaTech has had and continues to maintain successful long-term relationships with academic partners, including the Universities of Nottingham and Ulm. Dr. Simpson has received many awards including: the US Environmental Protection Agency (EPA) Presidential Green Chemistry Award (2015), the Sanitarium, NZ Innovator of the Year Award (2014), the Kea NZ World Class New Zealander in Science Award (2013), the Bio Spectrum Asia-Pacific Entrepreneur of the Year Award, the NZBIO Young Biotechnologist of the Year (2011) and the Ernst and Young Entrepreneur of the Year, New Zealand (2011).

Dr Josh Silverman

Calysta, USA

Dr Silverman is co-Founder and Chief Product and Innovation Officer of Calysta Inc. He is an experienced biotechnology entrepreneur with a history of successful development of novel technology platforms. Prior to founding Calysta he held positions at two public biotechnology companies, Maxygen, Inc. and Amgen, Inc. In addition, he has been involved in the founding and financing of five startup biotechnology companies (Avidia, Versartis, Diartis, Siluria, and Calysta). Dr. Silverman is generally interested in tools and applications of biological engineering as a robust and predictable engineering discipline. He is an advisor for several biotechnology companies and is a frequent speaker at scientific meetings. He has been directly involved in bringing three novel biopharmaceuticals from early phase research into clinical trials, with experience in both regulatory filings and drug manufacturing. He is an author on more than 10 scientific publications as well as over 30 patents and pending patent applications.

Dr Colin Murrell

University of East Anglia, UK

Dr Murrell is Professor in Microbiology in the School of Environmental Sciences and Director of the Earth and Life Systems Alliance at the University of East Anglia. He has wide ranging research interests centred round the bacterial metabolism of methane and other one carbon compounds. He has pioneered work on the physiology, biochemistry, molecular genetics and ecology of methanotrophs, encompassing regulation of gene expression by metals, microbial genomics, metagenomics, bioremediation, biocatalysis and industrial biotechnology. His research over the past 35 years has resulted in ~300 publications and six edited books. Dr Murrell is member of the Editorial Boards of Environmental Microbiology and The ISME Journal, and has Chaired Gordon Research Conferences on C1 Metabolism a& Applied and Environmental Microbiology. He is President-Elect of The International Society for Microbial Ecology, Member of the European Molecular Biology Organisation & Member of the European Academy of Microbiology & has been awarded an ERC Advanced grant of €2.5M to work on isoprene metabolism.

Professor Miriam A. Rosenbaum

RWTH Aachen University, Germany

Prof. Rosenbaum is a Junior professor at the Institute of Applied Microbiology at RWTH Aachen University. After studies in Biochemistry and a PhD in Environmental Chemistry from Greifswald University in 2006, Dr. Rosenbaum joined the group of Prof. Lars Angenent as a postdoc at Washington University in St. Louis and later as a research associate at Cornell University (from 2007-2011). The work in the Rosenbaum Lab is focused around bioelectrochemical systems and defined microbial mixed cultures. Her research interests are the investigation, understanding & manipulation of microorganisms in electrochemical interaction with electrodes and of inter-microbial relationships in these environments. While the overall goal is the development and advancement of new biotechnological applications based on bioelectrochemical systems, her group seeks to uncover the biochemical and physiological principles of the microbial systems they use.

Professor Christian Kennes

University of La Coruña, Spain

Professor Kennes is Professor of Chemical Engineering at the University of La Coruña in Spain. He undertook his Engineering studies in Brussels and did his PhD research both in Belgium and in France, being awarded a PhD-fellowship from the EU. His present research activities are largely related to environmental biotechnology and bioprocess engineering applied to biodegradation processes, biotransformation of pollutants to useful products, fermentation technology, waste gas treatment, and wastewater treatment. He has worked on several books focussing on biodegradation, bioconversion and gas-phase bioreactors and has given several keynote lectures on such topics. He is member of the Editorial Board of several SCI Journals, *e.g*. Journal of Chemical Technology & Biotechnology, Environmental Technology, Biofuel Research Journal. Over the past ten years, he has been the main organizer of the biennial International Conference on Biotechniques for Air Pollution Control and Bioenergy.

Dr Linsey Garcia-Gonzalez

Vito NV, Belgium

Dr. Linsey Garcia-Gonzalez holds a PhD in Bioscience Engineering and works as a senior scientist at the Flemish Institute for Technological Research (VITO). She is an expert in CO_2 fermentations and biocatalysis. She has extensive experience in the execution, management and coordination of multidisciplinary and multi-partner projects. She recently conducted a study for the Flanders Regional Government to assess the impact of Carbon Capture and Utilisation (CCU) on economic, technological and environmental level and to stimulate CCU in Flanders. She is part of the SCOT (Smart CO_2 Transformation) community and is member of the CO2CHEM and C1NET network. She is co-author of 18 SCI publications, 2 book chapters and 1 patent application.

Professor Peter Dürre

University of Ulm, Germany

Professor Dürre has more than 30 years' experience in the field of clostridial genetics. Following his PhD at the University of Göttingen, Germany, he did his postdoc at the University of California, Berkeley. He is currently Professor of Microbiology, Head of the Microbiology and Biotechnology Department, and Dean of the Faculty of Natural Sciences at Ulm University in Germany. Major research projects focus on regulation of acetone and butanol formation in *Clostridium acetobutylicum* and cell differentiation (spore formation) in clostridia. "omics"- technologies and system biology approaches are used to elucidate these complex metabolic networks. Several genomes of solventogenic clostridia have been sequenced. As both solvents are important feedstocks for the chemical industry, metabolic engineering of solvent-producing strains for industrial use is another important area of interest. Other projects are focused on medical issues. One aims at construction and application of clostridial recombinant endospores for cancer treatment, the other at identification of acne-causing enzymes in *Propionibacterium acnes* for selective inhibition and disease therapy. The genome of *P. acnes* has been sequenced.

Professor Stéphane Guillouet

INSA de Toulouse, France

Dr Stéphane Guillouet, Professor at the Engineer School INSA in Toulouse, is team leader of the Fermentation Advances and Microbial Engineering research team (30 people) at the Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés (LISBP, Toulouse, France). He was graduated in Industrial Microbiology at the Engineer School of the University of Marseille in 1989. He received his PhD in Biotechnology and Food Industry in 1996 at the Laboratoire des Sciences du Génie Chimique (INPL-Nancy). He then specialized in metabolic engineering at the Massachusetts Institute of Technology (MA, USA) for 3 years. He is currently developing his research activity at the LISBP for improving microbial performances within intensive bioprocesses through the modulation of the metabolic potentialities.

Dr Arren Bar Even

Max Planck Institute, Germany

Dr Bar-Even completed his Bachelor degree at the excellence program of the Technion, the Israeli Institute of Technology. As a Master's student in Bioinformatics at the Weizmann Institute of Science, he studied noise in gene expression. He then spent four years in the biotech industry, developing insect repellent agents for agriculture. Returning to academy to complete his PhD, he researched the design principles of cellular metabolism at the lab of Prof. Ron Milo at the Weizmann Institute of Science. In 2014 he opened his own lab at the Max Planck Institute of Molecular Plant physiology, conducting research on "Systems and Synthetic Metabolism".

ABSTRACTS OF ORAL PRESENTATIONS

How Acetogens Form Ethanol When Growing On Syngas

ROLF THAUER

Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse 10, D-35043 Marburg, Germany

Most acetogens can grow on H_2 and CO_2 or CO and CO_2 forming acetic acid as primary end product in the Wood-Ljungdahl pathway. Some acetogens can reduce the acetic acid further to ethanol involving ferredoxin-dependent carboxylate reductases (aldehyde:ferredoxin oxidoreductases). The energetics and kinetics of carboxylate reduction will be discussed.

Mock, J., Zheng, Y., Mueller, A. P., Ly, S., Tran, L., Segovia, S., Nagaraju, S., Köpke M., Dürre, P., and Thauer, R. K. (2015) Energy conservation associated with ethanol formation from H_2 and CO_2 in *Clostridium autoethanogenum* involving electron bifurcation. Journal of Bacteriology 197, 2965-2980.

Recycling Carbon: Gas Fermentation Enables Recycling Of Waste To Valuable Commodities

SEAN D. SIMPSON

LanzaTech Inc. 8045 Lamon Ave, Suite 400, Skokie, IL 60077. USA

LanzaTech has developed novel gas fermentation technology that captures and utilizes greenhouse gases. In contrast to traditional fermentation that use sugars as a substrate, gas fermentation utilizes C1 substrates such as carbon monoxide (CO) or CO_2. This enables access to a diverse range of feedstocks including industrial waste gases (e.g., steel mills) or syngas (e.g. generated from agricultural waste or municipal solid waste).

At the heart of the gas fermentation process is an acetogenic microbe capable of autotrophic growth due to unique redox chemistry and energy conservation mechanisms that do not exist in traditional hosts. Until recently, acetogenic organisms were considered genetically inaccessible, limiting their use as chassis organism for production of fuels and commodities. LanzaTech has been able to overcome these challenges and have developed a complete genetic toolbox for acetogens comprising genome editing tools, an extensive genetic parts library and automated strain engineering workflows.

Driven by systems biology characterizations, a genome scale model and a range of design tools and for acetogens were developed in order to accelerate strain engineering and adapt pathways developed and optimized in traditional hosts such as *E. coli* or yeast. These tools have been validated against hundreds of steady state fermentation runs. Using this platform, production of over 25 new molecules have been demonstrated from gas, in several cases exceeding the production rates and yields of native producers utilizing sugars.

Proprietary, scalable reactor designs and optimized process chemistry, ensure efficient, continuous, single-pass gas conversion with a high selectivity to the product of interest. The process has been successfully scaled up from the laboratory bench through in-lab and in-field pilot plants to fully integrated 100,000-gallon/year pre-commercial demonstration plants with a total of over 40,000 hours on stream. Currently, LanzaTech is constructing first commercial plants with partner ArcelorMittal in Belgium, Shougang Steel in China and China Steel in Taiwan.

Using Synthetic Biology to fix the Pantothenate Pathway in two Gas Fermenting Species of Clostridia

FLORENCE J. ANNAN

BBSRC / EPSRC Synthetic Biology Research Centre, The University of Nottingham, University Park, Nottingham, NG7 2RD

Clostridium autoethanogenum and *Clostridium ljungdahlii* are two industrially relevant gas fermenting anaerobic acetogens, which can use the wood-ljungdahl pathway and the mixed acid fermentation pathway to convert two greenhouse gases (carbon monoxide and carbon dioxide) into a variety of useful bulk and fine chemicals, traditionally derived from non-renewable resources. To increase the industrial attractiveness of producing chemicals via this route, the process has to be as economical as possible. One way to increase the economic viability of this process is to reduce the input costs. One of the facets of the process where costs can be reduced is in the media contents. It is important to characterise the essential vitamins required for growth as these can affect costs. Three vitamins which have been suggested to be essential are thiamine, biotin and pantothenate. The more efficient the media, the more economical the process will be and therefore will become more attractive as a mechanism for the sustainable production of the platform chemical than from fossil fuels leading us one step closer to decoupling our civilisation from oil.

Pantothenate (B5) is an essential nutrient from the B class of vitamins used in the synthesis of Co enzyme A. Here, using a mix of classical microbiological techniques we show that the two species are auxotrophic for the production of pantothenate and that pantothenate is an essential vitamin for the growth and viability of these two species. Using synthetic biology we attempt to “fix” this problem by determining which genes are essential in the pathway and introducing three “missing” genes into the two species in order to create two autotrophic strains.

-

Quantitative Proteomic Profiling of *Clostridium autoethanogenum* Strains

PHILLIP C WRIGHT

School of Chemical Engineering and Advanced Materials, Faculty of Science, Agriculture & Engineering, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK

Production of commodity chemicals and fuels via C1 gas fermentation is an emerging field in biofuel research. Industrially relevant gas fermenting acetogens such as *Clostridium autoethanogenum* have immense potential to utilise waste gases (CO and CO_2) without competing with food feedstocks. This thus represents an extremely versatile biological platform for the sustainable production of fuels (e.g. 2,3-butanediol and butanol and ethanol).

Although, characterising these microbes at the genomic and metabolomics levels provides new insights, the integrated proteomics approach can reveal further vital information about differential regulation of pathways and a more detailed understanding of biology in different stress conditions.

Therefore, we studied the effect of ethanol stress condition on the proteome of *Clostridium autoethanogenum* DSM10061. In total, 228 proteins were quantified as differentially regulated proteins during ethanol stress conditions (out of a total of 1040 quantified proteins with at least 2 unique peptides). Major metabolic pathways such as Wood-Ljungdahl, fermentation and TCA cycle were down regulated under ethanol stress conditions. Five alcohol dehydrogenases were significantly down regulated under ethanol stress and we concluded that ethanol induces feedback inhibition in this bacterium. The cellular responses of *C. autoethanogenum* to ethanol at metabolic and physiological levels possibly suggest targeted metabolic modeling and strain development to optimize biofuel production from waste gases by overcoming limitations imposed by the presence of ethanol.

Our current research is focused on studying the proteome of a re-sequenced strain of *C. autoethanogenum* DSM10061 under pH shift conditions at different CO concentrations. This ought to reveal major changes in metabolic pathways.

Developing a Modularized Toolset for CRISPR-based Technologies in *Clostridium autoethanogenum*

PETER ROWE[1], CHRIS HUMPHREYS[1], HE HUANG[2], LI QI[2], ANNE HENSTRA[1], KLAUS WINZER[1], YANG GU[2], NIGEL P. MINTON[1]

[1] *BBSRC / EPSRC Synthetic Biology Research Centre, The University of Nottingham, University Park, Nottingham, NG7 2RD*
[2]*Key Laboratory of Synthetic Biology, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences , Shanghai 200032, China*.

Clostridium autoethanogenum is an acetogen capable of producing ethanol, 2,3-butanediol and acetate from a syngas feedstock. This natural ability to convert gas to commodity chemicals has been utilised industrially, however, to date metabolic engineering for optimisation of this process has been constrained by drawbacks in genome editing technology.

In order to predict how carbon flux can be redirected to increase solvent production, the BBSRC sLoLa project GASCHEM has developed a genome scale model using data from batch and bioreactor fermentation. This model has provided many potential targets for strain optimization, although analysis of these targets has been limited by poor DNA transfer efficiency and genome editing tools in *C. autoethanogenum*. Both these issues have been addressed through the use of a novel conjugative donor strain to improve DNA transfer, which in turn enabled the use of CRISPR-Cas9 based tools.

As reported across a broad range of organisms, implementation of CRISPR-based technology has led to a major advancement in the speed and accuracy of mutant generation. In a recent publication we have shown the first instance of the application of such technology in an acetogen, *Clostridium ljungdahlii* [Huang H, Chai C, Li N, Rowe P, Minton NP, Yang S, Jiang W, Gu Y. ACS Synth Biol. 2016 Jun 15. DOI: 10.1021/acssynbio.6b00044]. Further to this, we also report the first use of CRISPR-Cas9 in *C. autoethanogenum*, with far faster mutant generation compared to conventional methods (ClosTron™ and Allelic Exchange). In order to achieve this, we have expanded the standardised pMTL80000 vector series, providing a new range of CRISPR modules enabling fast, efficient plasmid generation for many applications across the whole *Clostridium* genus.

Methane Biotechnology: A C1 Platform For Production Of Value-Added Feed And Chemicals

JOSH SILVERMAN

Calysta, Menlo Park, CA 94025, United States

Important progress has recently been made toward engineering a number of phototrophic, heterotrophic, and fermentative microorganisms for production of bio-based products, including protein for animal feeds. While phototrophic algae systems appear to have some advantages, scale-up to commercial production is currently challenging. Fermentation approaches are better characterized, but several limitations, most notably the increasing cost of sugar feedstocks, currently prevent the economical production of products from such systems. Exploiting alternative carbon feedstocks which are relatively inexpensive and domestically abundant represents an attractive alternative to more traditional systems.

One of the most abundant domestic carbon feedstocks is methane, sourced primarily from natural gas. Methane is the second most abundant carbon gas in the atmosphere and contributes >30x higher greenhouse gas effect per ton relative to CO_2. This implies that capturing methane sources will have a significant environmental benefit. Longer term, biomass-to-methane strategies may eventually enable a fully renewable carbon cycle if ‘green’ methane-based technologies as described in this proposal are developed. Current ongoing projects for methanotrophs include production of single cell protein, lactic acid, and omega-3 fatty acids from methane.

Catalytic And Metabolic Versatility In Methanotrophs

[1]ANDREW CROMBIE, [2]TOM SMITH & [1]J. COLIN MURRELL

[1]School of Environmental Sciences, University of East Anglia, Norwich, UK;
[2]Biomolecular Sciences Research Centre, Sheffield Hallam University, Sheffield, UK

Methane oxidation by methane oxidising bacteria (methanotrophs) is carried out by methane monooxygenase enzymes. The soluble methane monooxygenase (sMMO) is a diiron centre monooxygenase found in some but not all methanotrophs. It is an extremely versatile biocatalyst and although the natural substrate conversion is methane to methanol, sMMO catalyses the oxidation of well over 100 alkanes, alkenes and aromatic compounds, including halogenated compounds such as trichloroethene. Many years of development of genetic techniques for methanotrophs have enabled us to carry out site directed mutagenesis on the active site of sMMO to explore its catalytic utility and potential as an industrial biocatalyst.

Most methanotrophs grow only on methane as their sole carbon and energy source. Our studies on the facultative methanotroph *Methylocella silvestris* have shown that this unusual methanotroph can grow on a number of multi-carbon compounds including propane, another component of natural (thermogenically-derived) gas. *M. silvestris* contains sMMO and another soluble diiron centre monooxygenase called propane monooxygenase which enables it to grow on propane. We have elucidated the pathways of methane and propane metabolism in *M. silvestris* and studied their regulation using a combination of molecular physiology, biochemistry, genetic and postgenomic technologies. This has given insights into its potential competitiveness over other methanotrophs that only grow on methane in environments such as natural gas seeps where several gaseous alkanes are available as carbon and energy sources and this is now being explored in detail. The potential of *M. silvestris* and other facultative methanotrophs for use in biotechnology is also being examined through funding from The Leverhulme Trust.

Bioremediation Of Inorganic Pollutants By Methane-Oxidising Bacteria

THOMAS J. SMITH, ABDURRAHMAN ESWAYAH, SWAPNIKA CHALLA, SALAHELDEEN ENBAYA AND PHILIP H. E. GARDINER

Biomolecular Sciences Research Centre, Sheffield Hallam University, Sheffield S1 1WB.

Aerobic methane-oxidising bacteria are diverse and widespread in the environment and have been explored for their capacity as versatile biocatalysts for transformation of a wide range of hydrophobic organic molecules for bioremediation and synthetic organic chemistry. More recently, it has emerged that methanotrophs have substantial potential for bioremediation of inorganic pollutants. Here we demonstrate that laboratory strains and environmental isolates of methanotrophic bacteria are able to reduce hexavalent chromium to the less toxic and less bioavailable trivalent form, using methane as the source of electrons for the reduction. Similarly, methanotrophs are able to reduce toxic selenite (SeO_3^{2-}) to elemental selenium and, in certain instances, methylated selenium species. The former reaction, which is also dependent on methane, offers a new way to make nanoparticulate selenium with possible technological uses.

Metabolic Modelling To Support Synthetic Biology In C1net Organisms

DAVID FELL[1], MARK POOLMAN[1], ANDRZEJ KIERZEK[2], MEHDI KHOURY[2]

[1]Biological & Medical Sciences, Oxford Brookes University, Oxford OX3 0BP, UK;
[2]Microbial & Cellular Sciences, University of Surrey, Guildford GU2 7XH, UK

The aim of this Proof of Concept project was to create a genome-scale metabolic model of a C1 gas fermenting microorganism and demonstrate the applicability of predictive modeling to the design of new strains of interest to C1Net. We selected the methanotroph *Methylococcus capsulatus* (Bath) as the target organism, though in parallel we provided advice and guidance to the University of Nottingham in the construction of a model of *Clostridium autoethanogenum*. The draft model of *M. capsulatus* has been completed starting from the gene-enzyme-reaction mapping in BioCyc. It is fully mass-balanced for C, N, S and P and accounts for 965 reactions and 896 internal metabolites. The 63 external metabolites include 38 biomass precursors, and the rest are nutrients and metabolic products. Flux balance analysis of the model confirms that it is energetically consistent, in that it cannot show production of ATP or reduced cofactors in the absence of mass flow. The model can account for aerobic oxidation of substrates including glucose and methane to CO_2, and anaerobic conversion of glucose to acetate and CO_2, as well as production of biomass precursors (i.e. growth). Other products that can be formed form both glucose and methane include pyruvate, ethanol and succinate, and nitrate can be used as an oxidant. The model was initially generated with the ScrumPy package, and is available for download from http://mudshark.brookes.ac.uk both as a ScrumPy source file (a human-readable text file) and SBML format.

Industrially-Driven Discovery of C1-Utilising Microorganisms

YING ZHANG

BBSRC / EPSRC Synthetic Biology Research Centre, The University of Nottingham, University Park, Nottingham, NG7 2RD

The conversion of non-food, cellulosic feedstock to chemicals and fuels is proving challenging, and new solutions need to be found. Waste gases such as Syngas from steel mills (which contains carbon dioxide and carbon monoxide) and methane from anaerobic digestion and landfill are promising alternatives to the biomass required for the production of second generation biofuels and chemicals.

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.

In this short project, we aim to isolate new strains or species that can utilise C1 gases in the production of industrially-important chemicals. A number of methane consuming bacteria have been isolated from environmental samples and have been partially characterized.

Characterizing And Advancing *Clostridium Ljungdahlii* For Bioelectrochemical Applications

THOMAS M. KIRCHNER, RONNY UHLIG, MIRIAM A. ROSENBAUM

Institute of Applied Microbiology, RWTH Aachen University, 52074 Aachen, Germany

Cathodic microbial electro-transformations of CO_2 or target substrates to yield desired products is a highly fascinating but challenging research topic. In recent years, the capacity for cathodic electro-transformation of CO_2 has been claimed especially for several homoacetogenic bacteria, one of them being *Clostridium ljungdahlii*. We performed an extensive characterization of the cathodic performance of *C. ljungdahlii* and developed tools for its genetic modification for future target cathodic electro-transformations. Our results indicate that hydrogen plays a central role in electrode-to-microbe electron transfer, even when no electrocatalytic hydrogen evolution under abiotic conditions is observed. The local pH regime at the electrode interface and the presence of biological material at the electrode thereby play an important role. In N_2/CO_2-flushed bioelectrochemical systems, significant cell growth and acetate formation was observed at an applied electrode potential of ~700 mV vs. SHE, which allowed for abiotic and biotic hydrogen evolution. If this electrode potential was increased to -360 mV vs. SHE no cell growth, acetate consumption instead of formation, and no abiotic hydrogen evolution was observed, while low levels of biotic hydrogen evolution persisted. C13-labeling experiments and abiotic tests show that careful controls have to be included to understand the bioelectrochemical processes proceeding at these low redox potentials and low rates. Nevertheless, we see potential for the application of *C. ljungdahlii* in bioelectrochemical transformations and this presentation will also briefly summarize our efforts to a genetic advancement of this organism for future bioproductions.

Practical Approach To Flammability Hazard Problems In Gas Fermentation Research

JASBIR SINGH AND REHAN SHAH

Hazard Evaluation Laboratory Ltd, 9-10 Capital Business Park, Borehamwood, Hertfordshire, WD6 1GW

Gas fermentation involves the use of gases such as hydrogen and methane mixed with air or oxygen and this represents an explosive cocktail that is leading to much apprehension among research groups contemplating experimentation in conventional laboratory fume hoods.

This talk will present the main safety concerns in simple terms that can be readily understood by non-experts and then describe the range of options that are available for overcoming the problems such that research can be conducted without the need for explosion bunkers or other expensive modifications to common laboratories. The material will be drawn from accepted practice in chemical and petrochemical research laboratories where the use of flammable gases is common, even though the operating conditions (pressure and temperature) are much more extreme that for fermentation and related bio-processes.

The presentation will include an outline of the severity of potential hazards, the technical parameters that determine the severity and the surprising difference in hazard between the uses of air as opposed to pure oxygen. This will be followed by a description of the engineering solutions available that will enable research to be carried out safely, ranging from the importance of different equipment types, the role of proper controls and trips and the importance (and scope for) inventory limitation as a means of limiting the worst case scenario. Critical to achieving these objectives is the instrumentation and control system which runs the gas fermentation reactions.

The objective is to show that gas fermentation can be carried out safely and without excessive infrastructure cost and especially so if the control and instrumentation is selected at the outset.

C1 Gas Fermentation Lab In CPI: Scale-Down Flexibility Integrated With Safety Operations

VALENTINA MANGIAFRIDDA, SAUL ALONSO TUERO

Industrial Biotechnology and Biorefinery, Centre for Process Innovation, Wilton Centre, Wilton, TS10 4RF

The new C1 gas fermentation development laboratory is designed to perform fermentation process development work from 1 to 10L scale in a highly contained environment, offering an high cutting-edge facility to underpin the development of bio-based processes.

There is a gap identified by UK Government in gas fermentation development and as such this facility provides an opportunity to bring to the fore the economic, environmental and societal benefits of this technology. The aim of the laboratory is to provide industry and academia with a facility to test novel microorganisms, feedstock's and processes to gain process knowledge, while evaluating techno-economics and understanding scalability issues as a means of risk mitigation along the road to commercialisation.

The C1 Gas Fermentation laboratory in CPI addresses the commercial and environmental opportunity to utilise gaseous feedstocks in the production of bio-products by gas fermentation. Waste gasses such as CO2 from industrial activity and CH4 from landfill contribute to Greenhouse Gasses emissions. However, these waste streams together with syngas produced from gasification and pyrolysis of solid waste can be potential third generation feedstock's for bio-based production of chemicals and biofuels.

In order to fulfil such purpose and to offer the necessary flexibility of operation, the facility provides a broad range of bioreactors to perform batch, fed-batch and continuous operations along with the ability to undertake scale-down parallel DoE based fermentation optimisation approaches.

Central to the delivery of a safe operating environment for the use of hazardous gases was the design and construction of extracted cabinets into which fermentation equipment can be sited. A specific overview of the safety measure in place at the C1 Gas Fermentation lab in CPI will be given along with a parallel comparison with a recent explosion happened in a C1 Gas Fermentation Lab at the University of Manoa.

In-Situ Multiphase Compartmentalised Substrate Shuttle Bioreactor For Protein And Platform Chemicals

RICHARD M DINSDALE, ALAN J GUWY, GIULIANO PREMIER, JAIME MASSANET-NICOLAU, KATRIN FRADLER and AMANDEEP KAUR

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

There is significant interest in the utilization of biotechnology platforms for the production of “green” or sustainable chemicals which are not dependent of high grade organic substrates which compete with the production of food. One promising avenue is to use hydrogen and carbon dioxide based microbial metabolisms, as this route would be independent of the traditional energy and carbon supplying substrates such as molasses which can be used a human foodstuff. Hydrogen also can be produced electrolytically from renewable energy sources which have variable energy output such as wind power and thus could be used in areas where wind output is grid constrained for electricity production.

A bioreactor design which could produce a range of products including single cell protein (SCP), lipids and bioplastics, from aerobic microorganisms with hydrogen as the primary energy substrate without hydrogen mixing with oxygen to produce an explosive gas mixture has been designed and implemented. The first reactor compartment produces acetate from hydrogen and methane, the acetate is then extracted by membrane extraction and used for single cell protein and lipid production in a separate reactor

The acetate is currently being produced at a rate of 1.5 g/l/day from a hydrogen/carbon dioxide flow of 12 mls per minute hydrogen and 3 mls per minute of carbon dioxide. A feeding on demand system was implemented but a continuous flow based system with gas recycling gave better operational results. The in-reactor concentration was 13-15 g/l acetate.

The acetate utilizing process has been operated with pure cultures of *Cryptococcus curvatus* for lipid production and *Candida utilis* for single cell protein production. For single cell protein production 20% conversion efficiency of acetate to single cell protein has been determined.

Bioconversion Of CO-Rich Syngas/Waste Gas To Higher Alcohols By *Clostridium* In Bioreactors

CHRISTIAN KENNES

Environmental and Bioengineering Group, Centre for Advanced Scientific Research, Chemical Engineering Laboratory, University of La Coruña, E-15008-La Coruña, Spain

Several clostridial species are able to convert CO and CO_2:H_2, as well as mixtures thereof into organic acids (*e.g*., acetic acid, butyric acid). A few *Clostridium* spp. have also been shown to convert such gases into alcohols such ethanol; and more recently into higher alcohols such as butanol. Generally, bioconversion takes place in two stages, with production of organic acids first, followed by their subsequent bioconversion to alcohols.

Some of our recent studies focus on the production of ethanol and higher alcohols by *Clostridium carboxidivorans* in bioreactors. The wild type strain was shown to be able to produce mixtures of ethanol, butanol and also hexanol with either pure CO or mixtures of CO:CO_2:H_2 as substrates. Organic acids are produced first, *i.e.* acetic, butyric and hexanoic acids, followed by their conversion to ethanol, butanol and hexanol. A pH drop, resulting from acidogenesis, has been claimed to be required in order to stimulate solventogenesis in the ABE fermentation process in *Clostridium acetobutylicum* grown on sugars. However, the conversion of organic acids into alcohols by *Clostridium carboxidivorans* grown on CO-rich gases appears to take place both at high (slightly acidic) as well as low pH, although somewhat higher rates were found at lower pH. Although only few reports are available so far in the literature, concentrations of higher alcohols in those reports were generally well below 1 g/L for butanol and still lower for hexanol. In our first optimization studies, the wild type strain was able to simultaneously produce ethanol, as well as up to 2.4 g/L butanol and somewhat more than 1 g/L hexanol in a stirred tank bioreactor, although further improvements are needed in terms of cost-effectiveness. Similarly as in the ABE fermentation, high concentrations of alcohols end-up inhibiting the microbial activity. Inhibitory levels for ethanol and butanol in CO bioconversion were found to be of a similar order of magnitude as in the ABE process.

Valorization Of CO_2-Rich Off-Gases To Monomers And Polymers Through Biotechnological Process

LINSEY GARCIA-GONZALEZ, HELEEN DE WEVER

Flemish Institute for Technological Research, Separation and Conversion Technology, Boeretang 200, 2400 Mol, Belgium

Carbon dioxide or CO_2 is considered to be the major cause of climate change by its accumulation in the atmosphere and its greenhouse properties. To counteract climate change, most research focused in the past on Carbon Capture and Storage (CCS). Nowadays it is recognized that rather than just storing it, emitted CO_2 can be a valuable source of carbon for the production of commercially valuable products. This Carbon Capture and Utilization (CCU) approach provides much needed additional capacity in the move towards a low carbon economy. Clearly, CO_2 is the ultimate sustainable resource, available everywhere, in unlimited quantities, and forever.

This presentation focuses on the use of CO_2 as feedstock for the synthesis of biopolymers using biotechnology as core process. Two utilization forms will be discussed. VITO's activities in this area will be presented with results of selected projects and activities.

First, CO_2 can be utilized as renewable carbon source for the direct production of biopolymers via fermentation. As a case study, the use of *Cupriavidus necator* for the sustainable production of the biopolymer polyhydroxyalkanoate from CO_2 will be discussed. Test work encompassed optimizations with mock-up gas mixtures and real CO_2-containing off-gas. Biopolymers can also be produced in an indirect manner from CO_2 through the synthesis of chemical building blocks from CO_2. The conversion of CO_2 to different organic acids (such as acetic acid, lactic acid and succinic acid) was evaluated using either bacteria or enzymes as biocatalysts. We will end the presentation with an outlook on the potential and challenges of bioconversion processes for CO_2 valorization to biopolymers.

Optimizing Low Cost C1/C2 Compound Production And Fermentation From Biomass Solid Waste

MAHENDRA P RAUT

The ChELSI Institute, Department of Chemical and Biological Engineering, University of Sheffield, Mappin Street, Sheffield, S1 3JD, UK

The incomplete digestion of lignocellulosic biomass limits the production of biogas during anaerobic digestion. We proposed that the treatment of lignocellulosic biomass by composting prior to anaerobic digestion (AD) will lead to an enhanced recovery of biogas. The incorporation of a precomposting step will improve biomass digestion and will provide value added by-products such as VFAs that enhances biogas production during AD. These compounds can be further used as low cost feedstocks for the production of biofuels using C1/C2 fermenters.

The goal of this work is to develop a two stage “pre-composter cum AD” (PCAD) unit for recovery of value added C1/C2 compounds from lignocellulosic biomass. To achieve this goal, our challenge is to efficiently combine two stage processes- acetogenesis ((C2; CH_3COOH) during precomposting and methanogenesis (C1; CH_4 and CO_2) during AD-which requires unique conditions and different microbial consortia.

In this preliminary study, parameters such as carbon/nitrogen (C/N) ratio and volatile fatty acids (VFAs) and biogas (CH_4) were measured during pre-composting and anaerobic digestion.

Initial data demonstrate that the PCAD unit was successful with respect production of VFA during pre-composting and significant volume of biogas (approximately 60% CH_4) during AD. There was a significant reduction in the C/N ratio of biomass during pro-composting and this reflects efficiency of biomass digestion and improved biogas (CH_4) production as a final by-product.

Our current work is focused on studying the enzymatic and microbial activity within the PCAD unit at pilot scale. The outcome of our studies will lead to an enhanced recovery of C1/C2 compounds from both stages of the PCAD unit.

Analysis Of Sustainable Feedstock Options And Biotechnology Readiness For Developing Industrial Scale Fermentation To Produce Chemicals

REUBEN CARR

Ingenza Ltd., Wallace Building, Roslin BioCentre, Edinburgh, EH25 9PP

Ingenza is advancing with its commercial partners to transition engineered industrial microbial strains from feasibility to manufacturing scale operations. Choice of feedstock and efficiency in feedstock transformation is critical for commercial viability to manufacture these sustainable chemical products. This presentation highlights the current state of development, opportunities and future technological requirements for deploying any choice of feedstocks to fulfil the need of industrial biotechnological product of tomorrow's sustainable chemicals.

Microbubble Intensification Of Bioprocessing: Bioreactor Acceleration And Downstream Separations

PROFESSOR WILL ZIMMERMAN

Chemical and Biological Engineering, University of Sheffield

When fluidic oscillator driven, energy efficient microbubbles were invented in 2005, there were only two classes of industrial processes exploiting microbubbles - bioreactors and flotation separations. We have shown that several processes are enhanced or intensified with microbubbles, including liquid mixing, heat and mass transfer, particle separations, evaporation, condensation, distillation, and interfacial reactive distillation. This presentation will show how some of these features are being exploited for applications where no bubbles have been used before, highlighting microbubble distillation for ammonia stripping and other purposes. In respect to C1 fermentation, microbubbles have been used in anaerobic digesters to strip methane completely, resulting in higher metabolism and methane production (110% maximum observed increase in production rate over the control). Stripping is the inverse of the dosing requirement for methane gas fermenters. Since methane is practically insoluble in water, the effectiveness of microbubbles must be due to intermittent collisions with microbes and biomass where methane accumulates, rather than removal directly from the aqueous medium. Furthermore, with the very low rise time of <10 micron size microbubbles, the contact time is very long, with microbubbles leaving the liquid medium due to size growth due to methane uptake in AD. For gas fermenters, the analogous reason for microbubbles to leave is the extraction of CO_2. Since gas fermenters are typically strongly aerobic, a mixed dosing strategy of methane microbubbles and air / O_2 microbubbles should take advantage of our observed strong aeration rates in microbial bioreactors. Due to high aeration rates achievable, such as in yeast propagation, where dissolved oxygen levels up to saturation were achieved, there is no need for pure O_2 dosing to gas fermenters.

Autotrophic Acetogens: Genomics, Physiology, And Biotechnological Applications

PETER DÜRRE

Institute of Microbiology and Biotechnology, University of Ulm, 89069 Ulm, Germany

Autotrophic acetogenic bacteria employ the so-called Wood-Ljungdahl pathway for growth, forming naturally acetate, ethanol, and/or 2,3-butanediol from gaseous substrates such as CO_2 + H_2 or syngas (mostly a mixture of CO + H_2). To date, different acetogens are used in industrial applications in pilot and demonstration plants aiming at ethanol formation from different syngas sources. A major challenge is to reengineer these bacteria metabolically for formation of other interesting chemicals, allowing fermentation with an abundant, cheap carbon source and, in parallel, even consumption of greenhouse gases.

Genomes of a number of autotrophic acetogens have been sequenced and analyzed, among them *Clostridium ljungdahlii, C. aceticum, C. coskatii, C. ragsdalei*, and *Moorella thermoacetica/thermoautotrophica*. *C. ljungdahlii* is able to ferment either organic compounds or CO_2 + H_2 and syngas (CO + H_2). Experimental data and *in silico* comparisons revealed differences in energy metabolism. Unlike *M. thermoacetica*, no cytochromes and quinones are involved in energy generation, but instead an H^+-dependent Rnf system is present, analogous to *Acetobacterium woodii* with a Na^+-dependent Rnf system. Electroporation of *C. ljungdahlii* with plasmids bearing heterologous genes for butanol production was successful and formation of the biofuel could be demonstrated. Thus, *C. ljungdahlii* can be used as a novel microbial production platform based on syngas.

As the organism does not grow well on CO_2 + H_2 mixtures, *Clostridium aceticum* was chosen for this type of gaseous substrate. Expression of both, heterologous butanol- and acetone-forming enzymes could be demonstrated. *C. aceticum* does contain cytochromes, but no quinones, as verified by genome sequencing. *C. aceticum* can also use syngas as a carbon source.

Finally, *A. woodii* became a model organism for autotrophic acetogens forming acetate from CO_2 + H_2. CO does inhibit hydrogenase and CO_2 reductase/formate dehydrogenase. *A. woodii* was metabolically engineered to produce acetone in addition to acetate by introducing and overexpressing respective clostridial genes from *C. acetobutylicum*.

Metabolic Pathway Engineering In *Cupriavidus necator* As Platform For Biofuel And Chemicals Production From CO_2.

L. CREPIN, J. MARC, E. GROUSSEAU, F. FERREL, N. GORRET and STEPHANE E. GUILLOUET

Université de Toulouse; INSA, UPS, INP; LISBP, Ingénierie des Systèmes Biologiques et des Procédés 135 Avenue de Rangueil, F-31077 Toulouse, France

With the need to reduce consumption of petroleum-based products, diversified alternative fuels and bulk chemicals from renewable carbon sources have to be developed. The facultative chemolithoautotrophic bacterium *Cupriavidus necator* (also known as *Ralstonia eutropha*) is a metabolically versatile bioproduction platform organism. It is capable of utilizing many simple and complex carbon sources, especially carbohydrate, oils, fatty acids and CO_2, which can be derived from agro-industrial waste streams. *C. necator* is naturally able to divert a significant amount of carbon into poly-3-hydroxyalkanoates (PHA) biopolymers under unbalanced growth conditions by nutrient limitation (oxygen, nitrogen, phosphorus et al.), with adequate availability of carbon.

We developed a multidisciplinary strategy combining metabolic modelling, metabolic and biochemical engineering to design *Cupriavidus necator* as a platform for biofuel and chemical production. In this presentation, we will illustrate this topic with examples of strain engineering aiming at redirecting the carbon flow from PHAs towards the production of two types of molecules: isopropanol and alka(e)nes.

The engineered strains were evaluated first in terms of growth and production of isopropanol and hydrocarbons from fructose. Then trials of metabolite production from CO_2 and H_2 were successfully achieved in bioreactor. Kinetic characterization of the engineered strains under the different fermentation systems will be presented here.

Engineering Improved Ethylene Production In *C. necator*

RAJAN PATEL[1], ALEX VAN HAGEN[1], JOSEPH WEBB[2], CARRIE ECKERT[3], PAUL DALBY[4], NIGEL MINTON[1] and SAMANTHA BRYAN[1]

[1]BBSRC / EPSRC Synthetic Biology Research Centre, The University of Nottingham, University Park, Nottingham, NG7 2RD, [2] Department of Molecular Biology and Biotechnology, Sheffield University, Sheffield, UK, [3]NREL, Golden, Colorado, 80401, USA, [4]Department of Biochemical Engineering, UCL, London, WC1H 0AH, UK.

Global demand for ethylene is expanding at a rapid rate with production predicted to reach 200 million tonnes by 2020. Ethylene is currently produced from stream cracking or dehydrogenation of ethane, both of these produce large amounts of CO_2, which has a deleterious impact on the environment. We must find environmentally friendly alternatives to satisfy our energy requirements. *Cupriavidus necator* is a Gram-negative soil bacterium, capable of growing on CO_2 enabling low carbon fuels and chemicals to be produced with minimal environmental impact. We aim to engineer *Cupriavidus necator* as a platform for the production of hydrocarbon-based products such as ethylene. As an initial step we wanted to demonstrate that ethylene can be produced in *C. necator,* using the *efe* genes (ethylene forming enzyme) from *P. syringae pv. paseolicola,* which is sufficient for ethylene production in heterologous hosts and *Ralstonia solanacearum.* The *efe* genes were synthesised, cloned and expressed in *C. necator.* As proof of concept, we have generated ethylene from minimal media and from CO_2; we are now in the process of improving production through directed evolution and metabolic engineering.

3-Hydroxypropionic Acid (3-HP) Synthesis By *Cupriavidus necator H16*

KATALIN KOVÁCS, FREDERIK WALTER, DIEGO OROL GOMEZ, CHRISTIAN ARENAS, KLAUS WINZER AND NIGEL P. MINTON

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

Cupriavidus necator H16, also known as *Ralstonia eutropha* is a Gram-negative, non-spore forming bacterium found in both soil and water. It is facultatively chemolithoautotrophic, able to grow with organic substrates or H_2 and CO_2 under aerobic conditions. Its ability to grow on CO_2 as sole carbon source makes it an attractive chassis organism for the sustainable production of high value chemicals, such as 3-hydroxypropionic acid (3-HP), ethylene, propylene, isobutene and other higher carbon platform chemicals.

3-HP is an important building block for biorenewable polymers. It can be used for the sustainable production of acrylic acid, 1,3-propanediol, methyl acrylate, acrylamide, ethyl 3-HP, malonic acid and as a cross-linking agent for polymer coatings, metal lubricants and antistatic agents for textiles. 3-HP can be synthesised via glycerol, lactate, malonyl-CoA or β-alanine intermediates via at least seven different biosynthetic pathway.

The synthetic pathway for 3-HP production from β-alanine has been previously described in *E. coli* and yeast. In yeast, β-alanine was converted into malonic semialdehyde either by the action of β-alanine-pyruvate aminotransferase (BAPAT) or γ-amino butyrate transaminase (GABT), then further reduced into 3-HP by the action of either 3-hydroxypropionate dehydrogenase (HPDH) or 3-hydroxyisobutyrate dehydrogenase (HIBADH). Genome analysis of *C. necator* H16 revealed the presence of both BAPAT and GABT encoding genes for conversion of β-alanine to malonic semialdehyde, and the presence of HPDH and HIBDH for reduction of the semialdehyde to 3-HP. Several heterologous genes for both BAPATs and HIBADH/HPDHs (novel and characterized enzymes) have also been selected for individual expression in *C. necator* as well in combination with the corresponding enzymes for β-alanine conversion into 3-HP.

Modelling Central Carbon Metabolism Of *Acetobacterium woodii* DSM1030 Using A Genome-Scale Metabolic Model

NOAH MESFIN, DAVID FELL and MARK POOLMAN

Cell Systems Modelling Group, Dept. Biological and Medical Sciences, Oxford Brookes University, UK

Acetogens are microbes which produce acetate as a fermentation-product of anaerobic fermentation. They are diverse in their phylogeny and only have a metabolic feature in common called the Woods-Ljungdahl Pathway (WLP). WLP confers the ability of fixing atmospheric carbon dioxide into central metabolism in a non-photosynthetic way, using the electron bifurcation of molecular hydrogen or oxidation of carbon monoxide as a source of reducing potential.

We report the construction of a genome-scale metabolic model (GSM) of the model acetogen *Acetobacterium woodii* using a recently sequenced and annotated genome of strain DSM1030. An initial draft model was created using the Pathway/Genome Database from BioCyc, and then manually curated using current literature and other databases to fill gaps in the metabolic network and produce an analysis ready model. The model consists of 928 metabolites, 923 reactions and 25 transporters and can simulate growth on nearly every experimentally reported substrate. Growth is simulated using a lumped biomass equation devised from literature sources and reasonable estimates. Most substrates produce acetate as a by-product of growth but substrates such as 1,2 propandediol result in nonacetogenic growth with propionate and propanol as the fermentation-products.

Using the model, we predict routes through the metabolic network using different substrates and substrate combinations, and can subsequently calculate ATP yields of these substrates. We have applied this to *A.woodii* fermentations using methanol:formate as substrate, since these were the only fermentations reported in the literature providing a non-growth associated maintenance (NGAM) cost (given as methanol mM(gCell x hour)$^{-1}$). Using the calculated methanol:formate ATP yield we predict a NGAM for *A.woodii*.

The model is undergoing continual development and is currently being used for the purposes of metabolically engineering an industrially relevant acetogen *A.woodii* strain.

The Formate Bio-Economy Concept: Addressing Humanity's Grand Challenges

ARREN BAR-EVEN

Max Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, 14476 Potsdam-Golm, Germany

Our growing understanding of the design principles and biochemical logic that control the evolution and function of living organisms enables us to re-wire biological systems for our needs. In this talk, I shall discuss the formate bio-economy concept that put forward the idea that formic acid/formate can serve as an ideal mediator between the physicochemical and biological realms, i.e., formate can be synthesized efficiently using excess energy and then serve as sole source for microbial growth and chemical production. As the natural formate assimilation pathways are either inefficient or are constrained to organisms that are difficult to cultivate and engineer, an advantageous strategy is to adapt model industrial organisms to formatotrophic growth using synthetic, specially tailored formate-assimilation routes.

Several studies have begun to tackle this challenge by applying engineering principles to integrate existing enzymes into synthetic metabolic routes with promising characteristics, or, even more boldly, by evolving completely new enzymes to support efficient formate assimilation. I shall focus on our work, aiming to establish two synthetic formate assimilation pathways that are expected to have favorable properties. While the establishment of these synthetic routes is yet to be completed, our intermediate selection results suggest that both pathways could enable efficient growth on formate, demonstrating the capacity of metabolic engineering to tackle humanity's grand challenges via formate assimilation.

Upgrading The Toolbox For Fermentation Of (Crude) Syngas: From Syngas To Malic Acid

FLORIAN OSWALD, STEFAN DÖRSAM, MICHAELA ZWICK, NICOLAS VEITH, KATRIN OCHSENREITHER, ANKE NEUMANN and CHRISTOPH SYLDATK

Institute of Process Engineering in Life Science Section II: Technical Biology, Karlsruhe Institute of Technology (KIT), 76133 Karlsruhe, Germany

The bioliq® pilot plant at the KIT covers the complete process chain required for producing customized fuels from dry lignocellulosic biomass. The intermediate product, a tar-free, low-methane raw synthesis gas (syngas) is then used for further fuel synthesis. Since chemical synthesis has high requirements for gas purity and C/H ratio extensive conditioning of the crude syngas is necessary. Therefore the pilot plant is equipped with an innovative hot-gas cleaning system to remove impurities like HCl, H2S, COS, CS2, NH3, and HCN.

Acetogenic bacteria are able to grow on syngas as sole carbon and energy source under anaerobic conditions. They convert CO/CO2 and H2 to Acetyl-CoA and further to organic acids and alcohols using the reductive acetyl-CoA pathway. In contrast to catalysts used in chemical synthesis these bacteria can process a broad range of CO/CO2 to H2 ratios and tolerate impurities like sulphur or nitrogen compounds.

Suffering from energetic limitations, yields of C4-molecules produced by syngas fermentation are quite low compared with ABE fermentation using sugars as a substrate. Fungal production of malic acid on the other hand has high yields of product per gram metabolized substrate but is currently limited to sugar containing substrates. However, it was possible to show malic acid production by *Aspergillus oryzae* with acetate as sole carbon source. Using a sequential mixed culture approach, we interlinked anaerobic syngas fermentation with aerobic fungal fermentation for the production of high-value mailic acid. During the syngas fermentation *Clostridium ljungdahlii* was grown in 2.5 L stirred tank reactors under ammonia reduced conditions and sparged with 20 mL/min of artificial syngas, mimicking a composition of syngas from entrained flow gasification of straw (32.5 vol-% CO, 32.5 vol-% H2, 16 vol-% CO2 and 19 vol-% N2). After interlinking with the aerobic fungal fermentation, 22 % of the consumed syngas were converted to malic acid.

Functional Carbonic Anhydrase of *Clostridium autoethanogenum*

BART PANDER, KLAUS WINZER, NIGEL P. MINTON, ANNE M. HENSTRA

BBSRC / EPSRC Synthetic Biology Research Centre, The University of Nottingham, University Park, Nottingham, NG7 2RD

Carbonic anhydrases (CAs) are enzymes that catalyse the reversible conversion of carbon dioxide and water to bicarbonate and protons. CA activity the industrial relevant acetogen *Clostridium autoethanogenum* has not been described in literature. This clostridial acetogenic autotroph is used in industry to fix the waste gasses carbon monoxide and carbon dioxide into organic matter and valuable products such as ethanol and 2,3 butanediol via the acetogen specific Wood-Ljungdahl pathway. For a complete understanding of the carbon metabolism of these cells it is necessary to understand the role CAs play in these microorganisms.

We identified two putative CA genes in the genome of *C. autoethanogenum.* One codes for a β- carbonic anhydrase (β-CA) with little similarity to other known β-CAs. The other is a γ-carbonic anhydrase (γ-CA) with clear homology to other proteins in the γ-CA protein family. Both putative CA genes were heterologous expressed in *Escherichia coli* and purified. To determine CA activity of these purified enzymes a CA assay was developed using a 96-well plate reader with automated injection. The purified β-CA of *C. autoethanogenum* shows CA activity while the heterologous γ-CA seems not to be active. We showed that Caut- β-CA can complement a CA disrupted *E. coli* mutant reversing the phenotype to the *E.coli* WT. ClosTron (CT) mutants were generated for both CA genes in the genome of *C. autoethanogenum.* Metabolomics shows there is a subtle effect of the Caut- β-CA disruption on the metabolism. Further characterisation of the enzyme activity, gene overexpression and gene knock-out mutant strains is ongoing.

Carbamate Trapping—A New Tool For Understanding Protein-CO_2 Interactions

VICTORIA L. LINTHWAITE,[a] JOANNA M. JANUS,[a] DAVID R. W. HODGSON,[b] AND MARTIN J. CANN [a]

[a] *School of Biological and Biomedical Sciences, Durham University, South Road, Durham, DH1 3LE*

[b] *Department of Chemistry, Durham University, South Road, Durham, DH1 3LE*

Carbon dioxide (CO_2) is known for its role during metabolism,[1] however, there is very little known about its direct molecular interactions with cellular components. CO_2 combines rapidly but reversibly with amines, by the nucleophilic attack of an uncharged amine on CO_2, at physiological temperatures and pressures to form carbamates[2,3].

The presence of this post-translational modification has been demonstrated in a small number of key proteins, such as RuBisCO and haemoglobin, but remains unexplored in other systems. We demonstrate that we can identify new sites of CO_2 interactions within proteomes, using a chemical trapping technique combined with tryptic digest-MS analyses.

Initial validation experiments demonstrated effective carbamate trapping at NH_2 sites within model substrates acetyl-lysine, Lys-Gly and Phe-Gly, a tetra-peptide and haemoglobin. These results were confirmed using ESI-MS combined with ^{12}C and ^{13}C isotope incorporation. Initial screening of cellular lysates used ^{14}C-(CO_2) combined with scintillation counting to demonstrate that carbamates could be trapped under these conditions.

We are currently exploring *Arabidopsis thaliana* homogenates using these techniques, and we have identified at least 10 carbamylated proteins to date which we are validating via *in vitro* assays in the presence and absence of CO_2. This technology therefore represents the first general methodology for identifying CO_2 targets and is applicable to any cell type and organism.

1. Azzam, Z., *et.al.* (2010) The physiological and molecular effect of elevated CO_2 levels. *Cell Cycle* **9**, 1528-1532
2. Hampe, E. M., and Rudkevich, D. M. (2003) Exploring reversible reactions between CO_2 and amines. *Tetrahedron* **59**, 9619-9625
3. Terrier, P., and Douglas, D. J. (2010) Carbamino Group Formation with Peptides and Proteins Studied by Mass Spectrometry. *Journal of the American Society for Mass Spectrometry* **21**, 1500-1505

PMMO In Plants

VERENA KRIECHBAUMER

Plant Cell Biology, Department of Biological and Medical Sciences, Oxford Brookes University, Oxford OX3 0BP, UK

Methane is a potent greenhouse gas with a twenty times higher impact on global warming than carbon dioxide. Globally, over 60% of methane emissions come from human activities including industrial gas and petroleum systems, agricultural livestock, artificial wetlands, and landfills.

Methanotrophs are bacteria that feed on methane gas and they function as the only biological methane sink, thus performing a critical role in the global carbon cycle. Methanotrophs convert it to carbon dioxide, producing methanol as a by-product. For this, bacteria utilise an enzyme, the particulate methane monooxygenase (pMMO).

We aim to modify tobacco plants to produce pMMO, enabling them to turn methane into the less potent carbon dioxide. The by-product methanol can enhance plant growth and produce biomass for other processes. Ultimately plants could be grown for detoxification purposes on soil high in methane, e.g. wetlands, ex-landfill sites or rice paddy fields.

ABSTRACTS OF POSTER PRESENTATIONS

Construction And Evaluation Of A RBS Library For *Cupriavidus necator* H16

SWATHI ALAGESAN, NAGLIS MALYS, NIGEL P. MINTON

BBSRC / EPSRC Synthetic Biology Research Centre, The University of Nottingham, University Park, Nottingham, NG7 2RD

Cupriavidus necator H16 is a gram-negative hydrogen-oxidizing bacterium (1). It is capable of growing autotrophically by utilizing C1 gas such as carbon dioxide as the sole carbon source. Under certain growth conditions this microorganism produces and stores large quantities of polyhydroxyalkanoates (PHA). All above characteristics make *C. necator* an excellent chassis for the synthesis of useful products from synthesis gases (Syngas).

To facilitate metabolic engineering of *C. necator*, it is imperative to develop libraries of genetic parts (promoters, RBSs, terminators etc.). These genetic parts can then be applied for optimizing the synthetic metabolic pathways, by modulating the transcription and translation initiation rates. In this study, we have designed and constructed a Ribosome Binding Site (RBS) library for *Cupriavidus necator.* The library was developed using a DNA engineering method based on PCR and USER technologies (2). All constructs were tested in *C. necator* under control of PhaC promoter and using enhanced yellow fluorescent protein (eYFP) as a reporter.

The constructed library included unique RBS sequences spanning a range of translational activity. Enhanced gene expression was observed in constructs which had additional upstream elements such as a stem-loop structure and AU-rich regions, which contributed to improved mRNA stability and translation initiation rates. Selected RBS sequences were also tested with different promoters and, here for the first time, we report the implementation of functional L-rhamnose-inducible *RhaRS-rhaP*$_{BAD}$ system in *C. necator*.

References

1. Pohlmann, A. et al. (2006) Genome sequence of the bioplastic-producing "Knallgas" bacterium Ralstonia eutropha H16. *Nature Biotechnology*, 24: 1257-1262

2. Vaisvila, R., Bitinaite, J. (2013) Gene synthesis by assembly of deoxyuridine-containing oligonucleotides. *Methods in Molecular Biology*, 978: 165-171

C1 Gas Fermentation Lab In CPI: Scale-Down Flexibility Integrated With Safety Operations

VALENTINA MANGIAFRIDDA, SAUL ALONSO TUERO

Industrial Biotechnology and Biorefinery, Centre for Process Innovation, Wilton Centre, Wilton, TS10 4RF

The new C1 gas fermentation development laboratory is designed to perform fermentation process development work from 1 to 10L scale in a highly contained environment, offering an high cutting-edge facility to underpin the development of bio-based processes.

There is a gap identified by UK Government in gas fermentation development and as such this facility provides an opportunity to bring to the fore the economic, environmental and societal benefits of this technology. The aim of the laboratory is to provide industry and academia with a facility to test novel microorganisms, feedstock's and processes to gain process knowledge, while evaluating techno-economics and understanding scalability issues as a means of risk mitigation along the road to commercialisation.

The C1 Gas Fermentation laboratory in CPI addresses the commercial and environmental opportunity to utilise gaseous feedstocks in the production of bio-products by gas fermentation. Waste gasses such as CO2 from industrial activity and CH4 from landfill contribute to Greenhouse Gasses emissions. However, these waste streams together with syngas produced from gasification and pyrolysis of solid waste can be potential third generation feedstock's for bio-based production of chemicals and biofuels.

In order to fulfil such purpose and to offer the necessary flexibility of operation, the facility provides a broad range of bioreactors to perform batch, fed-batch and continuous operations along with the ability to undertake scale-down parallel DoE based fermentation optimisation approaches.

Central to the delivery of a safe operating environment for the use of hazardous gases was the design and construction of extracted cabinets into which fermentation equipment can be sited. A specific overview of the safety measure in place at the C1 Gas Fermentation lab in CPI will be given along with a parallel comparison with a recent explosion happened in a C1 Gas Fermentation Lab at the University of Manoa.

Engineering The Production Of 3-Hydroxypropionic Acid *Via* Malonyl-CoA Pathway In *Cupriavidus necator* H16

CHRISTIAN ARENAS, FREDERIK WALTER, KATALIN KOVACS, NIGEL P. MINTON, KLAUS WINZER

BBSRC / EPSRC Synthetic Biology Research Centre, The University of Nottingham, University Park, Nottingham, NG7 2RD

There is an urgent need to develop environmentally friendly and sustainable routes to fuels and chemicals which do not rely on fossil resources. Since efficient and cost-effective conversion of lignocellulosic waste materials remains problematic, gasification of biomass is increasingly considered an attractive alternative, as the resulting gaseous substrates can be utilised by a number of different bacteria as a source of carbon and energy and converted into interesting products. Here, we focus on the well-studied and genetically amenable 'Knallgas bacterium' *Cupriavidus necator*, which was chosen as a C1-chassis for the production of 3-hydroxypropionic acid (3-HP) and other fatty acid derivatives from CO_2 and H_2.

The first committed step in 3-HP synthesis is the carboxylation of acetyl-CoA to malonyl-CoA, a reaction that is catalysed by the enzyme acetyl-CoA carboxylase (ACC) and tightly controlled at various levels. The second step is the reduction of malonyl-CoA to 3-HP, a conversion catalysed by the bifunctional enzyme malonyl-CoA reductase (MCR) or, in some archaea, by the combination of two monofunctional enzymes which reduce malonyl-CoA first to malonate semialdehyde and then further to 3-HP. However, the generation of efficient and robust production strains remains a major challenge for metabolic engineering.

Genes encoding ACC subunits and MCRs from different bacteria and archaea were codon-optimised, assembled into functional operons and screened for efficient expression in *C. necator.* Strategies for establishing high-level 3-HP production and the resulting physiological and metabolic consequences for the host are currently being investigated.

Succinate Fermentation In *Clostridium autoethanogenum*

RONJA BREITKOPF, ALEXANDER T. WICHLACZ, SAMANTHA MCLEAN, SARAH SCHATSCHNEIDER, HUW EL. WILLIAMS, NEIL R. THOMAS, DAVE BARRETT, NIGEL P. MINTON and KLAUS WINZER

BBSRC / EPSRC Synthetic Biology Research Centre, The University of Nottingham, University Park, Nottingham, NG7 2RD

There is an increased need to generate fuels and platform chemicals in a more sustainable manner. One of the chemicals believed to have potential in a bio-based, circular economy is succinic acid. Already used in food and pharmaceutical market, it also functions as C4 building block and can therefore supply the basis for high value-added derivatives with applications in the technical and chemical industry.

Using the acetogenic bacterium *Clostridium autoethanogenum* as a microbial chassis, the proposed research aims to combine the utilisation of exhaust and waste fumes with the fermentative production of succinic acid. A prerequisite for this is a thorough understanding of the existing native metabolic route(s) to succinate, which is already generated by the organism in low amounts, as well as interconnecting pathways. This will be achieved through a combination of enzymatic studies, ^{13}C labelling experiments and gene inactivation/overexpression analyses.

Interestingly, provision of exogenous fumarate, a metabolite which other bacteria can convert to succinate acid in a single step, considerably increased growth of the organism without increasing the amount of succinate produced. However, this increase was only observed in the presence of other carbon and energy sources: addition of fumarate alone could not sustain growth. NMR analyses were therefore initiated to clarify the metabolic fate of fumarate. Investigations are still ongoing, but first results supported by these NMR analyses suggest a clear decrease in the culture supernatant accompanied by an increase in intracellular fumarate, suggesting that the compound is indeed taken up and co-metabolised in the presence of other carbon and energy sources.

A Key Role For Shared Pilot Facility To Deploy Gas Fermentation

FREDERIK DE BRUYN

Business Development Manager
Bio Base Europe Pilot Plant
Rodenhuizekaai 1, 9042 Gent, Belgium

Bio Base Europe Pilot Plant is a multipurpose pilot facility for the development, scale-up, and custom manufacturing of bio based products and processes. As a Research & Demonstration infrastructure, BBEPP has a wide range of state-of-the-art industrial equipment which covers the whole value chain from Biomass to Refined Product: biomass pretreatment, fermentation, downstream purification, bio-catalysis and explosion proof green chemistry.

Recently, much attention is going to carbon capture from large point waste sources such as fossil fuel power plants or steel mills. From an economical point of view, it is more interesting to use waste carbon dioxide to produce chemicals, rather than long term storage. Gas fermentation will play a key role in the production of biofuels and value chemicals, and this technology is currently undergoing intensive research and development. Despite many innovations in the field of strain engineering and reactor design, many processes are moving very slowly towards commercialization or don't even make it to pilot-scale.

The hurdles encountered during optimization and scale-up of gas fermentations are much more challenging and complex than those observed in classic bioprocesses. Besides technical issues, most companies or institutes typically don't have the necessary infrastructure, nor the skilled personnel and permits to run such pilot-scale tests. To obtain faster learning curves and shorter time to market, these activities are better outsourced. To this end, BBEPP is currently expanding its gas fermentation equipment and gives access and support to SMEs, large companies and research institutes to bring CCU technologies from a laboratory scale to a demonstration scale.

Modular Vectors For *Cupriavidus necator* H16 And Generation Of Restriction Negative Strain

MUHAMMAD EHSAAN, SAMANTHA BRYAN, STEPHAN HEEB, KLAUS WINZER and NIGEL MINTON

BBSRC / EPSRC Synthetic Biology Research Centre, The University of Nottingham, University Park, Nottingham, NG7 2RD

Cupriavidus necator H16 is a Gram negative, non-spore forming, obligate aerobic, and facultative chemolithoautothropic bacterium which can utilize H_2/CO_2 for growth under aerobic conditions and can reach high cell densities (>200g/l). There has long been interest in using Polyhydroxyalkanoates (PHAs) as biodegradable bioplastics that could serve as alternatives to petrochemical plastics. 3-Hydroxypropionic acid or 3-hydroxypropanoate (3-HP) is an important platform chemical and can be used to produce various chemicals in chemical industry. Despite the industrial importance of *C. necator* H16, sophistication in the gene tools are immature and in clear need of development which include gene transfer and maintenance of recombinant, replicative plasmids. In this study, we have constructed a set of modular vectors which can be used to metabolically engineer the strain as well as stably maintain the plasmids carrying synthetic genes/operons of interest having compatible replicons during fermentation. We are also in the process of creating a restriction negative strain which will enable us to use suicide vector for gene knock-ins and outs.

Biotechnological Applications Of Microorganisms Growing On C1 Compounds

MOHAMED A. EL-ESAWI

Botany Department, Faculty of Science, Tanta University, Egypt
Division of Crop Biotechnics, KU Leuven, B-3001 Leuven, Belgium

Biotechnology is a promising technology to form chemicals which could support biological processes. Microorganisms including bacteria could take up various materials which could be used in the production of chemicals, including C1 compounds. The required chemicals could be directly produced in one fermentation step. Here, we address the biotechnological applications of such microorganisms including bacteria growing on C1 compounds.

Identification Of Conditionally Essential Genes In *Cupriavidus necator* H16 Using TraDIS

MARCO GARAVAGLIA, KLAUS WINZER and NIGEL MINTON

BBSRC / EPSRC Synthetic Biology Research Centre, The University of Nottingham, University Park, Nottingham, NG7 2RD

Cupriavidus necator is a Gram-negative, aerobe, facultative chemolithoautotrophic bacterium of relevant biotechnological interest. However, our knowledge of *C. necator* as a biological system is still very limited.

The aim of the present study is to identify essential genes in *C. necator* strain H16 under different experimental conditions, using transposon-directed insertion site sequencing (TraDIS). To this end, a library consisting of over 1 million transposon mutants was constructed in *C. necator* H16. Delivery of the miniTn5*::tetC* transposon, harbouring a tetracycline resistance marker, to the *C. necator* H16 genome was achieved using a suicide modular vector (pMTL70115.1) carrying the gene encoding for a hyperactive transposase (TnpA).

The *C. necator* H16 transposon mutant library will be used to analyse gene essentiality under the following experimental conditions: heterotrophic growth in the presence of fructose as the sole carbon source and chemolithoautotrophic growth in gas mixture containing CO_2, H_2 and O_2. In addition, the transposon mutant library will be grown in the presence of high concentrations (up to 100mM) of 3-Hydroxypropionic acid (3HP), a platform chemical that can be converted into acrylic plastics and biodegradable polyesters. In this case, TraDIS will be used to identify *C. necator* H16 mutants exhibiting increased tolerance to 3HP, the production of which is currently being investigated in *C. necator* by our research group.

Altogether, the data obtained from the TraDIS analysis will significantly expand our knowledge of *C. necator* as a biological system and could aid in the design of metabolic engineering strategies, in the attempt to develop *C. necator* H16 into an improved microbial chassis for the production of industrially-relevant platform chemicals and fuels.

Novel Industrial Exploitation Of Methanotrophic Pathways

SATBIR KAUR GILL[1], PETER GARDNER[1] AND REUBEN CARR[1]

[1]*Ingenza Ltd., Wallace Building, Roslin BioCentre, Edinburgh, EH25 9PP*

Methane is one of the most abundant greenhouse gases, but it is also a source of energy recovery systems, heating, and transport for commercial applications. Biological conversion of methane by methanotrophic organisms could potentially provide an economical and environmentally responsible approach for the production of bulk and fine chemicals, as well as sustainable energy.

The conversion of methane in methanotrophs is carried by the oxidoreductase enzyme, methane monooxygenase enzyme (MMO) which catalyses the oxidation of methane to methanol. The aim of the current work is to explore the biology of MMOs and their mechanism of action to utilise methane. This will be carried out by taxonomy and genotyping of species specific MMOs. At present, the details of pathway for methane metabolism are missing, so bioinformatics approaches for the re-construction of the pathways will be utilised. The long term goal is to establish a knowledge base for methane utilisation to assist with the subsequent development of the methane as a commercially attractive feedstock option for the Industrial Biotechnology sector.

GASCHEM: Optimising C1 Gas Fermentation By The Acetogen *Clostridium autoethanogenum*

ANNE M. HENSTRA

BBSRC / EPSRC Synthetic Biology Research Centre, The University of Nottingham, University Park, Nottingham, NG7 2RD

Clostridium ljungdahlii and *Clostridium autoethanogenum* are model organisms for gas fermentation, a rapidly maturing technology for the production of fuels and chemicals from industrial waste gases. A natural process that captures CO, CO_2 and H_2, and converts them to products such as acetate, ethanol and 2,3 butanediol. Further engineering of these strains promises the production of diverse platform chemicals, that can be used as building blocks in the production of synthetic polymers. BBSRC's sLoLa GASCHEM seeks to understand and to engineer *C. autoethanogenum* metabolism using systems and synthetic biology approaches with the aim to improve and extend product streams.

Photoautotrophic Carbon Uptake By Microalgae For Biotransformations

RAHUL VIJAY KAPOORE, GLORIA PADMAPERUMA, JIM GILMOUR and SEETHARAMAN VAIDYANATHAN

ChELSI Institute, Advanced Biomanufacturing Centre, Department of Chemical & Biological Engineering, The University of Sheffield, Mappin Street, Sheffield S1 3JD.

Photoautotrophs rely on light for energy to fix atmospheric carbon dioxide. This is a natural phenomenon that has evolved over the years to accommodate naturally available levels of light and nutrients, including carbon dioxide. It can, in principle, be utilised effectively for the development of biochemical processes that are carbon neutral and environmentally sustainable. A better understanding of this natural phenomenon would help us in developing improved biomimetic systems for sustainable utilisation of carbon dioxide and its conversion to products of commercial value. Our interest is in studying the carbon dioxide uptake by one group of photoautotrophs (microalgae) in a controlled reactor environment. Microalgae are a versatile group of photoautotrophic microorganisms that have high metabolic diversity and the potential to route the fixed carbon to organic carbon of high value or bulk biofuel precursors, such as lipids. Carbon dioxide uptake by this group of organism is dependent on the interplay between chemical and biological equilibrium of dissolved carbon in the medium the organism is exposed to. We will discuss our recent observations on the kinetics of carbon dissolution and its uptake by selected microalgae and what this implies to the application of this group of photoautotrophs for CDU, or indeed for the development of biomimetic systems based on this natural phenomenon.

Assembly And Quantitative Evaluation Of Genetic Elements For Controlling Gene Expression In *Cupriavidus Necator*: Combinatorial Engineering For Chemical Production

NAGLIS MALYS, SWATHI ALAGESAN and NIGEL P. MINTON

BBSRC / EPSRC Synthetic Biology Research Centre, The University of Nottingham, University Park, Nottingham, NG7 2RD

Synthetic Biology Research Centre (SBRC) core research focusses on metabolic engineering with the use of synthetic biology tools to generate novel microbial strains for production of platform chemicals. As a chassis we use *Cupriavidus necator* H16, which can grow autotrophically by using carbon dioxide and hydrogen as sole carbon and energy sources, and directs a high proportion of carbon flux towards production of poly-3-hydroxybutyrate (PHB). The ability to use C1 gas as a feedstock and feasibility to redirect carbon flux from PHB to alternative chemicals opens opportunities for sustainable industrial biotechnology.

One of the major challenges of metabolic engineering is to maximize carbon flux towards desired metabolite. The balanced production of enzymes in synthetic metabolic pathways is required. Traditional approaches have been largely focused on the overexpression of rate-limiting enzymes. This simplistic approach can fall short when precise control of gene expression using different strengths of genetic elements (e.g. promoters) is required.

To address above, we developed library of constitutive promoters for *C. necator*. All promoter variants were seamlessly assembled using PCR- and USER-based DNA engineering technique. The promoter strengths were measured using yellow fluorescent protein as a reporter. The library extended the range of feasible expression levels based on low, medium and high strength, establishing at least four constitutive promoters that were the strongest yet to be employed in *C. necator*. Further, we aim to develop combinatorial engineering comprising transcriptional (e.g. promoter, transcription regulator) and translational (ribosome binding site) regulatory elements, and to utilize these synthetic biology tools and approaches for pathway assembly and production optimization.

Modelling Central Carbon Metabolism Of *Acetobacterium woodii* DSM1030 Using A Genome-Scale Metabolic Model

NOAH MESFIN, DAVID FELL and MARK POOLMAN

Cell Systems Modelling Group, Dept. Biological and Medical Sciences, Oxford Brookes University, UK

Acetogens are microbes which produce acetate as a fermentation-product of anaerobic fermentation. They are diverse in their phylogeny and only have a metabolic feature in common called the Woods-Ljungdahl Pathway (WLP). WLP confers the ability of fixing atmospheric carbon dioxide into central metabolism in a non-photosynthetic way, using the electron bifurcation of molecular hydrogen or oxidation of carbon monoxide as a source of reducing potential.

We report the construction of a genome-scale metabolic model (GSM) of the model acetogen *Acetobacterium woodii* using a recently sequenced and annotated genome of strain DSM1030. An initial draft model was created using the Pathway/Genome Database from BioCyc, and then manually curated using current literature and other databases to fill gaps in the metabolic network and produce an analysis ready model. The model consists of 928 metabolites, 923 reactions and 25 transporters and can simulate growth on nearly every experimentally reported substrate. Growth is simulated using a lumped biomass equation devised from literature sources and reasonable estimates. Most substrates produce acetate as a by-product of growth but substrates such as 1,2 propandediol result in nonacetogenic growth with propionate and propanol as the fermentation-products.

Using the model, we predict routes through the metabolic network using different substrates and substrate combinations, and can subsequently calculate ATP yields of these substrates. We have applied this to *A.woodii* fermentations using methanol:formate as substrate, since these were the only fermentations reported in the literature providing a non-growth associated maintenance (NGAM) cost (given as methanol mM(gCell x hour)$^{-1}$). Using the calculated methanol:formate ATP yield we predict a NGAM for *A.woodii*.

The model is undergoing continual development and is currently being used for the purposes of metabolically engineering an industrially relevant acetogen *A.woodii* strain.

Isolating Novel Carboxydotrophs

JAMES W. MURRAY

Department of Life Sciences, Imperial College, Exhibition Road, London, SW7 2AZ

Carboxydotrophs are able to use carbon monoxide as a carbon and energy source. They are of great biotechnological importance, as they can be grown on syngas, a possible product of cracking organic material. There are aerobic, anaerobic and microaerobic carboxydotrophas, mesophiles and thermophiles. We describe our efforts to isolate novel carboxydotrophs for biotechnology.

Constructing a Genome Scale Metabolic Model of *Clostridium autoethanogenum* for Metabolic Engineering

RUPERT NORMAN[1], SARAH SCHATSCHNEIDER[2], THOMAS MILLAT[1], NIGEL MINTON[1] AND CHARLIE HODGMAN[3].

[1]BBSRC / EPSRC Synthetic Biology Research Centre, The University of Nottingham, University Park, Nottingham, NG7 2RD, [2]Evonik Nutrition & Care, Germany. [3]School of Biosciences, Sutton Bonington, The University of Nottingham, UK

Global interest in development of sustainable industry has caused a revival in solventogenic bioprocess engineering. *Clostridium autoethanogenum*, an ethanol-producing autotrophic bacterium, provides the opportunity to develop renewable fermentation-based technologies for the synthesis of platform chemicals from industrial off-gas.

A genome scale metabolic network of *C. autoethanogenum* has been constructed through manual and programmatic curation of an automatically generated genome database to support synthetic biology approaches to metabolic engineering. Visualisation methods for network simulation are used to aid biological interpretation.

Engineering Synthetic RNA Devices To Expediate The Evolution Of Metabolite Producing Microorganisms

RAJAN PATEL[1], JAMIE TWYCROSS[1], JOSPEH WEBB[2], CARRIE ECKERT[3], NIGEL MINTON[1] and SAMANTHA BRYAN[1]

[1] BBSRC / EPSRC Synthetic Biology Research Centre, The University of Nottingham, University Park, Nottingham, NG7 2RD, [2] Department of Molecular Biology and Biotechnology, Sheffield University, Sheffield, UK, [3]NREL, Golden, Colorado, 80401, USA.

There is an urgent need for environmentally sustainable fuels, given the diminishing global reserves of crude oil coupled with the deleterious environmental impact of accumulating CO_2. We must find green alternatives to satisfy our energy requirements. The production of bulk chemicals via fermentation of CO_2 could lead to a 96% reduction in greenhouse gas production. *Cupriavidus necator* is a Gram-negative, hydrogen oxidising soil bacterium, which is capable of both heterotrophic and autotrophic growth on CO_2. It is capable of producing PHA's (polyhydroxyalkanoate), which can accumulate to 90% of dry cell weight, making it an attractive chassis for the production of fuels and chemicals. It has become evident in recent years that posttranscriptional regulation mediated by sRNA (small non-coding RNA) is critical to many cellular processes, thus making them powerful tools for metabolic engineering and synthetic biology. We aim to exploit this in *Cupriavidus necator* by engineering artificial sRNAs (Toehold Switches) as a platform for strain improvement for the production of hydrocarbon-based products such as ethylene. Multiple toehold switches have been designed and will be tested to modulate flux towards increased ethylene production.

Identification Of Target Knock Outs In *Cupriavidus Necator* Using Elementary Modes Analysis

NICOLE PEARCY[1], JAMES GILBERT[1], MARK POOLMAN[2], DAVID FELL[2], HASSAN HARTMAN[2] & JAMIE TWYCROSS[1,3]

[1] *BBSRC / EPSRC Synthetic Biology Research Centre, The University of Nottingham, University Park, Nottingham, NG7 2RD*
[2]*Cell Systems Modelling Group, Department of Biological and Medical Sciences, Oxford Brookes University, Gipsy Lane, Headington, Oxford, OX3 0BP, UK*
[3]*School of Computer Science, Computer Science Building, Jubilee Campus, University of Nottingham, Wollaton Road, Nottingham, NG8 1BB*

The autotroph bacterium *Cupriavidus necator* can fix CO_2 via the Calvin cycle and oxidize H_2 as its energy source. Under nutrient limitation, the bacterium directs excess carbon towards polyhydroxybutyrate (PHB), accumulating up to 90% of the cell's dry weight. The ability of *C. necator* to utilize these waste industrial gases and produce large quantities of PHB, make it an attractive host for providing sustainable routes to platform chemicals, which are traditionally synthesised from fossil fuels.

Through metabolic engineering, pathways for synthesising desirable chemicals, such as 3-hydroxypropionic acid (3HP), can be introduced into the non-native host. Experimental efforts then aim to identify metabolic interventions that redirect flux towards this pathway. Structural metabolic modelling, where metabolism is represented as a set of mass balanced reactions, is a highly valuable tool in microbial strain design. Elementary modes analysis (EMA) in particular, which computes all metabolic capabilities of the network in the form of non-decomposable steady state pathways, provides an efficient approach for identifying metabolic interventions for optimising the microbe.

In this work, we present a structural metabolic model of *C. necator* under lithoautotrophic conditions. The model consists of 84 reactions and 68 metabolites, comprised of the Calvin cycle, the electron transport chain, PHB synthesis and 15 non-native reactions for 3HP synthesis. Using EMA, we identified 10 optimal routes for producing 3HP, in terms of the maximum theoretical yield, energy requirements and minimal metabolic interventions. By comparing the reactions involved in optimal and non-optimal routes, we identified 20 possible target knockouts. To reduce the number of candidates, we considered all 2- and 3-combinations, and identified pyruvate dehydrogenase, aspartate decarboxylase and propionyl-CoA:succinate CoA transferase as the most efficient knockout strategy for redirecting flux towards 3HP.

Clean Gas Fermentation Feedstocks From MSW

DHIVYA PURI

Fiberight Ltd

Fiberight have created a circular economy solution to generate homogeneous value-added products from heterogeneous municipal solids waste (MSW). The process involves thermo-mechanically treating the waste to recover 2 main fractions: recyclables and biomass. This biomass is then further processed to generate two-key bio outputs: a soluble organic stream for biogas production via anaerobic digestion; and a clean solid ligonocellulose fibre (paper pulp) which is converted to platform-sugars via enzyme hydrolysis. These streams are then used as inputs for the production of various bio-based products such as bioresins, biofuels and bioenergy.

In addition to the main bio-based product streams, other valuable process by-products include: a lignin rich residual solid and a non-recyclable small plastics residue. Both these streams can be gasified to produce clean syngas. Thus, the single site production of low cost clean biomethane and syngas allows for the creation of ‘designer’ gas feedstocks that can be tailored for various gas fermenting microbes.

Fiberight have demonstrated the technological viability of their process over the last 3 years where a demonstration facility has been operating in Virginia, USA, with a MSW feed in rate of 2 tonnes per hour. The company has been carrying out research and development activities in the UK for the past 6 years and is now close to commercialising the process in both US and UK.

A Synthetic Approach To Bioconversion Of Carbon Dioxide To Formic Acid

MAGALI ROGER[1], MANUEL BLANK[1], JOE DUNSTER[2], WILLIAM GABRIELLI[3], FRASER BROWN[4], JOHN KING[2] and FRANK SARGENT[1]

[1]School of Life Sciences, University of Dundee, Dundee, Scotland
[2]School of Mathematical Sciences, University of Nottingham, Nottingham, England
[3]Sasol Technology (UK) Limited, St Andrews, Scotland
[4]Ingenza Limited, Roslin Biocentre Midlothian, Edinburgh, Scotland

The ability to transform gaseous CO_2 into useful compounds would contribute to the global requirement to reduce emissions and recycle waste. *Escherichia coli* can perform a mixed-acid fermentation of glucose to produce formate, acetate, lactate, ethanol and succinate. The formate can be further metabolised into CO_2 and H_2 by a metalloenzyme called formate hydrogenlyase (FHL). Thus, under physiological conditions, the FHL enzyme normally catalyses the production of hydrogen gas linked to formate oxidation. In this work, however, the challenge was to optimise the reverse reaction – something that is not normally attempted by *E. coli*. Using a combination of genetically engineered strains and optimised growth conditions, *E. coli* was shown to be able to generate formate from CO_2 in a H_2-dependent and FHL-dependent manner. Optimisation of the ideal reaction conditions, including testing external gas pressures and monitoring pH changes, was explored in detail. In parallel, a mathematical model of mixed-acid fermentation was constructed with a view to informing and guiding future laboratory experiments.

Further transformation of formic acid into other chemicals was also explored in this project. In keeping with the synthetic biology approaches already taken, a synthetic operon optimized for *E. coli* expression was designed that encoded NAD^+/NADH-dependent formaldehyde and methanol dehydrogenases systems from *Pseudomonas aeruginosa* and *Bacillus methanolicus*, respectively. The protein products were shown to be produced in an *E. coli* chassis and the ability of various engineered cells overproducing these enzymes to generate formaldehyde or methanol was tested.

This interdisciplinary, collaborative project provided proof of concept, at the bench scale, that synthetic biology approaches are appropriate for carbon capture and lays the foundations for new ventures in bioenergy research.

Production Of β-alanine As A Precursor Of 3-hydroxypropionate In *Cupriavidus necator* H16

FREDERIK WALTER, CHRISTIAN ARENAS, KATALIN KOVÁCS, KLAUS WINZER, NIGEL MINTON

BBSRC / EPSRC Synthetic Biology Research Centre, The University of Nottingham, University Park, Nottingham, NG7 2RD

The Gram-negative bacterium *Cupriavidus necator* H16 is able to grow lithoautotrophically with CO_2 and H_2 under aerobic conditions, and is a natural producer of the bioplastic poly-3-hydroxybutyrate (PHB). A known trigger for the diversion of carbon flux towards this internal energy storage compound is limited availability of certain nutrients, for example nitrogen.[1] Central to the exploitation of *C. necator* as a promising chassis organism for the development of microbial cell factories is the aim to redirect its internal overflow metabolism away from PHB, towards the production of desired chemicals. Production of chemicals non-natural to *C. necator* furthermore requires the introduction of synthetic biochemical pathways to convert supplied precursors to the final product.

One of the desired platform chemicals is 3-hydroxypropionate (3-HP), a versatile agent for chemical synthesis and a precursor of acrylic acid. Several biochemical pathways for its production were described in different organisms, of which the β-alanine pathway is regarded as the most favourable in terms of thermodynamics.[2] β-Alanine is an intermediate of the pantothenate and coenzyme A biosynthesis in *C. necator*. It can be converted to 3-HP by the consecutive action of beta-alanine-pyruvate aminotransferase (BABAT) and 3-hydroxypropionate dehydrogenase (HPDH). 3-HP production by recombinant *C. necator* strains could be demonstrated upon supplementation of growth media with β-alanine, showing that the terminal synthetic pathway has been implemented successfully.[3] Here we aim to establish a self-sufficient supply of β-alanine in *C. necator*, an essential step for sustainable production of 3-hydroxypropionate from gases.

[1] Ishizaki A, Tanaka K, Taga N. 2001. Microbial production of poly-D-3-hydroxybutyrate from CO_2. Appl. Microbiol. Biotechnol. 57:6–12.

[2] Kumar V, Ashok S, Park S. 2013. Recent Advances in Biological Production of 3-Hydroxypropionic Acid. Biotechnol Adv. 31(6):945-61.

[3] Kovacs K, unpublished

Shifting the balance: Metabolic pathway analysis and inhibitor design in *Clostridium autoethanogenum*.

ALEXANDER T. WICHLACZ & NEIL R. THOMAS

BBSRC / EPSRC Synthetic Biology Research Centre, The University of Nottingham, University Park, Nottingham, NG7 2RD

An ever-increasing area of research is the need for alternative routes to fuels and other high value products that are usually obtained from declining reserves of fossil fuels.[1] Crude oil is cracked to give not only vehicle fuels, but also gaseous fuels (butanes etc.), butylenes and numerous other products.

One route to these products is the use of acetogenic bacteria, microorganisms which generate bioethanol as a product of anaerobic respiration. One example is *Clostridium autoethanogenum*, which utilises carbon monoxide/dioxide as a one-carbon feedstock and through its metabolic network, produce some of the high value products we currently obtain from fossil fuels.[2] One of its main outputs is ethanol, but can also produce butyl butyrate (a potential jet fuel[3]), butadienes and butylenes which are used in synthetic rubbers and many other useful acidogenic and solventogenic products.

By designing isotopically labelled probes, the metabolic pathways in this acetogen can be investigated *via* flux analysis using 13C, 19F and 2H-labelling for mass spectrometry and NMR, followed by inhibitor design to transiently knock out enzymes in the network. This drives metabolic processes in certain directions by 'switching off' other branches of the network, which is comparable with a genetic engineering approach, where the gene can potentially not be knocked out.

Key references:

1. S. Shafiee and E. Topal, *Energ. Policy*, 2009, **37**, 181-189.
2. M. Kopke, C. Mihalcea, F. Liew, J. H. Tizard, M. S. Ali, J. J. Conolly, B. Al-Sinawi and S. D. Simpson, *Appl. Environ. Microbiol.*, 2011, **77**, 5467-5475.
3. C. J. Chuck and J. Donnelly, *Applied Energy*, 2014, **118**, 83-91.

Transposon Mutagenesis Of *C. autoethanogenum*

CRAIG WOODS, CHRISTOPHER HUMPHREYS, ANNE M. HENSTRA, KLAUS WINZER, NIGEL P. MINTON

BBSRC / EPSRC Synthetic Biology Research Centre, The University of Nottingham, University Park, Nottingham NG7 2RD

Forward genetics studies in *Clostridium autoethanogenum* aim to elucidate mechanisms of product formation and tolerance. A pool of transposon mutants can be screened for useful phenotypic traits to provide candidate genes for directed strain production. Initial product targets for this work are ethanol, 2,3-butanediol and isobutanol but the system can be applied to a variety of products of an industrial strain. The transposon library can also be used to undertake transposon directed insertion-site sequencing (TraDIS). TraDIS involves sequencing a transposon mutant library, 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. This will be of use for directed methods of strain production, to avoid wasteful attempts at knocking out essential genes. With a large enough mutant pool TraDIS can also be used to generate lists of genes advantageous or disadvantageous to a given growth condition.

Transposon mutants will be generated using the *mariner Himar1C9* transposase expressed via the *tcdR-tcdB* orthogonal expression system. The P_{tcdB} promoter is placed in front of the transposase and is active only in the presence of the *C. difficile* sigma factor TcdR. This conformation prevents unwanted transposition in *E. coli* storage and donor strains. *TcdR* is not present in wildtype *C. autoethanogenum,* but has been inserted into the genome using Allele-Coupled Exchange (ACE). The *TcdR* strain can then drive transposition from the plasmid-based transposon. One problem with transposon mutagenesis is that of multiple transposition events in the same genome, in this case it is not possible to attribute a single gene to the observed phenotype when screening. To increase the frequency of single transposon mutants, loss of the conditional transposon-carrying plasmid can be forced.

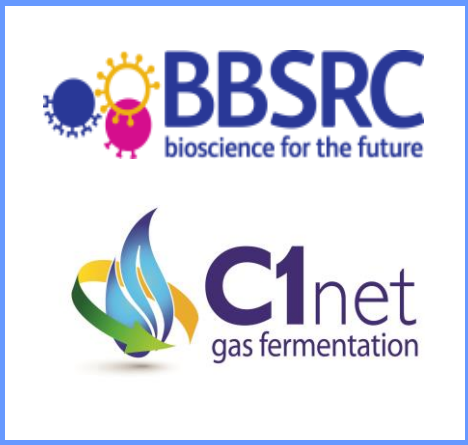
PARTICIPANTS

SURNAME	FIRST NAME	INSTITUTE	ROLE	EXPERTISE	EMAIL ADDRESS
Alagesan	Swathi	SBRC Nottingham, UK	PDRA	Synthetic Biology	Swathi.Alagesan@nottingham.ac.uk
Alonso Tuero	Saul	Centre for Process Innovation, UK	Fermentation scientist	Microbial fermentation	saul.alonso-tuero@uk-cpi.com
Annan	Florence	SBRC Nottingham, UK	PhD student	Clostridia, Gas fermentation	mrxfja@nottingham.ac.uk
Arenas	Christian	SBRC Nottingham, UK	PhD student	Clostridia, Gas fermentation	mrxca1@nottingham.ac.uk
Bar-Even	Arren	Max Planck Institute, Germany	Group Leader	Systems Biology	arren.be@gmail.com
Barnard	Ferlin	Pera Technology, UK	Account Manager	Funding Solutions	f.barnard@peratechnology.com
Bommareddy	Rajesh	SBRC Nottingham, UK	PDRA	Bioprocess Development	rajesh.bommareddy@nottingham.ac.uk
Breitkopf	Ronja	SBRC Nottingham, UK	PhD student	Microbiology, Gas Fermentation	mrxrb14@nottingham.ac.uk
Bryan	Samantha	SBRC Nottingham, UK	PDRA	Molecular Biology, Microbiology	samantha.bryan@nottingham.ac.uk
Burbidge	Alan	SBRC Nottingham, UK	Centre Manager	IB commercialisation	alan.burbidge@nottingham.ac.uk
Burgess	Gary	Bio Dynamic Ltd, UK	Technical Director	AD Engineering	gary.burgess@biodynamicuk.com
Carr	Reuben	Ingenza, UK	Team Leader	Bioprocessing, Manufacturing	r.carr@ingenza.com
Clark	Peter	Knowledge Transfer Network, UK	Network manager	Continuous Flow Chemistry	peter.clark@ktn-uk.org
Conradie	Alex	University of Nottingham, UK	Professor	Metabolic engineering, Bioprocessing	alex.conradie@nottingham.ac.uk
Crombie	Andrew	University of East Anglia, UK	PDRA	Molecular Biology, Microbial Genetics	a.crombie@uea.ac.uk
De Bruyn	Frederik	Bio Base Europe, Belgium	Business Developer	Scale-up fermentation	Frederik.De.Bruyn@bbeu.org
Dinsdale	Richard	University of South Wales, UK	Professor	Lithotrophs, Bioplastics	richard.dinsdale@southwales.ac.uk
Dixon	Nathan	SBRC Nottingham, UK	Research technician	Microbiology, Analytical Chemistry	nathan.dixon@nottingham.ac.uk
Duerre	Peter	University of Ulm, Germany	Professor	Anaerobes	peter.duerre@uni-ulm.de
Dunster	Joanne	University of Reading, UK	PDRA	Mathematical modelling, Colisytems	j.l.dunster@reading.ac.uk
Dynes	Louise	SBRC Nottingham, UK	Outreach Officer	Environmental Science	louise.dynes@nottingham.ac.uk
Ehsaan	Muhammad	SBRC Nottingham, UK	Research Fellow	Synthetic Biology, Microbiology	muhammad.ehsaan@nottingham.ac.uk
El-Esawi	Mohamed	Tanta University, Egypt and University of Nottingham, UK	Lecturer	Biotechnology	elesawi2005@yahoo.com
Fell	David	Oxford Brookes University, UK	Professor	Metabolic modelling	dfell@brookes.ac.uk
Gabrielli	William	Sasol Technology Ltd, UK	Principal Scientist	Chemistry	William.Gabrielli@eu.sasol.com
Garavaglia	Marco	SBRC Nottingham, UK	PDRA	Microbiology	mbzmg2@nottingham.ac.uk
Garcia-Gonzalez	Linsey	VITO NV, Belgium	Senior Scientist	Unit Separation, Conversion Technology	linsey.garcia-gonzalez@vito.be
Gianesella	Marco	Pera Technology, UK	Senior Energy Technologist	Energy, Process Engineering	m.gianesella@peratechnology.com
Gilbert	James	SBRC Nottingham, UK	Research Associate	Computational Modelling, Transcriptomics	james.gilbert@nottingham.ac.uk

Gill	Satbir	Ingenza Ltd, UK	Senior scientist	Molecular Biology	satbir.gill@ingenza.com
Gradley	Michelle	ZuvaSyntha, UK	Director Process Biotechnology	Microbiology, Genetics	michelle.gradley@zuvasyntha.com
Green	Edward	CHAIN Biotechnology, UK	Founder &Chief Scientific Advisor	Industrial Biotech	edward@chainbiotech.com
Grob	Ralph	Centre for Process Innovation, UK	Group Manager	Process Engineering	grobralph@gmail.com
Guentner	Bernhard	Syngip BV, The Netherlands	CEO	Strain development, Gas fermentation	info@syngip.com
Guillouet	Stéphane	INSA de Toulouse, France	Professor	Fermentation, Microbial Engineering	stephane.guillouet@insa-toulouse.fr
Harding	Emily	SBRC Nottingham, UK	Research Technician	Microbiology	emily.harding@nottingham.ac.uk
Hartman	Hassan	Oxford Brookes, UK	PDRA	Metabolic Modelling	hhartman@brookes.ac.uk
Hayes	Gary	Hgen Cleantech, UK	Chairman	Methanation of Hydrogen	info@hgencleantech.com
Henstra	Anne	SBRC Nottingham, UK	Research Fellow	Gas fermentation	anne.henstra@nottingham.ac.uk
Higgins	Ian	Calysta, UK	Analytical Scientist	Analytical Chemistry	ihiggins@calysta.com
Hodgson	David	Durham University, UK	Senior Lecturer	Physical, Organic & Biological Chemistry	d.r.w.hodgson@durham.ac.uk
Howarth	Rob	The University of Nottingham, UK	Business Engagement Executive	Business Development	rob.howarth@nottingham.ac.uk
Ibenegbu	Christopher	University of Bath, UK	PDRA	Biofuels	chrisibenegbu@yahoo.com
Infants	Alba	Karlsruhe Institute for Technology, Germany	PhD student	Gas fermentation	alba.infantes@kit.edu
Johannessen	Arild	Calysta, UK	Vice President, Nutrition	Fermentation, Process development	ajo@calysta.com
Juna	Azhar	Centre for Process Innovation, UK	Process Engineer	Syngas generation	azhar.juna@uk-cpi.com
Kapoore	Rahul	The University of Sheffield, UK	PDRA	Systems Biology, Sustainable Bioenergy	rahulkapoore@gmail.com
Kennes	Christian	University of La Coruña, Spain	Professor	Environmental biotechnology	kennes@udc.es
King	John	SBRC Nottingham, UK	Professor	Mathematical modelling	john.king@nottingham.ac.uk
Koelschbach	Janina	Chain Biotechnology, UK	Senior Scientist	Microbial biochemistry	Janina.Koelschbach@nottingham.ac.uk
Kovacs	Katalin	SBRC Nottingham, UK	PDRA	Microbiology, Synthetic Biology	Katalin.Kovacs@nottingham.ac.uk
Krabben	Preben	Green Biologics Ltd, UK	Senior Research Scientist	Clostridial physiology, Genomics	Preben.Krabben@greenbiologics.com
Kriechbaumer	Verena	Oxford Brookes University, UK	Research Scientist	Plant Biology & Biochemistry	vkriechbaumer@brookes.ac.uk
Little	Gareth	SBRC Nottingham, UK	PDRA	Clostridia, Genetic Manipulation	gareth.little@nottingham.ac.uk
Malys	Naglis	SBRC Nottingham, UK	Senior Research Fellow	Biochemistry, Synthetic Biology	Naglis.Malys@nottingham.ac.uk
Mangiafridda	Valentina	Centre for Process Innovation, UK	Lab Manager	Gas fermentation	valentina.mangiafridda@uk-cpi.com
Mansfield	Robert	SBRC Nottingham, UK	PhD student	Syngas fermentation	stxrpm@nottingham.ac.uk
McKechnie	Jon	University of Nottingham, UK	Lecturer	Techno-economic analysis	jon.mckechnie@nottingham.ac.uk
Mesfin	Noah	Oxford Brookes University, UK	PhD student	Metabolic modelling	14102280@brookes.ac.uk

Miles	Colin	BBSRC, UK	Head of Strategy	Industrial Biotechnology and Bioenergy	colin.miles@bbsrc.ac.uk
Millard	James	SBRC Nottingham, UK	PhD student	Molecular Microbiology	stxcjmi@nottingham.ac.uk
Millat	Thomas	SBRC Nottingham, UK	PDRA	Metabolic modelling	thomas.millat@nottingham.ac.uk
Minton	Nigel	SBRC Nottingham, UK	Professor	Gas fermentation	nigel.minton@nottingham.ac.uk
Minton	Jacque	SBRC Nottingham, UK	Network Manager	Biology	jacqueline.minton@nottingham.ac.uk
Mitra	Robin	Centre for Process Innovation, UK	Senior Program Manager	Bioprocess Development	robin.mitra@uk-cpi.com
Murray	James	Imperial College London, UK	Lecturer	Synthetic Biology	j.w.murray@imperial.ac.uk
Murrell	Colin	University of East Anglia, UK	Professor	Environmental Science	j.c.murrell@uea.ac.uk
Norman	Rupert	SBRC Nottingham, UK	PhD student	Synthetic Biology	stxron@nottingham.ac.uk
Ortega	David	SBRC Nottingham, UK	PhD student	Biochemistry	stxdo4@nottingham.ac.uk
Oswald	Florian	Karlsruhe Institute for Technology, Germany	PhD student	Anaerobic fermentation of syngas	florian.oswald@kit.edu
Pander	Bart	SBRC Nottingham, UK	PhD student	Gas fermentation	mrxbp@nottingham.ac.uk
Pasini	Martina	SBRC Nottingham, UK	Research Associate	Molecular and Synthetic Biology	martina.pasini8@gmail.com
Patel	Rajan	SBRC Nottingham, UK	PhD student	Industrial Biotechnology	stxrp11@nottingham.ac.uk
Patel	Vanisha	SBRC Nottingham, UK	PhD student	Mircobiology	mbxvp@nottingham.ac.uk
Pearcy	Nicole	SBRC Nottingham, UK	Research Associate	Metabolic networks, Modelling	Nicole.pearcy@nottingham.ac.uk
Princova	Diana	University of Nottingham, UK	MSc student	Synthetic Biology	diana.princova@hotmail.com
Puri	Dhivya	Fiberight, UK	R&D Engineer	Lignocellulose Anaerobic Digestion	dhivya.puri@fiberight.co.uk
Raut	Mahendra	University of Sheffield, UK	PDRA	Microbiology, Proteomics, Metabolomics	m.raut@sheffield.ac.uk
Rosenbaum	Miriam	RWTH Aachen University, Germany	Professor	Bioelectrochemical systems, Mixed cultures	Miriam.Rosenbaum@rwth-aachen.de
Rowe	Peter	SBRC Nottingham, UK	PhD student	Microbiology	msxpr@nottingham.ac.uk
Ruijssenaars	Harald	Corbion, The Netherlands	Principal scientist	Microbiology, Biochemistry	harald.ruijssenaars@corbion.com
Rumah	Bashir	SBRC Nottingham, UK	PhD student	Biochemistry	msxbr@nottingham.ac.uk
Ryan	John	SBRC Nottingham, UK	PhD student	Biotechnology	pcyjr1@nottingham.ac.uk
Sablitzky	Jan	SBRC Nottingham, UK	Centre PA	Event organisation	Janice.Sablitzky@nottingham.ac.uk
Saville	Renee	Calysta, USA	Senior scientist	Metabolic engineering	rsaville@calysta.com
Serna-Maza	Alba	University of Southampton, UK	Research fellow	Anaerobic digestion, Bioreactors	asm3g10@soton.ac.uk
Shah	Rehan	HEL Ltd, UK	Marketing Executive	Gas fermentation	rshah@helgroup.com
Silverman	Josh	Calysta, USA	Founder & CTO	Biotechnology	jsilverman@calysta.com
Simpson	Sean	LanzaTech, USA	Founder & MD	Gas fermentation	sean@lanzatech.com
Singh	Jasbir	HEL Ltd, UK	Director	Gas fermentation	jasbirsingh@helgroup.com
Smith	Tom	Sheffield Hallam University, UK	Professor	Methanotophs	t.j.smith@shu.ac.uk

Srodka	Zofia	Centre for Process Innovation, UK	Scientist	Microbial fermentation	zofia.srodka@uk-cpi.com
Stead	Christopher	SBRC Nottingham, UK	PhD student	Microbiology	msxcs3@nottingham.ac.uk
Strong	Philippa	SBRC Nottingham, UK	Molecular Biologist	Microbiology, Genetic manipulation	philippa.strong@nottingham.ac.uk
Tayler	Amy	Knowledge Transfer Network, UK	Manager	Industrial Biotechnology, Synthetic Biology	amy.tayler@ktn-uk.org
Thauer	Rolf	Max Planck Institute, Germany	Professor Emeritus	Methanogens	thauer@mpi-marburg.mpg.de
Tolhurst	Lucinda	Lucid Insight Ltd, UK	Director	Resource recovery, Waste utilisation	lucinda@lucid-insight.com
Twycross	Jamie	SBRC Nottingham, UK	Ass. Professor	Computer Modelling	jamie.twycross@nottingham.ac.uk
Uhlig	Ronny	RWTH Aachen University, Germany	Post doc	Molecular Biology	ronny.uhlig@rwth-aachen.de
Walter	Frederik	SBRC Nottingham, UK	PDRA	Metabolic engineering, Synthetic Biology	frederik.walter@nottingham.ac.uk
Wang	Yanming	SBRC Nottingham, UK	Research Associate	Fermentation, Bioprocess development	yanming.wang@nottingham.ac.uk
Wichlacz	Alex	SBRC Nottingham, UK	PhD student	Chemistry, Biochemistry	pcxatw@nottingham.ac.uk
Wimalasena	Tithira	Calysta, UK	Senoir Scientist	Fermentation, Synthetic Biology	tithiraw@calysta.com
Winzer	Klaus	SBRC Nottingham, UK	Associate Professor	Bacterial metabolism	Klaus.winzer@nottingham.ac.uk
Woods	Craig	SBRC Nottingham, UK	PhD student	Clostridia, Synthetic Biology	mrxcw4@nottingham.ac.uk
Wright	Phillip	Newcastle University, UK	Professor	Chemical Engineering	Phillip.wright@ncl.ac.uk
Zhang	Ying	SBRC Nottingham, UK	Senior Research Fellow	Metabolic engineering	ying.zhang@nottingham.ac.uk
Zimmerman	William	University of Sheffield, UK	Professor	Fluid and microbubble dynamics	w.zimmerman@shef.ac.uk



Acknowledgement

We acknowledge that the BBSRC through C1net supported this project
Grant Code BB/L013800/1