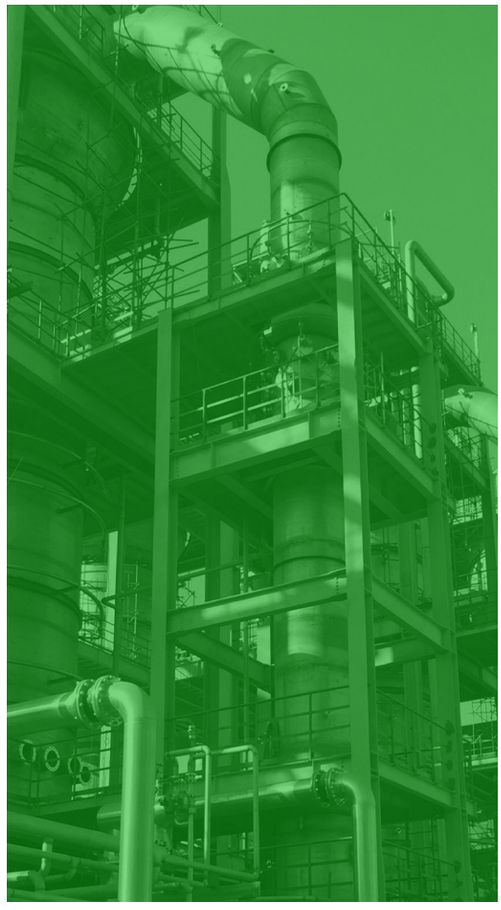
C1net Conference 4

20 - 23 January 2019

East Midlands Conference Centre,
University of Nottingham,
Nottingham,
UK

Programme and Abstracts



C1net gas fermentation

BBSRC bioscience for the future

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C1net Management Board

Nigel Minton (PI)	SBRC Nottingham, UK
David Fell (CoI)	Oxford Brookes University, UK
Rueben Carr	Ingenza Ltd, UK
William Gabrielli	Sasol UK Ltd, UK
Michelle Gradley	BioSyntha Technology Ltd, UK
Edward Green	Chain Biotech Ltd, UK
Arild Johannessen	Biosentrum AS, Norway
Preben Krabben	Centre for Process Innovation Ltd, UK
Stephen Poulston	Johnson Matthey, UK
Sean Simpson	LanzaTech, USA
Gary Smith	Invista, UK
Tithira Wimalasena	Calysta, 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
t. 0115 846 6287
e. jacqueline.minton@nottingham.ac.uk

Conference Support:

Janine Claber
Marick Communications,
18 Modwen Road,
Waters Edge Business Park,
Salford Quays,
Manchester,
M5 3EZ
m. 0161 877 7693
e. janine@marickcommunications.co.uk

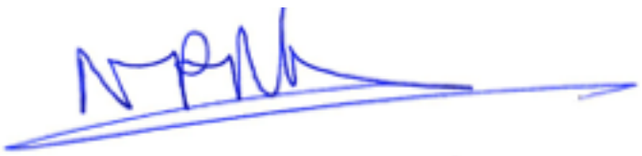
Welcome

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.

In the five year life-time from its inception 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 533 with members from all over the world; we have 479 followers on Twitter and have awarded a total of 18 POCs of £50,000 and 18 BIVs of £10,000. We have brought together 643 delegates at our conferences, trained 108 young scientists in our workshops and have reached out to the public at 43 Outreach events. In recognition of the great progress made and future potential of gas fermentation, it gives me great pleasure to announce, that the C1net will not end in March 2019, but will, thanks to BBSRC funding, evolve into CCnet (Carbon recycling: converting waste derived GHG into chemicals, fuels and animal feed). This new BBSRC-NIBB will not only continue the work done by C1net, but will also bring in those communities working on photosynthetic (cyanobacteria) and autotrophic CO_2 utilising chassis as well as exploring the potential of anaerobic digestion as a feedstock generator.

It is thus with a great feeling of optimism that I welcome you to this fourth C1net Conference.



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
University of Nottingham
Nottingham
NG7 2RJ

Oral Presentations

Oral presentations will be in the Conference Theatre. The length of oral presentations is scheduled for 15, 20 or 40 mins, within that presenters should allow 5 mins for discussion. All presentations should be prepared in a form of MS Power Point slide show and stored on USB sticks. The use of a personal computer or Mac is not possible. Your talk should be handed to the administration team on your arrival.

Poster Presentations

Poster presentations will be in the Atrium. 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.

Social Media

Our Twitter handle is @C1Net_NIBB and the conference hashtag is #C1netConf4.
We encourage all our delegates to share their attendance at the conference with their networks. Presenters please let your audience know if your work is confidential and should NOT be tweeted or recorded.

Data Protection

A photographer will be covering the event and the images may be used for promotional purposes in the future. If you have requested to opt out of this during the registration process / do not wish to be included in this, please collect a red lanyard from the registration desk. This lanyard will be used by the photographer to identify people who have requested not to be included in the event photographs.

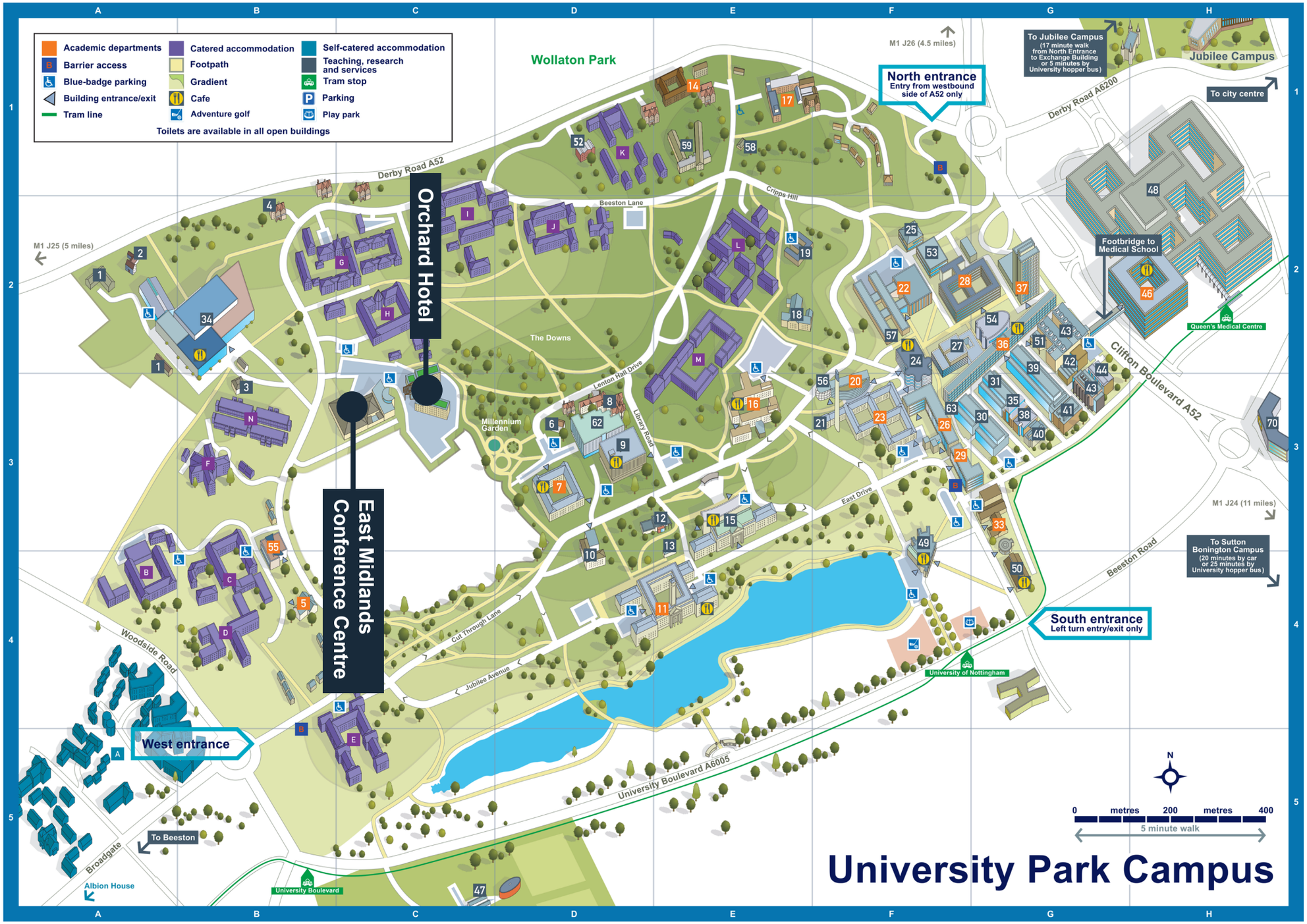
Social Events

*Welcome Reception & Dinner, Sunday 20 January, 19:00 & 20:00, Orchard Hotel
*Curry night, Monday 21 January, 19:30, Conference Theatre, EMCC
**Conference Dinner, Tuesday 22 January, 19:30, Conference Theatre, EMCC

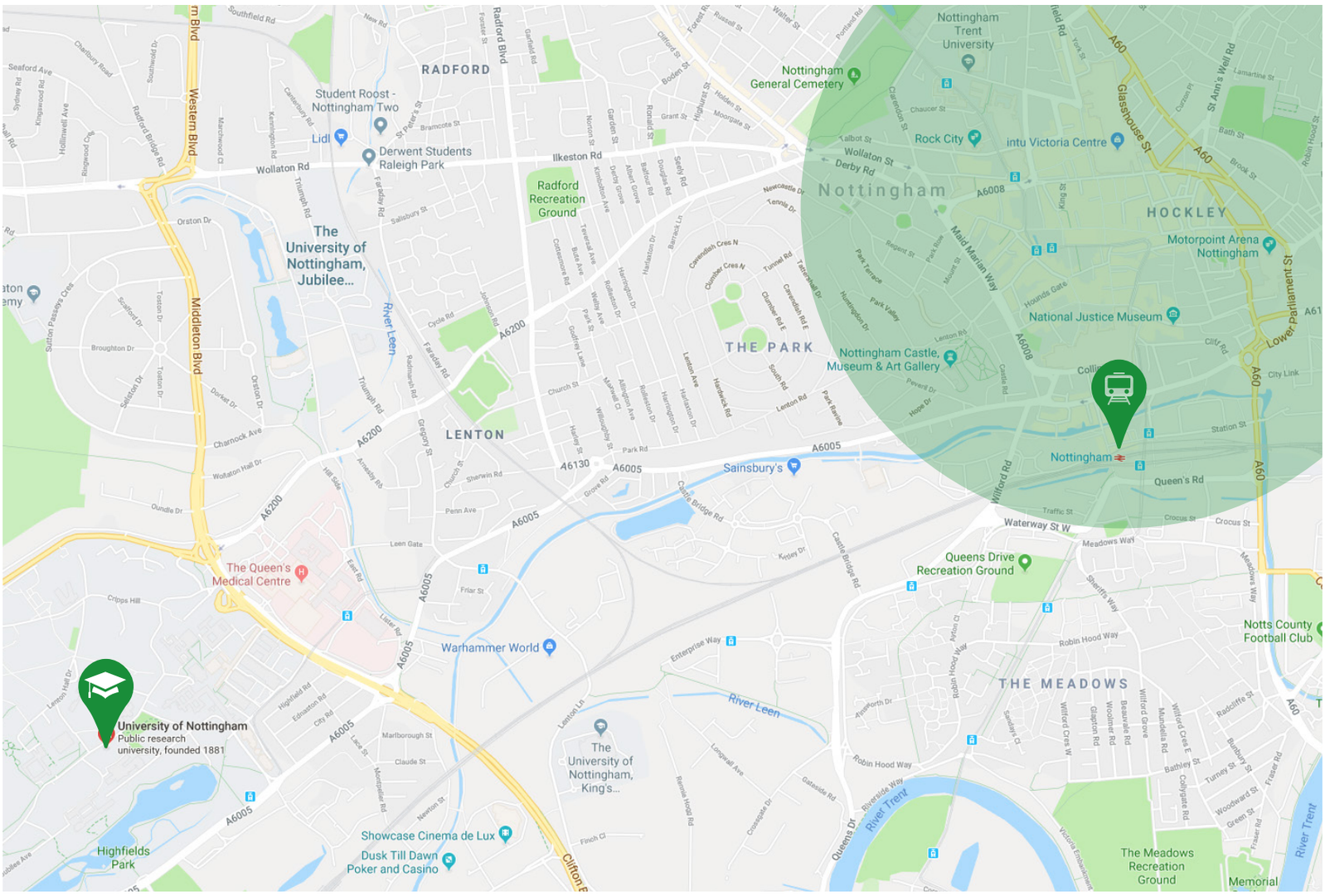
*For full delegates only
**For ALL delegates

Taxis

DG Taxis 0115 9500500 Trent Cars 0115 9505050 or ask the hotel.


Academic departments
Barrier access
Blue-badge parking
Building entrance/exit
Tram line
Catered accommodation
Footpath
Gradient
Cafe
Adventure golf
Self-catered accommodation
Teaching, research and services
Tram stop
Parking
Play park
Toilets are available in all open buildings
Wollaton Park
M1 J26 (4.5 miles)
North entrance
Entry from westbound side of A52 only
To Jubilee Campus
(17 minute walk from North Entrance to Exchange Building or 5 minutes by University hopper bus)
Jubilee Campus
To city centre
Derby Road A6200
Derby Road A52
Orchard Hotel
East Midlands Conference Centre
Beeston Lane
Cripps Hill
M1 J25 (5 miles)
Footbridge to Medical School
Queen's Medical Centre
The Downs
Lenton Hall Drive
Library Road
Millennium Garden
Clifton Boulevard A52
East Drive
M1 J24 (11 miles)
To Sutton Bonington Campus
(20 minutes by car or 25 minutes by University hopper bus)
Beeston Road
South entrance
Left turn entry/exit only
University of Nottingham
Cut Through Lane
Jubilee Avenue
Woodside Road
West entrance
University Boulevard A6005
To Beeston
Broadgate
Albion House
University Boulevard
N
0 metres 200 metres 400
5 minute walk
University Park Campus

Map of Nottingham



Green dot indicates Nottingham town centre

Directions: University of Nottingham to Nottingham train station/town centre

Walk to the tram stop located near the South Entrance or West Entrance and you can get direct trams to the train station for around £2.

Alternatively, a taxi will cost around £8.

Taxis

DG Taxis 0115 9500500 Trent Cars 0115 9505050 or ask the hotel.

Programme

MONDAY 21 JANUARY 2019		
SESSION 1	**CHAIR: Rolf Thauer *(Max Planck Institute, Germany)* Conference Theatre - EMCC**	
9:00-9.05	**Nigel Minton** *SBRC-Nottingham, UK*	Welcome
9:05-9:45	**Sean Simpson** *LanzaTech, USA*	INVITED TALK - Carbon recycling: commercializing a C1 fermentation process
9:45- 10:05	**In Seop Chang** *Gwangju Institute of Science and Technology, Republic of Korea*	Producing Nonnative Ethanol as a Major Product from Syngas Fermentation of an Engineered *Eubacterium limosum* KCTC1326BP
10:05-10:25	**Anja Wiechmann** *University of Frankfurt, Germany*	Hydrogen Cycling is Essential for Growth of the Acetogen *Acetobacterium woodii*
10:25-10:45	**Thomas Millat** *SBRC-Nottingham, UK*	Regulation of Metabolic Activity in *Clostridium autoethanogenum*
10:45-11:15	**COFFEE/TEA BREAK**	**Atrium - EMCC**
SESSION 2	**CHAIR: Sean Simpson *(LanzaTech, USA)* Conference Theatre – EMCC**	
11:15–11:55	**Volker Müller** *University of Frankfurt, Germany*	INVITED TALK - Physiological role and functional analyses of a novel enzyme for CO_2 reduction, the hydrogen-dependent CO_2 reductase
11:55-12:15	**Katalin Kovacs** *SBRC-Nottingham, UK*	Metabolic Engineering Of *Cupriavidus necator* H16 For The Sustainable Production Of C3 And C5 Monomers And Polymers
12:15-12:35	**Frank Bengelsdorf** *Ulm University, Germany*	Waste to Value - Production of the Bioplastic Poly(3-hydroxybutyrate) (PHB) and its Precursor 3-hydroxybutyrate (3-HB) from Waste gas
12:35- 12:55	**Matthias Beck** *Ulm University, Germany*	Conversion of CO_2 to the Platform Chemical 3-Hydroxypropanoic Acid (3-HP) Using Metabolically Engineered Strains of *Acetobacterium woodii*
12:55-14:00	**LUNCH**	**Atrium - EMCC**
SESSION 3	**CHAIR: David Fell *(Oxford Brookes University, UK)* Conference Theatre - EMCC**	
14:00-14:40	**Bryan Tracy** *White Dog Labs, USA*	INVITED TALK - Improving Biochemical Yields Through the Concurrent Consumption of Carbohydrates and C1 Gases
14:40-14:55	**Sandra Esteves** *University of South Wales, UK*	POC TALK - Novel PHA platform synthesized by anaerobic mixed bacteria in a single step gas fermentation
14:55-15:10	**Claudio Avignone Rossa** *University of Surrey, UK*	POC TALK - Microbial Electrosynthesis for the Capture and Transformation of CO_2 into Multicarbon Organic Compounds
15:10-15:25	**Ying Zhang** *SBRC-Nottingham, UK*	POC TALK - Reduce process carbon footprint-anaerobic fermentation of methane through reversion of methanogenesis
15:25-15:40	**Kamelia Boodhoo** *Newcastle University, UK*	POC TALK - Scalable engineering approaches for exploiting a novel biocomposite material applied to light-driven CO_2 absorption and utilization
15:40-17:30	**POSTERS AND REFRESHMENTS**	**Atrium - EMCC**

Programme

TUESDAY 22 JANUARY 2019		
SESSION 4	**CHAIR: Arild Johannessen *(NORCE Norwegian Research Centre AS)* Conference Theatre - EMCC**	
9:00-9:40	**Wolfgang Buckel** *Philipps-University, Marburg, Germany*	INVITED TALK - Electron Bifurcation in C1-Fermentations
9:40-10:00	**Charles Cotton** *Max Planck Institute, Germany*	Making Quantitative Sense of Electromicrobial Production
10:00-10:20	**Marie Schoelmerich** *University of Hamburg, Germany*	Regulation of Lactate Metabolism in the Acetogenic Bacterium *Acetobacterium woodii*
10:20-10:40	**Mohit Dalwadi** *University of Oxford, UK*	Relating Bacterial Properties To The Measured Effective Uptake in Gas Fermentation Using Multiscale Mathematical Modelling
10:40-11:15	**COFFEE/TEA BREAK**	**Atrium - EMCC**
SESSION 5	**CHAIR: Stephen Poulston *(Johnson Matthey Technology, UK)* Conference Theatre - EMCC**	
11:15-11:55	**Arren Bar-Even** *Max Planck Institute, Germany*	INVITED TALK - Engineering aerobic formate assimilation via the highly efficient reductive glycine pathway
11:55-12:10	**Robert Mansfield** *SBRC-Nottingham, UK*	POC TALK - Development of an assembly-based, recombineering pipeline for overcoming recalcitrance to DNA transfer caused by RM systems in C1 chassis
12:10-12-25	**Alex Grosse-Honebrink** *SBRC-Nottingham, UK*	POC TALK - High value uses of waste methane from landfill and biogas methane from anaerobic digestion
12:25-12:40	**William Zimmerman** *University of Sheffield, UK*	BIV TALK - Intensifying Fermentation, the microbubble way!
12:40-12:55	**Martin Warren** *University of Kent, UK*	POC TALK - Towards a Recombinant Pathway for Butanediol Biogenesis in Acetogens
12:55-14:00	**LUNCH**	**Atrium - EMCC**
SESSION 6	**CHAIR: Gary Smith *(Invista, UK)* Conference Theatre - EMCC**	
14:00-14:40	**E. Terry Papoutsakis** *University of Delaware, USA*	INVITED TALK - Direct cell-to-cell exchange of matter in a synthetic Clostridium syntrophy enables CO_2 fixation, superior metabolite yields, & an expanded metabolic space
14:40-15:00	**Diana Sousa** *Wageningen University, The Netherlands*	Tailored Microbial Consortia for Syngas Fermentation
15:00-15:20	**Romain Tuffou** *University of South Wales, UK*	Continuous Production of Acetate from Hydrogen and Carbon Dioxide Using Mixed Culture
15:20-15:40	**Kamran Jawed** *SBRC-Nottingham, UK*	Mixotrophic fermentation of *C. necator* H16 for PHA production
15:40-17:30	**POSTERS AND REFRESHMENTS**	**Atrium - EMCC**
17:00-19:00	**MANAGEMENT BOARD MEETING**	**Russet Suite**
19:30	**CONFERENCE DINNER**	**Conference Theatre – EMCC**

Programme

WEDNESDAY 23 JANUARY 2019		
SESSION 7	**CHAIR: Preben Krabben *(C1net Management Board)*- Conference Theatre - EMCC**	
9:00-9:40	**Byung-Kwan Cho** *Korea Advanced Institute of Science and Technology, Republic of Korea*	INVITED TALK - Systems and Synthetic Biology of Acetogenic Bacteria
9:40-10:00	**Muhammad Yasin** *COMSATS University Islamabad, Pakistan*	Microbial Bioprocessing: Optimum Reactor Design for Carbon monoxide (CO) Fermentation
10:00-10:20	**Jasbir Singh** *HEL Ltd, UK*	Gas fermentation of *Cupriavidus necator* H16 and variant at elevated pressure
10:20-10:40	**Reuben Carr** *Ingenza, UK*	Improving the Sustainability of Biobased Manufacturing
10:40-11:15	**COFFEE/TEA BREAK**	**Atrium - EMCC**
SESSION 8	**CHAIR: Tithira Wimalasena *(Calysta UK Ltd)* Conference Theatre – EMCC**	
11:15-11:55	**Lori Giver** *Calysta, USA*	INVITED TALK - FeedKind™ Protein: A Sustainable Approach to Meeting the Growing Protein Demand
11:55-12:10	**Dirk Holtman** *Dechema - Forschungs Institut (DFI), Germany*	Methanogens in Electrobiotechnology: electron transfer mechanism and reactor design
12:10-12:25	**Eun Yeol Lee** *Kyung Hee University, Republic of Korea*	Metabolic Engineering of Methanotrophs as Cell Factory for Methane-to-Chemicals Conversion
12:25-12:40	**Tatiana Spatola Rossi** *Oxford Brookes University, UK*	Bacterial methane monooxygenases in plants for methane detoxification
12:40-13:00	**Yue Zhang** *University of Southampton, UK*	Biomethanisation of CO_2 using H_2
13:00-13:10	**Nigel Minton** *SBRC-Nottingham, UK*	Closing remarks
13:10-14:30	**LUNCH AND DEPART**	**Atrium - EMCC**

POC PRESENTATIONS

POC-15-minton-C1net – Presented by Robert Mansfield
“Development of an assembly-based, recombineering pipeline for overcoming recalcitrance to DNA transfer caused by RM systems in C1 chassis”
PI – Nigel Minton, University of Nottingham, UK
Duration 12 months

POC-17-warren-C1net/ POC-23-warren-C1net – Presented by Martin Warren
“Towards a Recombinant Pathway for Butanediol Biogenesis in Acetogens”
PI - Martin Warren, University of Kent, UK
Duration 12 months and 6 months

POC-19-zimmerman-C1net/ BIV-7-zimmerman-C1net – Presented by Will Zimmerman
“Intensifying Fermentation, the microbubble way!”
PI – William Zimmerman, University of Sheffield, UK
Duration 12 months and 5 months

POC-20-boodhoo-C1net – Presented by Kamelia Boodhoo
“Scalable engineering approaches for exploiting a novel biocomposite material applied to light-driven CO2 absorption and utilization”
PI- Kamelia Boodhoo, Newcastle University, UK
Duration 7 months

POC-22-rossa-C1net – Presented by Claudio Avignone Rossa
“Microbial Electrosynthesis for the Capture and Transformation of CO2 into Multicarbon Organic Compounds “
PI - Claudio Avignone Rossa, University of Surrey, UK
Duration 6 months

POC-24-zhang-C1net – Presented by Alex Grosse-Honebrink
“High value uses of waste methane from landfill and biogas methane from anaerobic digestion”
PI – Ying Zhang, University of Nottingham, UK
Duration 7 months

ISCF-POC-13-esteves-C1net – Presented by Sandra Esteves
“Novel PHA platform synthesized by anaerobic mixed bacteria in a single step gas fermentation”
PI - Sandra Esteves, University of South Wales, UK
Duration 10 months

ISCF-POC-18-zhang-C1net – Presented by Ying Zhang
“Reduce process carbon footprint- anaerobic fermentation of methane through reversion of methanogenesis”
PI - Ying Zhang, University of Nottingham, UK
Duration 7 months

Invited Speakers

Invited Speakers

Arren Bar-Even
Research Group Leader
Max Planck Institute, Germany

Completed bachelor degree in the Excellence Program of the Technion, Israel Institute of Technology. Complete Master's degree in Bioinformatics at the Weizmann Institute of Technology, Israel. Completed PhD in Biochemistry at the Weizmann Institute of Technology, specializing in design principle of cellular metabolism. Since 2015, an Independent Research Group Leader at the Max Planck of Molecular Plant Physiology at Golm, Germany. Head of the Systems and Synthetic Metabolism lab. Specializes is applying biochemical logic principles for the rewiring for microbial central metabolism towards novel architectures to tackle humanity's grand challenges in food supply and sustainable production of carbon materials.

Dr. Wolfgang Buckel
Professor of Microbiology
Philipps-Universität, Germany

Dr. Wolfgang Buckel is Professor of Microbiology at Philipps-Universität, Marburg, Germany. He did his first Postdoc with H. Eggerer, LMU in collaboration with Sir John W. Cornforth, Sittingbourne, UK, and his second Postdoc with Horace Albert Barker, University of California at Berkeley, USA. Since then he has held positions at the Universität Regensburg and at the Philipps-Universität Marburg. He is a fellow of the Max-Planck-Society at the Max-Planck-Institute for Terrestrial Microbiology, Marburg. His main research interests are: Coenzyme A thioesters, enzyme mechanisms, stereochemistry, bioenergetics, anaerobic bacteria, radical enzymes, coenzyme B12, flavin-based electron bifurcation.

Invited Speakers

Dr. Lori Giver
Vice President of Biological Engineering
Calysta, USA

Dr Lori Giver is the Vice President of Biological Engineering at Calysta. She joined Calysta in 2013 and is responsible for R&D staff and project management. Prior to joining the company, Lori was with Codexis, Inc., where she held a number of positions including Vice President of Systems Biology, Senior Directory of Technology and Innovation, and Manager of Market Development. Prior to Codexis she was head of the Core Technology Group at Maxgyen, Inc. Dr. Giver has focused her career on the directed evolution of nucleic acids, proteins and organisms. She has chaired several conferences in the field including Enzyme Engineering XXI and Applications for Enzyme Technologies 2013. She is an author on more than 10 scientific publications and over 50 patents and patent applications. She received a Bachelor of Science degree in Molecular and Cell Biology from the University of California-Berkeley and Ph.D in Molecular Cell and Developmental Biology from Indiana University, Bloomington. Dr. Giver did her post-doctoral research at the California Institute of Technology in the laboratory of Dr. Frances Arnold.

Professor Volker Müller
Head of the Department of Molecular Microbiology and Bioenergetics
Goethe-University Frankfurt, Germany

Professor Müller is Head of the Department of Molecular Microbiology and Bioenergetics at Goethe University, Frankfurt. His research interest is the metabolism and biochemistry of anaerobic microorganisms with a focus on acetogenic bacteria. His group discovered how these bacteria make a living during autotrophic and heterotrophic growth, characterized the enzymes involved in bioenergetics, carbon and electron flow and redox homeostasis, and studies regulation of substrate utilization. The lab also uses archaea to study the metabolic processes that allow microbial life under extreme energy limitation and that couple CO_2 fixation to ATP synthesis. He directs a large research group of the German Research Foundation on *Acinetobacter baumannii* and an ERA-IB Network on industrial applications of acetogenic bacteria. He has co-authored more than 200 papers and was awarded in 2016 one of the prestigious Advanced Investigator Grants of the ERC to work on “Acetogenic bacteria: from basic physiology via gene regulation to application in industrial biotechnology”.

Invited Speakers

Eleftherios (Terry) Papoutsakis
Professor
University of Delaware, USA

Eleftherios (Terry) Papoutsakis is the Unidel Eugene DuPont Professor at the Dept. of Chem. & Biomolecular Engineering at the University of Delaware. Papoutsakis' group is active and has made important contributions in the areas of clostridia genetics and metabolic engineering and more recently synthetic methylotrophy. He received his undergraduate education at the National Technical University of Athens,Greece, and his PhD from Purdue University (USA). He has supervised over 60 PhD, 30 MS, and 31 postdoctoral students. He was elected to National Academy of Engineering (USA) in 2018; and is a Fellow of the American Chemical Society (ACS) and the American Academy of Microbiology (AAM). His awards include the 2017 ACS Murphree Award, the 2013 DIC Wang for Excellence in Biochemical Engineering Award (Amer. Inst. Of Chem. Engineers, AIChE), the 2012 James E. Bailey Award (AIChE); the 2010 Metabolic Engineering Award, & the 2005 Amgen Biochemical Engineering Award.

Dr Sean Simpson
Co-founder and Chief Scientific Officer
LanzaTech, USA

Dr. Sean Simpson is a Co-founder and Chief Scientific Officer of LanzaTech, a global leader in gas fermentation. Under Dr. Simpson's leadership, the company has established a broad and unique patent portfolio covering all areas of gas fermentation, including fermentation processes and microbes, gaseous feedstock handling, and product and waste handling. LanzaTech is experienced in technology commercialization, with commercial units in China and Belgium under development. Dr. Simpson has over 20 publications and 130 patents. He has received a number of awards including the 2015 US Environmental Protection Agency (EPA) Presidential Green Chemistry Award, the 2014 Sanitarium, NZ Innovator of the Year Award, the 2013 Kea NZ World Class New Zealander in Science Award, the 2013 Bio Spectrum Asia-Pacific Entrepreneur of the Year Award, the 2011 NZBIO Young Biotechnologist of the Year, and the 2011 Ernst and Young Entrepreneur of the Year, New Zealand.

Invited Speakers

Professor Rudolf Thauer
Professor
Max Planck Institute, Germany

Professor Thauer is a biochemist interested in the ecology and physiology of anaerobic bacteria and archaea. After his retirement 2008 as Professor at the Faculty of Biology of the Philipps-University Marburg and as Director of the Max Planck Institute for Terrestrial Microbiology in Marburg, he continued experimental work in the Max Planck Institute until end of 2014. Since then he has focused on theoretical studies on how strict anaerobes conserve energy. He is known primarily for his work on the biochemistry of methanogens. He received Lwoff Award of the Federation European Microbiology Societies (FEMS) in 2015, and before that numerous other honours including honorary doctorates from ETH Zurich, University of Waterloo and the University of Freiburg. In 1991 he became founding director of the Max Planck Institute for Terrestrial Microbiology in Marburg. Since 1984 he is member of the German National Academy of Science Leopoldina.

Bryan Tracy
CEO
White Dog Labs, USA

Bryan Tracy is the CEO and co-founder of White Dog Labs (WDL), Inc. WDL is developing and commercializing biotechnology solutions for the production of sustainable chemicals, protein, food ingredients, animal nutrition additives and transportation fuels. Prior to WDL, Bryan co-founded and led Elcriton, Inc., a synthetic biology company that worked with many of the world's largest chemical companies. Elcriton was founded with Eleftherios Papoutsakis and focused on clostridial solutions for biochemicals and fuels. Elcriton was acquired by WDL in 2014. Bryan's expertise is in microbial engineering, bioprocess product development and commercialization of new technologies. He has managed numerous government grants from various US agencies and the Binational Industrial Research and Development Foundation in Israel, and served as a technology to market consultant for the Department of Energy ARPA-E. He is a founding member of the American Chemical Society Biochemical Technology Leadership Roundtable, board chair of the Delaware Sustainable Chemistry Alliance, and previous board chair of the Forum for the Advancement of Minorities in Engineering (FAME). Outside of work, Bryan enjoys an active life with his wife Melissa and their two children Deirdre and Gabriel. Bryan received a BS in Chemical and Biomolecular Engineering from North Carolina State University and a PhD in Chemical and Biological Engineering from Northwestern University.

Industry Stands

Autichem

Autichem Ltd specialises in designing and manufacturing new equipment and processes for the pharmaceutical, fine chemical and energy sectors. Based at Thornton Science Park, Autichem has collaborated with the University of Chester on a number of projects focused on improving incumbent processing methodologies, with an emphasis on Carbon Capture. Autichem's patented DART Reactor was the enabling technology in several of these applications. Focusing on algae's capability to capture CO_2 by photosynthesis, they developed the DART algae bioreactor which in tests, captured over 91% of carbon emissions - 59% more than when tested against conventional gas sparging methods.

Name: David Morris
Address: Autichem Ltd, B49, Thornton Science Park, Poole Lane, Ince, Cheshire, CH2 4NU
Telephone: +44(0) 1244 568 944 **Mobile:** +44(0) 7720 968 520
Email: david@autichem.co.uk
Web: www.autichem.co.uk

Deep Branch BIOTECHNOLOGY

Transforming The Polluters Of Today Into The Producers Of Tomorrow

Deep Branch Biotechnology (DBB) has set out to transform the polluters of today into the producers of tomorrow. We are developing a robust gas-fermentation process targeted toward recycling the CO_2 rich waste-gas emissions from industries such as cement manufacturing, converting them into a harvestable microbial biomass. Our product, a high-quality single-cell-protein, represents a commercially valuable and environmentally conscious alternative to the fishmeal and soy-protein which today account for the major protein sources in animal-feeds worldwide.

Name: Rob Mansfield
Address: Deep Branch Biotechnology, 90 Middleton Boulevard, Nottingham, NG81AA
Telephone: +44(0)7732211998
Web: www.deepbranchbio.com

Industry Stands

better chemistry – faster

Founded in 1987 and based in North London, UK, HEL is an international company that specialises in chemical reactors, bioreactors and related data analysis tools for R&D in the pharmaceutical, chemical and biotechnology sectors. We have a broad product portfolio ranging from high performance single bioreactor systems to advanced integrated robotic, multi reactor systems. Our high pressure bioprocess platforms have been specifically designed for C1 gas fermentation and include an extensive range of features including sophisticated gas management controls. With standard and customised designs we can address virtually any C1 gas fermentation process.

Name: Gerard Gardner
Address: HEL, 9-10 Capital Business Park, Manor Way, Borehamwood, Hertfordshire, WD6 1GW
Telephone: +44 (0) 20 8736 0640 (Main)
Email: gerardgardner@helgroup.com
Web: www.helgroup.com

Lutra Ltd is a specialist biogas design and research consultancy. The director is Michael Chesshire who has more than 40 years of experience in AD across many sectors – sewage sludge, food waste and farm manure. Our design for biogas plants is based on our innovative below-ground plug-flow digester. Research work uses our 1500-litre pilot AD plant and is currently concentrating, in partnership with the University of Southampton, on the in-situ biomethanation of hydrogen and carbon dioxide.

Name: Michael Chesshire
Address: Lutra Ltd, Barrett's Mill, Woofferton, Ludlow, SY8 4AH
Email: lutra@lutra.eu
Web: www.lutra.eu

Abstracts of Oral Presentations

Carbon Recycling: Commercializing a C1 Fermentation Process

SEAN SIMPSON

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

World energy demand is expected to increase by up to 40% by 2035. As concerns about the economic and social impact of climate change grow, the need for strategies to minimize the emission of CO_2 through the production of the fuel, chemical and nutritional products demanded by societies intensifies. Gas fermentation allows the sustainable production of fuels, chemicals and nutrition products by recycling carbon from local, highly abundant, low-cost waste resources. The technology has been successfully demonstrated using a diverse range feedstocks that are composed of carbon monoxide (CO), carbon dioxide (CO_2) or methane (CH4), including waste gases from industrial sources (e.g., steel mills, or refineries), syngas generated from any biomass resource (e.g., unsorted and unrecyclable municipal solid waste, or agricultural waste) or biogas.

LanzaTech is pioneering the commercialization of a complete process platform to allow the continuous biological production of fuels and an array of chemicals intermediates from gases at scale. The first commercial plant demonstrating ethanol production from gas residues produced by the steel industry has been commissioned in China. Further are currently un commercial plants are in design or undre construction with the process having been demonstrated with live feeds of waste gas from numerous processes and industries

To expand the product production portfolio from these C1 fermentation processes, synthetic biology techniques have been developed for certain gas fermentation model organisms. The commercial deployment of bacteria able to produce different chemical intermediates from gases paves the way for the operation of “product flexible” conversion facilities that are able switch between final products by deploying a different bacteria in their bioreactors according to changing market dynamics. In this way gas fermentation is a vital bridge in the effort to create value from waste streams as part of an increasingly circular economic model.

Producing Nonnative Ethanol as a Major Product from Syngas Fermentation of an Engineered *Eubacterium limosum* KCTC1326BP

MUNGYU LEE[1], MUHAMMAD YASIN[1], JI YEONG JEONG[1], NURI JANG[1], ROBERT W. LOVITT[2], IN SEOP CHANG[1]

[1]School of Earth Sciences and Environmental Engineering, Gwangju institute of Science and Technology (GIST), Gwangju 61005, Republic of Korea
[2]College of Engineering, Swansea University, SA2 8PP, United Kingdom

Microbial processing of biomass-based syngas into marketable bioethanol is the most important aspect of C1 biorefinery. However, development of commercial syngas fermentation technology is limited by microbial characteristics as well as process constraints such as low microbial productivity, poor gas-liquid mass transfer efficiency and low cell concentration. Several efforts are made in the recent past to overcome these challenges through development of engineered strains and optimization of bioreactor systems. Our previous efforts were also contributed to overcome these process constraints by the employment of new design of gas diffusers to achieve high mass transfer rate and reactor operation to make cell concentration to be desired. In this study, we have used an engineered strain of *Eubacterium limosum* (Elm) KTCT1326BP (formerly, KIST612), able to produce nonnative product i.e., ethanol as a major product. Basically, the Elm has high CO conversion and acetate production rates compared to other reported strains. The strain autotrophically produce acetate as a main product but does not natively produce ethanol. Engineered Elm strain was constructed by heterologous expression of alcohol dehydrogenase, and confirmed its ethanol production significantly in vial test. The practicality of strain to produce a significant ethanol concentrations from syngas ($CO:CO_2$: 4:1) is assessed during a continuous microbial reaction in a cell-recycled bubble column reactor system. A continuous supply of dissolved nutrients and increase in CO mass transfer were used as controllable parameters to achieve high substrate consumption rate, and high cell and product concentrations. Ethanol to acetate ratio was increased during the operation, and significant amount of ethanol is obtained at the end of reactor operation. Obtained ethanol concentration is higher compared with several previously reported studies using different CO utilizing ethanol producers. These results would help to further optimize the process to bring down the acetate concentration to a level where ethanol concentration is competitive with other strains and commercially viable.

Hydrogen Cycling is Essential for Growth of the Acetogen *Acetobacterium woodii*

ANJA WIECHMANN, SARAH CIURUS AND VOLKER MÜLLER

Molecular Microbiology & Bioenergetics, Institute of Molecular Biosciences, Johann Wolfgang Goethe University Frankfurt/Main, Frankfurt, Germany

Acetogenic bacteria are characterised by the Wood-Ljungdahl pathway (WLP) of CO_2 fixation that allows lithotrophic growth on H_2+CO_2 or homoacetogenesis from organic substrates such as glucose. The first step in the methyl branch of the WLP is catalysed by the hydrogen-dependent CO_2 reductase (HDCR) that, *in vitro*, uses H_2 but also reduced ferredoxin as reductant. To determine, whether H_2 is essential for the WLP, we deleted genes encoding for the two major subunits of the electron-bifurcating hydrogenase in *Acetobacterium woodii*. The mutant no longer grew on H_2+CO_2 but also not on fructose. Growth on fructose could be restored by addition of H_2, indicating that the HDCR reaction did not run in the mutant. This is corroborated by the observation that addition of the product of the HDCR reaction, formate, also restored growth. Caffeate is reduced with NADH and can replace CO_2 as electron acceptor. Also, by addition of caffeate the mutant grew. This is expected since neither H_2, nor the HDCR are needed for caffeate reduction.

In summary, we present evidence that hydrogen cycling is essential for carbonate respiration in the model acetogen *Acetobacterium woodii*.

Regulation of Metabolic Activity in *Clostridium autoethanogenum*

THOMAS MILLAT, ANNE HENSTRA, RUPERT NORMAN, KLAUS WINZER And NIGEL MINTON

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

Recently, the acetogen *Clostridium autoethanogenum* has attracted academic and industrial interest due to its ability to convert waste gases (CO, CO_2) into value-added biochemicals and biofuels including ethanol and 2,3-butanediol. We conducted a series of experiments using a standardised continuous culture setup and sampling scheme to study the bacterium's response to changes in process conditions. The gathered multi-omics data were examined using an automated workflow for data processing and analysis to identify emerging patterns in the cellular response. Furthermore, a genome-scale model provides further insight into flux distributions and identifies targets for deliberate mutagenesis.

Grown on CO, *C. autoethanogenum* predominantly forms acetate and ethanol and in minor amounts 2,3-butanediol and lactate. It also produces H_2 and CO_2, the former being important for ATP generation during gas fermentation. With rising CO supply, we observed a metabolic switch from acidogenesis to ethanologenesis and rising levels of 2,3-BD and lactate. Importantly, our metabolite analysis indicated that ATP homeostasis neither triggers this phenomenon nor is a primary cellular objective. Transcriptomic activity suggested that CO utilization enforces the use of NADPH-dependent AlcDHs, in contrast to the NADH-dependent enzymes for the reduction of CO_2 and H_2, and confirmed that aldehyde ferredoxin oxidoreductase (AFOR) is the primary facilitator of ethanol formation. Interestingly, AFOR expression was strongly repressed with rising CO.

Our analysis revealed that thermodynamic regulation in combination with a change in growth limitation force the metabolic switch leading to significant changes in transcriptome and metabolome. Furthermore, constraints on hydrogen formation result in ethanol being the dominant product at high CO supply. Additionally, electron flow suggests that 2,3-butanediol and lactate are formed to compensate for the diminishing contribution of biomass formation.

Physiological Role and Functional Analyses of a Novel Enzyme for CO_2 Reduction, The Hydrogen-dependent CO_2 Reductase

VOLKER MÜLLER

Department of Molecular Microbiology and Bioenergetics, Institute of Molecular Biosciences, Goethe University, Frankfurt am Main, Germany

Acetogenic bacteria are characterized by a special pathway for CO_2 fixation, the Wood-Ljungdahl pathway (WLP). This pathway enables autotrophic growth on H_2 + CO_2 and is the only pathway of CO_2 fixation that is coupled to the synthesis of ATP. The electron carriers used are NADH and reduced ferredoxin, the latter for the CO dehydrogenase reaction and former for the methylene-THF dehydrogenase and the methylene-THF reductase. The first step in the methyl branch of the WLP in the model acetogens *Acetobacterium woodii* and *Thermoanaerobacter kivui* is catalyzed by the hydrogen-dependent carbon dioxide reductase (HDCR), a recently discovered solube enyzme containing four subunits.

To address the physiological role of the HDCR, the encoding genes were deleted from the chromosome of *T. kivui*. The mutant did no longer grow on hydrogen + carbon dioxide, but also not on fructose. Addition of formate restored growth on either fructose or hydrogen + carbon dioxide. To analyze the role of subunits in the catalytic mechanism of the HDCR, individual subunits and combinations thereof were produced in the HDCR mutant, purified and analyzed for reactions catalyzed and interaction partners. These studies gave the first insight into the path of electrons through the HDCR.

Metabolic Engineering Of *Cupriavidus necator* H16 For The Sustainable Production Of C3 And C5 Monomers And Polymers

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

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

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

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

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

Waste to Value - Production of the Bioplastic Poly(3-hydroxybutyrate) (PHB) and its Precursor 3-hydroxybutyrate (3-HB) from Waste gas

SEBASTIAN FLÜCHTER[1], FRANK R. BENGELSDORF[1], TINA BAUR[1], STEPHANIE FOLLONIER[2,3], BETTINA SCHIEL-BENGELSDORF[1], MANFRED ZINN[2] and PETER DÜRRE[1]

[1]Institut für Mikrobiologie und Biotechnologie, Universität Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany
[2]University of Applied Sciences and Arts Western Switzerland (HES-SO Valais), Institute of Life Technologies, Route du Rawyl 64, 1950 Sion, Switzerland
[3]Current Address: Lonza AG, Clinical Development-Microbial (USP), Lonzastrasse, 3930 Visp, Switzerland

The biopolymer poly(3-hydroxybutyrate) (PHB) is a fully biodegradable polymer and an attractive alternative to petroleum-based plastics. Synthesis gas (CO, H_2, CO_2) is a low-cost substrate for fermentations processes and a large-quantity waste gas in steel mill industry. We aimed for construction of gas-utilizing acetogens that produce PHB and its monomeric compound 3-hydroxybutyrate (3-HB).

The acetogens *Clostridium ljungdahlii* and *C. coskatii* were genetically engineered using two different plasmid-based production pathways for PHB and 3-HB. Recombinant strains were cultivated under heterotrophic and autotrophic conditions. Subsequently, production of 3-HB was analyzed using high performance liquid chromatography and PHB by various techniques such as gas chromatography, microscopy, nuclear magnetic resonance spectroscopy, differential scanning calorimetry, and gel permeation chromatography.

Recombinant strains of *C. ljungdahlii* and *C. coskatii* produced considerable amounts of PHB. PHB production in cells was visualized by microscopy. Thermal properties clearly showed all characteristics of PHB with high molecular weight. In contrast, successful synthesis of 3-HB was exclusively observed with engineered *C. coskatii.* Both, PHB and 3-HB proved to be stable products that can be further extracted, purified, and processed for a wide range of applications.

Production of the biopolymer and its precursor using recombinant acetogens opens the possibility of producing biodegradable plastic materials from cheap (waste) gases with low variability and large availability compared to other waste substrates.

Conversion of CO_2 to the Platform Chemical 3-Hydroxypropanoic Acid (3-HP) Using Metabolically Engineered Strains of *Acetobacterium woodii*

MATTHIAS H. BECK[1], JONATHAN BAKER[2], ANJA WIECHMANN[3], NIGEL P. MINTON[2], VOLKER MÜLLER[3], FRANK R. BENGELSDORF[1] AND PETER DÜRRE[1]

[1]: Universität Ulm, Institut für Mikrobiologie und Biotechnologie, Albert-Einstein-Allee 11, 89081 Ulm, Germany
[2]: BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), University of Nottingham, Nottingham, NG7 2RD, UK
[3]: Department of Molecular Microbiology and Bioenergetics, Institute of Molecular Biosciences, Johann Wolfgang Goethe University Frankfurt, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany

Acetobacterium woodii represents a promising candidate for the biotechnological production of high-value platform chemicals from CO_2. As such, 3-HP exhibits versatile applications as precursor for the production of acrylic acid, acrylamide, and poly(3-hydroxypropionate). The latter compound exhibits a prominent example for a biodegradable and biocompatible polymer with numerous possible implementations.

One major obstacle towards the production of 3-HP from CO_2 + H_2 using *A. woodii* strains is the native metabolism of lactate consumption. Lactate itself represents an important intermediate of the pursued 3-HP biosynthesis pathway.

The genes of the lactate dehydrogenase complex in *A. woodii* were deleted by allelic exchange using the *pyrE* gene as a counterselection marker. Several plasmids harboring a heterologous lactate dehydrogenase gene from *Leuconostoc mesenteroides* alone or in combination with the native pyruvate:ferredoxin oxidoreductase gene under the control of an inducible promoter were constructed. Further designed plasmids implied genes encoding the propionyl-CoA transferase (Pct), lactoyl-CoA dehydratase (Lcd) from *Clostridium neopropionicum*, and the enoyl-CoA hydratase (Ehy) from *Chloroflexus aurantiacus*. All plasmids were transformed into the respective *A. woodii* knock-out strain. Heterotrophic and autotrophic growth experiments were conducted in serum bottles followed by quantitative analysis of the fermentation products.

Growth experiments revealed that the engineered *A. woodii* strains were capable of producing up to 70 mM lactate from fructose and up to 10 mM lactate when cells were growing on CO_2 + H_2. Subsequently, the respective engineered *A. woodii* strain converted lactate into 3-HP (4 mM).

Via expression of heterologous 3-HP biosynthesis genes in *A. woodii*, lactate was produced from CO_2 + H_2 and subsequently successfully converted into 3-HP.

Improving Biochemical Yields Through the Concurrent Consumption of Carbohydrates and C1 Gases

BRYAN P. TRACY[1], EMILY E. CRAWFORD[1], JOHN R. PHILLIPS[1], PRADEEP C. MUNASINGHE[1], CARRISSA A. WIEDEL[1], BINIAM MARU[1], ILANA ALDOR[2], & SHAWN W. JONES[1].

[1]White Dog Labs, Inc., New Castle, DE, USA
[2]Delaware Innovation Campus, Air Liquide, Newark, DE, USA

Carbohydrate feedstocks are commonly more oxidized than the target metabolites of traditional fermentations. The classic example is ethanol from corn starch or sugar cane. Accordingly, there are physical limitations on mass and carbon yields, which can detriment economics and diminish life-cycle benefits. Occasionally the challenge is complicated by stoichiometry, for example the condensation and decarboxylation of acetyl-CoA to acetone, but the prevailing issue is the redox state per carbon mole of carbohydrate versus the desired products.

To overcome both limitations, we are developing a fermentation approach we call anaerobic, non-photosynthetic (ANP) mixotrophy, which employs clostridial acetogens that simultaneously consume organic (e.g., sugars) and inorganic (e.g., CO_2, CO, or H_2) substrates. We demonstrate that stoichiometry limitations can be overcome. For example, recombinant expression of an optimized acetone pathway in *Clostridium ljungdahlii* resulted in a strain that "refixed" CO_2 from decarboxylation of pyruvate to acetyl-CoA or acetoacetate to acetone. Mass yields of fructose to acetone increased to 43wt% compared to the theoretical, conventional maximum of 32wt%. ANP mixotrophy can also be practiced by adding exogenous, reduced gases such as H_2, CO and syngas mixtures to supplement the redox limitation of sugars, and generate near stoichiometric carbon yields to more reduced products. Recently, we have been demonstrating this potential with next-generation sugars, a diversity of clostridial acetogens, and consortiums of acetogens and non-acetogens.

Overall, ANP mixotrophy is a nascent but demonstrably robust and flexible platform with potential to maximize conversion of next-generation feedstocks, and combine conversion of low cost sugars and inexpensive reduced gases. The flexibility provides new strategies for biological conversion to be more economically viable with improved life-cycle outlooks.

Novel PHA Platform Synthesized By Anaerobic Mixed Bacteria in a Single Step Gas Fermentation

SANDRA R R ESTEVES

Wales Centre of Excellence for Anaerobic Digestion, Sustainable Environment Research Centre, University of South Wales, Pontypridd CF37 1DL

The project is developing the concept of a novel biotech process for the conversion of CO_2 into polyhydroxyalkanoates (PHAs) using anaerobic mixed cultures via single step fermentation; integrating with H_2 from renewable energy generation, gasification and industrial off-gases. Medical, packaging, chemical, textile and aquaculture food production are examples of sectors with a growing demand for these biomolecules.

The project aims to establish an understanding of communities' syntrophic interactions, metabolic pathways for PHA synthesis, and reduce bottlenecks for biopolymer accumulation, making a significant contribution towards the development of PHA synthesis using anaerobic metabolism. The project will also provide important insights into the PHA accumulation role in anaerobic bacteria as a stress response mechanism with a wider impact on other anaerobic biotech processes.

The project is being delivered as part of a collaboration between USW, Nitech Solutions and CSIC (Spain). Initial qPCR evaluations have determined that in typical anaerobic digesters, numerous bacteria feature PHA accumulating genes. A selection of anaerobic mixed cultures has been collected from full scale digesters operating on different feedstocks, using different process designs and inocula treatments have been carried out. These cultures will be evaluated in terms of PHA biosynthesis to establish which ones provide appropriate cultures for anaerobic PHA fermentation inoculants. High rate gas transfer reactors have been successfully established for mixed cultures where H_2 and CO_2 are converted to volatile fatty acids as well as CH_4. Future work will include specific co-fermentations using CO_2 and H_2 of a mixture of pure or mixed cultures in a single stage to achieve PHA production.

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

ROSA ANNA NASTRO and CLAUDIO AVIGNONE ROSSA

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

Microbial electrosynthesis (MES) or Electrofermentation (EF) can overcome stoichiometric and energy limitations, low yields, nutrient demands, and other issues found in conventional fermentation. In MES, the extracellular redox potential is modified by supplying electrons through the cathode, therefore displacing the intracellular redox balance required to obtain the desired fermentation products. It is then possible to utilize compounds of low degree of reduction (e.g. CO_2) as carbon sources to synthesize reduced molecules by supplying exogenous electrons to specific microorganisms. This electrochemical control redirects the metabolic and regulatory networks of single species as well as the metabolic interactions between species in co-cultures or multi-species communities.

We have assessed the effect of an external potential on the ability of an anodic biofilm composed by the binary consortium *Shewanella oneidensis* and *Pseudomonas aeruginosa* to provide electrons for CO_2 assimilation by *Clostridium saccharoperbutylacetonicum* present in the cathode. A potential of +3.00 V was applied to an MFC to allow the development of stable biofilms. At the steady state, potentials of +0.88 V and -0.215 V were observed at the anode and the cathode of the MFC. The evolution of both systems was monitored continuously for 30 days and the performance assessed in terms of Power Density (PD), Current Density (CD), Open Circuit Voltage (OCV). Polarization experiments were performed periodically, keeping the MFC at the maximum power, together with impedance, voltammetry and chronoamperometry analyses of the anodic biofilm. We measured the consumption of dissolved CO_2 and the presence of fermentation products in the cathodic suspension.

Our results showed an increased CD (from 1.24 to 31.9 fold) and of PD (from 1.19 to 58.0 fold) when the external potential is applied during the first 24 hours of operation, with a maximum of 721 mW/m^2 and 374.9 mA/m^2 at 1000Ω external load. In the cathode, *C. saccharoperbutylacetonicum* consumed approximately 50% of the dissolved CO_2 , and the chemical analysis of the fermentation products in the cathodic suspension showed peaks that can be associated to the production of acetate, butyrate and an unidentified compound with chemical characteristics generally associated to short-chain fatty acids. Full detail of the chemical analysis will be discussed.

We conclude that electrofermentation of CO_2 to produce valuable chemicals is a sustainable strategy that provides a viable alternative to current methods of chemical synthesis, that may contribute to the reduction of greenhouse gas emissions. The electrical input can be generated from renewable resources such as solar energy or wind power, supplementing the electrical power generated in the anode. If the system is fed with organic-rich feedstocks such as certain type of industrial wastewater, the overall output will be a decrease in the pollution levels and a reduction in CO_2 emissions, with the storage of energy under the form of covalent bonds in the organic compounds synthesized from captured CO_2.

Reduce Process Carbon Footprint- Anaerobic Fermentation of Methane through Reversion of Methanogenesis

CHRISTOPHER STEAD[1], RUTH CORNOCK[1], BASHIR RUMAH[1], RUEBEN CARR[2], YING ZHANG[1]

[1]BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), University of Nottingham, Nottingham, NG7 2RD, UK
[2]Ingenza Ltd, Roslin Innovation Centre, Charnock Bradley Building, Easter Bush Campus, Bush Farm Road, Roslin EH25 9RG

Use of methane as a biobased feedstock is attractive due to cost and wide scale availability. Furthermore methane is advantageous over other C1 feedstock choices which require energy input (typically hydrogen) to permit biological transformation to useful products. Fundamentally the chemical energy in methane is sufficient not to require energy dense co-substrate feeding as is the case for acetogenic options in bioprocessing to prepare useful products. However, current methane fermentation process using chassis Methanotrophs requires handling of oxygen/air, a health and safety risk of explosion. Moreover, every molar of methane fixed emits equal molar of CO_2, thus making the existing process high-carbon footprint economy.

We shall explore methane activation through reverse methanogenesis and focus on anaerobic methane fixation processes, as they confer higher energy and carbon yield efficiencies with lower CO_2 emissions than aerobic ones for converting methane into fuels and chemicals.

A recent example of applying a synthetic biology approach to take genetic information and gene coding sequences from metagenomics database sourced from anerobic methane utilizing environmental samples from the Black Sea to activate anaerobic methanotrophy growth potential into an archeal methanogen offers new promise in ability to preserve chemical energy of methane in carbon efficiency capability beyond thresholds current Methanotrophs can achieve [1]. This exciting development to date is limited with only a single working example published, this PoC project aims to extend the limited utility of anaerobic methane processing into the more conventional industrially relevant prokaryotic workhorse domain.

1. Soo, V.W., et al., Reversing methanogenesis to capture methane for liquid biofuel precursors. Microb Cell Fact, 2016. 15: p. 11.

Scalable Engineering Approaches for Exploiting a Novel Biocomposite Material Applied to Light-driven CO_2 Absorption and Utilization

THEA EKINS-COWARD[1], KAMELIA BOODHOO[1], SHARON VELASQUEZ-ORTA[1], MICHAEL FLICKINGER[2]

[1]Chemical Engineering, School of Engineering, Merz Court, Newcastle University, Newcastle Upon Tyne, NE1 7RU, UK
[2]Department of Chemical and Biomolecular Engineering, North Carolina State University, USA

A new, scalable, solar energy driven microbial gas absorber-converter technology is envisioned in this proof-of-concept project by applying a combination of advanced photoreactive biocomposite materials with two engineering approaches: 1) a continuous thin film flow absorber design based on a spinning disc concept and 2) an electrochemical processing system. *Chlorella vulgaris* cells were incorporated into a latex binder and a porous paper biocomposite. For the first time, the paper biocomposite used chitosan as a biopolymer bridging structure for efficient algal integration.

A spinning disc bioreactor (SDBR) using a 62 cm^2 biocomposite paper containing 75 mg chitosan, 10% MFC and $5.5x10^{10}$ cell loading demonstrated that high photoactivity (measured by pulse-amplitude-modulated (PAM) fluorescence) could be maintained at 300 rpm spin speed over 15 hours of operation, in the presence of bicarbonate in the liquid medium and 5% CO_2 in the gas phase. Interestingly, practically all *C. vulgaris* cells in the biocomposite successfully remained attached to the disc throughout the entire operating period. Overall, increased CO_2 biofixation in the SDBR with a much reduced biocomposite surface area compared to conventional photobioreactors and the high cell retention in the spinning biocomposite highlights the bioprocess intensification potential of the SDBR.

Electrochemical trials used immobilised living *C. vulgaris* on carbon foam electrodes using the latex binder. CO_2 conversion into organic carbon was evaluated via microbial electrosynthesis (MES) at a voltage of -800 mV vs Ag/AgCl. MES using suspended or attached living *C. vulgaris* sequentially increased the bulk liquid organic carbon content by 11.3±0.3 times, over 10 days. Resulting conversions were double with respect to blank experiments. This proof of concept showed that immobilised *C. vulgaris* can be used to convert CO_2 into organic carbon in a MES.

Electron Bifurcation in C1-Fermentations

WOLFGANG BUCKEL

Laboratorium für Mikrobiologie, Fachbereich Biologie,
Philipps-Universität, 35032 Marburg, Germany.

The reductions of CO_2 to formate and CO by H_2 are key reactions in C1-fermentations by acetogenic bacteria. These reductions work well at 10^5 Pa (1 bar) H_2, but in nature and biotechnology the partial hydrogen pressures are much lower. Thus at 10^3 Pa H_2, the reduction potential of H_2 increases by 60 mV from –414 to –354 mV, which is too high to efficiently reduce CO_2. To amplify the reductive power of hydrogen, acetogens use electron bifurcating hydrogenases (EBHase) which split the electron pair of H_2. The energy of one electron is lifted to that of ferredoxin (<–420 mV) at the cost of the other electron, which goes to the more positive acceptor NAD^+. The reduced ferredoxin is able to perform the reductions of CO_2, whereas NADH reduces formate to the methyl group of acetate.

Because no crystal structure of an EBHase is known, the mechanism of EB has been analysed using two enzymes from butyrate producing clostridia: the Etf-Bcd complex and the transhydrogenase Nfn. EtfAB and NfnAB contain two FAD each; one is the bifurcating FAD, which NADH or NADPH reduce to the hydroquinone, respectively. During bifurcation, the other FAD acts as acceptor of the first high potential electron. The remaining low potential semiquinone is very reactive and immediately reduces ferredoxin. Repetition of these processes, named FBEB, affords butyryl-CoA or NADH as well as two reduced ferredoxins. EBHases contain a [FeFe] H-cluster, several [2Fe-2S] and [4Fe-4S] clusters but only one flavin (FMN), which interacts with NADH. It has been proposed that the H-cluster functions as the bifurcating site. This controversial hypothesis is supported by a bifurcating formate dehydrogenase of similar structure, in which molybdopterin occupies the site of the H-cluster. Indeed, Mo and W have been speculated as primordial bifurcating centres, because, like flavins, they can exist in three oxidation states.

Ref.: Buckel, W., and Thauer, R.K. (2018). Chem Rev *118*, 3862-3886. Front Microbiol *9*, 401.

Making Quantitative Sense of Electromicrobial Production

CHARLES A. R. COTTON*, NICO J. CLAASSENS*, DENNIS KOPLJAR*+, ARREN BAR-EVEN **Equal contribution to the work*

Systems and Synthetic Metabolism Group, Max Planck Institute for Molecular Plant Physiology, Am Mühlenberg 1, Potsdam, 14476, Germany
+Deutsches Zentrum für Luft-und Raumfahrt e.V. (DLR), Institut für Technische Thermodynamik, Elektrochemische Energietechnik, Pfaffenwaldring 38-40, Stuttgart, 70569, Germany

Electromicrobial production offers a unique opportunity to displace fossil carbon with CO_2 and renewable energy as the ultimate feedstocks for carbon-based chemicals. Many competing strategies for CO_2 activation and transfer of reducing power from electrode to microbe have been proposed but it is still unclear which have the capacity to overhaul the chemical industry. To address this issue, we quantitatively analyze and compare different electromicrobial production approaches. First, we systematically survey the literature for microbial growth on a wide array of compounds that can be produced electrochemically, especially calculating two key properties: energetic efficiency for feedstock conversion and electron consumption rate. By further taking into account the efficiency with which these carrier compounds are generated, we show that only a few strategies can support efficient electromicrobial production. We further show that direct attachment of microbes to electrodes is highly constrained due to an inherent discrepancy between the rates of the electrochemical and biological processes, such that spatial decoupling is advantageous. Finally, we advocate for temporal decoupling to buffer the biological process from price-related fluctuations governing the electrochemical process; such decoupling can be best established using a soluble and easily stored electron carrier. Altogether, our quantitative perspective provides a data-driven roadmap towards economically and environmentally viable realization of electromicrobial production.

Regulation of Lactate Metabolism in the Acetogenic Bacterium *Acetobacterium woodii*

MARIE C. SCHOELMERICH,[1†] ALEXANDER KATSYV,[1] WOUNG SUNG,[1] VANESSA MIJIC,[1] ANJA WIECHMANN,[1] PATRICK KOTTENHAHN,[1] JOHATHAN BAKER,[2] NIGEL P. MINTON[2] & VOLKER MÜLLER[1]

[1]Molecular Microbiology and Bioenergetics, Goethe University Frankfurt, 60438 Frankfurt, Germany
[2]BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), University of Nottingham, Nottingham, NG7 2RD, UK
[†]current address: Microbiology & Biotechnology, University of Hamburg, Ohnhorststr. 18, 22609 Hamburg

Acetogenic bacteria compete in an energy-limited environment by coupling different metabolic routes to their central metabolism of CO_2 fixation. The underlying regulatory mechanisms are often still not understood. In this work, we analysed how lactate metabolism is regulated in the model acetogen *Acetobacterium woodii*. Construction of a *ΔlctCDEF* mutant and growth analyses demonstrated that the genes are essential for growth on lactate. Gene expression analyses revealed that the *lctBCDEF* genes form an operon that was expressed only during lactate metabolism. The *lctA* gene was cloned, expressed in *Escherichia coli* and purified. LctA bound to the intergenic DNA region between *lctA* and the *lct* operon in electromobility shift assays, and binding was revoked in the presence of lactate. Further restriction site protection analyses consolidated the lactate-dependent binding of LctA and identified the binding site within the DNA. Cells grew mixotrophically on lactate and another energy source and showed no diauxic growth. From these data, we conclude that the catabolic lactate metabolism is encoded by the *lct* operon and its expression is negatively regulated by the DNA-binding repressor LctA.

Relating Bacterial Properties To The Measured Effective Uptake in Gas Fermentation Using Multiscale Mathematical Modelling

MOHIT P. DALWADI[1,2], YANMING WANG[2], JOHN R. KING[2,3], NIGEL P. MINTON[2]

[1] Mathematical Institute, University of Oxford, Radcliffe Observatory Quarter, Oxford, OX2 6GG, UK
[2] Synthetic Biology Research Centre, University Park, University of Nottingham, Nottingham, NG7 2RD, UK
[3] School of Mathematics, University Park, University of Nottingham, Nottingham, NG7 2RD, UK

When scaling up gas fermentation experiments to industrial levels of production, it is important to avoid stagnant regions devoid of nutrient within a bioreactor. Mathematically modelling the nutrient transport within such vessels is a cheap and effective way to prevent this issue. One key challenge in implementing these models is relating properties between the bacterial and bioreactor lengthscales, for example, understanding how substrate diffusivity in cytoplasm affects the effective uptake.

In this talk, we present a systematic method to relate properties between the bacterial and bioreactor lengthscales. We discuss how the measured effective uptake can be used to understand key bacterial properties that are difficult to infer otherwise. Our model is very general and can be applied to many single-cell microorganisms, such as cyanobacteria, microalgae, protozoa, and yeast.

Engineering Aerobic Formate Assimilation via the Highly Efficient Reductive Glycine Pathway

ARREN BAR-EVEN

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

One-carbon compounds can be efficiently produced from a wide array of cheap and available resources to provide an alternative microbial feedstock for sustainable and affordable bioproduction. Acetogens support highly efficient anaerobic bioproduction using C1 feedstocks via the highly efficient reductive acetyl-CoA pathway. However, the product spectrum of acetogens is limited by several factors, the most predominant of which being energetic constraints, such that that aerobic C1-dependent bioproduction might provide a favourable alternative.

However, aerobic assimilation of reduced C1 compounds into central metabolism is limited to relatively inefficient routes with low biomass and product yields. An effective way to tackle this problem is to design and implement tailor-made pathways that could potentially transform any microbe into an efficient C1-metabolizer. Following this logic we have designed the reductive glycine pathway – an aerobic 'twin' of the anaerobic reductive acetyl-CoA pathway. In this synthetic route, formate is ligated to THF and further reduced to 5,10-methylene-THF. The glycine cleavage/synthase system (GCS) reacts 5,10-methylene-THF with CO_2, ammonia, and NADH to generate the C2 amino acid glycine. Finally, glycine is condensed with another 5,10-methylene-THF molecule to produce the C3 amino acid serine, which can be deaminated to provide pyruvate for cellular growth. The reductive glycine pathway thus represents the most efficient route for formate assimilation under aerobic conditions, and could further be used to assimilate other C1 compounds, such as methanol.

We are working to establish the reductive glycine pathway in multiple biotechnological organisms. In this talk I shall focus on our work in *E. coli* and show that a rational integration of native and foreign enzymes enables the THF and glycine cleavage/synthase systems to operate in the reductive direction, satisfying all cellular glycine and serine requirements from the assimilation of formate and CO_2. Importantly, the biosynthesis of serine from formate and CO_2 does not lower the growth rate, indicating high flux that is able to provide 10% of cellular carbon. Our findings assert that the reductive glycine pathway could support highly efficient aerobic assimilation of C1-feedstocks.

Development of an Assembly-based, Recombineering Pipeline for Overcoming Recalcitrance to DNA Transfer Caused by RM Systems in C1 Chassis

ROBERT MANSFIELD, ELIZABETH REDFERN, NIGEL MINTON

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

The development of effective DNA transfer protocols is essential for effective research and development in microorganisms of interest. Where effective gene transfer protocols cannot be established, a significant roadblock exists on the way to implementing the tools required for targeted genetic engineering. The success of gene transfer is affected by levels of natural recalcitrance to foreign DNA. Such recalcitrance stems in part from active protection mechanisms, such as restriction modification (RM) systems. Since the vast majority of microbes contain one or more RM system, the development of effective pipelines for overcoming these barriers is extremely valuable. This project set out to develop an assembly-based pipeline for overcoming RM systems in target strains thereby enabling DNA transfer. The proposed methodology centers around the mimicking of methylation patterns found in a target strain in order to protect transferred DNA. This is achieved through the development of bespoke *E. coli* methylation-donors, created using a Lambda-red mediated recombineering approach.

During this project we have successfully applied our pipeline to previously recalcitrant microbial strains, across multiple species relevant to both healthcare and biotechnology disciplines. This includes demonstrations of DNA transfer into organisms for which it was not previously possible. Additionally we have demonstrated significant efficiency improvements in strains where basic transfer had already been achieved. Substantial progress has also been made towards establishing DNA transfer in an industrially relevant, but previously untransformable, gas-eating bacteria, *Clostridium carboxidivorans* P7.

High Value Uses of Waste Methane from Landfill and Biogas Methane from Anaerobic Digestion

ALEXANDER GROSSE-HONEBRINK[1], RUTH CORNOCK[1], BASHIR RUMAH[1], CHRISTOPHER STEAD[1], ANDREW GODDARD[2], HELEN WEST[3], YING ZHANG[1]

[1]BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), University of Nottingham, Nottingham, NG7 2RD, UK
[2]Freeland Horticulture Ltd, Rosedale Nursery, College Road, Hextable, Kent, BR8 7LT, UK
[3]School of Biosciences, Environmental Biology, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Leicestershire, LE12 5RD, UK

Modern landfills are producing gas from decomposition of waste. This landfill gas accounts for the third largest source of human-made methane emissions. The gas is usually composed of a 1:1 ratio of methane and CO_2, with methane being a 25 times more potent greenhouse gas than CO_2. Traditional methods used in oil refineries, including flaring, are not suitable to landfill gas and solid phase biofilters have proven ineffective to abate the emissions. Similar is true for the gas from anaerobic digestion (AD). The gas with a 60 % methane concentration cannot be used directly as fuel and the contamination with H_2S leads to corrosion of mechanical components. As a consequence the AD gas is often flared locally.

Here we present a new and economical use for landfill and AD gas by using methanotrophic bacteria for purification. Methanotrophs are able to produce the carbon storage compound polyhydroxybutyrate (PHB) from methane. PHB is a polyhydroxyalkanoate (PHA), which can be used like polypropylene and polyethylene as a common plastic. The advantage of PHAs is their biodegradability in natural environments. Finally, with a value of $3 to $4 per kilogram this compound could be an economically viable product from methanotrophic gas conversion.

This project aimed to isolate new meso- and thermophilic methanotrophic species with the ability to produce PHB. This methanotroph culture collection has been screened on landfill and AD gases from different sources to find the most promising strains that grow on a wide range of methane concentrations with different contaminant compositions.

Intensifying Fermentation, The Microbubble Way!

WILLIAM B ZIMMERMAN[1], PRATIK DESAI[2]

[1]Department of Chemical and Biological Engineering, University of Sheffield, S1 3JD, Sheffield, U.K.
[2]Perlemax Ltd., 318 Broad Lane , Sheffield, S3 7HQ, U.K.

Fermentation faces three major operational challenges – internal mixing, mass transfer enhancement – therefore lower gas wastage, and separation of the product formed. Typically these three challenges are the limiting factors of the fermenter. Microbubbles can be used to eliminate or ameliorate these three limiting factors due to the substantially higher surface area to volume ratios that result in improved mass transfer as well as mixing. They can also be used to separate components *in situ* without substantially changing the temperature of the liquid mixture being used. Metabolites and products are usually the inhibitors for processes. Zimmerman proposed that removing these inhibitors would then result in an improved process as the microorganisms can be grown without being limited by the inhibitor. Desai designed the fermenter which could be used for this process integrating spargers designed for microbubble generation capable of introducing nutrients and separating components simultaneously. It also included an integrated solids separation process so that the biomass could be separated post fermentation.

For this BIV, Desai and Zimmerman proposed to build a novel microbubble fermenter system (the DZ Fermenter), and investigate for application to gas fermentation. The DZ fermenter is capable of propagation, biochemical product separation, and biomass separation using the same vessel but based on simple tuning parameters. The increase in propagation is approximately between 10%-400% based on the strain in terms of rates, and total biomass can go as high as 1000 times for final biomass achievable (strain dependent). The biomass can be separated in the DZ Fermenter and unoptimised removal recoveries of 95% have been achievable in 10 minutes of processing time. The typical cycle for dissolved air flotation – a much more power consumptive conventional process – is ~24 minutes for the three separate stages of dosing with flocculant and coagulant, flocculation, and flotation separation, with the same levels of recovery.

Since this was a BIV, a proposed next stage project is in the works for a larger grant proposal amongst the partners. Consortial strains from a typical wastewater were used in order to feed biogas and concomitantly increase the growth rate for these strains. Preliminary results will be showcased in the presentation.

Towards a Recombinant Pathway for Butanediol Biogenesis in Acetogens

[1]MARTIN WARREN, and [2]MICHELLE GRADLEY

[1]*School of Biosciences, University of Kent, Canterbury, Kent, UK, CT2 7NZ*
[2]*ZuvaSyntha, 22 Watton Road, Knebworth, Hertfordshire, UK, SG3 6AH*

The significant contribution of petrochemical-derived commodity chemicals to increasing carbon dioxide levels has rightly promoted research into alternative production methods to make these chemicals from renewable or sustainable sources. The best example of this is the biological production of 1,3-propanediol, which is produced by engineered *E. coli* strains and has completely replaced the petrochemical-derived synthesis. In this project we aimed to develop a biological synthesis for 1,3-butanediol (1,3-BDO) in an acetogen. 1,3-BDO is a molecule of considerable commercial potential. In collaboration with industry, we sought to develop novel technologies to produce 1,3-BDO by building on patented knowledge held by both our industrial partner, ZuvaSyntha, and the University of Kent. The project aimed to identify the best optimal combination of enzymes for product formation in terms of yield and efficiency. This project involved the application of synthetic biology approaches, enzyme evolution and the development of technologies to encourage metabolic channelling. The recombinant pathway is seen to work well in *E. coli* and is currently being cloned into a suitable compatible plasmid system to allow transformation into acetogens.

Direct Cell-to-cell Exchange of Matter in a Synthetic *Clostridium* Syntrophy Enables CO_2 Fixation, Superior Metabolite Yields, and an Expanded Metabolic Space

ELEFTHERIOS TERRY PAPOUTSAKIS & KAMIL CHARUBIN

Department of Chemical and Biomolecular Engineering & Delaware Biotechnology Institute, University of Delaware, Newark, DE 19711 USA

In microbial fermentations to produce metabolites, at least 33% of the sugar-substrate carbon is lost as CO_2 during pyruvate decarboxylation to acetyl-CoA, which is typically the starting point for metabolite biosynthesis. Previous attempts to reduce this carbon loss focused on engineering a single organism aiming to achieve that goal. In nature, most microorganisms live in complex communities where syntrophic interactions result in superior resource utilization. Here we show that a synthetic *Clostridium* syntrophy consisting of a solventogen *C. acetobutylicum*, which converts carbohydrates into a variety of chemicals, and an acetogen *C. ljungdahlii* which fixes CO_2. This syntrophic system performed robustly to achieve better efficiencies than either organism alone by improving the carbon recovery into metabolites to achieve almost theoretical yields as defined by electron availability. Our data shows that in this syntrophic co-culture, the two organisms exchange metabolites directly, frequently leading to interspecies cell-wall/membrane fusion events, which we probed using advanced electron microscopy tools including correlative fluorescence/TEM/SEM microscopy and electron tomography. Such fusion events have not been previously reported and may be more frequent than is currently appreciated in natural consortia. In addition, this syntrophy results in novel biosynthetic pathways/ capabilities that neither of the two organisms alone possesses. This and other syntrophies expand the metabolite space by generating metabolites that neither organism can produce alone. Syntrophic cultures offer a flexible platform for metabolite production with superior carbon recovery that can also be applied to electron-enhanced fermentations enabling even higher carbon recovery. *Supported by the National Science Foundation through the NSF grant (CBET-1511660), the IGERT fellowship (1144726), and the Army Research Office (W911NF-17-1-0343).*

Tailored Microbial Consortia for Syngas Fermentation

MARTIJN DIENDER, IVETTE PARERA OLM, JOÃO C. MOREIRA, ALFONS J.M. STAMS, DIANA Z. SOUSA

Laboratory of Microbiology, Wageningen University & Research, Wageningen, the Netherlands

Syngas is one of the most flexible feedstocks for biotechnological processes: it can be produced from any carbon-containing material including renewable biomass resources and wastes. Anaerobic microorganisms are able to utilize syngas, but the natural products of this conversion are generally limited to acetate and ethanol. At our research group, we aim at broadening the variety of products that can be produced from syngas by developing tailored anaerobic microbial consortia.

We established a co-culture of *Clostridium autoethanogenum*, a well-known carboxydotrophic acetogen, together with *Clostridium kluyveri*, a bacterium employing the reverse β-oxidation pathway. *C. autoethanogenum* uses syngas to produce a mixture of acetate and ethanol. *C. kluyveri* subsequently uses these products to perform chain elongation. This results in a co-culture producing a mixture of C4 and C6 acids and alcohols using syngas as a sole substrate. Using a transcriptomics approach we have attempted to unravel the functioning of this co-culture and how its production spectrum can be optimized. Results indicate that production of hydrogen by *C. kluyveri* stimulates the metabolism of *C. autoethanogenum*, resulting in more alcohol formation. This has a subsequent effect on the production levels of C4 and C6 products by *C. kluyveri*.

More recently, a second synthetic co-culture composed of an *Acetobacterium* sp. and *Pelobacter propionicus* was constructed to convert CO to propionate. The objective is to produce odd-numbered fatty-acids by adding *C. kluyveri* to this co-culture. Proteomics analyses were also performed to get insights on the physiology and mechanisms of microbial interaction in this system.

Overall, our results show the potential of using synthetic mixed cultures for the conversion of syngas to chemicals. Besides, we highlight the need to understand well microbial interactions in order to optimize product yields and titres.

Continuous Production of Acetate from Hydrogen and Carbon Dioxide Using Mixed Culture

ROMAIN TUFFOU, JAIME MASSANET-NICOLAU, RICHARD DINSDALE, ALAN GUWY.

Sustainable Environment Research Centre, University of South Wales, Pontypridd, CF37 4BD, UK

With the environmental concerns and health issues linked to fossil fuels, attention has been drawn toward alternative energy sources. The use of sun or wind as energy sources is increasing but storing the electricity from these sources and balancing supply with demand is challenging. One way to do this is the electrolysis of water to produce hydrogen. This hydrogen can be transformed in a more dense form of energy such as methane or acetate. Homoacetogenic bacteria found in anaerobic digesters have the ability to convert H_2 and CO_2 into acetate without need for sterility and represent an inexpensive inoculum compared to pure culture.

A continuously stirred tank reactor (CSTR) with a working volume of 3.5L and a headspace of 0.5L was inoculated (5% v/v) with anaerobically digested sludge collected from a local sewage treatment works. The reactor was supplemented with feed medium composed of macro nutrients and trace metal solution, as well as silicon antifoaming agent. A blend of 80% H_2 and 20% CO_2 was continuously injected into the reactor at a flow rate of $3L.h^{-1}$, the gas composition was continuously monitored with online gas sensors (H_2, CO_2 and CH_4), and the exhaust gas flow rate was monitored with a positive displacement gas flow meter. The content of the fermenter was kept at 30°C ± 0.5°C by a thermostatically controlled heating pad and the pH of the liquid phase was kept at 6 by the automated addition of 2M NaOH whenever the pH fell below this level.

The acetate concentration reached 16 g/L after 42 days. The experiment was ended when it became clear that no more acetate was being produced. Based on these results, a continuously operating reactor was set up for acetate production using the same conditions as the batch experiment. The hydraulic retention time was set to 72 hours and the daily production reached 550 mg/L/d. This is the first reported reactor to produce acetate solely from homoacetogenesis with sewage sludge in continuous mode.

Mixotrophic Fermentation of *C. necator* H16 for PHA Production

KAMRAN JAWED, NIGEL P. MINTON, KATALIN KOVACS

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

Cupriavidus necator previously known as *Ralstonia eutropha* is a gram negative facultatively chemolithoautotrophic bacterium that can grow on various organic substrates or CO_2 and H_2 under aerobic conditions. It has been a model organism for producing diverse polymers belonging to polyhydroxyalkanoates (PHAs) family.
In this study we have explore the capability of *C. necator* to grow mixotropically with volatile fatty acids (VFAs) as complementary carbon source, to produce diverse range of PHA.

Firstly, toxicity assay for individual VFAs (Formic acid, Acetic acid, Lactic acid, Propionic acid, Butyric acid and Valeric acid) were performed to check the tolerance of *C. necator*. Further, a new strategy of simultaneous feeding of VFAs mix and CO_2 as carbon sources in a controlled manner was investigated. Growth kinetics and substrate utilization reveals the preference of VFAs for the growth. All the VFAs were completely utilized after 24 hours of cultivation in bioreactor, when we cultivated *C. necator* mixotropically i.e. CO_2, VFAs and H_2 as carbon and energy source. Formic acid and lactic acid were co-utilized first and totally exhausted after 12 and 22 hours of cultivation, respectively. The concentration of acetic acid increased up to the 6 hours and then began to consumed. All the VFAs, except formic acid were co-consumed after 12 hours of cultivation. Interestingly, the CO_2 level was constant throughout the cultivation, which indicates that there was no co-utilization of CO_2 along with VFAs mix. However, when we cultivated *C. necator* on CO_2 and H_2 and injected the VFAs mix at the log phase of cultivation, we found drastic shifting in substrate utilization i.e. VFAs over CO_2. The uptake of Propionic acid, Butyric acid and Valeric acid was very slow throughout of the cultivation. In both strategy, PHA was produced as poly(3-hydroxybutyrate) in the initial phase of cultivation while copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate in the later stage.

Further bioprocess engineering for co-utilization of CO_2 along with VFAs (produced from AD digestate) will help to reduce the amount of H_2 per unit product mass, thereby achieving better economic feasibility.

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Systems and Synthetic Biology of Acetogenic Bacteria

BYUNG-KWAN CHO

Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Republic of Korea

Acetogenic bacteria are considered to be the most efficient microorganism for fixing C1 compound as they gain energy from operating the pathway, in contrast to the other C1 compound-fixing bacteria that spend energy during the uptake. Acetogenic bacteria are present in 23 different genera with over a hundred strains isolated from diverse habitats. The Wood-Ljungdahl pathway in the microorganism converts C1 into acetyl-CoA, which is an important cellular precursor that is converted into biochemicals. Despite the potential to reduce C1 compound in the atmosphere and industrial waste gases, lack of a systemic understanding, complex layers of regulation system, and inefficient electron delivery has limited the construction of a cellular factory optimized for producing the desired chemical. To overcome the limitation, molecular level insight has been obtained via multi-layered genome-scale analyses. The results revealed functional genes required for C1 compound fixation and their regulatory systems. Integration of the information-rich data types with synthetic biology tools facilitates the construction of optimal C1 fixing and biochemical-producing cellular factories.

Microbial Bioprocessing: Optimum Reactor Design for Carbon monoxide (CO) Fermentation

MUHAMMAD YASIN[1], NULEE JANG[2], MUNGYUE LEE[2], IN SEOP CHANG[2]

[1]Bioenergy & Environmental Sustainable Membrane Technology (BEST) Research Group, Department of Chemical Engineering, COMSATS University Islamabad (CUI), Lahore Campus, Pakistan
[2]School of Earth Sciences and Environmental Engineering, Gwangju institute of Science and Technology (GIST), Gwangju 61005, Republic of Korea

Microbial bioprocessing of CO has emerged as a potential alternative to the first generation feedstock based biorefinery. Nevertheless, poor solubility and mass transfer of CO, less lucrative cell density, and low microbial productivities are the big hurdles, yet to climb over, for technology commercialization. Scale-up of syngas fermentation can be realized by operating the bioreactor at optimum substrate conditions. This requires a fixed overall volumetric mass transfer coefficient (k_La) of the reactor. However, a reactor operated at a constant k_La will support up to certain cell concentrations. Hence, predicting the reactor conditions that make a happy case for maintaining both the optimum substrate levels and high cell concentrations in the reactor are strongly required. This talk will primarily discuss the strategies to resolve kinetic and mass transfer limitations during syngas fermentation, and ways to predict the optimum conditions to achieve high microbial productivities through an ideal bioreactor operation.

Gas Fermentation of *Cupriavidus necato*r H16 and Variant at Elevated Pressure

JASBIR SINGH** and GERARD GARDNER**,
TUCK SENG WONG*, KANG LAN TEE*

***HEL Ltd, Borehamwood, Herts, England*
**Department of Chemical & Biological Engineering, University of Sheffield, England*

The commercial feasibility of many bio-processes can depend on how fast gas transfer takes place. This is especially true if gas solubility is poor, for example when working with gases such as hydrogen and methane in the context of gas fermentation for the production of fuels and chemicals from waste gas. The engineering solution to poor gas transfer is limited to mass transfer coefficient (kLa) increase through changes in sparger and stirring arrangement. This offers very limited scope for improvement and therefore many potentially interesting processes can be rendered uneconomic. A much more effective alternative is to operate the bio-reactor at elevated pressure as this can in principle increase gas transfer rate several-fold without any changes to sparging or agitation.

This presentation will discuss data from a mini-bioreactor platform operating at a range of pressures, used for fermentation of *C. necato*r H16 wildtype and its v6C6 variant using gas mixture $CO_2/H_2/O_2$. Results with mixed carbon sources - gluconate and CO_2, as well as glycerol and CO_2 - will also be presented.

Improving the Sustainability of Biobased Manufacturing

REUBEN CARR[1], FRASER BROWN[1], PHILIP WEYRAUCH[1], ALISON ARNOLD[1], MAGALI ROGER[2], FRANK SARGENT[2] and DAVID SMITH[3]

1. *Ingenza Ltd, Roslin Innovation Centre, Charnock Bradley Building, Easter Bush Campus, Bush Farm Road, Roslin, Midlothian, EH25 9RG*
2. *School of Natural & Environmental Sciences, Devonshire Building 5.12C, Newcastle University, Newcastle upon Tyne, NE1 7RU*
3. *Drochaid Research Services, Purdie Building, North Haugh, St Andrews, Fife, KY16 9ST*

Ingenza is developing cutting edge biobased manufacturing routes for several industrial end-user chemical company customers. To improve process sustainability and provide a greater choice of feedstock options, Ingenza has been working in collaboration with Newcastle University and Drochaid Research Services on establishing novel proprietary bioprocessing routes.

Building on earlier C1net PoC results, with support from Zero Waste Scotland's Circular Economy Investment Fund, our collaboration has demonstrated improved circularity using typical fermentative carbon dioxide off gas that arises during biobased chemical manufacturing. The off gas carbon dioxide is recovered and consumed in fermentative preparation of a platform biobased chemical in a two stage bioprocess. Concerted use of renewable power-derived green hydrogen nearly doubles the weight quantity of carbon dioxide that can be assimilated into the biobased product formed.

This proprietary technology achieves a further stepped improvement in the sustainability profile of biobased chemicals manufacturing to maximise feedstock and energy utilisation whilst also abating biogenic fermentative GHG emission. The demonstration here gives evidence of Ingenza's commitment to deliver cost effective and scalable bioprocesses for the manufacture of chemicals, biologics, pharmaceuticals and biofuels from sustainable sources.

FeedKind™ Protein: A Sustainable Approach to Meeting the Growing Protein Demand

LORI GIVER

Calysta, Inc., Menlo Park, CA

The rapidly growing demand for livestock, tied to rising populations and standards of living, has placed increasingly unsustainable pressure on current sources of high protein feeds. FeedKind protein provides a sustainable source of protein that is comparable in many respects to high-quality fishmeal, a critical ingredient for aquaculture. FeedKind protein is sustainably produced via non-GMO fermentation of methane, which has minimal impacts on water and land use and does not compete with any aspect of the human food chain.

FeedKind protein represents a high-quality, sustainable protein source which is well tolerated across multiple livestock species. For aquaculture in particular, FeedKind *Aqua* protein has been demonstrated to perform significantly better than fishmeal in promoting growth and health in Atlantic salmon. The composition of FeedKind *Aqua* protein is comparable to fishmeal, and superior to many novel protein sources under development. The FeedKind production process has been validated at commercial scale in conjunction with European Union product approval.

Methanogens in Electrobiotechnology: Electron Transfer Mechanism and Reactor Design

DIRK HOLTMANN; FLORIAN MAYER; FRANZISKA ENZMANN

DECHEMA-Forschungsinstitut, Industrial Biotechnology, Theodor-Heuss-Allee 25, 60486 Frankfurt am Main, Germany

Since fossil sources for fuel and platform chemicals will become limited in the near future, it is important to develop new concepts for energy supply and production of basic reagents for chemical industry. One alternative to crude oil and fossil natural gas could be the biological conversion of CO_2 to methane *via* methanogenic archaea. This process has been known from biogas plants, but recently, new insights into the methanogenic metabolism, technical optimizations and new technology combinations were gained, which would allow moving beyond the mere conversion of biomass. In biogas plants, steps have been undertaken to increase yield and purity of the biogas, such as addition of hydrogen or metal granulate. Furthermore, the integration of electrodes led to the development of microbial electrosynthesis (MES). The idea behind this technique is to use CO_2 and electrical power to generate methane *via* the microbial metabolism. The aim of our work is one hand to get a deeper understanding of underlying electron transfer mechanism and on the other hand to develop scalable reactors to transfer the results in real applications.

So far, only for a few methanogens the type of electron transfer is known or proposed but it is unclear how the electrons are taken up exactly and which proteins are involved. In our studies we used a hydrogenotrophic strain belonging to the order *Methanococcales* and tested its ability for MES of methane. This strain, not described so far to take up electrons, produced methane by MES between potentials of -850 and -1000 mV (vs. Ag/AgCl). The coulombic efficiency was more than 80 %. Surprisingly, gas analysis revealed unusual results dealing with a possible new mechanism of electron transfer in this strain. Additionally real-time qPCR analyses under MES conditions identified a gene/protein which might be responsible for the unusual electron transfer in this methanogenic strain.

Unfortunately, there is still a lack of well characterized, scalable reactor systems that are capable of hosting different bioelectrochemical processes, linking lab scale research to industrial application. Here, we introduce a two-chamber bioelectrochemical bubble-column reactor (one liter working volume), which can be used as microbial fuel cell as well as for microbial electrosynthesis and is especially advantageous for processes with gaseous substrates. It is designed flexible in terms of electrode material and area, membrane material and area, and capable of hosting continuous processes. It is a promising replacement of lab-scale H-cells for wider screening possibilities with regard to industrial applications

Metabolic Engineering of Methanotrophs as Cell Factory for Methane-to-Chemicals Conversion

EUN YEOL LEE

Department of Chemical Engineering, Kyung Hee University, Yongin-si 17104 & C1 Gas Refinery Research Center, Republic of Korea

Nowadays, methane has attracted much attention as a low-priced next-generation carbon feedstock for industrial biotechnology. Compared to chemical methods, bioconversion of methane-to-chemicals can be conducted at ambient condition without extensive energy consumption. Methanotrophs are microorganism that can utilize methane for their cell growth as sole carbon and energy source. Bioconversion of methane to value-added chemicals using methanotrophs can valorize low-priced methane and mitigate methane gas as greenhouse gas causing global warming. Recently, genetic tools and metabolic engineering approaches have been applied for development of engineered methanotrophic strain. In this presentation, metabolic engineering of methanotrophs for the production of 2,3-butanediol, succinate and etc. from methane will be demonstrated. The technical challenges and issues of methanotrophs as a platform cell factory for methane bioconversion will be also discussed.

Bacterial Methane Monooxygenases in Plants for Methane Detoxification

TATIANA SPATOLA ROSSI & VERENA KRIECHBAUMER

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

Methane is a greenhouse gas which has contributed in approximately 20% to the increase in global warming since pre-industrial times, and is experiencing a new atmospheric rise since 2007. Globally, over 60% of methane emissions come from human activities including industrial gas and petroleum systems, agricultural livestock, artificial wetlands, and landfills.
Methanotroph bacteria feed on methane using the enzyme particulate methane monooxygenase (pMMO), the only known natural catalyst capable of breaking the C-H bond, which converts methane into the less potent carbon dioxide and methanol. These microorganisms however present slow growth and require structures such as biofilters or bioreactors to be cultivated industrially.
Plants contribute significantly to methane conduit into the atmosphere in wetlands and crop cultivars such as rice, two main sources of global methane emissions, by transporting it through porous tissues and releasing it by transpiration. Our aim is to clone the genes encoding for pMMO enzymes into tobacco plants, and ultimately produce plants capable of metabolising methane, which could be used for *in situ* detoxification in soils producing high amounts of the greenhouse gas such as wetlands, ex-landfill sites or rice paddy fields.

Biomethanisation of CO_2 using H_2

YUE ZHANG, SONIA HEAVEN, CHARLES BANKS

Water and Environmental Engineering Group, Faculty of Engineering and Physical Sciences, University of Southampton, Southampton, SO17 1BJ, UK

The biological methanisation of CO_2 using methanogenic archaea is a promising process for a range of applications, from biogas upgrading to increase the efficiency of carbon utilisation in waste feedstocks, to helping balance the electricity grid by providing a means to convert surplus energy from intermittent renewables into a storable biofuel which is compatible with current infrastructure. Its greatest future potential, however, is in mitigating CO_2 emissions by carbon capture and utilisation.

In practical terms there are two main system configurations for CO_2 biomethanisation: in situ where H_2 is added directly to an anaerobic digester fed on organic waste or wastewater; and ex situ where CO_2 and H_2 are fed directly to a fermenter. In situ conversion is of interest to the water industry, which already has many digesters and even upgrades biogas for grid injection at some UK sites. In a conventional digester with H_2 addition, a methane production rate of 4 m^3 m^{-3} digester day^{-1} is a good improvement on typical rates in commercial plant. If the technology can be made simple and cheap enough, it could also be used in applications such as on farm digestion. Digester pH control is a critical issue for in situ application, as pH increases when the partial pressure of CO_2 reduces in the headspace. To what extent this affects the digester operation depends on the buffering capacity of the digester as well as the baseline pH level when biomethanisation has not been introduced.

Ex situ conversion is regarded as more suitable for handling higher rates of gas input, as the inhibition effect of H_2 on the digestion of organic material can be ignored in ex situ processes. Unlike most microbial fermentations, however, the reactants and product of this process are solely gases; therefore gas liquid mass transfer plays an important role for high volumetric productivity. Although rates of more than 100 m^3 CH_4 m^{-3} fermenter day^{-1} have been achieved in short term experiments using pure cultures, fermenter volumes required for carbon capture and utilisation would be excessive for the big CO_2-producing industries, such as incineration, cement production or power generation, which produce hundreds or thousands of tonnes of CO_2 per day at a single plant. For the purposes of carbon capture and utilisation, more work therefore needs to be done to translate CO_2 biomethanisation technologies in terms of scale and affordability to make them more competitive than thermochemical catalytic processes.

Abstracts of Poster Presentations

POSTERS - 21 JANUARY 2019

Board No.			
1	Arenas-Lopez	Christian Arenas-Lopez	Exploring strategies to improve CO_2 fixation in *Cupriavidus* necator H16
2	Avignone Rossa	Claudio Avignone Rossa	Microbial Electrosynthesis for the Capture and Transformation of CO_2 into Multicarbon Organic Compounds
3	Baur	Tina Baur*	Waste to Value - Production of the Bioplastic Poly(3-hydroxybutyrate) (PHB) and its Precursor 3-hydroxybutyrate (3-HB) from Waste gas
4	Bjorck	Charlotte Bjorck*	Capture and utilisation of biogas components by a mixed methanotroph and microalgae culture
5	Bourgade	Barbara Bourgade*	Commodity Chemicals Production in *Moorella thermoacetica*
6	Chalmers-Brown	Rhiannon Chalmers-Brown*	Anaerobic Treatment of Blast Furnace Gases
7	Dietrich	Helge Dietrich*	Homologous Overproduction and Mutagenesis to Unravel the Function of the Hydrogen-Dependent CO_2-Reductase
8	Fink	Christian Fink*	Establishing Genetic Tools for the Thermophilic Methanogen *Methanothermobacter thermautotrophicus* ΔH
9	Flaiz	Maximilian Flaiz*	Heterologous Expression of Genes for 1,4-Butanediol Production with the Acetogen *Eubaterium limosum*
10	Flickinger	Michael Flickinger	Continuous Gas Processing without Bubbles using Thin Liquid Film Bioreactors Containing Biocomposite Biocatalysts
11	Gascoyne	Joshua Gascoyne*	Biosynthetic Pathway Engineering in *Cupriavidus* necator Towards 1,3-butanediol Production from Carbon Dioxide
12	Gilbert	James Gilbert	Gsmodutils: A Python Based Framework For Test-driven Genome Scale Metabolic Models
13	Gomez	Diego Orol Gomez*	3-Hydroxypropionic acid (3-HP) production by *Cupriavidus necator* H16 via the β-alanine pathway
14	Gude	Christian Gude*	Metabolic engineering of *Cupriavidus necator* for the production of aminovaleric acid and hydroxyvaleric acid
15	Infantes	Alba Infantes*	Evaluation of “Impure” Syngas from The Karlsruhe Bioliq Plant and Other Sources as Substrate in Syngas Fermentation
16	Jeong	Ji-Yeong Jeong*	Heterologous Expression of Alcohol Dehydrogenase Enables Concentrated Production of Non-Native Ethanol from Carbon Monoxide in Acetogen, *Eubacterium limosum* KCTC1326BP
17	Kim	Ji-Yeon Kim*	Development of Optimised CRISPR/Cas9 System for Acetogenic Bacterium, *Eubacterium limosum* KIST612
18	Klask	Christian-Marco Klask*	Investigating the RNF-complex and Its Impact on the Energy Metabolism of the Acetogen *Clostridium ljungdahlii*
19	Lee	Mungyu Lee*	Simultaneous Gas- and Cell-Recycled Continuous Carbon Monoxide Fermentation Under Open-, Closed- and Mixed Circuit to Boost-up Biomass and Product Titer Using Acetogen Strain, *Eubacterium limosum* KIST612
20	Locker	Jessica Locker*	Engineering *Cupriavidus necator* for the Production of 3-Hydroxypropionic Acid
21	Malys	Naglis Malys	Functional genetic elements for controlling gene expression and production of chemicals in *Cupriavidus necator* H16
22	McCusker	Christopher McCusker*	Metabolic engineering of *Cupriavidus necator* H16 for overproduction of δ-Aminolevulinic acid

*PhD students

POSTERS - 22 JANUARY 2019

Board No.			
1	McGregor	Callum McGregor*	Development Of A Versatile Plasmid Addiction System For Use In *Cupriavidus necator* H16
2	McLeod	Carmen McLeod	Circling Sustainability and Responsibility: Exploring Synergies between the Circular Economy, Synthetic Biology and Responsible Research and Innovation
3	Mesfin	Noah Mesfin*	Metabolic Modelling of *Acetobacteirum woodii* Metabolism
4	Millard	James Millard	ENGICOIN: Engineered microbial factories for CO_2 exploitation in an integrated waste treatment platform
5	Millat	Thomas Millat	Robust Analysis of Intracellular Metabolite Concentrations Measured During the Gas-Induced Metabolic Shift in *Clostridium autoethanogenum*
6	Molitor	Bastian Molitor	Genome-scale Investigations of *Methanothermobacter thermautotrophicus*
7	Montarnal	Amaury Montarnal*	Study of the molybdopterin Biosynthesis pathway of *Methanococcus maripaludis*, a methanogens able to grow on CO_2/H_2
8	Nick	Saskia Nick*	Production of Acetone Using Recombinant *Acetobacterium woodii* Strains
9	Norman	Rupert Norman	Construction of a Predictive Genome Scale Model for Clostridium *autoethanogenum* by Incorporation of Biological and Physical Regulation
10	Öppinger	Christian Öppinger	On the Diversity of 5,10-Methylene-THF Reductases in Acetogenic Bacteria.
11	Osundeko	Olumayowa Osundeko	Methane upgrading by novel continuous extraction
12	Oswald	Florian Oswald*	Intermediate Hydrogen Formation by Heterotrophically Growing *Acetobacterium woodii*
13	Park	Byeonghyeok Park*	Comparative Genomic Analysis of Carbon Monoxide Dehydrogenase and Its Gene Cluster
14	Pearcy	Nicole Pearcy	A Genome Scale Model of *Cupriavidus necator* for Platform Chemical Production
15	Pugh	Richard Pugh*	Bio-refining of Coke Oven Gas – The Removal and Recovery of Sulfur
16	Roger	Magli Roger	Efficient Hydrogen-dependent Carbon Dioxide Reduction by *Escherichia coli*
17	Schwarz	Fabian Schwarz*	Whole Cell Catalysis for Hydrogen Storage and Biohydrogen Production Using a Thermophilic Acetogen
18	Tibaldero	Giorgia Tibaldero*	Maximizing the CO_2 Assimilation Efficiency of the Endogenous CBB Pathways in Cupriavidus necator H16
19	Tomi-Andrino	Claudio Tomi-Andrino*	The Use of Thermodynamics and Biomass Assembly to Further Constrain Metabolic Flux Analysis Based Methods: Toward Monitoring Bacterial Metabolism
20	Valgepea	Kaspar Valgepea	H_2 Drives Metabolic Rearrangements in Gas-fermenting Clostridium *autoethanogenum*
21	Winzer	Klaus Winzer	The Genetic Basis of 3-Hydroxypropanoate Metabolism in *Cupriavidus necator*

*PhD students

Exploring Strategies to Improve CO_2 Fixation in *Cupriavidus necator* H16

CHRISTIAN ARENAS-LOPEZ, GIORGIA TIBALDERO, KATALIN KOVACS, NIGEL P. MINTON

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

Six different pathways for carbon dioxide fixation have been described in bacteria, the most studied of which is the Calvin-Benson-Bassham (CBB) cycle. Three rounds of this cycle convert 3 molecules of CO_2 to one molecule of glyceraldehyde-3-phosphate (G3P) where ribulose-1,5-biphosphate carboxylase/oxygenase, commonly known as RubisCO, is the enzyme involved in the first major step of carbon fixation.

Bacteria, like eukaryotes, contain subcellular structures that function to compartmentalize certain metabolic steps or reaction sequences. The prototype bacterial organelle is the carboxysome, a polyhedral microcompartment found in cyanobacteria and in many chemoautotrophs. The carboxysome consists of a thin protein shell that surrounds a core composed of RubisCO and impedes diffusion of CO_2 out of it. The resulting localized high concentration of the RubisCO substrate in the microcompartment interior enhances CO_2 fixation by the catalytically rather inefficient RubisCO.

α-carboxysomes have been succesfully expressed and its activity is currently being tested in *Cupriavidus necator* strain H16, which was chosen as a chassis organism for the current investigation as it can grow to high cell densities on CO_2/H_2 due to the presence of CBB cycle. Moreover, the large subunit of RubisCO has been identified to be an important determinant for its sequestration into α-carboxysomes in *H. neapolitanus*. Therefore, a mutated large subunit of the *C. necator* RubisCO has been engineered and its role in carboxysome formation is currently being investigated.

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

ROSA ANNA NASTRO and CLAUDIO AVIGNONE ROSSA

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

Microbial electrosynthesis (MES) or Electrofermentation (EF) can overcome stoichiometric and energy limitations, low yields, nutrient demands, and other issues found in conventional fermentation. In MES, the extracellular redox potential is modified by supplying electrons through the cathode, therefore displacing the intracellular redox balance required to obtain the desired fermentation products. It is then possible to utilize compounds of low degree of reduction (e.g. CO_2) as carbon sources to synthesize reduced molecules by supplying exogenous electrons to specific microorganisms. This electrochemical control redirects the metabolic and regulatory networks of single species as well as the metabolic interactions between species in co-cultures or multi-species communities.

We have assessed the effect of an external potential on the ability of an anodic biofilm composed by the binary consortium *Shewanella oneidensis* and *Pseudomonas aeruginosa* to provide electrons for CO_2 assimilation by *Clostridium saccharoperbutylacetonicum* present in the cathode. A potential of +3.00 V was applied to an MFC to allow the development of stable biofilms. At the steady state, potentials of +0.88 V and -0.215 V were observed at the anode and the cathode of the MFC. The evolution of both systems was monitored continuously for 30 days and the performance assessed in terms of Power Density (PD), Current Density (CD), Open Circuit Voltage (OCV). Polarization experiments were performed periodically, keeping the MFC at the maximum power, together with impedance, voltammetry and chronoamperometry analyses of the anodic biofilm. We measured the consumption of dissolved CO_2 and the presence of fermentation products in the cathodic suspension.

Our results showed an increased CD (from 1.24 to 31.9 fold) and of PD (from 1.19 to 58.0 fold) when the external potential is applied during the first 24 hours of operation, with a maximum of 721 mW/m^2 and 374.9 mA/m^2 at 1000Ω external load. In the cathode, *C. saccharoperbutylacetonicum* consumed approximately 50% of the dissolved CO_2 , and the chemical analysis of the fermentation products in the cathodic suspension showed peaks that can be associated to the production of acetate, butyrate and an unidentified compound with chemical characteristics generally associated to short-chain fatty acids. Full detail of the chemical analysis will be discussed.

We conclude that electrofermentation of CO_2 to produce valuable chemicals is a sustainable strategy that provides a viable alternative to current methods of chemical synthesis, that may contribute to the reduction of greenhouse gas emissions. The electrical input can be generated from renewable resources such as solar energy or wind power, supplementing the electrical power generated in the anode. If the system is fed with organic-rich feedstocks such as certain type of industrial wastewater, the overall output will be a decrease in the pollution levels and a reduction in CO_2 emissions, with the storage of energy under the form of covalent bonds in the organic compounds synthesized from captured CO_2.

Waste to Value - Production of the Bioplastic Poly(3-hydroxybutyrate) (PHB) and its Precursor 3-hydroxybutyrate (3-HB) from Waste Gas

SEBASTIAN FLÜCHTER[1], FRANK R. BENGELSDORF[1], TINA BAUR[1], STEPHANIE FOLLONIER[2,3], BETTINA SCHIEL-BENGELSDORF[1], MANFRED ZINN[2] and PETER DÜRRE[1]

[1]Institut für Mikrobiologie und Biotechnologie, Universität Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany
[2]University of Applied Sciences and Arts Western Switzerland (HES-SO Valais), Institute of Life Technologies, Route du Rawyl 64, 1950 Sion, Switzerland
[3]Current Address: Lonza AG, Clinical Development-Microbial (USP), Lonzastrasse, 3930 Visp, Switzerland

The biopolymer poly(3-hydroxybutyrate) (PHB) is a fully biodegradable polymer and an attractive alternative to petroleum-based plastics. Synthesis gas (CO, H_2, CO_2) is a low-cost substrate for fermentations processes and a large-quantity waste gas in steel mill industry. We aimed for construction of gas-utilizing acetogens that produce PHB and its monomeric compound 3-hydroxybutyrate (3-HB).

The acetogens *Clostridium ljungdahlii* and *C. coskatii* were genetically engineered using two different plasmid-based production pathways for PHB and 3-HB. Recombinant strains were cultivated under heterotrophic and autotrophic conditions. Subsequently, production of 3-HB was analyzed using high performance liquid chromatography and PHB by various techniques such as gas chromatography, microscopy, nuclear magnetic resonance spectroscopy, differential scanning calorimetry, and gel permeation chromatography.

Recombinant strains of *C. ljungdahlii* and *C. coskatii* produced considerable amounts of PHB. PHB production in cells was visualized by microscopy. Thermal properties clearly showed all characteristics of PHB with high molecular weight. In contrast, successful synthesis of 3-HB was exclusively observed with engineered *C. coskatii.* Both, PHB and 3-HB proved to be stable products that can be further extracted, purified, and processed for a wide range of applications.

Production of the biopolymer and its precursor using recombinant acetogens opens the possibility of producing biodegradable plastic materials from cheap (waste) gases with low variability and large availability compared to other waste substrates.

Capture and Utilisation of Biogas Components by a Mixed Methanotroph and Microalgae Culture

CHARLOTTE E. BJORCK[a], PAUL D. DOBSON[b], JAGROOP PANDHAL[a].

[a] Department of Chemical and Biological Engineering, University of Sheffield, Sheffield, S1 3JD, UK
[b] Scruffy Biotech Ltd., Glossop, Derbyshire, UK

The co-culture of methane oxidising communities (MOCs) with microalgae offers the potential to simultaneously utilise methane and carbon dioxide constituents of biogas by conversion to biomass.

Proposed in either sequential or simultaneous batch culture, the methane component satisfies the carbon and energy requirements of the methanotrophic bacterial communities, and through aerobic metabolic processes releases carbon dioxide as a waste product. Released into the culture medium, carbon dioxide is photosynthetically fixed by the microalgae, producing biomass and dissolved oxygen, which is then available for methane oxidation by the MOCs.

Interest in this transformation is driven by greenhouse gas (GHG) mitigation strategies, through capture and utilisation, in addition to addressing the commercial viability of anaerobic digesters (AD) in remote locations. The potential to diversify biogas utilisation beyond heat and electricity generation by producing a higher value product, offers the possibility to monetise unutilised waste streams, avoiding the negative environmental impacts of waste disposal, and may also allow specific AD processes to be economically viable.

Common in nature, synergistic relationships have been observed between bacterial methane oxidising communities and microalgae in a number of locations including freshwater lakes. By isolating and investigating methanotroph-microalgal communities, we hope to exploit the symbiotic relationship, with the benefits associated with a naturally occurring microbial community.

Commodity Chemicals Production in *Moorella thermoacetica*

BARBARA BOURGADE, AHSAN ISLAM

Department of Chemical Engineering, Loughborough University, Loughborough, LE11 3TU, United Kingdom

Most commodity chemicals are currently derived from the petrochemical industry. However, the detrimental environmental footprint of petrochemical industries due to the emissions of greenhouse gases and the imminent shortage of petroleum resources urge us to find alternative methods to generate chemicals. Several bio-based alternatives have arisen in the past decade. Amongst these alternatives, gas-fermenting bacteria, such as acetogens, allow to simultaneously produce chemicals and reduce greenhouse gases. Indeed, under autotrophic conditions, these acetogens convert C1 gases (CO and CO_2) into acetyl-CoA and other industrially relevant products, such as acetate or ethanol, using the Wood-Ljungdahl pathway. While several mesophilic acetogens have been engineered to produce non-native metabolites, thermophilic acetogens are advantageous in an industrial context to limit contamination and upstream gas cooling.

The thermophilic acetogen *Moorella thermoacetica* produces acetate during gas fermentation. Although acetate is industrially relevant, there is an interest in expanding the spectrum of compounds that *M. thermoacetica* can produce. To do so, the two computational tools From Metabolite to Metabolite and Metabolic Route Explorer have been used to design synthetic pathways for several target compounds, such as ethylene glycol or 1,2-propanediol.

Experimental implementation in this organism might be challenging as the available genetic tools are still limited. Although successful genetic modifications have been published, transformation efficiency still seems suboptimal. While attempting to improve transformation efficiency, one of the designed synthetic pathways for the production of ethylene glycol will be introduced in *M. thermoacetica*. While implementation will be prioritised in *M. thermoacetica*, the pathways have been designed for introduction in any acetogen.

Anaerobic Treatment of Blast Furnace Gases

RHIANNON CHALMERS-BROWN, RICHARD DINSDALE, JAIME MASSANET-NICOLAU, GARETH LLOYD

Sustainable Environment Research Centre, Faculty of Computing, Engineering and Science, Glyntaff, University of South Wales, Pontypridd, CF37 4BE, UK

The aim of the research was to determine the volatile fatty acid (VFA) production of a mixed microbial culture when fed on synthetic industrial off-gas streams. Anaerobic bacterial cultures are well studied and widely utilised for C1 fermentation although the application of easily accessible, cost effective mixed cultures to industry has not yet been thoroughly explored. Whilst pure cultures have their advantages, they are often challenging to culture and maintain, particularly with complex gas mixtures.

Synthetic Blast Furnace Gas (BFG) was used to produce acetic acid and other long chain VFAs using a mixed microbial culture from a domestic waste water treatment anaerobic digestion plant. Headspace gas chromatography (HSGC) analysis showed that up to 117 mgL^{-1} acetic acid had been produced with batch reactors using a non-heat-treated inoculum and nutrients, stirred and maintained at 30^0C for 3 days. Longer chain VFAs including propionic acid and butyric acid were also observed. Micro gas chromatography analysis of the final headspace composition showed the CO_2 concentration had decreased by 98.73%. A steady drop in headspace pressure over the three days was observed using an Oxitop pressure measurement system. VFAs could be used to produce bioplastics, polymers, coatings and paints. Acetic acid is a favourable output for industrial production due to its versatility and wide application whilst offering a flexible range of downstream products without complex genetic engineering and high process maintenance costs. Further work will include the sampling and testing of BFG from steel works at Port Talbot to determine whether the mixed culture can directly treat industrial waste gases without pre-treatment, followed by development of the process for continuous operation on industrial scale. The results to date are an excellent starting point for the development of mixed culture fermentation processes for the low-cost treatment of carbon rich industrial waste gases.

Rhiannon Chalmers-Brown, Research Student, University of South Wales, Rhiannon.Chalmers-Brown@southwales.ac.uk

Homologous Overproduction and Mutagenesis to Unravel the Function of the Hydrogen-Dependent CO_2-Reductase

HELGE M. DIETRICH and VOLKER MÜLLER

Molecular Microbiology & Bioenergetics, Institute of Molecular Biosciences, Johann Wolfgang Goethe University Frankfurt/Main, Frankfurt, Germany

The first step in CO_2 reduction as carried out by some acetogenic bacteria is catalyzed by a hydrogen-dependent CO_2-reductase (HDCR) that consists of four subunits. HydA2 is the hydrogenase subunit, FdhF the formate dehydrogenase and HycB3 and HycB4 are two small iron-sulfur proteins assumed to be involved in electron transfer. To adress the function of the complex and the role of individual subunits during catalysis, the *hdcr* genes from *Thermoanaerobacter kivui* were expressed in a Δ*hdcr*-strain of *T. kivui*. The purified HDCR was as active as the enzyme purified from the wild type. Individual subunits were deleted and the effect on the HDCR reaction determined. These studies led to the first insights into the role of individual subunits of the HDCR.

Establishing Genetic Tools for the Thermophilic Methanogen *Methanothermobacter thermautotrophicus* ΔH

CHRISTIAN FINK, LARGUS T. ANGENENT, BASTIAN MOLITOR

Environmental Biotechnology Group, Center for Applied Geosciences, University of Tübingen, Tübingen, 72074, Germany

The thermophilic methanogen *Methanothermobacter thermautotrophicus* is already used in a commercially available biomethanation process as an energy storage and carbon recycling system. This microbe is suitable for such a process because of a relatively high tolerance towards oxygen, the possibility of intermittent gas feeding without compromising the biocatalyst, and high volumetric methane production rates. Furthermore, the high optimum growth temperature reduces cooling needs for the bioreactor. For the optimization of the biomethanation process different strategies can be used. First, the reactor design, process parameters, and media compositions can be optimized. Second, the microbe itself can be optimized through laboratory evolution or genetic modification. So far, no techniques for the genetic modification of *M. thermautotrophicus* were published in the literature. Few reports on approaches with low efficiency for available for the closely related species *Methanothermobacter marburgensis*, such as transduction using the specific phage ΨM2, and transformation by genomic DNA of a 5-Fluoruracil-resistant strain using natural competence. together with modern seamless cloning techniques and the existing genetic systems for mesophilic methanogens, we will establish genetic tools for *M. thermautotrophicus*.

To accomplish this, we first developed self-integrating DNA constructs that carry a puromycin resistance cassette. Additionally, we are constructing shuttle plasmid vectors for *Escherichia coli* and *M. thermautotrophicus* in a modular fashion that will make it easy to test various origins of replications and resistant markers. The self-integrating constructs and the shuttle plasmid vectors enable us to test DNA-transfer systems to generate gene deletion mutants and express foreign genes in *M. thermautotrophicus*, respectively. Afterwards, the genetic toolbox can be expanded to include regulated promoters, marker genes, and CRISPR-based methods.

Heterologous Expression of Genes for 1,4-Butanediol Production with the Acetogen *Eubacterium limosum*

MAXIMILIAN FLAIZ, FRANK R. BENGELSDORF, AND PETER DÜRRE

Institut für Mikrobiologie und Biotechnologie, Ulm University, Albert-Einstein-Allee 11, 89081 Ulm, Germany. maximilian.flaiz@uni-ulm.de

The strict anaerobic, acetogen *Eubacterium limosum* is a promising bacterial candidate for the recombinant production of chemicals from non-petrochemical resources. *E. limosum* is genetically accessible and able to metabolize the C1 carbon sources CO, CO_2, and methanol. As a fluid, methanol has several advantages compared to the commonly used C1 gases. Methanol does not suffer from gas to liquid mass transfer problems and it can be stored as well as transported easily. Moreover, methanol can be produced from syngas, which in turn can be derived from biomass or is part of the waste stream from industrial processes.

The aim of this study is the production of the platform chemical 1,4-butanediol (BDO) from methanol using recombinant *E. limosum* strains. Therefore, the heterologous genes *abfD* (encodes 4-hydroxybutyryl-CoA dehydratase) and *adhE2* (encodes bifunctional alcohol/aldehyde dehydrogenase) are intended to be expressed in *E. limosum*. The gene *adhE2* originates from *Clostridium acetobutylicum* and the gene *abfD* originates either from *C. aminobutyricum*, *C. beijerinckii*, or *C. kluyveri*.

The genes *adhE2* and the three homologues of *abfD* were amplified and cloned into suitable plasmids under the control of the tetracycline inducible *tet* promoter. Plasmids were successfully transformed into *E. limosum*. The resulting strains *E. limosum* [pMTL83251_P_{tet}_AA_CAMI], *E. limosum* [pMTL83251_P_{tet}_AA_CBEI], and *E. limosum* [pMTL83251_P_{tet}_AA_CAMI] were characterized in growth experiments using methanol as carbon source.

The analysis and validation of the data obtained from growth experiments will reveal, whether the production of BDO is feasible in *E. limosum*. Moreover, the experiments might unveil, which of the three selected *abfD* genes leads to the highest amount of BDO from methanol.

Continuous Gas Processing without Bubbles using Thin Liquid Film Bioreactors Containing Biocomposite Biocatalysts

MICHAEL C. FLICKINGER[1], RYAN R. BARTON[1], CHRISTOPHER DURAN[1], ADAM WALLACE[1], MARK J. SCHULTE[1], ORLIN D. VELEV[1], NILAY CHAKRABORTY[2], MICHAEL GUARNIERI[3], KAMELIA BOODHOO[4]

[1]Dept. Chemical & Biomolecular Engineering (CBE), North Carolina State University, Raleigh, NC 27695 USA, [2]Dept. Mechanical Engineering, University of Michigan, Dearborn, MI 48128 USA , [3]National Renewable Energy Laboratory, Golden, Colorado 80401 USA, [4]Chem. Eng. Adv. Matls., Newcastle University, Newcastle Upon Tyne, NE1 7RU, UK

Continuous microbial gas processing without bubbles is possible with countercurrent thin liquid film bioreactors. Mass transfer power input can be minimized by using thin liquid films with laminar wavy flow (Re <200) in contact with highly concentrated live, non-growing microbes stabilized in a porous biocomposite biocatalyst. This composite materials/reactor design approach can increase k_La values to 1 to 1.5 x 10^3 h^{-1}, increase secreted product concentration, and significantly decrease gas-liquid mass transfer energy input, process volume and water use. Microbial specific activity is also increased for some organisms compared to suspension in liquid media. Paper-based biocomposite biocatalysts have a rough hydrophilic surface resulting in 150 to 300 µm thick falling liquid films. Paper roughness enhances gas-liquid mass transfer and has been simulated using a 2D finite element (FEM) CFD model. The paper also serves as a separation device - the secreted products are released into the falling liquid film. We investigated gas-liquid mass transfer using a ~0.04 m^2 prototype cylindrical paper falling film bioreactor (FFBR). Experimental model systems investigated include *Clostridium ljungdahlii* OTA1 for absorbing CO, *Methylomycrobium alkaliphilum* 20Z for CH_4, and algae for CO_2. Critical are generation of coating microstructure, microbe adhesion or entrapment in paper, preservation of microbe viability in a non-growth state, surviving osmotic shock and desiccation tolerance for dry storage. Spatially correlated Raman microspectroscopy and hyperspectral imaging are being developed to monitor the distribution of residual water surrounding and within the cells in the biocomposite. A spinning disk bioreactor (SDBR), that enhances mass transfer by reducing liquid film thickness to <100 µm with wave induced turbulent flow using centrifugal force (1000 x g) is being investigated with algae to intensify CO_2 absorption.

Biosynthetic Pathway Engineering in *Cupriavidus necator* Towards 1,3-butanediol Production from Carbon Dioxide

JOSHUA L. GASCOYNE, RAJESH BOMMAREDDY, STEPHAN HEEB, NAGLIS MALYS

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

1,3-butanediol is an important commodity chemical used in the production of insecticides and fragrances. The optically active form (*R)*-1,3-butanediol is used as a key intermediate for β-lactam antibiotic production with a higher demand of (*R)*-1,3-butanediol required for synthesis of synthetic antibacterial agents. Chemical synthesis from petrochemical resources often produces a racemic mixture of *R*- and *S*- forms, whereas enzyme-driven production can achieve a high optical purity of (*R)*-1,3-butanediol.

Engineering microorganisms capable of utilising waste greenhouse gases such as CO_2 for the production of butanediols provides a promising solution, reducing crude oil consumption and atmospheric CO_2 levels. The widely studied facultative lithoautotrophic bacterium *Cupriavidus necator* is an ideal candidate due to its capability of reaching high cell densities and widely understood mechanism of using CO_2 as the sole carbon source with H_2 and O_2 as energy sources. Through expression of heterologous enzymes *C. necator* has been successfully engineered to produce 1,3-butanediol heterotrophically and autotrophic fermentation. Further research into optimisation of growth conditions combined with targeted gene deletions has resulted in greater carbon flux towards 1,3-butanediol production.

Gsmodutils: A Python Based Framework for Test-driven Genome Scale Metabolic Models

JAMES PETER GILBERT, NICOLE PEARCY, RUPERT NORMAN, THOMAS MILLAT, KLAUS WINZER, JOHN KING, CHARLIE HODGMAN, NIGEL MINTON and JAMIE TWYCROSS

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

Genome scale metabolic models are increasingly important tools for industrial biotechnologists that enable simulation of the steady state behaviour of microorganisms. Models of this form can include thousands of reactions involving pathways that only become relevant in specific simulation settings. However, despite their widespread use, power and the availability of tools to aid with the analysis of reconstructions, little methodology is suggested for their continued management. For example, updated genome annotations can result in significant changes to model structure. As their usage becomes more wide spread within systems and synthetic biology applications, high quality reusable models are integral to the design, build and test cycle of industrial biotechnology.

As part of an ongoing effort to improve the reproducibility of systems biology models and simulations, we have adopted a test-driven development methodology for the continuous integration of genome scale metabolic reconstructions. Based around the COBRApy stack, we have developed the gsmodutils modelling framework which places an emphasis on well-defined reusable test cases. Crucially, the software provides management utilities for the differences between models allowing users to examine how different strain designs impact a wide range of system behaviours, minimising error between versions of reconstructions.

This software framework is open source and freely available from https://github.com/SBRCNottingham/gsmodutils

3-Hydroxypropionic Acid (3-HP) Production by *Cupriavidus necator* H16 via the β-alanine Pathway

DIEGO OROL, KATALIN KOVACS and NIGEL MINTON

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

Microbial fermentation for production of high-value products such as chemicals and biofuels could become the key technology in our efforts to reduce our dependence on fossil energy and reduce the global carbon-containing waste gases.

Cupriavidus necator H16 (*C. necator*) is a Gram-negative, non-spore forming bacterium found in aerobic and anaerobic, non-halophilic environments. It is a facultatively chemolithoautotrophic organism, able to grow with organic substrates, H_2 and CO_2 under aerobic conditions. It has not been recognized as pathogen therefore, *C. necator* has been selected as a suitable candidate for the industrial production of green chemicals from CO_2.

3-Hydroxypropionic acid (3-HP) is one of these potential chemicals which might be obtained by microbial fermentation in a sustainable manner. This product is a platform chemical which can be converted into a wide variety of acids, biodegradable polyesters among other highly valued industrial products.

According to the metabolic modelling previously described and consulted in *E.coli* and *S. cerevisiae*, the most energy efficient metabolic pathway for 3-HP production is the one proceeding via the β-alanine intermediate (Borodina, Kildegaard et al. 2015).

We aim to gain insight into the potential of the β-alanine metabolism and the optimization of each metabolic step in this pathway in order to generate a 3-HP producing *C. necator* strain able to obtain commercially viable amounts of 3-HP, using CO_2 as sole carbon source.

Metabolic Engineering of *Cupriavidus necator* for the Production of Aminovaleric Acid and Hydroxyvaleric Acid

CHRISTIAN J.E. GUDE, ALEXANDER CONRADIE, NIGEL P. MINTON, KATALIN KOVACS

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

Rising demand for value-added chemicals and environmental concerns associated with petrochemical synthesis routes make it necessary to develop new solutions for the production of chemicals in microorganisms. This work aims to add the production of 5-Aminovaleric acid (5AVA), a Nylon-5 precursor, and 5-hydroxyvaleric acid (5OHV), a polyester precursor, to the portfolio of bio-based chemicals we can produce in an environmentally friendly way and will introduce to recent achievements of metabolic engineering towards a more carbonneutral future for plastic production.

Cupriavidus necator is a rod-shaped gram-negative, strictly aerobic and facultatively lithoautotrophic β-proteobacterium. It was first described as “Knallgasbakterium” (“Oxyhydrogen bacterium”) when it was first isolated, for its ability to generate reduction equivalents from molecular hydrogen with oxygen as main electron acceptor. It the capability to grow on a very broad range of industrially relevant carbon and energy sources, including sugars, glycerol and, owing to its lithoautotrophic metabolism, CO2 and H2. Its remarkable flexibility for utilizing different nutrient and energy sources and its capability to fix carbon through the Calvin-Benson-Bassham (CBB) cycle make *C. necator* the ideal host to eventually produce 5AVA from waste gases as sole carbon source.

Two new potential pathways are shown alongside the research that has been conducted so far in their establishment. Our first novel approach of 5OHV synthesis in *C. necator* builds on its ability to produce 3HP-CoA through an established synthetic pathway, namely the beta-alanine route. From 3-HP-CoA, a reversed beta oxidation cycle extents the chain length from C3 to C5, leading to 5OHV. The second pathway shows the production of 5AVA from lysine with efforts undertaken to transfer the excellent lysine production capabilities of *Corynebacterium glutamicum* to *C. necator*, for an efficient production of 5AVA from central metabolism and syngas.

Evaluation of "Impure" Syngas from The Karlsruhe Bioliq Plant and Other Sources as Substrate in Syngas Fermentation

ALBA INFANTES, MICHAELA ZWICK, ANKE NEUMANN

Institut für Bio- und Lebensmitteltechnik 2, Technische Biologie, Karlsruhe Institute of Technology (KIT), Fritz-Haber-Weg 4, 76131 Karlsruhe

To advance towards an environmentally friendly bioeconomy, renewable, alternative energy sources and chemical building blocks must be developed. To achieve this, a promising process is the use of acetogenic fermentation of syngas to obtain commodity chemicals.

The ability of this microorganisms to withstand some impurities contained in the syngas and their flexibility to use different mixtures of CO and/or CO_2 and H_2 makes them an attractive alternative to chemical processes.

Most studies have used pure syngas, containing only a fixed composition of CO, CO_2 and H_2 or a mixture thereof. Because of this, it is still not well understood how "impure" syngas could impact the performance of the process due to the combined effect of various impurities, together with varying gas composition.

In this study, the focus is to investigate the effect of various biomass-derived syngas, produced and bottled directly in a gasification plant, and hence, containing different impurities.

The final syngas product is expected to contain the following main impurities: methane (CH_4), ethane (C_2H_6), ethylene (C_2H_4), acetylene (C_2H_2), tar components like benzene, and hydrogen sulphide (H_2S). As well, traces of other C3, C4, C5 and C6 components can be found. Nonetheless, the exact amount and species of the impurities can fluctuate depending on the syngas source.

Moreover, the varying amounts of CO, CO_2 and H_2 in the "impure" syngas are the result of different biomass sources, with their intrinsic natural variability, being used in the gasification process.

In this project, *Clostridium ljungdahlii*, a well-known, well-characterised acetogen is used to assess the impact of different biomass-derived syngas. The established, robust fermentation system provides continuous monitoring of the gas consumption, and so the effects of the different gases compositions can be exhaustively monitored.

The fermentation tests are currently ongoing and the results will be presented in this work.

Heterologous Expression of Alcohol Dehydrogenase Enables Concentrated Production of Non-Native Ethanol from Carbon Monoxide in Acetogen, *Eubacterium limosum* KCTC1326BP

JIYEONG JEONG[1], JIYEON KIM[1], and IN SEOP CHANG[1]

[1] School of Earth Sciences and Environmental Engineering, Gwangju Institute of Science and Technology (GIST), Gwangju 61005, Republic of Korea

Acetogens are beneficial bio-catalysts to use in the resource recovery technology from waste gas (synthesis gas). The major product of acetogens is acetate, and some of them are able to produce alcohols. Several efforts are being made to construct genetic toolbox in order to engineer acetogens. However, up to now, the successful genetic engineering system is limited to a few acetogens. *Eubacterium limosum* (Elm) KCTC1326BP (formerly, KIST612) is a butyrate-producing acetogen that the genomic data are available and the metabolic properties with bioenergetics were well studied.

In this research, we developed the foreign gene expression system for Elm strain preferentially. The compacted size of shuttle vector (pELM) was constructed for the strain by modifying pJIR418 that the stable replication was confirmed. In addition, the transcriptomics-based candidate selection of native constitutive promoter and the β-glucuronidase reporter gene assay enabled to sort one strong and constitutive native promoter (H2) for the strain. Then, based on the genetic system of the strain, the recombinant vector (pECPH2::*adhE*), that the heterologous gene encoding bi-functional aldehyde/alcohol dehydrogenase (AdhE) with H2 promoter was inserted in pELM, was constructed and it was introduced into the strain. The successful transformation and expression of heterologous AdhE gene enabled to produce non-native ethanol from the strain.

The AdhE transformants were cultivated on autotrophic CO-substrate condition. As a result of product profiling, the transformants produced ethanol by showing clear re-assimilation of acetate without any butyrate production. Interestingly, the transformants produced 27 mM of ethanol consuming 12.9 mmols of CO with complete re-assimilation of acetate, in CO fed-batch condition. In conclusion, we successfully engineered the Elm strain to ethanol producer and identified the prominent production of ethanol with acetate-assimilation in CO-specific condition.

Keywords: Acetogen, Constitutive native promoter, Heterologous gene expression, Acetate-assimilatory ethanol production

Development of Optimised CRISPR/Cas9 System for Acetogenic Bacterium, *Eubacterium limosum* KIST612

JI-YEON KIM*, JIYEONG JEONG*, MINSEOK CHA, JINSUNG JEON and IN SEOP CHANG

School of Earth Sciences and Environmental Engineering, Gwangju Institute of Science and Technology (GIST), 123 Cheomdangwagi-ro, Buk-gu, Gwangju 61005, Republic of Korea

*Both authors contributed equally to this work.

Increasing interest in acetogen for the production of such valuable products (e.g., butanol, ethanol, and 2,3-butandiol) requires the development of new genetic tools for these atypical production organisms. Herein, we develop CRISPR/Cas9 system for the acetogen, *Eubacterium limosum* KIST612 that can metabolise synthesis gas (syn-gas) components, H_2, CO and CO_2 via Wood-Ljungdahl pathway, producing acetate and butyrate as end-products. In this study, *E.coli-Clostridium/Eubacterium* shuttle vectors were used for carrying Cas9 and sgRNA. Initially, in order to see whether Cas9 has a toxicity to the strain or not, we expressed Cas9 protein using native promotors of *Eubacterium.* Expression of Cas9 did not show inhibitory effect on the cellular growth of *E. limosum* KIST612 indicating that CRISPR/Cas9 system can be developed for the genetic tool box. In order to develop optimised system of CRISPR/Cas9, a couple of sgRNAs (e.g., *pyrF, blaI*) were designed to assess ability of Cas9 and target gens in *E. limosum* KIST612. This study not only makes an efficient way of metabolic engineering for producing biochemical and biofuels but also provides valuable guidance and essential references for genome editing in the use of *E. limosum* KIST612 strain that have not been reported on genes editing toolbox.

Investigating the RNF-complex and Its Impact on the Energy Metabolism of the Acetogen *Clostridium ljungdahlii*

CHRISTIAN-MARCO KLASK, BASTIAN MOLITOR, LARGUS T. ANGENENT

Environmental Biotechnology Group, Centre for Applied Geoscience, University of Tübingen, Tübingen, 72074, DE

The biotechnological use of acetogenic bacteria provides a promising platform to convert waste gases or syngas into bio-based fuels and chemicals. These bacteria use the Wood-Ljungdahl pathway (WLP) to fix carbon under autotrophic conditions. However, the WLP is a non-ATP generating pathway. One mole of ATP is invested for the activation of one mole of CO_2 in the methyl branch of the WLP, while one mole of ATP is regenerated during the dephosphorylation of acetyl-phosphate into acetate. The membrane-located RNF-complex plays a critical role in the energy conversion and maintenance in these bacteria. The only way an acetogen such as *C. ljungdahlii*, can generate ATP for its anabolism under autotrophic conditions is based on the RNF-complex activity. The RNF-complex shares high similarities with respiratory oxidoreductases, and was first characterized in ***R****hodobacter sphaeroides* while investigating its **N**itrogen **F**ixation pathway. The RNF-complex in *C. ljungdahlii* catalyzes the oxidation of reduced ferredoxin and the subsequent reduction of NAD^+ while protons are transferred over the membrane. The proton gradient is then used to drive an ATP synthase.

The implementation of new metabolic pathways, especially for higher value fermentation products, such as butanol or hexanol, is often strongly dependent on the availability of sufficient cellular energy in the form of ATP. Elucidating the RNF-complex in detail is one of the key elements to reveal the full potential of *C. ljungdahlii* for biotechnology. Therefore, different recombinant strains overexpressing *rnf* genes were generated and tested for growth under heterotrophic and autotrophic conditions. In addition, a full RNF knock-out mutant will be generated and complemented by an inducible *rnf*-gene expression system. This will further our knowledge on the metabolism of acetogens, and provide a powerful molecular tool to fine-regulate the RNF activity of *C. ljungdahlii* in bioprocesses.

Simultaneous Gas- and Cell-Recycled Continuous Carbon Monoxide Fermentation Under Open-, Closed- and Mixed Circuit to Boost-up Biomass and Product Titer Using Acetogen Strain, *Eubacterium limosum* KIST612

MUNGYU LEE, MUHAMMAD YASIN, NURI JANG, IN SEOP CHANG

School of Earth Sciences and Environmental Engineering, Gwangju institute of Science and Technology (GIST), Gwangju 61005, Republic of Korea

Synthesis gas (syngas) can be produced from gasification of various carbon-rich feed-stocks, such as fossil and renewable biomass. Syngas can be converted into chemicals and biofuels through biological conversion called “syngas fermentation”. Syngas fermentation could be less sensitive of inject gas mixture and cost effectiveness due to energy requirement compared to chemical conversion using precious metal catalysts. However, for the real scale operation and production of products to be desired, several issues should be settled and optimised, including low mass transfer, low cell biomass, and product concentration. In this study, three operation modes were tested to convert CO using *Eubacterium limosum* (Elm) KIST612 strain called ‘Closed circuit Simultaneous Gas and Cell recycled (C-SGCR)’, ‘Open circuit Simultaneous Gas and Cell Recycled (O-SGCR)’ and ‘Mixed circuit Simultaneous Gas and Cell Recycled (MC-SGCR)’. Three reaction systems were independently operated and comapred to see how the parameters (Dilution rate, *D*; Gas-liquid mass transfer, *GL-MT*) are affected the reaction performances. All reactions were tested in bubble column reactor (BCR) employed *ex-situ* membrane to apply continuous cell recycling mode. During the reactors operation, Elm was cultivated on carbonate-buffered basal medium (CBBM), and gaseous mixture ($CO:CO_2$: 4:1) was used as model syngas. C-SGCR was operated in Gas Fed-batch mode with gasbag, O-SGCR was operated with Gas cylinder with constant pressure of 14.7 psig to maintain the partial pressure of CO in the system. MC-SGCR was converted to Gas cylinder from gas fed-batch mode during the operation. Furthermore, MC-SGCR initially operated with high Dilution rate (***D***) than growth rate to boost the quick biomass increase in the reactor to retain activated microorganisms in high optical density (OD). These comparison of three operation would help to optimise and economically operate early steps of reactor for high biomass and acetate production.

Engineering *Cupriavidus necator* for the Production of 3-Hydroxypropionic Acid

JESSICA LOCKER, CHRISTIAN ARENAS, KATALIN KOVACS, KLAUS WINZER

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

3-hydroxypropionic acid (3-HP) is a 3-carbon molecule that is regarded as a significant precursor for renewable bioplastics and industrial chemicals. It can be reduced, esterified, dehydrated and oxidized into useful products used in industrials related to cosmetics, adhesives and textiles. Potential commercial routes for chemical synthesis are impeded by unwanted by-products, high start-up costs or unacceptable environmental consequences.

Due to unsatisfactory chemical methods for its production, it has become a noteworthy target for biological manufacture. Many enzymatic pathways for biological synthesis of renewable 3-HP have been proposed. These have been demonstrated in a range of organisms such as *Escherichia coli*, *Klebsiella pneumoniae* and *Saccharomyces cerevisiae*. However, all of these organisms have require substrates that compete with existing food supplies.

Cupriavidus necator is a facultative chemolithoautotrophic proteobacterium that has been described as a 'Knallgas' (H_2-oxidizing) microorganism. It has the ability to use only H_2 and CO_2 as sole sources of carbon and energy, alongside (or as an alternative to) more complex organic substrates. It has evolved the ability to survive in both aerobic and anaerobic conditions, due to its natural environment of soil and freshwater where oxygen levels can be variable. The ability of *C. necator* to grow to a high cell density and its metabolic versatility has made it a prime chassis for commodity chemical creation.

By inserting the 2-step malonyl-CoA pathway into *C. necator*, biodegradable 3-HP could be produced from waste gas rather than non-renewable resources. The first reaction of this pathway is the conversion of the central metabolite acetyl-CoA to malonyl-CoA, which is catalysed by acetyl-CoA carboxylase (Acc). The second step is the reduction of malonyl-Co-A to 3-hydroxypropanoic acid. This is catalysed by a malonyl-CoA reductase (Mcr) found for instance in members of the genus *Chloroflexus*, where it has a role in carbon fixation as part of the 3-HP cycle.

By introducing this pathway into *C. necator,* 3-HP may be created using CO_2 and H_2 as sole sources of carbon and energy, helping to solve the related issues of climate change and environmental plastic accumulation.

Functional Genetic Elements for Controlling Gene Expression and Production of Chemicals in *Cupriavidus necator* H16

NAGLIS MALYS, SWATHI ALAGESAN, ERIK K.R. HANKO, MUHAMMAD EHSAAN, KLAUS WINZER, NIGEL P. MINTON

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

Development and quantitative evaluation of functional genetic elements such as constitutive and inducible promoters, as well as ribosome binding sites (RBSs), plays an important role in synthetic biology. In this study, we design, build and test promoters and RBSs for controlling gene expression in the model lithoautotroph *Cupriavidus necator* H16. Using isoprene production as an example, we investigate if and to what extent chemical compound yield correlates to the level of gene expression of product-synthesising enzyme. By applying of inducible promoters for controlling expression of isoprene synthase gene *ispS*, we achieve isoprene yields that exhibit a significant correlation to the gene expression levels. However, when the impact of designed RBSs on the gene expression is evaluated. a second-order polynomial relationship is observed between the RBS activities and isoprene yields. This study presents quantitative data on regulatory genetic elements and expands the genetic toolbox of C1 gas-fixing bacterium *C. necator*.

Metabolic Engineering of *Cupriavidus necator* H16 for Overproduction of δ-Aminolevulinic Acid

CHRISTOPHER MCCUSKER, ALEX CONRADIE[1], ELAINE O'REILLY[2] AND KATALIN KOVACS[3]

[1]Sustainable Process Technologies Group, Faculty of Engineering. B53 Coates Building, University Park, University of Nottingham, NG7 2RD, UK
[2] School of Chemistry, University Park, University of Nottingham NG7 2RD, UK
[3]BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), University of Nottingham, Nottingham, NG7 2RD, UK

δ-Aminolevulinic acid (ALA), an endogenous, 5-carbon, non-protein amino acid, is the single precursor in the biosynthesis of arguably the most significant molecules in the biosphere, namely haems, chlorophylls, cytochromes, bilins, and vitamin B_{12}. ALA and chemically synthesised derivatives are routinely applied in medicine as photodynamic therapy agents, as dietary supplements, and ALA can act as both a plant growth promoter and a selectable herbicide. The chemolithoautotroph *Cupriavidus necator* H16, a ubiquitous soil and freshwater β-proteobacterium, is of great significance due to its' natural ability to fix CO_2 via the Calvin-Benson-Bassam (CBB) cycle as the sole source of carbon, and ability to oxidise molecular H_2 as the sole source of electron donor. The overall aim of this project is to overproduce ALA in *C. necator* H16 using CO_2 and H_2 via heterologous expression of the C_4 pathway for ALA biosynthesis. The project will also exploit strategies already underway at the SBRC in the modification of the Calvin cycle and tricarboxylic acid (TCA) cycle in *C. necator* H16 to maximise flux to ALA biosynthesis. Alongside this work, we intend to engineer the ALA synthase enzyme to improve production of ALA.

Acknowledgements: The authors gratefully acknowledge the financial support of the University of Nottingham SynBio Doctoral Training Programme BB/M008770/1.

Development of a Versatile Plasmid Addiction System for use in *Cupriavidus necator* H16

CALLUM MCGREGOR, KATALIN KOVACS, NIGEL MINTON

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

Direct conversion of CO_2 to useful and valuable commodity chemicals is seen as a way of reducing environmental damage associated with current chemical manufacturing processes which often use fossil fuels as a feedstock. The conversion can be further enhanced by using microorganisms capable of fixing CO_2 from the atmosphere as microbial cell factories to facilitate the conversion of CO_2 into more diverse compounds.

Cupriavidus necator H16 (*C. necator*) is a gram-negative, hydrogen-oxidizing bacteria capable of chemolithoautotrophic growth. That is, it can grow using CO_2 and H_2 as sole carbon and energy sources respectively. *C. necator* is already recognised as the model organism for polyhydroxyalkanoate (PHA) accumulation and under the appropriate conditions can accumulate up to 90% of its cell dry weight (CDW) as PHA. Due to its capacity for CO_2 fixation and its propensity for genetic modification, *C. necator* is considered an industrially interesting organism for large-scale microbial production of platform chemicals from CO_2.

In order for such a process to be viable, high productivity is required. Genomic integration is a stable means of expression, however productivity is typically reduced in comparison to plasmid-based expression due to lower copy number. On the other hand, plasmid expression in large-scale fermentation suffers from plasmid loss, and antibiotics are disadvantageous as they must be removed from the end product. Additionally, antibiotic supplementation still does not always ensure plasmid retention. Therefore, plasmid addiction systems can be used to improve stability and retain high productivity.

We have developed a plasmid addiction system using a strain of *C. necator* defective in pantothenate biosynthesis. Our initial results show an extremely stable system capable of sustaining production of compounds directly from CO_2.

Circling Sustainability and Responsibility: Exploring Synergies between the Circular Economy, Synthetic Biology and Responsible Research and Innovation

CARMEN M. McLEOD

Interdisciplinary Responsible Research & Innovation Group, BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), University of Nottingham, Nottingham, NG7 2RD, UK

The Circular Economy (CE) is an emerging concept that is proving increasingly popular amongst policymakers, industry and business globally. CE incorporates sustainability goals that are based on an economy that promotes the continuous circulating and recovering of resources, rather than a linear economy focussed on making, using and disposing. A key case that is often highlighted by proponents of CE, is the potential for industrial waste to become a sustainable and renewable resource, reflected in recent UK policy such as the 'sustainable circular biotechnology' strategy. In particular, synthetic biology has been forecast by policy makers to revolutionise the national bioeconomy, with feedstocks from waste gases playing a key role in these expected environmental and economic benefits. The promotion of synthetic biology as a vehicle towards a more sustainable future by UK and EU policy makers, has also been closely tied to the science governance framework, Responsible Research and Innovation (RRI). These concepts of CE, sustainable bioeconomy and RRI share similar elements – especially the idea of circulating resources and responsibility – and appear to position synthetic biology in particular as a form of what has been described by some analysts as 'sustainability-orientated innovation'.

However, some researchers have highlighted the lack of agreement over definitions and applications of CE, and the challenges, as well as opportunities that are associated with mobilising CE. Using documentary analysis drawn from an ongoing social science project in the SBRC, this poster will present key findings about the emerging discourses of sustainability in the context of synthetic biology, as they relate to the concepts of CE and RRI. This analysis aims to contribute to a broader understanding about the linkages between these concepts, and what implications this could have at both national and international policy levels.

Metabolic Modelling of *Acetobacteirum woodii* Metabolism

NOAH MESFIN, DAVID FELL AND MARK POOLMAN

Cell Systems Modelling Group, Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, OX3 0BP

Acetogens are microbes which produce acetate as a fermentation by-product. They are diverse in their phylogeny but have a metabolic feature in common called the Woods-Ljungdahl Pathway (WLP), which confers the ability to fix carbon dioxide via a non-photosynthetic route. Electrons for this process are derived from diverse substrates including molecular hydrogen and carbon monoxide. The ability of acetogens to utilise components of syngas (H_2, CO, CO_2) make them an attractive target for metabolic engineering for industrially relevant products such as 3-hydroxypropionic acid (HPA). We have previously reported the construction of a genome-scale metabolic model of the model acetogen *Acetobacterium woodii* using a recently sequenced and annotated genome of strain DSM1030. The model consists of 836 metabolites, 909 reactions and 84 transporters and can account for growth on diverse substrates reported in the literature. We identified the reactions used to catabolise fifteen single substrates and substrate combinations, and used this to construct a sub-model representing a core set of energy producing catabolic pathways. We then introduced heterogenous reactions to allow for the production of platform chemicals. Elementary modes analysis of this extended sub-model was applied to further decompose the metabolic network into unique sets of the smallest functioning sub-networks, to investigate the impact of producing novel compounds on the bioenergetics of *A.woodii*. With CO_2 and H_2 as substrates, we find six elementary modes which produce HPA. One elementary mode produces HPA as a sole by-product with a net positive ATP yield representing potential growth supporting HPA production. The genome scale-model reflects in vivo observations, and is being used to gain insight and engineer *A.woodii* metabolism.

ENGICOIN: Engineered Microbial Factories for CO_2 Exploitation in an Integrated Waste Platform

C. JAMES MILLARD, JONATHAN P. BAKER, NIGEL P. MINTON

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

The ENGICOIN consortium consists of 12 partner organisations (universities and companies) from across Europe. The aim of the ENGICOIN project is to design and prototype an integrated biorefinery in which biogas from a variety of waste sources is utilised for the production of valuable chemicals. In principle, the biorefinery will accept waste gases from anaerobic digesters, wastewater treatment plants and landfill sources. With the complete assimilation of CO_2 from mixed gas waste streams, the biorefinery will also serve to purify methane for downstream use.

One of the three organisms to be employed within the biorefinery is *Acetobacterium woodii*, a Gram-positive anaerobe which is capable of assimilating inorganic carbon in the form of CO_2 and methanol. One of the SBRC's contributions to the ENGICOIN refinery will be the generation of a modified *A. woodii* strain for the production of acetone (wild-type *A. woodii* primarily produces acetate), as well as the development of a minimal medium for the economical growth of *A. woodii* under industrial conditions.

Through the use of genetic tools developed in-house for the modification of *A. woodii*, an acetone-producing strain has been generated through the addition of acetone production genes from *Clostridium acetobutylicum*. Characterisation of acetone production in lab-scale bioreactors is currently underway. Alternative metabolic pathways for the generation of acetone are also being investigated. Large *A. woodii* transposon mutant libraries have previously been generated at the SBRC, and the gene essentiality data generated in those experiments will be used to guide the genetic interventions conducted during this project. Additionally, Adaptive Laboratory Evolution (ALE) of *A. woodii* is currently underway, with the objective of improving acetone tolerance so that product toxicity does not limit the product concentrations to be achieved in the final bioprocess.

Robust Analysis of Intracellular Metabolite Concentrations Measured During the Gas-Induced Metabolic Shift in *Clostridium autoethanogenum*

THOMAS MILLAT[1], SALAH ABDELRAZIG[2], LAUDINA SAFO[2], RUPERT NORMAN[1], ANNE HENSTRA[1], KLAUS WINZER[1], DAVE BARRETT[2], NIGEL MINTON[1]

[1] *BBSRC/EPSRC Synthetic Biology Research Centre (SBRC)*
[2] *Centre for Analytical Bioscience*
University of Nottingham, Nottingham, NG7 2RD, UK

The metabolic shift from acetogenesis to ethanologenesis in *C. autoethanogenum* is characterised by significant changes on all levels of biological organisation. Using a standardized experimental setup, we studied the intra- and extracellular alterations in response to changing CO supply using high-throughput methods. The amount of gathered data and different qualitative and quantitative characteristics of the data sets made it mandatory to develop automated methods for data processing and data analysis to support a meaningful interpretation and graphical representation of our results. Here, we outline selected features applied for the estimation of steady-state parameters using experimentally measured intracellular metabolite concentration as an example.

We conducted gas-shift experiments to elucidate the changes in metabolite composition in cells of *C. autoethanogenum* grown in continuous culture. Metabolite samples were collected on a daily base and subsequently analysed using a newly developed method. Considering time courses of about 100 metabolites and 7 phases representing different CO inflows, around 2000 steady-state concentrations were to estimate. Thus, the development of an automated approach was the only practical solution. Here, a major challenge is the identification of outliers that could strongly misguide the numerical analysis. Towards this end, we combined a robust nonlinear fitting method for parameter estimation and outlier identification with a pooled averaging for the calculation of mean and standard deviation of the independent experiments.

Our approach offers a robust method to estimate the steady-state metabolite concentrations. Our analysis unravels that some metabolite concentrations possess a strong dependence on the supplied CO and the growth phase supporting the notion of a significant change in cellular composition during the transition from carbon to non-carbon growth limitation.

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Genome-scale Investigations of *Methanothermobacter thermautotrophicus*

ISABELLA CASINI[a], CHRISTIAN FINK[a], DARIA EVSEEVA[b], ANDREAS DRÄGER[b], DANIEL HUSON[b], LARGUS T. ANGENENT[a], BASTIAN MOLITOR[a]

[a] Environmental Biotechnology Group, Center for Applied Geosciences, University of Tübingen, 72074 Tübingen, Germany;
[b] Wilhelm Schickard Institute for Computer Science, University of Tübingen, 72076 Tübingen, Germany

Renewable energy sources are well-established parts of the electricity mix for many countries. However, an imbalance between production and consumption makes storage of electric power necessary. Pure cultures of acclimated, but non-genetically modified *Methanothermobacter* species are utilized in Power-to-Gas processes for storage of renewable electric power. In this bioprocess, hydrogen (from electrolysis of water) and carbon dioxide (from biogas or industrial off gases) are converted to methane with high process stability and at high conversion rates. The resulting biomethane can be introduced into the existing natural gas grid for storage and distribution.

The utilization of pure culture fermentation for Power-to-Gas processes is already progressed from a process engineering point of view. However, the detailed understanding of the underlying biological processes is necessary to harness the full potential of this promising biotechnology and to implement chemical production routes besides methane. Therefore, differences in metabolic versatility in various *Methanothermobacter* strains are being investigated by comparative genomics. Together with genome-scale metabolic modeling, this will provide a platform for the analysis of systems biological data sets. Genetic tools will enable to test hypotheses resulting from metabolic modeling, fermentation, and systems biology.

Study of The Molybdopterin Biosynthesis Pathway of *Methanococcus maripaludis,* a Methanogen Able to Grow on CO_2/H_2.

AMAURY MONTARNAL, MINYEONG YOO, NAGLIS MALYS, PHILIPPE SOUCAILLE.

Synthetic Biology Research Centre, School of Life Sciences, University of Nottingham, Centre for Biomolecular Sciences, University Park, Nottingham, NG7 2RD, UK

Methanococcus maripaludis is a methanogenic organism able to use C1 compound to produce methane. The glyceraldehyde-3-phosphte: ferredoxin oxidoreductase (GAPOR) plays a key role in the central metabolism of this microorganism by performing dual substrate electron-transfer reaction, the oxidation of glyceraldehyde-3-phosphate to 3-phosphoglycerate and reduction of ferredoxin. Active GAPOR requires a specific molybdenum cofactor consisting of a molybdenum atom coordinated by two pyranopterin moieties.

This cofactor is different from the main product of the *E. coli* molybdopterin biosynthesis pathway which is substituted with two guanosine monophosphate.
The *M. maripaludis* molybdenum cofactor biosynthesis pathway has not been characterized so far.

The aim of this study is to establish the enzymes involved in molybdenum cofactor biosynthesis pathway. The GAPOR gene expressed in *E. coli* along with different combinations of *M. maripaludis* genes will be used to identify the enzymes involved in molybdenum cofactor biosynthesis.

Production of Acetone Using Recombinant *Acetobacterium woodii* Strains

TERESA SCHOCH[1], CATARINA ERZ[1], SABRINA HOFFMEISTER[1], SASKIA NICK[1], FRANK R. BENGELSDORF[1], PETER DÜRRE[1]

[1] *Universität Ulm, Institut für Mikrobiologie und Biotechnologie, Albert-Einstein-Allee 11, 89081 Ulm, Germany*

Acetone is an important platform chemical for industrial applications. Acetone can be produced by the cumene process, which depends on fossil resources. A further option to produce acetone is gas fermentation using recombinant autotrophic acetogenic bacteria. One of these organisms is *Acetobacterium woodii*, which is capable of using CO_2 and H_2 as carbon and energy source via the Wood-Ljungdahl pathway. However, *A. woodii* lacks an acetone production pathway. Therefore, the genes encoding thiolase (*thlA*), acetoacetyl-CoA:acetate/butyrate-CoA transferase subunits A and B (*ctfA/B*) and the acetoacetate decarboxylase (*adc*) from *Clostridium acetobutylicum* were assembled on a plasmid pJIR750_act, and the respective gene cluster termed ASO. In addition, the constitutive thiolase promoter (P_{thlA}) from *C. acetobutylicum* was cloned upstream of the ASO. pJIR750_act was used to introduce recombinant acetone production in *A. woodii* (Hoffmeister et al., 2016). The ASO was modified to study further the acetone production capabilities of the respective recombinant *A. woodii* strains.

Therefore, the genes *ctfA/ctfB* and *thlA* from *Clostridium scatologenes* were used to replace the genes from *C. acetobutylicum* in the ASO. Since *C. scatologenes* habors two copies of corresponding gene, two respective plasmids termed pJIR750_ac3t3 and pJIR750_ac4t4 were constructed. The plasmid pJIR750_ac3t3 contains *thlA* (EG59DRAFT_00774) as well as *ctfA/ctfB* (EG59DRAFT_00772/ EG59DRAFT_00773) and was used to obtain the strain *A. woodii* [pJIR750_ac3t3]. The other *thlA* gene copy with the locos tag EG59DRAFT_01004 and the other *ctfA/ctfB* copy with the locos tag EG59DRAFT_01002/EG59DRAFT_01003 were used to clone the plasmid pJIR750_ac4t4. The plasmid pJIR750_ac4t4 was used to construct the recombinant strain *A. woodii* [pJIR750_ac4t4].

The effect of the new ASOs regarding acetone production by the newly constructed recombinant *A. woodii* strains was examined by performing heterotrophic and autotrophic growth experiments.

Construction of a Predictive Genome Scale Model for *Clostridium autoethanogenum* by Incorporation of Biological and Physical Regulation

RUPERT NORMAN[1,2], THOMAS MILLAT[1], HASSAN HARTMAN[3], MARK POOLMAN[2], DAVID FELL[3], KLAUS WINZER[1], NIGEL MINTON[1], CHARLIE HODGMAN[2]

[1] BBSRC/ EPSRC Synthetic Biology Research Centre (SBRC), University of Nottingham, Nottingham, NG7 2RD, UK
[2] School of Biosciences, University of Nottingham, Sutton Bonington, LE12 5RD, UK.
[3] School of Life Sciences, Oxford Brooks University, Oxford, OX3 0BP, UK.

Recently, *C. autoethanogenum* has attracted academic and industrial interest because of its ability to convert syngas components (CO, CO_2 & H_2) into valuable platform chemicals, including ethanol and 2,3-butanediol - a jet fuel additive. Developing the metabolic conversions catalysed by *C. autoethanogenum* into an efficient bioprocess requires the accurate prediction of optimal metabolic steady states, which in turn necessitates the construction of a genome-scale model (GSM). Whereas the process of (automated) construction of a GSM has been greatly improved in recent years, incorporation of biological and physical regulation is often sparse and usually based on information from different, often unrelated, species.

We have successfully constructed a predictive model consisting of approximately 800 reactions and metabolites. An initial analysis proved that our model reproduced experimentally observed specific growth rates and growth yields. Furthermore, elementary flux modes analysis confirmed the availability of metabolic routes for all products. However, product distribution and formation activity disagreed with experimental results. Thus, we investigated the effect of biological and physical features on the model predictions. We found that consideration of the experimentally observed growth regimes is crucial to obtain qualitatively correct results. Furthermore, the external pH restricting acetate efflux and a limited H_2 production shift the metabolic flow towards ethanol and 2,3-BD.

We constructed the first genome-scale model of *C. autoethanogenum* that can simulate the formation of all experimentally observed products. Here, the incorporation of organism-specific experimental information representing biological and physical regulation was important to derive a predictive model that can be applied for the optimization of the production of industrially relevant chemicals from waste gases combining synthetic biology and bioprocess engineering.

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On the Diversity of 5,10-Methylene-THF-Reductases in Acetogenic Bacteria

CHRISTIAN ÖPPINGER, FLORIAN KREMP and VOLKER MÜLLER

Molecular Microbiology & Bioenergetics, Institute of Molecular Biosciences, Johann Wolfgang Goethe University Frankfurt/Main, Frankfurt, Germany

Acetogenic bacteria grow lithotrophically on H_2+CO_2 but the energetics of this reaction are at the thermodynamic limit of life. Energy (ATP) is conserved by ferredoxin-dependent respiratory chains. Ferredoxin is a low potential electron donor that can be reduced by electron bifurcation with H_2 as electron donor. A second, electron-bifurcating reaction of the pathway may be the reduction of methylene-THF with NADH, a reaction exergonic enough (ΔG_0' = -23 kJ/mol) to be coupled to ferredoxin reduction. Indeed electron-bifurcation by MTHFR is often assumed on theoretical grounds but has never been demonstrated. We have purified the MTHFRs from *Acetobacterium woodii, Clostridium ljungdahlii, Eubacterium limosum* KIST612 and *Sporomusa ovata.* They all differ in genomic organization and subunit composition. The core complex consists of MetF and MetV, that catalyses methylene-THF reduction. In *A. woodii*, an NADH binding and oxidizing subunit is connected to the core-unit enabling the use of NADH as reductant. This subunit is missing in the enzyme from *C. ljungdahlii* and *E. limosum* and those enzymes do not use pyridine nucleotides as electron donors, but ferredoxin could be used as electron donor. In none of these enzymes, electron-bifurcation can be demonstrated. The MTHFR of *S. ovata* has a HDR module connected to the core enzyme, but also this enzyme does not reduce ferredoxin.

Although the subunit composition of MTHFRs in acetogenic bacteria is very different and reflects the use of different electron donors, electron bifurcation could not be demonstrated in any of these enzymes.

Methane Upgrading By Novel Continuous Multistage Bioprocess

OLUMAYOWA OSUNDEKO[1*], DAVID MORRIS[2*], STEVE WILKINSON[1]

[1] *Department of Chemical and Engineering, University of Chester, CH2 4NU, UK*
[2] *Autichem Limited, Thornton Science Park, Chester, CH2 4NU, United Kingdom*

[*] Corresponding Authors: o.osundeko@chester.ac.uk; david@autichem.co.uk

The presence of CO_2 and H_2S and water vapour in biogas reduce its relative calorific value and its usability for gas to grid distribution. Therefore, there is need for additional processing to remove these gases by upgrading the quality of the methane in the biogas. However, upgrading adds to the costs of biogas production and It is therefore important to have an optimized upgrading process in terms of low energy consumption, high efficiency and cost. A continuous multistage bioprocess developed in collaboration with Autichem Ltd, has already demonstrated that it can increase the efficiency of delivering CO_2 to algae with no gas escape and thereby providing a pathway for the commercial implementation of CO_2 capturing using algae. This system was deployed for the study on methane upgrading using algae. In this experiment, a mixture of pure CO_2 and CH_4 gas respectively were added into the integrated bioreactor system containing algae culture. The initial result showed that about 86% of CO_2 was removed from the mixture of the gases. Therefore could increases the calorific value of biogas. A further optimization of this process and further test with biogas from anaerobic digestion are currently underway.

Intermediate Hydrogen Formation by Heterotrophically Growing *Acetobacterium woodii*

FLORIAN OSWALD and VOLKER MULLER

Molecular Microbiology & Bioenergetics, Institute of Molecular Biosciences, Johann Wolfgang Goethe University Frankfurt/Main, Frankfurt, Germany

Acetobacterium woodii is one of the model organisms for acetogenic bacteria. The metabolic pathways of *A. woodii* for conversion of organic and inorganic carbon sources into acetic acid are widely understood. An essential element of acetogenic metabolism is the reductive acetyl-CoA or Wood-Ljungdahl-Pathway (WLP) for recycling of NAD^+ and ferredoxin as well as carbon fixation. One of the initial steps in the WLP is the reduction of carbon dioxide into formic acid. In *A. woodii* this reaction is catalyzed by the hydrogen dependent carbon dioxide reductase (HDCR). Results from analysis of purified HDCR show, that this enzyme requires molecular hydrogen as electron donor and does not accept NADH but purified HDCR can also use reduced ferredoxin. Though, the rate of ferredoxin dependent carbon dioxide reduction into formic acid is much lower compared to the hydrogen dependent reaction. However, to date no experimental proof exists that molecular hydrogen is formed when *A. woodii* grows on organic carbon sources.

We established four 2 L-scale stirred tank reactor systems with product analysis, online off-gas analysis and gas flow control. With this set-up it is possible to cultivate *A. woodii* with gas flow rates as low as 0.005 vvm and investigate if hydrogen can be detected in the off-gas. Our results from cultivations with constant sparging of N_2+CO_2 (80 vol-% + 20 vol-%) at 0.005 vvm and fructose as source of carbon and energy show that *A. woodii* forms low amounts of molecular hydrogen. This is the first experimental report that shows hydrogen formation from fructose by wild type cultures of *A. woodii*. These studies are consistent with the hypothesis that hydrogen is the electron carrier that combines the oxidative and reductive branch of acetogenesis.

Comparative Genomic Analysis of Carbon Monoxide Dehydrogenase and Its Gene Cluster

BYEONGHYEOK PARK, SEUNGWOO BAEK, DONGMIN YANG, IN-GEOL CHOI

Computational and Synthetic Biology Laboratory, College of Life Sciences & Biotechnology, Korea University, Seoul, 02841, Republic of Korea

Carbon monoxide dehydrogenase (CODH) is one of the ancient enzymes that involve in diverse C1 compound metabolisms such as acetogenesis, methanogenesis and hydrogenesis. Because of its ecological and industrial importance, molecular phylogenetic and structural analyses based on sequences of CODHs have been reported. However, the functional understanding of CODH in genomic context is less known, and the molecular function of CODH is not predicted solely by its sequence information. Many CODHs form operon-like gene clusters with various genes involved in C1 metabolisms. Those commonly found genes with CODHs are often regulated together. Therefore, the cellular and molecular functions of CODHs can be understood more in details in the context of the gene clusters involved. In this study, through a comparative analysis of 332 CODH gene clusters from 199 non-redundant CODH-retaining genomes, we classified the gene clusters of CODH and predicted their functional features in genomic context. Although a large number of CODHs were predicted to carry well-known molecular functions such as acetogenesis with its gene clusters, independent CODHs were also identified. In addition, we confirmed that there is a correlation between phylogenetics of the CODH gene sequence and its gene clusters, but also observed obvious exceptions. We anticipate that our studies will provide insight into the functions of CODH and its evolution, and provide an improved clue for the industrial application of CODH.

A Genome Scale Model of *Cupriavidus necator* for Platform Chemical Production

NICOLE PEARCY[1], JAMES GILBERT[1], THOMAS MILLAT[1], MARK POOLMAN[2], DAVID FELL[2], HASSAN HARTMAN[2], JOHN KING[3], ALEX CONRADIE[4], SAMANTHA BRYAN[4], KLAUS WINZER[1], NIGEL MINTON[1] & JAMIE TWYCROSS[1,5]

[1]*BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), University of Nottingham, Nottingham, NG7 2RD, UK*

[2]*Cell Systems Modelling Group, Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, OX3 0BP, UK*

[3]*School of Mathematical Sciences, University of Nottingham, Nottingham, NG7 2RD, UK*

[4]*Faculty of Engineering, University of Nottingham, Nottingham, NG7 2RD, UK*

[5]*School of Computer Science, University of Nottingham, Nottingham, NG8 1BB, UK*

The autotroph bacterium *Cupriavidus necator* fixes CO_2 via the Calvin cycle and oxidise 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. Its ability to utilize these waste gases to produce large quantities of PHB makes it an attractive host for providing sustainable routes to platform chemicals, which are currently synthesised from fossil fuels.

In the SBRC, we are using a newly constructed genome scale model of *C. necator* as a tool for simulating the bacterium's metabolic behaviour to genetic modifications, such as gene insertions and deletions to guide ongoing experimental work to improve production of platform chemicals, e.g. ethylene. We describe here the construction and validation of the new model, which accounts for 1284 genes, 1237 reactions and 1329 metabolites. Using flux balance analysis, we demonstrate that the model is capable of predicting biomass yields and intracellular fluxes that are consistent with experimental observations.

We propose a new strategy for improving the production of ethylene. It fully couples the formation of this widely used organic compound to biomass formation by inactivation of two reactions. Transforming *C. necator* into an efficient CO_2-consuming ethylene producer could overcome the currently used CO_2-emitting steam cracking. SBRC experimentalists are currently testing these knockouts in vivo.

Bio-refining of Coke Oven Gas – The Removal and Recovery of Sulfur

RICHARD PUGH

SERC, University of South Wales, Cemetery Road Glyntaff, Pontypridd, CF37 4BD

TATA Steel is one of the largest steel producers in Europe producing almost 5 million tonnes of slab steel per year. With world population and industry constantly increasing in size the demand for this raw material is constantly growing, for this TATA runs 24 hours a day all year round.

In integrated steel works coke is a vital step in the steel making process, steel plants possess coke oven batteries, each with 42 ovens, these are charged with blended coal. Each oven is heated to over 1300^{o}C for up to 17 hours this has the capability of producing 22000 kg of coke every 12 minutes this equates to approximately 42000 m^3/h (15000kg) of Coke oven gas (COG) produced. Having a nonstop - high throughput industrial process, if left untreated the waste gas emissions can have detrimental effects on the environment.

The research conducted utilises the waste gas coming from the coke ovens in TATA's industrial process the aim being to remove potentially harmful concentrations of chemicals found in the exhaust fumes. This project evaluates the possibility of an industrial scale plant using a laboratory scale model using an environmentally friendly bacterial process.

To make this possible an airlift reactor was designed to incorporate the gas into the bacterial consortium. The gas stream will contain a vast number of contaminants in high concentration such as Carbon monoxide, Methane, Ethylene, along with small quantities of Benzole (Benzene and Toluene), Naphthalene and Hydrogen cyanide, this adds a level of complexity to be able to keep the bacteria alive in these conditions and still remove Hydrogen sulfide in a high capacity.

This method utilises the bacteria present in wastewater, which can be used to convert the H_2S in the gas into elemental sulfur, this will mean less damage to the metalwork in the TATA plant while also reducing the effect of H_2S on the environment. What makes this method more suitable for large industry is the use of mixed culture inoculum; this will be able to withstand the harsh conditions and rapid changes that could be encountered in gas leaving the plant.

Efficient Hydrogen-dependent Carbon Dioxide Reduction by *Escherichia coli*.

MAGALI ROGER[1], FRASER BROWN[2], WILLIAM GABRIELLI[3], DAVID SMITH[4], AND FRANK SARGENT[1]

[1]School of Natural and Environmental Sciences, Newcastle University, Newcastle Upon Tyne NE1 7RX, UK.
[2]Ingenza Limited, Roslin Biocentre Midlothian, Roslin EH25 9R, UK
[3]Sasol Technology (UK) Limited, St Andrews KY16 9ST, UK.
[4]Drochaid research services Ltd, St Andrews KY16 9ST, UK.

H_2-dependent reduction of CO_2 to formic acid offers a promising route to greenhouse gas sequestration, carbon abatement technologies, H_2 transport and storage, and the sustainable generation of renewable chemical feedstocks. Several chemical processes exist for the reduction of CO_2 to formic acid, but these technologies requires the use of expensive catalysts and extreme reaction conditions. In contrast, biological catalysts, which work under mild conditions and with high specificity, provides a "greener" system for the conversion of CO_2 to formic acid.

The formate hydrogenlyase (FHL) enzyme from *Escherichia coli* oxidizes formic acid to CO_2 coupled to proton reduction. In addition to its role in bio-H_2 production, it has been demonstrated that it can operate *in vivo* and *in vitro* as a H_2-dependent CO_2 reductase (HDCR), paving the way for its exploitation in CO_2 capture technology.

By using a bioprocess engineering approach, we recently demonstrated that the FHL can operate as a highly efficient CO_2 reductase under controlled CO_2 and H_2 gas pressure. Using intact whole cells, the pressurised system converted 100% of gaseous CO_2 while producing >500 mM formic acid.

To further improve the system, we are seeking to design strains optimised for the HDCR activity during bacterial growth. Finally, in keeping with the synthetic biology approaches already taken, a synthetic carbonate uptake and conversion pathway was designed and incorporated into *E. coli*. The protein products were shown to be produced and the ability of various engineered cells to fix CO_2 assayed.

Whole Cell Catalysis for Hydrogen Storage and Biohydrogen Production Using a Thermophilic Acetogen

FABIAN M. SCHWARZ and VOLKER MÜLLER

Molecular Microbiology & Bioenergetics, Institute of Molecular Biosciences, Johann Wolfgang Goethe University Frankfurt/Main, Frankfurt, Germany

In times of climate change and increasing atmospheric CO_2 concentrations there is a strong need for alternative renewable energy sources. However, the energy needs to be stored and transported. Molecular hydrogen has been considered as a promising alternative energy carrier that can be produced from renewables like wind and solar by electrolysis. To bypass the limitations of molecular hydrogen, the transient conversion of H_2 (and CO_2) into so-called liquid organic hydrogen carriers such as formic acid has already gained increased attention.

Recently a new group of enzymes were discovered in the two acetogenic bacteria *Acetobacterium woodii* and *Thermoanaerobacter kivui* which catalyzes the direct hydrogenation of CO_2 to formic acid with exceptional high rates, the hydrogen-dependent CO_2 reductases (HDCRs). These enzymes are promising biocatalysts for the storage of molecular hydrogen in form of liquid formic acid as well as for the production of molecular hydrogen from formic acid. Furthermore, it was already shown for *A. woodii*, that even whole cells can be used for the described reactions. To design a more efficient storage/production platform for molecular hydrogen and to take advantage of using whole cells from a thermophilic organism, we studied the thermophilic acetogen *T. kivui* for its biotechnological application in hydrogen storage and biohydrogen production. Here, *T. kivui* reached with 170 mmol g_{CDW}^{-1} h^{-1} the highest specific formate production rates of wild-type strains in closed batch conditions mentioned in literature. The reversibility of this reaction (qH_2 of 185 mmol g_{CDW}^{-1} h^{-1}) makes *T. kivui* a promising candidate for two challenging reactions.

Maximizing the CO_2 Assimilation Efficiency of the Endogenous CBB Pathways in *Cupriavidus necator* H16

GIORGIA TIBALDERO, NAGLIS MALYS, NIGEL MINTON and KATALIN KOVÁCS

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

CO_2 assimilation and fixation is at the core of every process aiming to design industrial carbon-neutral production routes for a low-carbon future. Waste gases from industry such as steel manufacturing, oil and natural gas refining can be potentially used as renewable source for the production of alternatives to petroleum-based solutions.

The model organism *Cupriavidus necator* H16 is able to produce biodegradable thermoplastics by growing on CO_2 and H_2 as a sole carbon and energy source. CO_2 assimilation in *C. necator* is mediated by the endogenous Calvin-Benson-Bassham (CBB) cycle encoded by two almost identical *cbb* operons. Both are tightly regulated by the LysR-type regulatory protein CbbR located at the 5' end of the chromosomal copy of the *cbb* operon. Unfortunately, the role and identity of positive and negative CbbR's effector molecules are still not well understood in *C. necator*.

The aim of my project is to investigate LysR independent transcription of the *cbb* operon driven by either the constitutive promoter P*trp*, the arabinose inducible promoter P_{BAD} or the P_{tdcB}/TcdR UoN patented orthologous inducible system in *C. necator*. To further improve the CO_2 fixation ability, we introduce the controllable expression of the *cso* operon from *Halothiobacillus neapolitanus,* encoding self-assembling, proteinaceous prokaryotic organelles involved in the carbon concentrating mechanism (CCM) of several cyano- and chemoautotrophic bacteria. Improved CO_2 fixation efficiency of strains is evaluated using our state-of-the-art fermenters under autotrophic aerobic conditions and by Q-Exactive LC-MS metabolomics.

Acknowledgements

The authors gratefully acknowledge the financial support of the BBSRC Doctoral Training Programme BB/M008770/1

The Use of Thermodynamics and Biomass Assembly to Further Constrain Metabolic Flux Analysis Based Methods: Toward Monitoring Bacterial Metabolism

CLAUDIO TOMI-ANDRINO [1,2,3]; THOMAS MILLAT[2]; KLAUS WINZER[2]; JOHN KING[3]; PHILIPPE SOUCAILLE[2,4,5,6,7]; DAVID A. BARRETT[1]; KIM DONG-HYUN[1].

[1]Advanced Materials and Healthcare Technology Division, School of Pharmacy, University of Nottingham, Nottingham, United Kingdom.
[2]BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), University of Nottingham, Nottingham, NG7 2RD, UK
[3]BBSRC/EPSRC Synthetic Biology Research Centre, School of Mathematical Sciences, University of Nottingham, Nottingham, United Kingdom.
[4]INSA, UPS, INP, LISBP, Université de Toulouse, Toulouse, France.
[5]INRA, UMR792, Toulouse, France.
[6]CNRS, UMR5504, Toulouse, France.
[7]Metabolic Explorer, Biopôle Clermont-Limagne, Saint Beauzire, France.

Metabolic engineering in the post-genomic era is characterised by the development of new methods for metabolomics and fluxomics, supported by the integration of genetic engineering tools and mathematical modelling. Fluxomics focuses on the quantification of the conversion rates through enzyme-catalysed reactions, which depend on both enzyme abundance and activity. Given the scarcity of high quality kinetics data, most approaches consist in modelling the metabolic fluxes. Particularly, constraint-based stoichiometric models have been deeply studied, where a pseudo steady-state for intracellular metabolites is assumed. Two main frameworks stand out: (i) flux balance analysis (FBA), which is normally applied to genome-scale models and optimises the flux distribution for an objective function (e.g. maximization of biomass yield), and (ii) metabolic flux analysis (MFA), which is used for smaller metabolic networks and requires more experimental measurements. These approaches have proven to provide satisfactory results – however certain limitations can be identified: (i) no objective function is always valid, and (ii) isotopic labelling experiments for autotrophic metabolism is technically highly demanding.

In order to overcome the intrinsic limitations, this study aims at developing an MFA-based method accounting for thermodynamic feasibility of reactions (TMFA) which will be calibrated by the assembly of biomass. To do so, the core *Escherichia coli* metabolic model (subset of iAF1260) was used, along with published metabolomics data (absolute quantification of intracellular metabolites), biomass composition and bioenergetics information. Finally, the results were compared with available flux data obtained with isotopic tracers (heterotrophic metabolism) to assess the suitability of this novel approach.

H_2 Drives Metabolic Rearrangements in Gas-fermenting *Clostridium autoethanogenum*

KASPAR VALGEPEA[1], RENATO DE SOUZA PINTO LEMGRUBER[1], TANUS ABDALLA[2], STEVE BINOS[3], NOBUAKI TAKEMORI[4,5], AYAKO TAKEMORI[4], YUKI TANAKA[5], RYAN TAPPEL[2], MICHAEL KÖPKE[2], SÉAN DENNIS SIMPSON[2], LARS KELD NIELSEN[1] AND ESTEBAN MARCELLIN[1,6]

[1]Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland, St. Lucia, Australia; [2]LanzaTech Inc., Skokie, USA; [3]Thermo Fisher Scientific, Bio21 Institute, The University of Melbourne, Parkville, Australia; [4]Proteo-Science Center, Ehime University, Ehime, Japan; [5]Advanced Research Support Center, Ehime University, Ehime, Japan; [6]Queensland Node of Metabolomics Australia, The University of Queensland, St. Lucia, Australia

There is an imperative in many industrial processes to use waste streams to make products. Gas-fermenting acetogens offer a potential solution and several commercial gas fermentation plants are currently under construction. As energy limits acetogen metabolism, supply of H_2 should diminish substrate loss to CO_2 and facilitate production of reduced and energy-intensive products. However, the effects of H_2 supply on CO-grown acetogens have yet to be experimentally quantified under controlled growth conditions.

Here, we quantify the effects of H_2 supplementation by comparing growth on CO, syngas, and a high-H_2 CO gas mix using chemostat cultures of *Clostridium autoethanogenum*. Cultures were characterised at the molecular level using metabolomics, proteomics, gas analysis, and a genome-scale metabolic model (GEM). CO-limited chemostats operated at two steady-state biomass concentrations facilitated co-utilisation of CO and H_2. We show that H_2 supply strongly impacts carbon distribution with a four-fold reduction in substrate loss as CO_2 (61% vs. 17%) and a proportional increase of flux to ethanol (15% vs. 61%). Notably, H_2 supplementation lowers the molar acetate/ethanol ratio by five-fold. At the molecular level, quantitative proteome analysis showed no obvious changes leading to these metabolic rearrangements suggesting the involvement of post-translational regulation. Metabolic modelling showed that H_2 availability provided reducing power via H_2 oxidation and saved redox as cells reduced all the CO_2 to formate directly using H_2 in the Wood-Ljungdahl pathway. In combination with proteomics, modelling also showed that ethanol was synthesised through the acetaldehyde:ferredoxin oxidoreductase (AOR) activity.

Our quantitative molecular analysis revealed that H_2 drives rearrangements at several layers of metabolism and provides novel links between carbon, energy, and redox metabolism advancing our understanding of energy conservation in acetogens.

The Genetic Basis of 3-Hydroxypropanoate Metabolism in *Cupriavidus necator*

CHRISTIAN ARENAS-LOPEZ, JESSICA LOCKER, FREDERIK WALTER, TOBIAS BUSCHE[*], JÖRN KALINOWSKI[*], NIGEL P. MINTON, KATALIN KOVACS & KLAUS WINZER

BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), University of Nottingham, Nottingham, NG7 2RD, UK
[*]Centre for Biotechnology, Bielefeld University, D-33615 Bielefeld, Germany

3-hydroxypropionic acid (3-HP) is a promising platform chemical that can be produced by microorganisms via several metabolic routes. Here, we report on the native 3-HP metabolism of *Cupriavidus necator*, a promising chassis for the production of fatty acid derivatives from CO_2 and H_2. When testing the organism's tolerance towards 3-HP, we noticed that the compound was rapidly metabolised and used as a source of carbon and energy. This was a highly undesirable trait that required elimination. Putative genes responsible for 3-HP degradation were identified by bioinformatic means and confirmed by transcriptomic analyses of cell grown in the absence and presence of 3-HP. Candidate genes included three putative (methyl)-malonate semialdehyde dehydrogenases (*mmsA1*, *mmsA2* and *mmsA3*) and two putative dehydrogenases (*hpdH* and *hbdH*). All five genes were inactivated through in-frame deletion to generate a strain unable to metabolise 3-HP. Interestingly, we found that only a triple Δ*mmsA1*Δ*mmsA2*Δ*mmsA3* knock-out strain could no longer grow and utilise 3-HP as the sole source of carbon and energy. This strain was also unable to co-metabolise 3-HP when grown in the presence of additional carbon sources such as fructose and CO_2/H_2 and thus represents an ideal chassis for autotrophic 3-HP production. Further growth experiments revealed main roles for *mmsA1*, *mmsA2* and *mmsA3* in β-alanine, 3-HP and valine breakdown, respectively.

Participants

SURNAME	FIRST NAME	INSTITUTE	ROLE	EXPERTISE
Akaluka	Cynthia	University of Nottingham, UK	PhD student	Metabolic engineer, Molecular biologist
Arenas	Christian	University of Nottingham, UK	Post Doc	Clostridium, Gas fermentation
Avignone Rossa	Claudio	University of Surrey, UK	Reader	Systems Microbiology
Baker	Jonathan	University of Nottingham, UK	PDRA	Synthetic biology
Bar-Even	Arren	Max Planck Institute, Germany	Research Group Leader	Metabolic Engineering, Synthetic Biology
Baur	Tina	Ulm University, Germany	PhD student	Metabolic engineering
Beck	Matthias	Ulm University, Germany	PhD student	Acetogenic bacteria
Bengelsdorf	Frank	Ulm University, Germany	Senior Scientist	Metabolic engineering
Bjorck	Charlotte	University of Sheffield, UK	PhD student	Methanotrophs
Bogat	Kamilla	Calysta Ltd, UK	Fermentation assistant	Continuous fermentation
Bommareddy	Rajesh	University of Nottingham, UK	PDRA	BioProcess Development
Boodhoo	Kamelia	Newcastle University, UK	Reader	Sustainable Process Technology
Bourgade	Barbara	Loughborough University, UK	PhD student	Meatbolic engineer, Molecular Biology
Buckel	Wolfgang	Philipps-University, Marburg, Germany	Professor	Microbial Biochemistry
Burbidge	Alan	University of Nottingham, UK	Centre Manager	IB commercialisation
Carr	Reuben	Ingenza, UK	Head of Chemical Biology	Bioprocessing and fermentation
Cartman	Stephen	Invista Ltd, UK	Technical Capability Leader	Metabolic Engineer, Microbial Physiologist
Chalmers-Brown	Rhiannon	University of South Wales, UK	PhD student	Biochemical Engineering, Carbon Capture
Chang	In Seop	Gwangju Institute of Science and Technology, Republic of Korea	Professor	Metabolic Engineer
Chesshire	Michael	Lutra Ltd, UK	Director	Anaerobic digetsion, Process engineering
Cho	Byung-Kwan	Korea Advanced Institute of Science and Technology, Republic of Korea	Associate Professor	Synthetic Biology, Systems Biology
Claassens	Nico	Max Planck Institute of Molecular Plant Physiology		Metabolic engineer
Cornack	Ruth	University of Nottingham, UK	Research Technician	Molecular Biology
Cotton	Charles	Max Planck Institute, Germany	PostDoc	Biochemist
Dalwadi	Mohit	University of Oxford, UK	PDRA	Mathematical modelling
Dietrich	Helge	Goethe University, Frankfurt, Germany	PhD student	Biochemistry
Dimitriou	Ioanna	University of Nottingham, UK	Assistant Professor	Biprocess design, Modelling, CO2 utilisation
Dürre	Peter	University of Ulm, Germany	Professor	Anaerobes
Dykstra	James	Wageningen University, The Netherlands	PhDstudent	Metabolic engineer, Microbial physiologist, Molecular biologist
Dynes	Louise	University of Nottingham, UK	Outreach Officer	Outreach
Esteves	Sandra	University of South Wales, UK	Professor	Metabolic engineering, Molecular biology
Fell	David	Oxford Brookes University, UK	Professor	Modeller
Fink	Christian	University of Tübingen, Germany	PhD student	Methanogens, Genetic engineering
Flaiz	Maximilian	Ulm University, Germany	PhD student	Anaerobic digestion, Biochemistry, Metabolic engineer
Gardner	Gerard	HEL Ltd, UK	Sales director	Gas fermentation
Gascoyne	Joshua	University of Nottingham, UK	PhD student	Microbiology
Gilbert	James	University of Nottingham, UK	Research Associate	Computational Modelling, Transcriptomics
Gitlesen	Susanne	NORCE Norwegian Research Centre AS	Special Advisor	Methane fermentation
Giver	Lori	Calysta, USA	VP, R&D	Biological engineering
Gradley	Michelle	SciVerita, UK	Director of Process Biotechnology	Microbiology/Genetics
Green	Edward	CHAIN Biotechnolgy, UK	Founder & Chief Executive	Clostridia
Grob	Ralph	Jacobs Consultancy, UK	Senior Consultant	Biotechnology
Grosse-Honebrink	Alex	University of Nottingham, UK	Research fellow	Methanothrophs
Gude	Christian	University of Nottingham, UK	PhD student	Metabolic engineering, Molecular biology
Hadley Kershaw	Eleanor	University of Nottingham, UK	Research fellow	Social Scientist
Hanko	Erik	University of Nottingham, UK	PhD student	Metabolic engineering, Synthetic biology
Holtman	Dirk	Dechema - Forschungs Institut, Germany	Group leader	CO2 Conversion, Biotechnology
Humphries	Christopher	University of Nottingham, UK	PDRA	Synthetic Biology
Infantes	Alba	Karlsruhe Institute for Technology, Germany	PhD student	Gas fermentation
Jawed	Kamran	University of Nottingham, UK	PhD student	Metabolic Engineer, Molecular Biology

SURNAME	FIRST NAME	INSTITUTE	ROLE	EXPERTISE
Jeong	Ji-Yeong	Gwangju Institute of Science and Technology, Republic of Korea	PhD student	Fermentation, Synthetic Biology
Johannessen	Arild	NORCE Norwegian Research Centre AS	Managing Director	Fermentation, Process development
Jones	Zofia		Bioinformatician	Systems Biology, Constraint based Models
Kelly	Ciaran	University of Newcastle, UK	PDRA	Bacterial Fermentation, Synthetic Biology
Kennes	Christian	University of La Coruna, Spain	Professor	Chemical Engineering
Kerr	Josh	University of Nottingham, UK	Technician	Fermentation Technologist
Kim	Ji-Yeon	Gwangju Institute of Science and Technology, Republic of Korea	PhD student	Anaerobic Digestion, Biochemist
Kissinger	Matt	Synata Bio Inc, USA	Senior Research Scientist	Fermentation, Metabolic Engineer, Microbial Physiologist
Klask	Christian-Marco	University of Tübingen, Germany	PhD student	Syngas fermentation
Kovacs	Katalin	University of Nottingham, UK	PDRA	Microbiolgy, Synthetic Biology
Krabben	Preben	C1net, UK		Clostridial physiology, Genomics
Kremp	Florian	Goethe University, Frankfurt, Germany	Masters Student	C1 metabolism
Kumar	Vinod	Cranfield University, UK	Lecturer	Synthetic Biology
Lee	Eun Yeol	Kyung Hee University, Republic of Korea	Professor	Chemical Engineer
Lee	Mungyu	Gwangju Institute of Science and Technology, Republic of Korea	PhD student	Anaerobic digestion, Biochemist, Chemical Engineer
Locker	Jessica	University of Nottingham, UK	PhD student	Synthetic biology
Malys	Naglis	University of Nottingham, UK	Senior Research Fellow	Biochemistry, Systems and Synthetic Biology
Mansfield	Robert	University of Nottingham, UK	PDRA	Microbial engineering
McCusker	Christopher	University of Nottingham, UK	PhD student	Biotechnology, Microbiology
McGregor	Callum	University of Nottingham, UK	PhD student	Synthetic biology
McLeod	Carmen	University of Nottingham, UK	PDRA	Responsible Research and Innovation
Menchavez	Russel	University of Nottingham, UK	PhD student	Industrial Biotechnology
Mesfin	Noah	Oxford Brookes University, Uk	PhD student	Metabolic modelling
Miles	Colin	BBSRC	Chief Scientific Advisor	Industrial Biotech
Millard	James	University of Nottingham, UK, UK	PDRA	Molecular Microbiology
Millat	Thomas	University of Nottingham, UK	PDRA	Metabolic modelling
Minton	Nigel	University of Nottingham, UK	Director SBRC Nottingham	Synthetic Biology, Clostridia
Minton	Jacque	University of Nottingham, UK	Network Manager	
Molitor	Bastian	University of Tübingen, Germany	Postdoc	Syngas fermentation
Montarnal	Amaury	University of Nottingham, UK	PhD student	Biochemist, Engineer
Morris	David	Autichem Ltd, UK	Engineer	Reactor design
Müller	Volker	University of Frankfurt, Germany	Professor	Acetogenesis, Syngas fermentation
Neumann	Anke	Karlsruhe Institute for Technology, Germany	PDRA & Lecturer	Syngas fermentation
Nick	Saskia	Ulm University, Germany	PhD student	Biochemist, Metabolic Engineer
Norman	Rupert	University of Nottingham, UK	PDRA	Synthetic Biology
Olsson	Lisbeth	Chalmers University of Technology, Sweden	Professor	Syngas fermentation
Omorotionmwan	Bunmi	University of Nottingham, UK	PhD student	Metaboloic engineer, Molecular Biology, Synthetic Biology
Öppinger	Christian	Goethe University, Frankfurt, Germany	PhD student	Molecular Biology
Orol Gomez	Diego	University of Nottingham, UK	PhD student	Biocemist, Metabolic engineer, Molecular Biologist
Osundeko	Olumayowa	University of Chester, UK	Research Scientist	Anaerobic digetion, Biochemistry, Chemical engineer
Oswald	Florian	Goethe University Frankfurt, Germany	PhD student	Anaerobic fermentation of syngas
Pander	Bart	Deep Branch Biotechnology Ltd, UK	Co-founder	Gas fermentation
Papapietro	Mariano	Calysta Ltd, UK	Fermentation assistant	Gas fermentation
Papoutsakis	E.Terry	University of Delaware, USA	Professor	Chemical & Biomolecular Engineering
Park	Byeonghyeok	Korea University, Republic of Korea	PhD student	Bioinformatician, Synthetic Biology, Systems Biology
Patel	Vanisha	University of Nottingham, UK	PhD student	Mircobiology
Pearcy	Nicole	University of Nottingham, UK	Research Associate	Metabolic networks, Genome Scale Modelling
Piatek	Pawel	Chalmers University of Technology, Sweden	PDRA	Synthetic biology, Biochemistry
Poulston	Stephen	Johnson Matthey Technology, UK	Research Scientist	Syngas production and Processing
Pugh	Richard	University of South Wales, UK	PhD student	Biological Desulphurisation
Roger	Magli	University of Newcastle, UK	PDRA	Molecular Biology

SURNAME	FIRST NAME	INSTITUTE	ROLE	EXPERTISE
Rossi	Tatiana Spatola	Oxford Brookes University, UK	PhD sudent	Metabolic engineering, Molecular Biologist
Rossi	Ricardo	Calysta, UK	Research Assistant	Molecular Biology, Fermentation
Ruijssenaars	Harald	Corbion Global	Principal scientist	Microbiology, Biocehmistry
Rumah	Bashir	University of Nottingham, UK	PhD student	Biochemistry
Sargent	Frank	University of Newcastle, UK	Professor	Bactreial enzymes and metabolism
Schilling	Boris	Givaudan AG, Switzerland	Head of External Opportunities	Biochemistry, Project management
Schoch	Teresa	Ulm University, Germany	Masters Student	Metabolic engineering
Schoelmerich	Marie	University of Hamburg, Germany	Postdoc	Anaerobic digestion, Biochemist, Synthetic Biologist
Schwarz	Fabian	Goethe University, Germany	Masters Student	C1 metabolism
Simpson	Sean	Lanzatech, USA	Co-founder & Chief Scientific Officer	Gas Fermentation
Singh	Jasbir	HEL Ltd, UK	Director	Gas Fermentation
Smith	Gary	Invista, UK	VP Biotechnology R&D	Industrial Biotechnology
Soucaille	Philippe	University of Nottingham, UK	Professor	Metabolic Engineering
Sousa	Diana	Wageningen University, The Netherlands	Associate Professor	Anaerobic digestion, Microbial Physiologist, Process engineer
Stead	Christopher	University of Nottingham, UK	PhD student	Microbiology
Thauer	Rolf	Max Planck Institute, Germany	Professor	Methanogens
Tibaldero	Giorgia	University of Nottingham, UK	PhD student	Sythetic Biology
Tomecka	Monika	uFractions8 Ltd, UK	Executive Director	Biochemistry, Genetics
Tomi Andrino	Claudio	University of Nottingham, UK	PhD student	Microbiology
Tracy	Bryan	White Dog Labs	CEO	Renewable chemicals and fuels
Tuffou	Romain	University of South Wales, UK	PhD student	Biotechnology
Turco	Federico	University of Nottingham, UK	PhD student	Biotecnology
Valgepea	Kaspar	University of Tartu, Estonia	Research fellow	Bioinformatician, Systems Biology
Van Hagen	Alexander	University of Nottingham, UK	PhD student	Molecular Biology, Microbiology, Synthetic Biology
Velasquez Orta	Sharon	Newcastle University, UK	Lecturer	Chemical engineer
Wang	Yanming	University of Nottingham, UK	PDRA	Fermentation, Bioprocess development
Warren	Martin	University of Kent, UK	Professor	Biochemistry
Wiechmann	Anja	University of Frankfurt, Germany	PDRA	Molecular Biology, Bioechnology
Wimalasena	Tithira	Calysta UK Ltd	Senior Scientist	Fermentation, Microbiology, Synthetic biology
Winzer	Klaus	University of Nottingham, UK	Associate Professor	Bacterial metabolism
Yadav	Kavita	University of Nottingham,UK	PDRA	Biochemist, Metabolic Engineer, Microbial Physiologist
Yasin	Muhammad	COMSATS University Islamabad, Pakistan	Assistant Professor	Anaerobic digerstion, Chemical Engineer, Scale-up
Yeboah	Edwin	University of Nottingham, UK	PhD student	Molecular Biologist, Synthetic Biologist
Yoo	Minyeong	University of Nottingham, UK	Postdoc	Bacterial physiology
Zhang	Yue	University of Southampton, UK	Lecturer	Anaerobic digestion, Process engineering
Zhang	Ying	University of Nottingham, UK	Senior Researcher	Methanogens
Zhuang	Shiwen	Calysta, UK	Fermentation Engineer	Methane fermentation
Zimmerman	William	University of Sheffield, UK	Professor	Fluid dynamics

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Acknowledgement

We acknowledge that the BBSRC through C1net supported this project

Grant Code BB/L013800/1

C1net gas fermentation

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