C1net

Conference 3

5-7 November 2017
Nottingham, UK

Programme and Abstracts

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
Robin Mitra	Centre for Process Innovation Ltd, UK
Sean Simpson	LanzaTech, USA
Bob Tooze	Sasol UK Ltd, 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

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

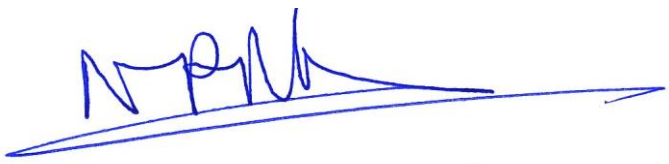
WELCOME

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.

Since its initiation in March 2014, C1net has created a cross-disciplinary community of academics and industrialists working together to achieve the networks goals. Much progress has been made: Membership currently stands at 424 with members from Europe, India, USA, Russia, China, South Korea and New Zealand, we have 373 followers on Twitter and a total of 16 POC awards of £50,000 have been made. Additionally, this year we were able to award 5 summer vacation studentships.

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



Nigel P Minton
On behalf of the C1net management board

GENERAL INFORMATION

CONFERENCE VENUE AND ACCOMMODATION

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

ORAL PRESENTATIONS

Oral presentations will be in the Conference Theatre. The length of oral presentations is scheduled for 20 or 30 min (check programme), within that presenters should allow 5 min for discussion. All presentations should be prepared in a form of MS Power Point slide show and stored on USB sticks or CD/DVD. The use of a personal computer or Mac is not possible. This can be done anytime, by handing it to the duty IT technician, but at least 2-3 hours before your session or the evening before for early morning presentations.

ELEVATOR PITCHES

Elevator pitches will be in the Conference Theatre. They should no longer than 3 minutes with 2 PowerPoint slides (or soap box pitch if you prefer). All pitches were submitted to the conference organiser in advance, before 23 October 2017 and will be pre-loaded for you.

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.

DATA PROTECTION

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

SOCIAL EVENTS

Welcome Dinner, Sunday, 5 November 2017, 19:00, Conference Theatre, EMCC
Conference Dinner, Monday, 6 November 2017, 20:00, Conference Theatre, EMCC

TAXIS

DG Taxis 0115 950 0500 or ask the hotel.

PROGRAMME

SUNDAY 5th November 2017 - ARRIVAL AT ORCHARD HOTEL (Check-in from 14:00)		
19:00	**Registration/Reception**	**Atrium - East Midlands Conference Centre (EMCC)**
20:00	**Dinner**	**Conference Theatre - EMCC**
MONDAY 6th November 2017		
SESSION 1 - Chair: Rolf Thauer (Max Planck Institute, Germany) – **Conference Theatre - EMCC**		
09.00 – 09.05	**Jacque/Nigel Minton** *SBRC-Nottingham, UK*	Welcome
09.05 – 09:35	**Volker Müller** *Goethe-University Frankfurt, Germany*	**INVITED TALK** - How to Make a Living by producing Acetate from Carbon Dioxide and Hydrogen
09.35 – 09:55	**Jonathan Baker** *SBRC-Nottingham, UK*	Development and Application of Efficient Forward and Reverse Genetic Tools in *Acetobacterium woodii*
09:55 – 10:15	**Noah Mesfin** *Oxford Brookes University, UK*	Using A Metabolic Model of *Acetobacterium woodii* for Insights into its Utility for Biotechnological Purposes
10:.15 – 10:35	**Fabian Schwarz** *Goethe University, Frankfurt, Germany*	Hydrogen Storage Using Acetogens as Biocatalysts: Hydrogen-Dependent CO_2-Reductase from a Thermophilic Acetogen
10:35 - 11:00	**Coffee/Tea Break**	**Atrium - EMCC**
Session 2 - Chair: Sean Simpson (Lanzatech, USA) – **Conference Theatre EMCC**		
11:00 – 11:30	**Guido Saracco** *Italian Institute of Technology (IIT), Italy*	**INVITED TALK** - Sustainable Chemical Production Ecosystems based on CO_2 and Combined Photo-, Electro- and Biochemical Processes
11:30 – 11:50	**Mahendra Raut** *University of Sheffield, UK*	Optimizing the Hydrolysis Process by Pre-Composting to Enhanced Biomethane Production by Anaerobic Digestion from Lignocellulosic Waste
11:50 – 12:10	**Nuri Azbar** *Ege University, Turkey*	CO_2 Utilisation via a Novel Anaerobic Bioprocess Configuration with Simulated Gas Mixture and Real Stack Gas Samples
12.10 – 12:30	**Konstantinos Asimakopoulos** *Technical University Denmark*	Assessment of Syngas Biomethanation by Mixed Microbial Consortia in a Trickle-bed Reactor
12:30 – 14:00	**Lunch**	**Atrium - EMCC**
Session 3 - Chair: David Fell (Oxford Brookes University, UK) – **Conference Theatre - EMCC**		
14:00 – 14:30	**Sean Simpson** *Lanzatech, USA*	**INVITED TALK** - Fuel and Chemical Production by Gas Fermentation at Scale
14:30 – 14:50	**Stephen Wilkinson** *University of Chester, UK*	Design and Analysis of Bioreactors for Maximum CO_2 Capture and Utilisation
14:50 – 15:10	**Jasbir Singh** *HEL Ltd, UK*	Enhanced Process Economics of C1 Gas Fermentations Through Elevated Pressure Operation – Presentation of Principles and Application Data
15:10 – 15:30	**Vera Salgado** *SBRC-Nottingham, UK*	**POC TALK** - Exoelectrogenic Intensification of CO_2 / H_2 Fermentations using O_2 as Final Electron Acceptor
15:30 - 16:00	**PITCHES**	Gerard Gardener (HEL Ltd) Andrew Goddard (Freeland Horticulture Ltd) Alex Finney (University of Dundee) Lucy Montgomery (National Non-Food Crops Centre) Koen Quataert (Bio Base Europe Pilot Plant) Steve Skill (Greenskill Ltd)
16:00 – 18:00	**POSTERS/ Refreshments**	**Atrium - EMCC**
18:00 – 19:30	**Management Board meeting**	MB only – Meeting Room (TBC) Orchard Hotel
20:00	**Dinner**	**Conference Theatre - EMCC**

TUESDAY 7th November 2017		
Session 4 - Chair: Tithira Wimalasena (Calysta UK Ltd) – **Conference Theatre EMCC**		
09.00 – 09:30	**Linsey Garcia-Gonzalez** *VITO NV, Belgium*	**INVITED TALK** - Carbon Capture and Utilization (CCU) in Flanders: current status, challenges and way forward
09.30 – 09:50	**Karel De Winter** *Bio Base Europe Pilot Plant, Belgium*	A Key Role for Shared Pilot Facility to Deploy Gas Fermentation
09:50 – 10:10	**Thomas Smith** *Sheffield Hallam University, UK*	Developing obligate aerobic methanotrophs for biotechnology
10:10– 10:30	**Robert Manfield** *SBRC-Nottingham, UK*	**POC TALK** - *Eubacterium limosum*: Maximising reaction productivity through protein scaffolding with cohesion-dockerin domains
10:30 – 11:00	**Coffee/Tea Break**	**Atrium - EMCC**
Session 5 - Chair: Edward Green (Chain Biotech, UK) – **Conference Theatre EMCC**		
11:00 – 11:30	**Alexander Steinbüchel** *University of Münster, Germany*	**INVITED TALK** - *Ralstonia eutropha* as production strain utilizing CO_2 and CO.
11:30 – 11:50	**Dirk Holtman** *Dechema - Forschungs Institut, Germany*	Expanding the Product Spectrum of Microbial Electrosynthesis – Engineering of *Cupriavidus necator* to produce the Sesquiterpenoid α-Humulene from CO_2
11:50 – 12:10	**Anne Henstra** *SBRC-Nottingham, UK*	Transcriptional Changes Underpinning Shifts In Acetate, Ethanol, 2,3 Butanediol And Lactate Ratios In Gas Fermentation By *Clostridium autoethanogenum*
12:10 – 12:30	**Rupert Norman** *SBRC-Nottingham, UK*	Construction and Analysis of a Genome-Scale Metabolic Model of *Clostridium autoethanogenum*
12:30 – 14.00	**Lunch**	**Atrium - EMCC**
Session 6 - Chair: Nigel Minton (SBRC-Nottingham, UK) – **Conference Theatre EMCC**		
14:00 – 14:30	**Christopher Brigham** *University of Massachusetts, Dartmouth, USA*	**INVITED TALK** - Autotrophic *Ralstonia eutropha*: going "under the bonnet" of a microbial chassis to deliver multiple bioproducts
14:30 – 14:50	**Christian Arenas** *SBRC-Nottingham, UK*	The Genetic Basis of 3-Hydroxypropanoate Metabolism in *Cupriavidus necator* H16
14:50 – 15:10	**Frank Sargent** *University of Dundee, UK*	**POC TALK** - Hydrogen-dependent CO2 reduction by *Escherichia coli*
15:10 – 15:30	**Samantha Bryan** *SBRC-Nottingham, UK*	Engineering Improved Ethylene Production in *Cupriavidus*
15:30 – 15:45	**Nigel Minton** *SBRC-Nottingham, UK*	Wrap up and Next Steps
15:45 - 17:00	**Refreshments/ Depart**	**Atrium - EMCC**

POC TALKS – These are oral presentations by the recipients of BBSRC-NIBB C1net "Proof of Concept" funding.

POC PRESENTATIONS

POC-7-minton-C1net – oral – presented by Robert Mansfield
Maximising reaction productivity through protein scaffolding with cohesion-dockerin domains
PI – Nigel Minton, University of Nottingham
12 months

POC-8-sargent-C1net – oral – presented by Frank Sargent
A Synthetic Approach to bioconversion of carbon dioxide to formic acid
PI – Frank Sargent, University of Dundee
6 months

POC-10-winzer-C1net – poster – presented by Klaus Winzer
Novel aerobic chassis for the conversion of mixed CO/CO2 feedstocks
PI – Klaus Winzer, University of Nottingham
12 months

POC-12-conradie-C1net – oral – presented by Vera Salgado
Exoelectrogenic Intensification of CO2/ H2 Fermentations using 02 as Final Electron Acceptor
PI – Alex Conradie, University of Nottingham
6 months

ELEVATOR PITCHES (order of presentation)

Gerard Gardener (HEL Ltd)
gerardgardner@helgroup.com

Andrew Goddard (Freeland Horticulture Ltd)
andrew@freelandhorticulture.co.uk

Alex Finney (University of Dundee)
a.j.finney@dundee.ac.uk

Lucy Montgomery (National Non-Food Crops Centre)
l.montgomery@nnfcc.co.uk

Koen Quataert (Bio Base Europe Pilot Plant)
koen.quataert@bbeu.org

Steve Skill (Greenskill Ltd)
Steve@greenskill.co.uk

INVITED SPEAKERS

Professor Rudolf Thauer

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.

Dr Christopher Brigham

University of Massachusetts, Dartmouth, USA

Dr Brigham received his Ph.D. from Tufts University School of Medicine in Boston, MA, USA and has previously worked as a Research Scientist at Massachusetts Institute of Technology. At University of Massachusetts Dartmouth, his group focus on bioproduction of sustainable, fermentation-based chemicals and materials. He is well-versed in the discipline of metabolic engineering, and has been involved in constructing novel microbial metabolic pathways to synthesize value added products from surplus or waste carbon feedstocks. Using the bacterium *Ralstonia eutropha* (a.k.a *Cupriavidus necator*) and other industrially relevant species (e.g., *Rhodococcus opacus*), his group produces biopolymers and biofuels from a variety of carbon-containing waste streams. Dr Brigham's educational focus is towards the understanding of the metabolism of industrially relevant organisms like *R. eutropha, R. opacus* and others with the goal of rationally designing high-productivity production strains.

Dr Linsey Garcia-Gonzalez

Vito NV, Belgium

Dr. Garcia-Gonzalez holds a PhD in Bioscience Engineering. As senior scientist at VITO she is responsible for the valorization of CO_2 as feedstock towards polymers and chemical building blocks using biotechnological processes. Also as programme manager for joint industry/government initiative “Catalisti”, she is responsible for sidestream valorization, renewable chemicals and the strategic theme Carbon Capture and Utilisation (CCU). She has extensive experience in the execution, management and coordination of multidisciplinary and multi-partner projects. She recently conducted a study for the Environment, Nature and Energy Department of the Flemish Region to assess the impact of CCU on an economic, technological and environmental level and to stimulate CCU in Flanders. She is part of the SCOT (Smart CO2 Transformation) community, member of the CO2CHEM and C1NET network. She is co-author of 23 SCI publications, 2 book chapters and 1 patent application.

Professor Volker Müller

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 earlier this year, was awarded 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”.

Professor Guido Saracco

Instituto Italiano di Tecnologia, Italy

Professor Saracco graduated and received his PhD in Chemical Engineering at Politecnico di Torino where he is now a full professor in Industrial and Technological Chemistry. He is author of over 200 papers on chemical reaction engineering and separation processes; has contributed to the founding and co-ordination of numerous research laboratories at his University (Catalysis Environmental, Hydrogen and Fuel Cells, Biosolar Lab, Graphene @ PoliTo) and has been scientific lead of many national and EU projects (SYLOC-DEXA; BIOFEAT; HyTRAN, MOREPOWER; FLEXHEAT; ATLANTIS). He is a member of the European Energy Research Alliance Advance Materials and Processes for Energy Applications section, with responsibility for the Low Temperature Heat Exploitation program. Additionally, he is responsible for the Working Group Processes Scale-Up and Industrialization as a member of the European Cluster on Catalysis.

Dr Sean Simpson

LanzaTech, USA

Dr. 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.

Professor Alexander Steinbüchel

University of Münster, Germany

Professor Steinbüchel is Professor of Microbiology at the Institute of Microbiology, University of Münster, Germany. His research links basic research and applied studies with the aim to establish new or improve already existing biotechnological processes, to obtain novel products and to provide them in sufficient amounts for characterization. His research focuses on synthesis of biopolymers (e.g. polyesters), natural flavours (e. g. vanillin) and other chemicals (e. g. lipids) as well as on the biodegradation of polymers and their utilization (e.g. natural and synthetic rubbers). Professor Steinbüchel has contributed more than 410 publications of original papers in peer reviewed journals and is also Editor in Chief of the scientific journals “Applied Microbiology and Biotechnology” and “AMB Express”.

ABSTRACTS OF ORAL PRESENTATIONS

How to make a living by producing acetate from carbon dioxide and hydrogen

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 mechanism of ATP synthesis has been unravelled only recently. The model acetogen *Acetobacterium woodii* has a respiratory chain that contains a Na^+-motive ferredoxin:NAD-oxidoreductase (Rnf) and a Na^+-F_1F_O ATP synthase that are electrically connected by a transmembrane electrochemical Na^+ gradient. The energy barrier for the hydrogen-dependent reduction of ferredoxin, the reductant for the respiratory enzyme, is overcome by flavin-based electron bifurcation (FBEB). Rnf and FBEB are membrane-bound and soluble processes, respectively, for redox homeostasis in acetogens. Both processes are reversible and indeed, many acetogenic pathways require Rnf to drive the endergonic reduction of ferredoxin with NADH as reductant by reverse electron transport. Likewise, FBEB may overcome energetic barriers in hydrogen production from NADH or in lactate oxidation.

Some acetogens do not have the *rnf* genes. Instead, genes for an energy-converting hydrogenase (Ech) are present. One such organism is *Thermoanaerobacter kivui*. We have studied energy metabolism in *T. kivui* and will provide data that the Ech complex is indeed the (only) respiratory enzyme in this organism. The end product of this respiration is molecular hydrogen and the bioenergetic implications will be discussed.

We conclude that acetogens use chemiosmotic energy conservation in addition to substrate level phosphorylation and can be grouped into two groups. Both groups use reduced ferredoxin as electron donor for the respiratory enzyme, that is reduced with H_2 by FBEB. One group, with *A. woodii* and *Clostridium ljungdahli* as the model strains, use Rnf as respiratory enzyme, the other, with *T. kivui* and *Moorella thermoacetica* as model strains the Ech complex instead.

Development and Application of Efficient Forward and Reverse Genetic Tools in *Acetobacterium woodii*

JONATHAN P. BAKER[1], JAVIER SAEZ[1], SHEILA INGEMANN JENSEN[2], ANJA WIECHMANN[3], FRANK BENGELSDORF[4], MATTHIAS BECK[4], ELVIRA MARÍA FERNÁNDEZ-SANCHIS[5], VOLKER MÜLLER[3], ALEX TOFTGAARD NIELSEN[2], PETER DÜRRE[4], MANFRED BALDAUF[5], SEAN SIMPSON[6], NIGEL P. MINTON[1].

[1]Clostridia Research Group, BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), University of Nottingham, Nottingham, NG7 2RD, UK; [2]The Novo Nordisk Center for Biosustainability, Kogle Allé 6, 2970 Hoersholm, Denmark. [3]University of Frankfurt, Molekulare Mikrobiologie & Bioenergetik Institut für Molekulare Biowissenschaften, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany. [4]Institut für Mikrobiologie und Biotechnologie, Albert-Einstein-Allee 11, 89081 Ulm, Germany. [5]Siemens AG, Guenther-Scharowsky-Str. 1, 91056 Erlangen, Germany. [6]LanzaTech, 3-5 Horndean Road, RG12 0XQ Bracknell, UK.

Acetobacterium woodii represents one of the most studied and, therefore, best understood anaerobic acetogens. Currently the CO2CHEM consortium (comprising the Universities of Ulm, Frankfurt and Nottingham, together with the Novo Nordisk Foundation Center for Biosustainability, Siemens and LanzaTech) seek to exploit its potential as a chassis for chemical and fuel manufacture through ERA-IB funding. Essential to this goal are the availability of effective gene tools for genome engineering, both by directed and random mutagenesis. These systems have been implemented through adherence to our recently published roadmap for gene system development [1].

As a first step we established a high efficiency transformation system for *A. woodii*, allowing for the subsequent use of suicide vectors to create a *pyrE* knockout strain. This uracil auxotroph provides the background for an ACE knockout system which has been exemplified through phenotypic studies of substrate pathway gene deletions.

An orthogonal expression system based on the unique *Clostridium difficile* sigma factor TcdR, has been shown to act exclusively on the toxin *tcdA/B* gene promoters and so are not recognized by *E. coli* RNA polymerase. To ensure expression solely in the target host (*A. woodii*), the *tcdR* gene was introduced into the *A. woodii* genome using an ACE cargo vector at the *pyrE* locus. This allowed for the use of *tcdB* to drive a mariner transposon system on a suicide vector to create a library of mutants for both phenotypic and high-throughput genome sequencing studies; further increasing our understanding of this important chassis strain.

[1] Minton NP, Ehsaan M, Humphreys CM, Little GT, Baker J, Henstra AM, Liew F, Kelly ML, Sheng L, Schwarz K, Zhang Y. A roadmap for gene system development in *Clostridium*. *Anaerobe*. 2016; **41:**104-112.

Using A Metabolic Model Of *Acetobacterium woodii* For Insights Into Its Utility For Biotechnological Purposes

NOAH MESFIN[1], SHEILA INGEMANN JENSEN[2], ALEX TOFTGAARD NIELSEN[2], DAVID FELL[1] AND MARK POOLMAN[1]

[1]*Department of Biological and Medical Sciences, Oxford Brookes University, Oxford*
[2]*Novo Nordisk Foundation Centre for Biosustainability, Technical University of Denmark, Denmark*

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 121 substrate pair combinations, and used this to construct a sub-model representing a core set of energy producing catabolic pathways. We then introduced heterologus reactions to allow for production of HPA. 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. 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 growth supporting HPA production. Our analysis provides evidence for the potential of non-acetate dependent growth of *A.woodii*.

Hydrogen Storage Using Acetogens as Biocatalysts: Hydrogen-Dependent CO_2-Reductase from a Thermophilic Acetogen

FABIAN SCHWARZ and VOLKER MÜLLER

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

Reduction of CO_2 to formic acid is the first step in the Wood-Ljungdahl pathway of CO_2 fixation. In contrast to most acetogens that use a reduced cofactor such as NADPH or reduced ferredoxin as electron donor for a formate-dehydrogenase[1] catalyzed CO_2 reduction to formate, *Acetobacterium woodii* has an enzyme that directly use hydrogen gas as electron donor for CO_2 reduction, the hydrogen-dependent CO_2-reductase (HDCR)[2]. The HDCR of *A. woodii* can be used to store hydrogen in the form of formate and, thus, can contribute to solve the problem to store energy produced from renewable sources such as wind. Homologs of the genes encoding the HDCR from *A. woodii* are also present in the thermophilic acetogen *Thermoanaerobacter kivui*. To proof the existence of the enzyme, it was purified from cell paste of *T. kivui* grown on pyruvate to apparent homogenity. The enzyme contained the subunits predicted from the DNA structure. Interestingly, the HDCR of *T. kivui* is much more active than the enzyme from *A. woodii* and has superior stability properties. The enzyme catalyzed H_2-dependent CO_2-reduction as well as the reverse reaction and is thus a promising candidate for future applications in hydrogen storage.

1. Maia LB, Moura JJ, Moura I. 2015. Molybdenum and tungsten-dependent formate dehydrogenases. J Biol Inorg Chem **20:**287-309

2. Schuchmann K, Müller V. 2013. Direct and reversible hydrogenation of CO_2 to formate by a bacterial carbon dioxide reductase. Science **342:**1382-1385

Sustainable Chemical Production Ecosystems based on CO_2 and Combined Photo-, Electro- and Biochemical Processes

GUIDO SARACCO[a,b], SIMELYS HERNANDEZ[a,b]

(a) Istituto Italiano di Tecnologia, Centre for sustainable Future Technologies, Corso Trento 21, Italy
(b) Politecnico di Torino, Department of Applied Science and Technology, Corso Duca degli Abruzzi 24, Italy.

Sustainable production of chemicals requires the use of renewable material and energy sources. Among them the use of CO_2 as a raw material alongside with organic wastes, as well as the use of solar energy will be of major importance.

The European roadmap (http://ec.europa.eu/clima/roadmap2050) for CO_2 anthropogenic emissions reduction targets a 40% decrease in CO_2 emissions for 2030 compared to 1990 levels, and of an 80% decrease by 2050. The recent agreements of Paris (December 2015) essentially confirmed this perspective. Particularly, 195 countries have signed these agreements with the aim to keep global warming of the Earth's surface well below 2 °C when compared to pre-industrial period.

This result cannot be reached without a radical technological innovation that is based on materials, processes and new technologies that, integrated into intelligent grid management, will bring renewable energy in the core of new production ecosystems.

Governmental actions and promoted by key stakeholders will target not only the sequestration of CO_2 but also its re-use/conversion to synthesize compounds, materials, fuels alternative to fossil ones. On top of this, the development (already ongoing) of conversion of renewable energy into electricity (photovoltaics, wind, etc.) and the storage of this latter (through lithium and post-lithium batteries) will remain fundamental aspect of the present worldwide scenario.

Based on recent research efforts by the authors on either bio-based or photo-electrochemical approaches to sustainable production processes involving CO_2 or organic wastes, this presentation provides a comprehensive overview of the international research on the most promising technologies in this area, with a special focus on those involving microorganisms. In particular, approaches based on natural (including synthetic & systems biology) and artificial (including PV-driven electrolysis or photo-electrolysis) technologies will be critically analysed and compared.

Optimizing the hydrolysis process by pre-composting to enhanced biomethane production by anaerobic digestion from lignocellulosic waste

MAHENDRA P RAUT, JAGROOP PANDHAL and PHILLIP C WRIGHT [1]

The ChELSI Institute, Department of Chemical and Biological Engineering, University of Sheffield, Mappin Street, Sheffield, S1 3JD, UK
[1]*School of Engineering, Faculty of Science, Agriculture & Engineering, Newcastle University, Devonshire Building, Newcastle upon Tyne, NE1 7RU*

Hydrolysis by pretreatment of lignocellulosic material for increased biogas production is required for *in vitro* anaerobic digestion (AD) development. Existing hydrolysis methods such as chemical, physical or enzymatic approaches are energy intensive. Thus, the development of simple and cost effective biological pretreatment workflows for lignocellulosic biogas production is of general interest.

However, biological pretreatment often suffers low hydrolysis rates, due to a lack of suitable microbial consortia and the low nutrient value of lignocellulosic waste. This has resulted in underperformance of AD. Therefore, in order to develop biological pretreatment, extensive research on testing and seeking an adaptive microbial consortium is necessary.

In this study, we optimized biological hydrolysis of barley straw (BS) as a pre-composting stage (step I) with and without applying adaptive and enriched microbial consortia followed by AD (step II). The pre-composting was carried out for 40 days to achieve hydrolysis. This predigested material was subjected to AD for 30 days.

The efficiency of pre-composting was monitored by microbial activity, carbon/nitrogen (C/N) ratio, total cellulase activity and lignin peroxidase activity. Biogas (CH_4) was measured during AD.

Our results demonstrate that by using an adaptive microbial consortium, significant reduction in C/N ratio was achieved by higher cellulase and microbial activity. This indicates a significant increase in the hydrolysis process during pre-composting. This was successfully reflected in a significant volume of biogas production (approximately 1L) with 32 % CH_4 content within 15 days of AD.

With the perspective of the “pre-composter cum AD” (PCAD) unit development, the outcome of our study proves that a two stage AD is a promising and cost effective way for lignocellulosic biogas generation.

CO_2 Utilisation via a Novel Anaerobic Bioprocess Configuration with Simulated Gas Mixture and Real Stack Gas Samples

DAGLIOGLU, S.TUGCE[1], KARABEY, BURCIN[2], OZDEMIR, GUVEN[2], AZBAR, NURI[1-3]

[1]Ege University, Center for Environmental Studies, 35100 Bornova-Izmir TR
[2]Ege University , Faculty of Science, Biology Department, 35100 Bornova-Izmir, TR
[3]Ege University, Faculty of Engineering, Bioengineering Department, 35100 Bornova-Izmir, TR

In this study, amongst the various CO_2 capture methods, anaerobic bioconversion of CO_2 into bio-methane using a novel bioprocess configuration (HYBRID bioreactor) was studied under mesophilic conditions. Thereby, a sustainable and environment-friendly model solution for industries with high CO_2 emissions was presented. Varying ratios of H_2/CO_2 gas mixture and volumetric feeding rates were investigated. The maximum methane production of 5.4 m^3 CH_4/m^3 $_{reactor}$/d was achieved at a H_2/CO_2 ratio of 4:1 and loading rate of 1.33 m^3 gas/$m^3_{reactor}$/d. It was determined that H_2 conversion rate is about 96%. For demonstration purpose, real stack gas sample from a petrochemical industry was also tested under optimized operation conditions in the study. No inhibitory effect from stack gas mixture was observed. The study showed an environmentally friendly and sustainable solution for industries such as petrochemical industry in order to produce extra energy while capturing their waste CO_2. Furthermore, this approach is feasible for the enrichment of biogas content in all biogas plants.

H_2 consumption showed that the novel anaerobic HYBRID bioreactor configuration of the bioreactor was successful for gas hold-up and gas solubility. CO_2 and H_2 conversion to bio-methane reached 86% without any need for external organic matter or trace element addition and gas recirculation. The experiments with real stack gas showed no toxic effect. The system would be an environmental friendly and sustainable solution for the petrochemical industry, which has H_2 in the stack gas. For the economic sustainability of the proposed system, hydrogen should be provided from either a waste source as in our case or should be produced via sustainable method such as electrolysis of water using renewable energy sources (wind or solar energy).

Acknowledgments

The authors wish to thank TUBITAK-CAYDAG under the grant No 115Y455 and Ege University Scientific Research Project Fund under the grant no 15/CSUAM/003 for the financial support of this study. The data presented in this article was produced within the project above, however it is only the authors of this article who are responsible for the results and discussions made herein.

Assessment of Syngas Biomethanation by Mixed Microbial Consortia in a Trickle-bed Reactor

KONSTANTINOS ASIMAKOPOULOS, HARIKLIA GAVALA, IOANNIS V. SKIADAS

Department of Chemical and Biochemical Engineering, Technical University of Denmark, DK-2800 Kongens Lyngby Denmark

Syngas or synthesis gas is a mixture of gases consisting mainly of CO, CO_2 and H_2. It can be converted to liquid or gaseous biofuels through chemical or biological processes. Conventional catalytic processes like Fischer-Tropsch present high operational costs as they require high temperatures and pressures and high supply cost for the catalysts. One promising alternative is the microbial fermentation processes which take place under mild conditions that entail low energy and infrastructure costs. In addition, the microbes are relatively cheap and they do not demand a fixed H_2/CO ratio as the chemical catalysts do.

However syngas bioconversion to biofuels faces important challenges that should be circumvented before the process is scaled up. The main bottlenecks are the mass transfer of sparingly soluble syngas compounds to the water based microbial cultures and the relatively low growth rate of the microbes that leads to insignificant productivity rates. Towards this direction we designed lab scale experiments with trickle bed reactors and enriched anaerobic sludge as their inoculum. The use of mixed microbial consortia renders strictly sterile operation unnecessary and enhances the adaptability of the microbial community to sudden changes of the operational conditions.

The goal of this study is to assess several operational parameters such as liquid recirculation rate, hydraulic retention time and gas flow with reference to the methane productivity, the substrate conversion yield and the composition of the gas exiting the reactor as well as shedding more light to the phenomena occurring in mixed microbial consortia syngas biomethanation.

Fuel and Chemical production by gas fermentation at scale

DR SEAN SIMPSON
Chief Scientific Officer and Founder

LanzaTech
8045 Lamon Avenue, Suite 400, Skokie, IL; USA

As a result of anthropogenic climate change there is a growing move to develop technologies that enable non traditional, sustainable or waste feedstocks to be used for the productin of fuels chemicals and power. Technologies allowing power production with no terminal release of greenhouse CO_2 are now mature and out-compete traditional power generation processes economically. This advance not only impacts the power sector, but has also enabled an accelerated transition to sustainable electrical mobility, thus challenging the role of hydrocarbons as the dominant source of road transportation fuel. Even with the advance of electrical mobility, it is evident that a source of carbon is still required for the aviation sector and for the production of chemicals. Gas fermentation enables a broad range of high volume, low value waste streams to be transformed into both fuels and chemicals. These gas feedstocks either exist as direct by-products of essential industrial processes or through the gasification of agricultural and societal solid waste streams into syngas. In this way gas fermentation is a vital bridge in the effort to create value from waste and enable the perpetual capture of greenhouse carbon in valuable materials. 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 facilities are currently under construction with the process having been demonstrated with live feeds of waste gas from numerous processes and industries

Design and Analysis of Bioreactors for Maximum CO_2 Capture and Utilisation

STEPHEN WILKINSON[1], DAVID MORRIS[2]

[1]Department of Chemical Engineering, University of Chester, Thornton Science Park, Chester CH2 4NU
[2]Autichem Ltd., Building B49, Thornton Science Park, Chester CH2 4NU

Aerated bioreactors are typically designed with the focus on providing enough gas (oxygen for respiration, carbon dioxide for photosynthesis) to optimize growth conditions rather than on absorbing as much of the supplied gas as possible. This means that only a small fraction of the gas is typically utilized and most of the supplied gas is lost in the off-gas. If the aim is to maximize the utilization of the gas for carbon capture and utilization (Sayre, 2010) then the absorption of gas into the liquid medium must be increased. This can be achieved by longer gas residence times and/or larger interfacial areas.

The creation of smaller bubbles with either mechanical agitation to break up bubbles or by using novel sparging technology (Mahmood et al., 2015) are two ways of doing this. There have also been efforts to design novel reactor systems using tubular or rotating components in multi-phase systems (Hardin, et al., 2001, Singh and Sharma, 2012).

In this work we identify key engineering principles of reactor design for efficient gas utilization. We also compare an analysis of a standard airlift reactor (with bubble size as the key design parameter) with results for a novel horizontal reactor system and a two stage bioreactor system.

Hardin, M.T., Howes, T. and Mitchell, D.A., 2001. Residence time distributions of gas flowing through rotating drum bioreactors. Biotechnology and bioengineering, 74(2), pp.145-153.

Mahmood, K.A., Wilkinson, S.J. and Zimmerman, W.B., 2015. Airlift bioreactor for biological applications with microbubble mediated transport processes. Chemical Engineering Science, 137, pp.243-253.

Sayre, R., 2010. Microalgae: the potential for carbon capture. Bioscience, 60(9), pp.722-727.

Singh, R.N. and Sharma, S., 2012. Development of suitable photobioreactor for algae production–A review. Renewable and Sustainable Energy Reviews, 16(4), pp.2347-2353.

Enhanced Process Economics of C1 Gas Fermentations Through Elevated Pressure Operation – Presentation of Principles and Application Data

JASBIR SINGH and GERARD GARDNER

Hel Ltd, Borehamwood, Herts, 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 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 used to screen and then develop bacterial strains at elevated pressure, allowing process economics to be directly improved through higher production rate and better yield. Substantial increases is solubility and mass flux will be demonstrated while at the same time achieving fine control of dissolved oxygen profile to suit different bacterial strains. Through operating at under 100ml in each bio reactor, successful scale up to multi-litre volume will also be demonstrated to show that scale-up from laboratory scale is possible and reliable.

Exoelectrogenic Intensification of CO_2 / H_2 Fermentations using O2 as Final Electron Acceptor

RAJESH BOMMAREDDY, VERA SALGADO & ALEX CONRADIE

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

Valorisation of C1 carbon feedstocks at low temperature, low pressure and high catalytic selectivity is highly desirable from a processing cost perspective. As such, the metabolic conversion of CO_2 / H_2 to higher value compounds in bioreactors, with O_2 as final electron acceptor, offers significant opportunities. However, given the flammability of hydrogen in oxygen atmospheres, operational safety considerations abound. Safety constraints limit the intensification of fermentation, impacting adversely on capital gearing. An alternate bioreactor design, alleviating the oxygen mass transfer constraint associated with a flammable gas mixture, is required.

A novel exoelectrogenic bioreactor concept using *C. metallidurans* as host has been established, providing for CO_2 fixation using H_2 as electron donor at the anode and O_2 as final electron acceptor at the cathode. The exoelectrogenic reactor thus introduces an anode as intermediary electron acceptor. Laboratory scale experiments were undertaken in continuous cultivation over several hundred hours, demonstrating stable steady state operation. The exoelectrogenic bioreactors using *C. mettallidurans* demonstrated comparable electrical current generation to *Geobacter sulfurreducens* as control. The current contribution from the electro-activity of hydrogen was negligible. Moreover, the specific CO_2 fixation rate per m^2 of anode compared favourably to the specific CO_2 fixation rate in high cell density suspended culture.

Therefore, the exoelectrogenic bioreactor concept design (1) obviates the need for flammability considerations, (2) lifts O_2 mass transfer restrictions and (3) offers an intensification strategy underpinned by an immobilised culture. Future work aims to scale up the exoelectrogenic reactor concept to further demonstrate the effective intensification of CO_2 fixation in this novel reactor configuration.

This is a POC ORAL PRESENTATION - by the recipient of BBSRC-NIBB C1net "Proof of Concept" funding.

Carbon Capture and Utilization (CCU) in Flanders: current status, challenges and way forward

LINSEY GARCIA-GONZALEZ, HELEEN DE WEVER*

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

Carbon Capture and Utilization or CCU includes processes in which CO2 from point sources is being used as feedstock for the synthesis of new molecules with an economic value. This approach can contribute to the reduction of the greenhouse gas CO_2 in the atmosphere, which is considered one of the main causes for climate change. Although several CCU technologies are developed, the implementation of CCU on an industrial scale is rather limited in Flanders. To map the most promising new valorization pathways for CO_2 as raw material/feedstock with a view to their application in the Flemish Region and to define how CCU can be stimulated, the Environment, Nature and Energy Department of the Flemish Region commissioned a study to VITO and DNV GL.

In this presentation, an overview of the CO_2-utilization projects of research centers and the industrial CCU-initiatives in Flanders will be given. Following, 4 cases will be discussed more in detail based on 5 criteria to assess which contribution they could make to emission reductions in the Flemish Region. The general aim of this analysis is to compare the different cases, to identify the strengths and to evaluate how weaknesses could be overcome. The criteria that will be discussed include the actual contribution of a CCU technology to emission migitation (criterion 1), the economic feasibility of the process (criterion 2), the potential scale of application in Flanders (criterion 3), the level of technology development (TRL) (criterion 4) and system analysis (criterion 5). Finally, specific policy recommendations to encourage the application of CO_2 in Flanders will be presented.

*Corresponding author: Heleen De Wever, heleen.dewever@vito.be, Tel +32 14 33 69 32

A key role for shared pilot facility to deploy gas fermentation

KAREL DE WINTER

Bio Base Europe Pilot Plant, Rodenhuizekaai 1, 9042 Ghent, Belgium

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

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

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

Developing obligate aerobic methanotrophs for biotechnology

THOMAS J SMITH

Biomolecular Sciences Research Centre, Sheffield Hallam University, Howard Street, Sheffield S1 1WB, UK

Obligate aerobic methane oxidising bacteria offer many opportunities to produce valuable products using methane from fossil or biogas sources as the feedstock. Also, the catalytically highly versatile soluble methane monooxygenase (sMMO) can be used to insert oxygen functionality into a wide range of hydrocarbons and other small hydrophobic molecules, which may be useful both to make high-value chemicals and in bioremediation. Engineering of sMMO for specific biotransformations has proven challenging because attempts to express the enzyme in *Escherichia coli* and other common expression hosts have been unsuccessful.

A homologous expression system has been developed to enable expression of recombinant sMMO derivatives in a methane oxidising bacterium in which the chromosomal copy of the sMMO genes is deleted. This has been used to probe the function of a number of active-site residues in the enzyme to complement biochemical studies performed on the wild-type and has also indicated a number of residues that are responsible for controlling the precision of regioselectivity with mono- and di-aromatic substrates. This work has led to a mutant with improved regioselectivity in oxygenation of biphenyl, thus providing proof-of-principle for tailoring the properties of sMMO for specific biotransformations.

It has also been shown that certain aerobic methanotrophs can transform chromium and selenium species. *Methylococcus capsulatus* (Bath) reduces chromium (IV) to the less toxic and less bioavailable chromium (III) via a reaction that depends upon methanotrophic metabolism and results in the chromium (III) accumulating in the particulate fraction of the culture. Selenite is transformed by *Mc. capsulatus* (Bath) and *Methylosinus trichosporium* to elemental selenium nanoparticles, which may have commercial applications, as well as methylated selenium species.

Eubacterium limosum: Maximising reaction productivity through protein scaffolding with cohesion-dockerin domains

ROBERT MANSFIELD**,** GEORGINA BATES, JAMES MILLARD, CHRISTOPHER HUMPHREYS, ELIZABETH REDFERN, KATALIN KOVÁCS, NIGEL P. MINTON

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

Several studies have used the dockerin cohesin domains of clostridial cellulosomes to construct synthetic scaffolds which enhanced cascade catalysis. In the current study we sought to use a previously constructed BB2-standardized library of cohesin and dockerin domains from *Clostridium thermocellum* and *Clostridium cellulolyticum* [*Biotechnol. Biofuels* 2013; **6:**117] to improve the productivity of an exemplar pathway in acetogens. The primary chassis selected was *Eubacterium limosum* on the basis that it is able to produce butyrate/*n*-butanol in addition to acetate/ethanol, and is capable of growing on the C1 feedstock methanol in addition to CO and CO_2. Unlike the latter gases, methanol does not suffer from mass transfer issues in fermenters and is more easily stored and transported. It can be made from many sustainable feedstocks, including biomass, MSW, biogas, waste CO_2, and even renewable electricity.

A prerequisite was the establishment of effective genetic systems. This was achieved through the implementation of our published 'roadmap' for gene system development [*Anaerobe* 2016; **41:**104-112]. The complete genome sequence of *E. limosum* ATCC-8486 was determined, through a combination of Pac-Bio and Illumina paired-end sequencing, and fully annotated using the Integrated Microbial Genomes pipeline; transformation by electroporation was established and then optimised through inactivation of a type I restriction systems using CRISPR/Cas9.

Extension of the native *E. limosum* product spectrum was achieved through the development and implementation of plasmid borne synthetic isopropanol production operons (IPO), encoding thiolase A, CoA-transferase, acetoacetate decarboxylase from *C. acetobutylicum* ATCC 824, and secondary alcohol dehydrogenase from *Clostridium beijerinckii* NRRL B593. Enzyme scaffolding of the IPO enzymes, using synthetic mini-cellulosomes, did not result in improved isopropanol titres in *E. limosum* or *Clostridium autoethanogenum*.

This is a POC ORAL PRESENTATION - by the recipient of BBSRC-NIBB C1net "Proof of Concept" funding.

Ralstonia eutropha as production strain utilizing CO_2 and CO.

ALEXANDER STEINBÜCHEL, DANIEL HEINRICH AND MATTHIAS RABERG

Institut für Molekulare Mikrobiologie und Biotechnologie, Westfälische Wilhelms-Universität, Corrensstraße 3, D-48149 Münster

Ralstonia eutropha strain H16 is a Gram-negative bacterium ubiquitously found in soils and has been intensively studied for about 60 years in academia and industry. It is probably the best studied 'Knallgas' bacterium capable of chemolithoautotrophic growth with hydrogen as electron donor and carbon dioxide as carbon source. First applications of *R. eutropha* aimed at chemolithoautotrophic production of single cell protein (SCP) as food and feed and at the production of various thermoplastic polyhydroxyalkanoates (PHA) which are intracellularly accumulated as storage compound of carbon and energy. Various value added chemicals significantly extended recently the range of products synthesized by *R. eutropha*. Beside C1-compounds it utilizes also a broad range of renewable heterotrophic resources and high cell density cultivations can be done. The substrate utilization range can be further extended by metabolic engineering. The complete annotated genome is available and omics studies were also done. Altogether, this qualifies *R. eutropha* strain H16 to become a production platform strain for a large variety of products.

To enable the utilization of syngas by this strain, a *cox*-sub-cluster of the carboxydotrophic *Oligotropha carboxidovorans* OM5 was expressed. The expression of these genes enabled *R. eutropha* to oxidize CO to CO_2 for the use as carbon source, but the recombinant strains remained dependent on H_2 as external energy supply. With this extension of the bacterium's substrate range, growth in CO-, H_2- and CO_2-containing artificial synthesis gas atmosphere was enhanced, and PHA synthesis was increased by more than 20%. With this study, progress regarding the efficient conversion of waste gases into value added products with *R. eutropha* has been made while concomitantly providing novel insights into the hitherto scarcely studied CO metabolism of *R. eutropha* which could be described as 'carboxyhydrogenotrophic'.

Expanding the Product Spectrum of Microbial Electrosynthesis – Engineering of *Cupriavidus necator* to produce the Sesquiterpenoid α-Humulene from CO_2

ANNE SYDOW, SONJA FAUST, THOMAS KRIEG, ANDREAS PANNEK, DIRK HOLTMANN

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

In microbial electrosynthesis, carbon dioxide is fixed by using energy supplied by electrodes. Besides building up biomass, so far mainly low value organic acids and biofuels are produced. Genetic tools are limited for microorganisms used in the field of microbial electrosynthesis. To date, heterologous gene expression for the production of high-value products from carbon dioxide is rare.

Cupriavidus necator is one of the exceptions, because it is genetically accessible and able to fix carbon dioxide with electrochemically produced hydrogen. *C. necator* is well known as production strain in biotechnological processes. It is well known to produce and sequester polyhydroxyalkanoates (PHA). PHA can accumulate to levels around 90% of the cell's dry weight under excess of carbon source and limitation of other nutrients such as phosphate or nitrogen source. The latest results show that the strain can be engineered to produce different industrial important bulk chemicals (e.g. fatty acids, isopropanol or alkanes).

Here we present the production of α-humulene by a genetically modified *C. necator* strain. In the last decade, the monocyclic sesquiterpenoid is in the focus of research due to its therapeutic uses. α-Humulene is a powerful anti-inflammatory and analgesic agent with additional anti-bacterial and anticancer properties.

The facultative chemolithoautotrophic bacterium *C. necator* produces the C15 compound after heterologous expression of the respective terpene synthase gene. Water electrolysis provides the strain with electron donor hydrogen and electron acceptor oxygen, respectively. By using the indirect extracellular electron transfer approximate 10 mg/L α-humulene, corresponding with 5 mg per gram cell dry weight, is produced. Productivity is increased by the introduction of a heterologous mevalonate pathway, which intensifies the precursor flux to the humulene synthase.

For the first time, chemolithoautotrophic and electroautotrophic terpene production is presented.

Transcriptional Changes Underpinning Shifts In Acetate, Ethanol, 2,3 Butanediol And Lactate Ratios In Gas Fermentation By *Clostridium autoethanogenum*

ANNE M. HENSTRA[1], THOMAS MILLAT[1], RUPERT O. J. NORMAN[1], BART PANDER[1], SALAH ABDELRAZIG[1], LAUDINA SAFO[1], FREDERIK WALTER[1], MAHENDRA RAUT[2], SARAH SCHATSCHNEIDER[1], LOUISE SEWELL[1], MICHAEL KÖPKE[3], KLAUS WINZER[1], T. CHARLIE HODGMAN[1], DAVID A. BARRETT[1], PHILLIP C. WRIGHT[2], SÉAN D. SIMPSON[3], NIGEL P. MINTON[1]

[1]SBRC Nottingham, Centre for Biomolecular Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD, UK
[2]ChELSI Institute, Department of Chemical and Biological Engineering, University of Sheffield, Sheffield, S1 3JD, UK
[3]LanzaTech Inc., Skokie, IL 60077, USA

Clostridium autoethanogenum represents one of the most promising acetogen chassis for the industrialisation of Gas Fermentation. Acetate, lactate, ethanol and 2,3-butanediol are natural products of gas fermentation by *C. autoethanogenum*. Production of acetate and ethanol are fairly well understood based on recent advances in scientific research. Various studies revealed little remarkable change in transcriptome composition when cultures were forced to shift production of acetate to ethanol. It was proposed that the product shift is mainly due to thermodynamic drivers. Production of lactate and 2,3 butanediol is less well understood. Lactate seems to appear under stress and 2,3 butanediol late in batch fermentations.

We found conditions of growth that help explain the production of lactate and 2,3 butanediol. Analysis of the transcriptome revealed changes in expression of lactate dehydrogenase and in the metabolic pathway to 2,3-butanediol. Also changes in the expression of various alcohol dehydrogenases and aldehyde:ferredoxin reductase was observed under certain conditions, that indicate alternative mechanisms to thermodynamics that drive product shift.

Construction and Analysis of a Genome-Scale Metabolic Model of *Clostridium autoethanogenum*

[1]RUPERT NORMAN, [1]THOMAS MILLAT, [2]SARAH SCHATSCHNEIDER, [1]ANNE HENSTRA, [3]HASSAN HARTMAN, [4]MARK G POOLMAN, [4]DAVID A FELL, [1]KLAUS WINZER, [1]NIGEL P MINTON and [5]CHARLIE HODGMAN.

[1]BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), University of Nottingham, Nottingham, NG7 2RD, UK. [2]Evonik Nutrition and Care GmbH, Kantstr. 2, 33798 Halle-Kinsbeck, Germany. [3]Genomics England, London. [4]Department of Biological and Medical Sciences, Oxford Brookes University, Gipsy Lane, OX3 0BP. [5]School of Biosciences, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Leicestershire, LE12 5RD.

Recent international directives promoting the reduced consumption of fossil fuels have warranted methods for effective carbon recycling. Subsequently, *Clostridium autoethanogenum* has attracted academic and industrial interest due to its ability to convert syngas components (CO, CO_2 & H_2) into valuable platform chemicals, including ethanol and 2,3-butanediol - 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).

We have successfully constructed a predictive model, suitable for the integration of omics data sets and prediction of gene knock-out targets, consisting of 795 reactions and 786 metabolites. Our model-simulated growth yields agree well with experimentally observed specific growth rates, while elementary modes analysis (EMA) confirms the availability of metabolic routes for acetate, ethanol, lactate and butanediol production. Elevated ethanol production is predicted to result from a reduction in pH levels. Similarly, we found that the switch from acetate to ethanol production occurs with increasing CO uptake rates under non-carbon limited conditions, finally leading to lactate production as a consequence of electron stress. Our results are consistent with trends observed in continuous cultures.

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

Autotrophic *Ralstonia eutropha*: going "under the bonnet" of a microbial chassis to deliver multiple bioproducts

CHRISTOPHER BRIGHAM, JINGNAN LU, CLAUDIA SANTOS GAI, ANTHONY SINSKEY

The Calvin Benson Bassham cycle of *Ralstonia eutropha* has been well studied in the hopes of producing fermentative bioproducts using carbon dioxide as the main carbon source. *R. eutropha*, a model organism of polyhydroxyalkanoate (PHA) bioplastic synthesis, has been shown to produce PHA autotrophically. There is an interest in producing other products like biofuels from CO_2, and *R. eutropha* can be engineered for this purpose. One challenge is to alter the downstream product of autotrophic metabolism from PHA to a biofuel molecule, such as isobutanol. The Ehrlich pathway of isobutanol synthesis can be expressed in *R. eutropha* by the addition and expression of a couple of heterologous genes. With the PHA synthesis pathway eliminated and the isobutanol synthesis pathway inserted, engineered *R. eutropha* cells will produce isobutanol and, to a lesser extent, 3-methyl-1-butanol. The metabolic engineering challenge is now to increase titers of the biofuel product. Another challenge for production of biofuels using autotrophic cultures is the handling of the gas mixtures. The mixture of oxygen and hydrogen needed to supply autotrophic cultures can be an explosion risk, and novel methods of delivery of these gases would be welcome for autotrophic cultures at any scale. A novel type of bioreactor has been designed that will separate oxygen and hydrogen but still deliver both gases to growing (and isobutanol-synthesizing) *R. eutropha* cells.

The Genetic Basis of 3-Hydroxypropanoate Metabolism in *Cupriavidus necator* H16

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

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

Different pathways for the production of 3-hydroxypropionic acid (3-HP) have been proposed, one of which proceeds via malonyl-CoA. The first committed step in this pathway is the carboxylation of acetyl-CoA to malonyl-CoA, a reaction that is catalysed by the enzyme acetyl-CoA carboxylase (ACC). The second step is the reduction of malonyl-CoA to 3-HP, a conversion catalysed by the bifunctional enzyme malonyl-CoA reductase (MCR) or, in some archaea, by the combination of two monofunctional enzymes which reduce malonyl-CoA first to malonate semialdehyde and then further to 3-HP. The aim of the work presented here was to enable 3-HP production from C1-feedstocks such as CO_2, using the autotrophic bacterium *Cupriavidus necator* as a chassis.

When testing *C. necator* for its tolerance towards 3-HP, it was noted that it can utilise the compound as the sole source of carbon and energy. Several genes involved in the degradation of 3-HP were subsequently identified and inactivated, resulting in a strain unable to grow on this compound. Moreover, the *phaCAB* operon, responsible for the production of PHB, has been knocked out to redirect carbon flow towards the production of 3-HP.

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

Hydrogen-dependent CO_2 reduction by *Escherichia coli*

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

[1]School of Life Sciences, University of Dundee, Dundee, Scotland
[2]Ingenza Limited, Roslin Biocentre Midlothian, Edinburgh, Scotland
[3]Sasol UK, St Andrews, Scotland

The *Escherichia coli* formate hydrogenlyase (FHL) complex is normally produced under fermentative conditions and has a physiological role to couple formate oxidation to hydrogen production. The enzyme is membrane-bound and consists of a molybdenum-dependent formate dehydrogenase linked to a [NiFe]-hydrogenase *via* two Fe-S-cluster-containing subunits. This catalytic domain is anchored to the cytoplasmic face of the inner membrane *via* an integral membrane domain. The genetics of FHL are well understood and genetic engineering has allowed the intact enzyme to be isolated. Moreover, evolutionary biologists hypothesise that progenitors of FHL may have been important enzymes on early Earth, where the 'reverse' activity could have connected H_2 oxidation to CO_2 reduction resulting in the generation of bioavailable formic acid.

Early work suggested FHL could operate in reverse, but at low efficiency. It is now reported that, under newly discovered reaction conditions, the *E. coli* FHL can be harnessed to rapidly and efficiently catalyse 100% conversion of gaseous carbon dioxide to formic acid. By placing gaseous CO_2 and H_2 mixtures under pressure (up to 10 bar), the efficiency of the reverse reaction was significantly enhanced and found to generate >0.5 M formic acid in solution in a few hours. Given *E. coli* is a widely exploited biotechnology workhorse, the potential for bolting this system into other existing bioprocesses is therefore very high. Conversion of gaseous CO_2 to liquid formate would be an acceptable solution in itself to current carbon capture challenges. Formate will be easier to store and transport than CO_2. Indeed, the concept of a formate bioeconomy has recently been mooted: sodium formate is already a tradable commodity and formic acid can be used as an energy store or a feedstock for synthesis of many other biochemical products.

This is a POC ORAL PRESENTATION - by the recipient of BBSRC-NIBB C1net "Proof of Concept" funding.

Engineering Improved Ethylene Production in *Cupriavidus*

RAJAN PATEL[1], SOPHIE VAUD[1], ALEX VAN HAGEN[1], NICOLE PEARCY[1], PIERRE REITZER[1], CARRIE ECKERT[2], PAUL DALBY[3], NIGEL MINTON[1] ALEX CONRADIE[4] and SAMANTHA BRYAN[1]

[1]BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), University of Nottingham, Nottingham, NG7 2RD, UK, [2]NREL, Golden, Colorado, 80401, USA, [3]Department of Biochemical Engineering, UCL, London, WC1H 0AH, UK, [4]Department of Chemical and Environmental Engineering, Faculty of Engineering, University of Nottingham, Nottingham, NG7 2RD.

Ethylene is a small hydrocarbon gas, widely used in the chemical industry. Its annual worldwide production currently exceeds 150 million tonnes, surpassing any other organic compound. Ethylene is currently produced from steam cracking of ethane which produces vast quantities of CO2, contributing to global warming. Ethylene is the monomer for the most common plastic, polyethylene, and annual global production is approximately 80 million tons. Therefore, unlocking a sustainable or carbon neutral alternative to ethylene production is imperative. We aim to engineer *Cupriavidus* sp as a platform for the production of ethylene utilising two different pathways, the ethylene forming enzyme (EFE) from *P. syringae pv. Paseolicola* and the Yang cycle from plants (ACO/ACS).We have already demonstrated that ethylene can be produced in *C. necator* by expressing the *efe* gene from *P. syringae* pv phaseolicola. Utilising a minimal salts medium containing different carbon sources, including fructose, gluconate, glycerol and xylose we were able to generate 300 $nmol/L^{-1}/h^{-1}$ of ethylene. Double the ethylene production seen in genetically modified *E. coli*. We were also able to produce 50nmol/ethylene/OD_{600}/ml utilising the plant pathway considerably more than previously reported. We are currently utilising a combination of metabolic engineering, systems biology and directed evolution coupled with a unique attenuation strategy, to generate a robust couple between product synthesis and biomass growth.

ABSTRACTS OF POSTER PRESENTATIONS

Adopting gas based fermentation processing at Ingenza

REUBEN CARR, PETER GARDNER, SATBIR GILL, ALISON ARNOLD,

Ingenza Ltd, Wallace Building, Roslin Biocentre, Roslin, Midlothian, EH25 9PP

Ingenza are the UK's leading synthetic biology and industrial biotechnology company. The company provides integrated molecular biology, fermentation and chemistry disciplines to serve its customers and clients in the chemicals, biofuels, pharmaceuticals and biologics sectors. Choice of feedstock elected to use in any biobased chemicals manufacturing has a significant impact on the overall process economics and ultimate profitability to transition into manufacturing scale operations. Recently the company has completed an Innovate UK sponsored feasibility study to examine use of methanotrophic production systems and their suitability to process alternate biobased feedstocks. The output from this work has now broadened Ingenza's technology offering of alternative cost effective feedstock options it can offer to its current and future customer base.

Implementation of Inducible Promoter Systems for the Obligate Anaerobe *Acetobacterium woodii*

MATTHIAS H. BECK and PETER DÜRRE

Institute of Microbiology and Biotechnology, Albert-Einstein-Allee 11, University of Ulm, 89075 Ulm, Germany

Acetobacterium woodii represents a promising candidate for the biotechnological production of high-value platform chemicals from CO_2. This can be achieved via the introduction and expression of biosynthesis genes in *A. woodii* and thus shifting its metabolic pathway towards the production of the desired compound. However, pathway engineering sometimes faces major problems since the constitutive expression of some genes appears to be detrimental to the organism.

One solution to overcome this problem is the application of inducible promoter systems. The objective of our studies was the establishment of such systems in *A. woodii* allowing the expression of genes which cannot be expressed constitutively. In addition, the respective system should exhibit a proportionality of the gene expression level in accordance with the applied inducer dose.

Several constitutive and inducible promoter systems were cloned into a Gram-positive/Gram-negative shuttle vector controlling the *gusA* gene (encoding the β-glucuronidase from *Escherichia coli*). The respective promoter systems were analyzed in recombinant *A. woodii* cells grown on fructose via recording the specific β-glucuronidase activity of the cell lysates.

Among several substances tested, *A. woodii* exhibited moderate tolerance to anhydrotetracycline, thus a concentration of 200 ng/ml did not impede growth when added during the exponential growth phase and thereby allowing the usage of a tetracycline inducible promoter system. After induction, GusA assays revealed the activity of this promoter system in a concentration-dependent manner. Only evanescently low activities could be detected when cells were not induced, which is in accordance with a relatively tight regulation of this promoter system. Our data suggest the tetracycline inducible promoter system as a feasible system for *A. woodii* and thus a very promising tool for the controlled expression of genes normally causing problems when constitutively expressed.

Continuous Isopropyl Alcohol Production from CO_2 by *Cupriavidus necator*

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

[1]BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), University of Nottingham, Nottingham, NG7 2RD, UK [2]Faculty of Engineering, University of Nottingham, Coasts Building, University Park, Nottingham, NG7 2RD, UK

Isopropyl alcohol (isopropanol or IPA) is a mature platform chemical currently produced from petrol chemical processes. A biochemical route for IPA production from CO_2 would make the process more sustainable, and expand the market into greener applications. *Cupriavidus necator*, capable of chemolithotrophic growth on CO_2, H_2 and air, was modified to host the IPA production pathway.

IPA pathway comprising of genes, atoAD (acetoacetate acetyl CoA transferase) from *E. coli*, ADC (acetoacetate decarboxylase) from *C. acetobutylicum* and sADH (secondary alcohol dehydrogenase) from *C. bejerinckii,* was constructed as an operon on plasmid (pBBR) under arabinose inducible promoter. Sequences of phaC (PHB synthase) were selected for homologous recombination with the IPA operon in between the homologous arms of phaC or between upstream sequences of phaC gene and downstream sequence of phaB (acetoacetyl CoA reductase) gene. Chromosomal integration was performed by replacing either the phaC gene alone or the phaCAB operon on chromosome 1. Successful knock off of phaC gene or phaCAB operon and knock in of the IPA pathway was verified using PCR and DNA sequencing. IPA production was confirmed from two IPA variants (one with only phaC knock off and other with phaCAB knock off) grown on CO_2.

A phosphorous limiting chemostat gas fermentation process was developed to characterise the IPA production strains at lab scale safely. H_2 was introduced to the bioreactor through a separate sparger. The headspace O_2 concentration was monitored and feedback controlled at the set point of 4% (below the O_2 percentage corresponding to the upper explosive limit of H_2). A proportional–integral controller was set up to regulate the air flow rate by the headspace O_2 concentration. This improved the oxygen transfer rate, and allowed high cell density (> 50 g/L) at steady state. The IPA concentration during the steady state is expected to exceed 5 g/L, which translates to the productivity of 0.1 g/(L·h).

Biorefining of Steel Manufacturing Co-Production Gases

RHIANNON PETA CHALMERS-BROWN

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

The research aim is to design, build and optimise a reactor that could be used by the steel industry to reduce the environmental impact of the release of greenhouse gases into the atmosphere and benefit from the use or re-sale of products formed in the process. 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.

The study investigates whether an anaerobic stirred tank reactor can be used to treat blast furnace gas (BFG) using a mixed culture collected from a wastewater treatment plant. Ethanol, a common product of existing biorefining technologies, can be used as a stand-alone fuel or to replace Methyl Tertiary Butyl Ether, an anti-knocking agent used in engine fuel. This results in a reduction of CO and NOx, although carbon is re-released to the atmosphere as CO_2 on combustion. Ideally, carbon captured from waste off-gases would be converted to useful products and stored in the form of coatings or chemicals to reduce the amount of GHGs being released into the atmosphere. Acetate 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. Batch studies were used to determine the capability of the bacteria to produce acetate from BFG ratios with the view to then optimising the continuous stirred tank reactor. The batch reactions used the Oxitop pressure gauge system to establish whether the gases were consumed by the mixed culture, therefore resulting in a drop of pressure and the production of acetic acid. The process shows immense potential as a waste treatment process as well as a valuable addition to the chemical and steel industries.

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

Thermophilic and Mesophilic Acetogens as Production Platform for Enzymes

HELGE M. DIETRICH, CHRISTIAN ÖPPINGER, MIRKO BASEN
and VOLKER MÜLLER

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

Many enzymes of acetogens that are important for energy conservation as well as product formation are waiting for their molecular characterization[1,2]. Hence, a site-directed mutagenesis system is a prerequisite. Unfortunately, heterologous overproduction of active acetogenic enzymes in hosts such as *Escherichia coli* prooved to be impossible. This is due to the presence of unusual cofactors that those hosts are not able to produce. Therefore, the establishment of a homologous production system is of prime interest.

We have established a procedure to plate *Acetobacterium woodii* with high efficiency and to grow the bacteria on agar plates. We also established a transformation protocol and will demonstrate the homologous production of an alcohol dehydrogenase and an electron-bifurcating enzyme with retention of full activity.

The plating procedure as well as a procedure to introduce DNA was also established for the thermophilic bacterium *Thermoanaerobacter kivui*. The genes encoding the hydrogen-dependent CO_2 reductase (HDCR) were cloned into a pMU-based vector and expressed under the control of the S-Layer promotor. This led to the production of active HDCR. The enzyme was purified by affinity chromatography using an engineered His-Tag.

1. Poehlein A, Schmidt S, Kaster AK, Goenrich M, Vollmers J, Thürmer A, Bertsch J, Schuchmann K, Voigt B, Hecker M, Daniel R, Thauer RK, Gottschalk G, Müller V. 2012. An ancient pathway combining carbon dioxide fixation with the generation and utilization of a sodium ion gradient for ATP synthesis. PLoS One **7**:33439

2. Hess V, Poehlein A, Weghoff MC, Daniel R, Müller V. 2014. A genome-guided analysis of energy conservation in the thermophilic, cytochrome-free acetogenic bacterium *Thermoanaerobacter kivui***.** BMC Genomics **15**:1139

Improving Energetics in Acetogenic Carbon Dioxide Fermentation

CATARINA ERZ and PETER DÜRRE

Institute of Microbiology and Biotechnology, Albert-Einstein-Allee 11, University of Ulm, 89075 Ulm, Germany

Increasing the supply of fuel as well as reduction of greenhouse gas emissions and thereby preventing crop-based feedstocks represents a worldwide goal. Gas fermentation using carbon dioxide as feedstock is a sustainable alternative to crop-based production of fuels and chemicals and becomes more and more important, thus gas fermentation technology is evolving rapidly. This technique uses a broad spectrum of carbon-rich waste gases such as synthesis gas (syngas) containing mainly carbon monoxide (CO), hydrogen (H_2), and carbon dioxide (CO_2).

There is a certain group of bacteria, acetogenic bacteria (acetogens), that use this waste gas as sole carbon and energy source without requiring light or oxygen. Thereby, reducing equivalents for reduction of CO_2 is gained from molecular H_2 or CO_2, or a mixture thereof. The products formed from CO_2 and H_2 as energy and carbon source differ in acetogens. Acetate is often the sole end-product, while ethanol occurs as by-product in some cases. In fact, formation of products is dependent of three main factors: the enzymatic portfolio of the organism, the energy state of the cell (ATP content), and the redox balance of the fermentation. Hence, these factors do also have an impact on products generated by metabolic engineering, especially if they are energy-demanding.

Our aim is to form other products, such as acetone, diols, or olefins from CO_2 and H_2 using acetogens. However, these products do not lead to a positive energy balance in these bacteria. We want to transfer additional energy-generating modules into *Rhodobacter* nitrogen fixation (Rnf)-complex possessing acetogens including energy-converting hydrogenase (Ech)-complex or methylene-THF reductase. Furthermore, acetone production in *Acetobacterium woodii* from CO_2 and H_2 (Hoffmeister et al., 2016) might be further enhanced by exchanging enzymes responsible for acetone formation with enzymes showing better kinetics.

An Unusual Formate Hydrogenlyase in *Pectobacterium atrosepticum*

ALEX J. FINNEY, SARAH COULHURST & FRANK SARGENT

Molecular Microbiology, Medical Sciences Institute, University of Dundee, Dow Street, Dundee, DD1 5EH, UK

A multitude of microorganisms possess the ability to metabolise molecular hydrogen (H_2). Hydrogenases are the major contributors to microbial hydrogen metabolism. These enzymes catalyse the reversible conversion of molecular hydrogen to protons and electrons ($H_2 \leftrightarrow 2H+ + 2e-$). Increasingly these enzymes are being utilised for biotechnological applications such as hydrogen fuel cells but they also represent promising drug targets. Hydrogenases are categorised by their active site architecture. One well studied group is termed the [NiFe]-hydrogenases, which all harbour a complex Ni-Fe-CO-2CN active site in the 'large' subunit and have three Iron-Sulfur clusters within a 'small' subunit.

Much of the basic understanding of these complex enzymes derives from the study of native *Escherichia coli* hydrogenase enzymes. Within *Escherichia coli* genome are two operons encoding two distinct [NiFe]-hydrogenase enzymes, Hydrogenase 3 and 4, able to form Formate Hydrogenlyase (FHL) complexes with Formate dehydrogenase (FdhF). Similarity between membrane domains of Hydrogenase 4 and Respiratory Complex 1 suggest an interesting new link between membrane potential and hydrogenase activity. Unfortunately, work on *E. coli* Hydrogenase 4 is currently impossible in laboratory conditions, a new model organism for Hydrogenase 4 FHL complexes is required.

Through data mining of *Enterbacteriaceae*, a putative Hydrogenase 4 encoding operon was identified as part of a large gene cluster within the *Pectobacterium atrosepticum* SCRI1043 genome. This large gene cluster encodes two hydrogenases orthologous to Hydrogenase 2 and Hydrogenase 4 from *E. coli* as well as Hyp family maturase enzymes, an FdhF orthologue and a putative Ni^{2+} transporter. Here we show initial characterisation of *P. atrosepticum* hydrogenases using genetic and biochemical approaches. To our knowledge this is the first time any Hydrogenase 4 orthologue has been shown to produce molecular hydrogen.

Engineering of *Cupriavidus necator* for the Production of Butanediols 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

Butanediols are widely used in the synthesis of polymers, specialty chemicals and important chemical intermediates. 1,3-butanediol is of particular interest due to the optically active form (*R)*-1,3-butanediol that is used for synthesis of industrial chemicals and as a key intermediate for beta-lactam antibiotic production. Chemical synthesis often produces a racemic mixture of *R*- and *S*- forms, whereas the bio-based 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 the expression of heterologous enzymes in combination with gene deletions, we engineer *C. necator* H16 for production of 1,3-butanediol.

BioFlux Capacitor – An open source software suite for the integration of retrosynthesis methods with genome scale metabolic models

JAMES GILBERT[1], NICOLE PEARCY[1] AND JAMIE TWYCROSS[2]

[1] BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), University of Nottingham, Nottingham, NG7 2RD, UK
[2]School of Computer Science, University of Nottingham, Nottingham, NG8 1BB

The expression of heterologous enzymatic reactions for the production of platform chemicals in microbial species is a field of immense economic, scientific and social potential. However, the size and scale of the universal network of metabolism leads to huge potential pitfalls for the selection of pathways *in silico*. Consequently, retrosynthesis methods have become an increasingly popular technique for the selection of entirely novel routes towards natural products. Whilst these approaches can be successful in finding viable routes, current software is limited in a number of ways. For example, methods are often proprietary, with algorithms being hidden from the wider scientific community limiting future advancements from being made with improvements shared with the wider scientific community. Similarly, much of the software available fails to provide good integration with specific microbial chassis of interest, with users being limited to standard strains.

In this work we present BioFlux Capacitor, an open source software tool we are developing that integrates retrosynthesis algorithms with genome scale models and powerful constraints-based methods for the prediction of steady state fluxes. This tool exploits distributed computing to predict potential routes for chemicals in condition dependent scenarios such as growth under nutrient limitation. Results are compared using maximum theoretical yields, pH dependent thermodynamics and nutrient demands. Furthermore, pathways are exportable as cobra models to facilitate further analysis such as the discovery of potential gene knock-out and amplification targets. Preliminary results are presented for potential pathways in t-he industrially relevant *Cupriavidus necator.*

A 3-Hydroxypropionic Acid-Inducible System for Gene Expression Control in *Cupriavidus necator*

ERIK. K. R. HANKO, NIGEL P. MINTON, NAGLIS MALYS

BBSRC/EPSCR Synthetic Biology Research Centre, Centre for Biomolecular Sciences, University Park, University of Nottingham, Nottingham, NG7 2RD, UK

Inducible gene expression systems are essential tools in synthetic biology and metabolic engineering. They are frequently applied to fine-tune enzyme levels in rationally designed biosynthetic pathways or to monitor *in vivo* metabolite concentrations. 3-hydroxypropionic acid (3-HP) is an important platform chemical used as a precursor for production of added-value compounds such as acrylic acid. Metabolically engineered yeast, *Escherichia coli*, cyanobacteria and other microorganisms have been developed for the biosynthesis of 3-HP. *Cupriavidus necator* is a Gram-negative, chemolithoautotrophic bacterium which is naturally capable of accumulating large amounts of poly-3-hydroxybutyrate (PHB). It is considered to be a suitable chassis organism for the production of 3-HP from C1 gas.

Attempts to overproduce this compound in recombinant *Pseudomonas denitrificans* revealed that 3-HP is consumed by this microorganism using the catabolic enzymes encoded by genes *hpdH*, *hbdH* and *mmsA*. 3-HP-inducible systems controlling the expression of these genes have been predicted in proteobacteria and actinobacteria. Here, we identify and characterise 3-HP-inducible promoters and their corresponding LysR-type transcriptional regulators from *Pseudomonas putida* KT2440. A newly-developed modular reporter system proved possible to demonstrate that *Pp*MmsR/P_{mmsA} and *Pp*HpdR/P_{hpdH} are orthogonal and highly inducible by 3-HP in *E. coli* (12.3- and 23.3-fold, respectively) and *C. necator* (51.5- and 516.6-fold, respectively). We investigate the kinetics and dynamics of the *Pp*HpdR/P_{hpdH} switchable system in response to 3-HP and show that it is also induced by both enantiomers of 3-hydroxybutyrate. These findings pave the way for use of the 3-HP-inducible system in synthetic biology and biotechnology applications.

A Synthetic Pathway to Isopropanol from C1 Gas

CHRISTOPHER M. HUMPHREYS, ROBERT MANSFIELD, PETER ROWE, KLAUS WINZER, NIGEL P. MINTON

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

The future sustainable production of chemicals and fuels from non-petrochemical sources, while at the same time reducing greenhouse gas (GHG) emissions, represent two of society's greatest challenges. Microbial chassis able to grow on waste carbon monoxide (CO) and carbon dioxide (CO2) can provide solutions to both. The development of advanced genetic tools in parallel with accurate metabolic modelling has allowed us to reach a stage where these chassis may be modified to diversify their range of products to more industrially relevant chemicals and fuels, and redirect carbon flux towards these pathways.

Clostridium autoethanogenum is an exceptionally industrially relevant chassis, currently used for ethanol production at industrial scale within Lanzatech's commercial process, and is capable of natively producing ethanol, acetate, 2,3-butanediol and lactate. Through controlled expression of a synthetic operon comprising five genes from related species (*C. acetobutylicum* and *C. beijerinckii*), we have demonstrated inducible isopropanol production under heterotrophic and autotrophic growth conditions.

The development of a CRISPR-based in-frame deletion system within this organism has also enabled the generation of a wide range of combinatorial gene knockouts in key solventogenic and metabolic pathways. Product spectrum analyses of these strains will allow validation and refinement of genome scale metabolic models produced within the SBRC, and potentially give rise to non-intuitive combinatorial knockout strains optimised toward the production of isopropanol.

Interactions Between Yeast and Microalgae For Biofuel Production

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

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

Microorganisms such as oleaginous yeasts and microalgae are high lipid producers that can be used to produce biodiesel. They have potential for biofuel production to help achieve a circular CO_2 economy. However, biodiesel production from these microorganisms cannot yet be commercialised due to low lipid yields and high associated costs.

Due to the plasticity of cell-cell interactions, the synergistic interactions that can occur during the co-culture of these two microorganisms has great potential in enhancing lipid yields for biofuel production. A novel co-culture technique on agar plates was devised to investigate the interactions between these two microorganisms. Investigation into effects of gaseous exchange and diffusible molecules released by microorganisms are achievable by this technique.

Changes in optical density reading showed that higher growth of yeasts could enhance the specific growth rate of microalgal growth from 0.019 ± 0.04 day^{-1} in monocultures to 0.26 ± 0.03 day^{-1} in co-cultures by gaseous exchange. In contrast, during investigation into effects of diffusible molecular exchange, the enhanced microalgal growth was postulated to be more dependent on a higher number of yeast cells available. Preliminary experiments using direct infusion electrospray ionisation mass spectrometry were accomplished to allow identification of possible metabolites and molecules being exchanged by diffusion in agar. The understanding of their interactions will be useful for applications in synthetic biology such as synthetic engineering of these microorganisms.

Establishment of a protocol for Genetic Manipulation of the Acetogenic Bacterium *Clostridium aceticum*

MICHAEL M. KARL and PETER DÜRRE

Institute of Microbiology and Biotechnology, Albert-Einstein-Allee 11, University of Ulm, 89075 Ulm, Germany

Clostridium aceticum was the first isolated autotrophic acetogen. It is able to use gases such as syngas and H_2+CO_2 via the Wood-Ljungdahl pathway and forms acetate. Therefore, *C. aceticum* is an interesting biocatalyst for the production of biofuels and biochemicals. Like *C. ljungdahlii* and *Acetobacterium woodii* it uses an Rnf complex to pump cations for energy conservation. Unlike those two organisms, *C. aceticum* also contains a cytochrome. The role of cytochromes during autotrophic growth is unclear. To elucidate the function of these cytochromes a toolkit for genetic engineering was developed.

The whole genome of *C. aceticum* revealed several annotated restriction endonucleases. To test the ability of those endonucleases regarding degradation of the plasmid to be transformed, a restriction assay with cell free extract of *C. aceticum* was performed. This assay revealed a distinct digestion pattern, which could be associated with the *Mva*I/*Bcn*I endonuclease family protein (CACET_c27460).

To overcome this potential transformation barrier a shuttle-vector without *Mva*I recognition sites was designed. The resulting plasmid pMK83 showed no more degradation during the restriction assay. This plasmid was then successfully transformed into *C. aceticum* using electroporation.

The major obstacle for successful electroporation was identified through a restriction assay. With the constructed plasmid pMK83 the restriction-/modification-system of *C. aceticum* could be circumvented, which allowed us to find a suitable protocol for genetic manipulation of this organism. So far, this is the first reported, stable, and reproducible transformation protocol for *C. aceticum* and is the basis for elucidating the role of cytochromes in this organism.

Regulation of Substrate Utilization in Acetogenic Bacteria

ALEXANDER KATSYV and VOLKER MÜLLER

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

Acetogenic bacteria are metabolically very versatile. They can oxidize a wide variety of organic substrates to acetate, carbon dioxide and electrons[1]. The latter are then converted in the Wood-Ljungdahl pathway to an additional acetate[2]. Many acetogens can also grow mixotrophically, but how the substrates are recognized and how the expression is regulated is largely unknown. With the advent of genome sequencing and omics studies the molecular basis of substrate utilization can now be addressed.

Lactate is a carbon and energy source for acetogenic bacteria such as *Acetobacterium woodii*[3]. The lactate-utilization genes are clustered together with a potential lactate transporter on the genome of *A. woodii*. Upstream of the *lct*-genes is a gene encoding a potential GntR-like transcriptional regulator. To address its function in regulation of *lct*-gene expression, the gene was cloned and expressed. The heterologously produced protein was purified by affinity chromatography to apparent homogeneity. The purified lctA regulator was mixed with DNA from the intergenic region upstream of the *lct*-genes and electrophoretic mobility shift assays indeed revealed that the protein bound to this DNA region. Further studies also showed that lactate could abolish the bond between lctA and the intergenic region. This is the first transcriptional regulator identified in *A. woodii*.

1. Balch WE, Schoberth S, Tanner RS, Wolfe RS. 1977. *Acetobacterium*, a new genus of hydrogen-oxidizing, carbon dioxide-reducing, anaerobic bacteria. Int J Syst Bact **27**: 355-361

2. Müller V. 2003. Energy conservation in acetogenic bacteria. Appl Environ Microbiol **69**: 6345-6353

3. Weghoff MC, Bertsch J, Müller V. 2015. A novel mode of lactate metabolism in strictly anaerobic bacteria. Environ Microbiol **17**:670-677

Hydrogenogenesis from Formate by the Acetogen *Acetobacterium woodii*

PATRICK KOTTENHAHN, KAI SCHUCHMANN, VOLKER MÜLLER

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

Acetogenic bacteria such as *Acetobacterium woodii* and *Thermoanaerobacter kivui* have an enzyme that directly uses hydrogen gas for the reduction of CO_2 [1,2]. This enzyme, the hydrogen-dependent CO_2 reductase or HDCR, contains a hydrogenase module, a formate dehydrogenase module and two small electron-transferring subunits. The HDCR catalyzes H_2-dependent CO_2 reduction to formate as well as hydrogen (and CO_2) formation from formate. Thus, the enzyme is of considerable biotechnological interest in, for example, hydrogen storage and hydrogen formation from formate.

Whole cells are mostly superior over purified enzymes as industrial biocatalysts. Therefore, we have developed a whole cell system based on *A. woodii* to convert hydrogen to formate. Here we describe the reverse reaction, hydrogenogenesis from formate in *A. woodii*. We present an uncoupled whole cell system able to convert formic acid to hydrogen gas without production of acetate. The kinetics of the process as well as the dependence of the rate of hydrogen formation on physical and chemical parameters will be discussed.

1. Schuchmann, K., Müller, V. (2013). Direct and reversible hydrogenation of CO_2 to formate by a bacterial carbon dioxide reductase. Science **342**:1382-1385

2. Hess, V., Poehlein, A., Weghoff, M.C., Daniel, R., Müller, V. (2014) A genome-guided analysis of energy conservation in the thermophilic, cytochrome-free acetogenic bacterium *Thermoanaerobacter kivui*. BMC Genomics 15:1139

Metabolic engineering of *Cupriavidus necator* H16 for production of platform chemicals

KATALIN KOVÁCS, CHRISTIAN ARENAS, DIEGO OROL, JESSICA LOCKER, CHRISTIAN GUDE, CALLUM MCGREGOR, GIORGIA TIBALDERO, KLAUS WINZER and NIGEL P. MINTON

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

There is an urgent need to develop technologies for the sustainable production of platform chemicals form cheap and renewable sources. The Synthetic Biology Research Centre (SBRC) Nottingham has set out the challenging task of engineering *Cupriavidus necator* strain H16 as the main microbial factory for the production of C2 (i.e. ethylene), C3 (i.e.3-hydroxypropionic acid) and higher carbon (C5 and polymeric compounds) platform chemicals from CO_2 as sole carbon source. To do so, we are using an integrative approach, making use of our in-house genome scale model (GSM), our unique gas fermentation facility and expertise in metabolic engineering.

Our first target platform chemicals is 3-hydroxypropionic acid or 3-HP, which can be converted to acrylic acid, methyl acrylate, acrylamide, 3-hydroxypropionaldehyde (3-HPA), and into poly(3-HP) and other biodegradable polymers. Biological synthesis of 3-HP proceeds through several metabolic pathways; two of these were considered for engineering in *C. necator* H16, as they are the most thermodynamically favourable routes. To date, the pathway proceeding via beta-alanine proved to be the most successful. Synthetic pathways were constructed to increase the carbon flux towards beta-alanine synthesis as well as synthetic pathways for the conversion of beta-alanine to 3-HP. Further synthetic pathways for conversion of 3-HP to higher carbon compounds (i.e.C5) are also being constructed and tested. In parallel, production of 3-HP polymer and p(3-HP)-poly(3-hydroxybutyrate) or p(3-HP)-p(PHB) co-polymers are being investigated.

As 3-HP is our main intermediate for the synthesis of the above mentioned products, we used the GSM to assess available strategies to further increase 3HP production in *C. necator.* Coupling 3-HP production to growth via the glutamate/glutamine synthesis pathways is one such strategy and requires overexpression and deletion of competing pathways. These pathways are described and presented on the poster along with the results achieved to date.

Methanol Metabolism in *Acetobacterium woodii* and *Eubacterium limosum*

FLORIAN KREMP, DENNIS LITTY and VOLKER MÜLLER

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

Acetogenic bacteria are used as industrial plattforms for the conversion of C_1 substrates such as in syngas (CO_2, CO) to acetate or ethanol[1]. Methanol is the C_1 substrate of acetogens that is of considerable biotechnological interest. Industrially it is produced chemically from, for example, syngas and then used as building block in chemical syntheses. Alternatively, methanol can be converted by acetogenic bacteria to acetate and ethanol[2]. *Eubacterium limosum* and *Acetobacterium woodii* are very robust acetogenic methanol converters. The methyl group is channeled by the action of O-demethylases to tetrahydrofolic acid (THF) and the resulting methyl-THF is disproportionated. One quarter is oxidized to CO_2 by a reversal of the methyl branch of the Wood-Ljungdahl pathway, yielding 6 electrons, one ATP and one CO_2 per methyl group. The electrons are then used to reduce the one CO_2 produced plus two additional CO_2 to CO, which is combined with three more methyl groups and CoA to three acetyl-CoA. The biochemistry and the energetics of the pathway is described.

The first step in methyl group oxidation is highly endergonic and its driving force is unknown. We purified the enzyme, the methylene-THF reductase, from methanol-grown cells to apparent homogenity. The enzyme catalyzed NADH-dependent methylene-THF reduction but not methyl-THF oxidation coupled to NAD reduction. Electron bifurcation using reduced ferredoxin as co-reductant was ruled out[3]. We will present an alternative model how the energetic barrier in methyl group oxidation will be overcome.

1. Bertsch J, Müller V. 2015. CO metabolism in the acetogen *Acetobacterium woodii*. Appl Environ Microbiol **81**:5949-5956

2. Sharak Genthner BR, Bryant MP. 1987. Additional characteristics of one-carbon-compound utilization by *Eubacterium limosum* and *Acetobacterium woodii*. Appl Environ Microbiol **53**:471-476

3. Bertsch J, Öppinger C, Hess V, Langer JD, Müller V. 2015. Heterotrimeric NADH-oxidizing methylenetetrahydrofolate reductase from the acetogenic bacterium *Acetobacterium woodii*. J Bacteriol **197**:1681-1689

Pressurised Anaerobic Digestion of Algae for Higher Energy Biogas

ZHIXUAN LIANG and STEPHEN WILKINSON

Department of Chemical Engineering, University of Chester Thornton Science Park, Pool Lane, Ince, Chester, CH2 4NU

Biogas generation from wastewater, organic wastes and marine algae has received more and more attention since it is an important potential source of renewable energy. Depending on the waste composition and operating parameters of the reactors, the methane content in biogas production generally varies from 50% to 65% with most of the remainder being carbon dioxide. The biogas is typically 'sweetened' to increase the methane content by removing the carbon dioxide which increases the calorific value and makes combustion more efficient. Conventional methods to does this include additional processing steps such as absorption of carbon dioxide using water or amines. We propose a new method of high pressure operation in which the biogas produced has higher methane content due to the higher solubility of carbon dioxide in the fermentation medium compared to that of methane. We have investigated the effect of pressure on biogas production and our results demonstrate that the headspace pressure has only a slight negative correlation on the total amount of methane produced. This suggests that our approach might be advantageous for small scale production of biogas suitable for direct combustion for local energy needs.

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.

Engineering Isoprenoid Bioproduction in the Facultative Autotroph *Cupriavidus necator*

NAGLIS MALYS, SWATHI ALAGESAN, ERIK K.R. HANKO, MARCO GARAVAGLIA, NIGEL P. MINTON

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

Isoprenoid natural products are well known for their use in medicine, food and agriculture industries. Furthermore, some isoprenoids, such as isoprene, can be developed for application as platform chemicals. The isoprene is emitted naturally by plants in large quantities, whereas its current industrial production is based on thermal cracking of oil products. As a sustainable alternative, the microbial fermentation-based process is required for isoprene production. *Cupriavidus necator* H16 is a gram-negative hydrogen-oxidizing bacterium, which is capable of growing autotrophically by utilizing carbon dioxide as a sole carbon source. We engineer *C. necator* for the bioproduction of isoprenoids.

Metabolic Engineering of Cupriavidus Necator H16 for Production of Polyhydroxyalkanoates from CO_2

CALLUM MCGREGOR, KATALIN KOVACS, NIGEL MINTON

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

Plastics are a globally important commodity, with uses in virtually all sectors of the modern world. This is reflected by the production of approximately 322 million tonnes of plastic in 2016. Two key disadvantages of plastics are their derivation from fossil fuels, the reserves of which are finite, and their recalcitrant nature, which leads to waste plastic persisting in nature, causing damage to the environment. An alternative to conventional plastics is clearly necessary.

Polyhydroxyalkanoates (PHAs) are polymeric carbon storage materials accumulated by a variety of microorganisms. PHAs are biodegradable yet retain properties similar to conventional plastics. Furthermore, PHAs are known to accumulate to very high levels in certain bacteria. Large-scale production of PHAs from bacteria could provide a renewable source of biodegradable plastic.

Cupriavidus Necator is the model organism for the study of PHA accumulation. The major PHA species accumulated in *C. necator* is poly(3-hyroxybutyrate) (P(3HB)). P(3HB) has been seen to occupy up to 90% cell dry mass in *C. necator*. Additionally, *C. necator* is able to grow and accumulate P(3HB) using CO_2 as a sole carbon source.

Unfortunately, the properties of P(3HB) are poor. Commercial applications for P(3HB) are therefore limited. Diversifying PHA composition greatly affects material properties. While P(3HB) is a brittle material, poly(3-hydroxypropionate) (P(3HP) resembles an opaque film. Copolymers such as P(3HP-4HB) can range from clear films to firmer plastics depending on the monomer ratio. PHAs containing low 3(hydroxyhexanoate) content have been found to have adhesive properties.

Improved properties and growth on cheap carbon sources will enhance the commercial viability of PHAs. We aim to produce PHAs of varied compositions from CO_2 through genetic and metabolic engineering of *C. necator*. Our initial work has produced small amounts of P(3HP-HB) via an engineered pathway using β-alanine as a precursor to 3HP.

Metabolic shift in *Clostridium autoethanogenum*

THOMAS MILLAT, ANNE M. HENSTRA, KLAUS WINZER, NIGEL P. MINTON

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

Recent international demands for a global reduction of fossil fuel consumption and effective carbon recycling facilitate activities towards the development of biotechnological approaches addressing these important objective. Subsequently, the acetogen *Clostridium autoethanogenum* has attracted academic and industrial interest due to its ability to convert waste gases (CO, CO_2 and H_2) into value-added biochemicals and biofuels including ethanol and 2,3-butanediol. To transform *C. autoethanogenum*'s natural capabilities into an efficient and reliably controllable bioprocess, an improved understanding on external constraints and cellular adaptions orchestrating the metabolic activity of the cells is required.

We studied the response of a continuous culture to changing environmental conditions with a focus on process-relevant parameters like CO uptake and external pH. Grown on CO as sole carbon and energy source, *C. autoethanogenum* predominantly forms the liquid products acetic and ethanol and in minor amounts 2,3-butanediol and lactic acid. Furthermore, hydrogen and carbon dioxide are produced, with the first one is important for ATP generation during gas fermentation. We recorded the alterations caused by systematic changes to the process parameters using a multi-omics approach combined with an automated workflow for data processing and data analysis.

Our results indicate that the CO uptaken by the cells modulates the metabolic flow towards the liquid products. Importantly, the transition from carbon-limited growth to non-carbon-limited growth accompanied by further increasing levels of CO supply induces a metabolic switch from acidogenesis to ethanologenesis resulting in a significant rearrangement on transcriptional and metabolomic activity. Furthermore, thermodynamic constraints on the formation of hydrogen ultimately restricts *C. autoethanogenum's* ability to adapt to further increasing CO amounts or changing inter- and intracellular conditions.

How to Make Clostridia Smell Great Again – Higher-Alcohol Production from Syngas with a Co-culture

BASTIAN MOLITOR[a], HANNO RICHTER, MARTIJN DIENDER[b], DIANA Z. SOUSA[b], AND LARGUS T. ANGENENT[a]

[a] *Environmental Biotechnology Group, Center of Applied Geosciences, University of Tübingen, 72074 Tübingen, Germany;*
[b] *Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands*

Carboxydotrophic bacteria (CTB) have received attention due to their ability to synthesize commodity chemicals from producer gas and synthesis gas (syngas). CTB have an important advantage compared to chemical catalysts because of a high product selectivity. However, the product spectrum of wild-type CTB is narrow. Our objective was to investigate whether a strategy of combining two wild-type bacterial strains into a single, continuously fed bioprocessing step would be promising to broaden the product spectrum. Here, we have operated a syngas-fermentation process with *Clostridium ljungdahlii* and *Clostridium kluyveri* with in-line product extraction through gas stripping and product condensation within the syngas recirculation line.

The main products from *C. ljungdahlii* fermentation were ethanol (vinous odor) and acetate (pungent, sour odor). An estimated 2/3 of the total ethanol produced was utilized by *C. kluyveri* to chain elongate with the reverse β-oxidation pathway, resulting in *n*-butyrate (obnoxious, unpleasant, rancid odor) and *n*-caproate (goat-like odor). *C. ljungdahlii* likely reduced the produced carboxylates to their corresponding alcohols with the reductive power from syngas. This resulted in the longer-chain alcohols *n*-butanol, *n*-hexanol, and *n*-octanol (all sweet, fruity odor). These alcohols, in combination with the produced carboxylic acids, may also have formed small amount of esters in the condensed solution, resulting in a fruity and pleasant odor. The continuous production of the longer-chain alcohols occurred only within a narrow pH spectrum of 6-6.3 due to the pH discrepancy between the two strains. To develop this co-culture idea into a promising biotechnology production platform, novel bacteria should be isolated with matching pH optima of ~5-5.5. Such an optimized co-culture would be able to produce mixtures of alcohols, such as *n*-butanol, *n*-hexanol, and *n*-octanol, resulting in a great smelling condensate.

Flavin-Based Electron Bifurcation: Role in Physiology of Acetogens and Insights into the Reaction Mechanism

CHRISTIAN ÖPPINGER and VOLKER MÜLLER

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

ATP and the transmembrane electrochemical ion potential across the cytoplasmic membrane are the two energy currencies of living cells. These two currencies are exchanged by the enzyme ATP synthase that balances both currencies thermodynamically. Both currencies are used to drive endergonic reactions such as involved in chemical, osmotic and mechanical work of a living cell.

In 2008, a novel type of coupling endergonic and exergonic reactions was discovered, Flavin-based electron bifurcation[1]. This process describes the coupling of an exergonic with an endergonic redox-reaction, often used to drive the energetic "uphill" transport of electrons from H_2 or NAD(P)H to ferredoxin. Acetogenic bacteria such as *Acetobacterium woodii* have an electron-bifurcating hydrogenase[2], an electron-bifurcating lactate dehydrogenase[3] and an electron-bifurcating caffeyl-CoA reductase[4]. The physiological significance of these enzymes is discussed.

The caffeyl-CoA reductase from *A. woodii* was produced heterologously in *Escherichia coli* using pET-based plasmids. The enzyme was purified to apparent homogeneity and catalyzed electron- bifurcation from NADH to caffely-CoA and ferredoxin with a specific activity of 2.1 U/mg. Structure-based, site-directed mutagenesis revealed residues involved in electron flow. Those studies gave fundamental insights into the mechanism of a novel coupling mechanism in living cells.

1. Li F, Hinderberger J, Seedorf H, Zhang J, Buckel W, Thauer RK. 2008. Coupled ferredoxin and crotonyl coenzyme A reduction with NADH catalyzed by the butyryl-CoA dehydrogenase/Etf complex from *Clostridium kluyveri*. J Bacteriol **190**:843-850

2. Schuchmann K, Müller V. 2012. A bacterial electron-bifurcating hydrogenase. J Biol Chem **287**:31165-31171

3. Weghoff MC, Bertsch J, Müller V. 2015. A novel mode of lactate metabolism in strictly anaerobic bacteria. Environ Microbiol **17**:670-677

4. Bertsch J, Parthasarathy A, Buckel W, Müller V. 2013. An electron-bifurcating caffeyl-CoA reductase. J Biol Chem **288**:11304-11311

Increasing Mass Transfer In Syngas Fermentation: Effects Of Elevated Pressure

FLORIAN OSWALD[1], INA KATHARINA STOLL[2], MICHAELA ZWICK[1], SOPHIA HERBIG[2], NICOLAOS BOUKIS[2], ANKE NEUMANN[1], JÖRG SAUER[2] AND CHRISTOPH SYLDATK[1]

[1]Institute of Process Engineering in Life Science Section II: Technical Biology,
[2] Institute of Catalysis Research and Technology
Karlsruhe Institute of Technology (KIT), 76133 Karlsruhe, Germany

Syngas-fermentation is the biological conversion of synthesis gases (mixtures of H_2, CO_2 and CO) with specialized anaerobic bacteria. *Clostridium ljungdahlii* is one of those bacteria, an acetogen well investigated for the fermentation of synthesis gas under ambient pressure. The natural main products are acetic acid and ethanol. Although syngas fermentation is a process with first industrial applications, there are still essential problems to be addressed.

One main bottleneck in gas fermentation is gas-liquid mass transfer of low soluble gas components like H_2 and CO. Traditional ways of increasing mass transfer rate in aerobic cultivations like increasing stirrer speed, aeration rate or molar percentage of desired compounds in the gas stream only work in a narrow field of performance. Henry's-Law provides another possibility to overcome these limitations in increasing the pressure within the bioreactor to increase liquid saturation concentrations.

In the 1990s, the University of Arkansas investigated the production of ethanol with *Clostridium ljungdahlii* from a mixture of H_2 and CO. In this study, the pressure was up to 11 bar. A strong influence of the process pressure on composition of the products has been demonstrated. In another study of Younesi et al. (2005), the production of ethanol and acetate on a mixture of H2/CO/CO2 has been studied. The maximum product ratio of ethanol and acetate was achieved at the maximum experimental pressure of 1.8 bar.

The aim of our experiments is to study the fermentation of H_2 and CO_2 mixtures (without CO) under elevated pressure. Therefore we built a high pressure semi batch-stirred-tank reactor system with continuous gas feed of H_2 and CO_2 as well as pH measurement, control and adjustment and on-line analytics of off-gas. So far we are able to investigate pressures of up to 7 bar.

The Effect of Nutrient Limitation on the Product Formation *Clostridium autoethanogenum*

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

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

The industrial application of *Clostridium autoethanogenum* has made it the focus of attention in recent years. This bacterium is an anaerobic facultative autotroph that can utilise both carbon monoxide or carbon dioxide and hydrogen as its carbon and energy source. It produces acetate, ethanol, 2,3-butanediol and lactate natively and has been engineered to produce non-native products amongst which isopropanol. Microorganisms can often be steered towards specific production of target products by applying specific feeding strategies. To study this effect phosphate limitation was applied under several different carbon source regimen. On one specific carbon source, rhamnose, two previously not described native products were formed: 1,2 propanediol and 1-propanol. The production of these products is affected by phosphate limitation, initial carbon source concentration and headspace composition. Next to this discovery these products the effect of phosphate limitation was further studied in continuous stir tank reactors using carbon monoxide as carbon source. Phosphate limitation in this case leads to higher ethanol and 2,3-butanediol production and lower acetate and biomass production. LC-MS reveals that the metabolome is enriched in branched chain amino acids and malate, fumarate and succinate under phosphate limitation.

Investigating the use of Classifiers and Feature Selection Methods for the Prediction of Essential Genes

VANISHA PATEL, JAMES GILBERT and JAMIE TWYCROSS

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

There are a range of laboratory methods which are used to inactivate potentially essential genes, with essentiality being determined by organism survival. While experimental techniques are reliable, they are also expensive and time consuming.

An alternative way is to combine computational techniques and biological features to identify essential genes. There have been many different features proposed for computational prediction, but this can result in more complex and less generalizable models. Feature selection methods can solve this problem by identifying and removing features that do not contribute to the accuracy of the model.

In this study we investigate the accuracy of different machine learning classifiers, focusing on Decision trees, Support Vector machines – Linear and Radial, Random forest, Logistic regression, Neural networks, Nearest neighbours and Naive Bayes. Building and testing the models on 32 bacterial species from the Database of Essential Genes. We also look at the following features selection methods: Recursive Feature Elimination, Sequential Feature Selection and Least Absolute Shrinkage and Selection Operator, and study the accuracy of the subsets selected.

We have demonstrated that across Logistic regression, Decision trees and Linear Support Vector Machines a subset of between 19 to 30 features has the same accuracy as all 61 features. In general, one would aim to find a simpler model. Our results indicate that a reduced feature set produced no significant decrease in classification accuracy.

Investigation of Redox-sensing transcriptional repressor REX deletion in *Clostridium autoethanogenum*

SIMONA PENTASSUGLIA[a], CHRISTOPHER M. HUMPHREYS, PETER ROWE, YANMING WANG, GUIDO SARACCO[a], NIGEL P. MINTON

BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), University of Nottingham, Nottingham, NG7 2RD, UK
[a] Center for Sustainable Futures (CSF)@Polito, Istituto Italiano di Tecnologia, Corso Trento 21, 10129 Torino, Italy

Due to the dilemma related to diminishing fossil fuel reserves coupled with the environmental impact resulting from CO_2 accumulation into the atmosphere, the development of alternative and sustainable bioenergy solutions is required. Among them, gas-fermentation of C1 feedstocks performed by microorganisms, represents a promising opportunity for the production of industrially-relevant low-carbon fuels and commodity chemicals. In this scenario genetic engineering approaches play a key role in improving biocatalysts in terms of biological performance and product spectrum.

Acetogenic bacteria such as *Clostridium autoethanogenum*, are able to ferment gas using CO_2+H_2 and CO as sole energy and carbon sources. Under autotrophic growth conditions *C. autoethanogenum* predominantly produces acetate and ethanol, along with a small amount of 2,3 butanediol (2,3-BDO) and lactate. The effects of the deletion of the gene encoding REX, a redox-sensing transcriptional repressor, whose regulons are involved in energy metabolism and fermentation processes, have been investigated. The deletion has been carried out by a CRISPR-Cas9 genome editing tool developed within the SBRC.

As preliminary characterisation, the REX mutant has been cultivated autotrophically in serum bottles with CO as the sole carbon source and the fermentation products analysed by HPLC. Future perspectives include the characterisation of these stains in a CSTR *(Continuous-Flow Stirred-Tank Reactor)* in order to evaluate their effect on product formation, and therefore relevance to future industrial applications.

Essential genes for 3-hydroxypropionic acid tolerance in Methanotrophs

BASHIR RUMAH, YING ZHANG AND NIGEL P. MINTON

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

3-Hydroxypropionic acid (3-HP) is an important platform chemical and its biosynthesis has been established in *E.coli, C. necator* and many other gram negative bacteria through metabolic engineering of these organisms. One of the phenotypic characteristics required by these organisms to synthesize 3-HP is there tolerance to it. Methane-oxidising bacteria (Methanotrophs) show low tolerance to 3-HP. Here we present the use of TraDis to create a library of mutants that enable the identification of essential genes during growth on relatively high 3-HP concentrations. Ten methanotrophs were isolated from landfill sites, wetlands, cow manure and lakes. These were characterised and their genomes sequenced using PacBio and Illumina sequencing. A miniTn5 Transposon with kanamycin resistant gene was cloned into a vector containing R6K origin of replication. This was transformed into *E.coli* S17-1 λPir. *E.coli* was used to conjugate two different methanotroph isolates (Iso10 and Iso12). Inverse PCR was carried out on gDNA of transconjugants. Transposon Insertion points were identified in each specie which was shown to be random. A library of one million mutants is being created which will be grown on different concentrations of 3-HP to identify the genes involved with 3HP tolerance in methanotrophs. This study exemplifies the importance of mobile genetic elements in metabolic engineering applications.

Development of CRISPR/Cas9-based genome editing tools in *Clostridium autoethanogenum*

FRANCOIS SEYS, NIGEL P. MINTON

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

Clostridium autoethanogenum is a biocatalyst of interest for fermentation of CO, CO_2 and H_2 gases (a.k.a. gas fermentation) into valuable products such as plastics and fuels. Developing gas fermentation technology is of prime importance to increase UK energy security and to mitigate the CO_2 emissions of its industry. However, the extensive genetic engineering effort in *C. autoethanogenum* is still hampered by the lack of effective tools for convenient, multiplex genome editing tools.

The purpose of this PhD will be to implement state-of-the-art CRISPR/Cas9-based genome editing tools, mainly in C*. autoethanogenum* but also in other C1 fermenting organisms such as *Eubacterium limosum*. Three CRISPR/Cas9-based genome editing tools will be developed. Namely, the fusion of a Cas9 nickase and an activation-induced cytosine deaminase (AID) ortholog (PmCDA1) into a so-called Target-AID complex to enable targeted, multiplex point mutagenesis; the creation of a targeted transcriptional repressor based on a nuclease-deficient Cas9; and, finally, the introduction of a heterologous bacterial Non-Homologous-End-Joining (NHEJ) pathway in *C. autoethanogenum* to improve recombination efficiency upon CRISPR/Cas9-based mutagenesis. Together, those three strategies should enable quick modification and regulation of several genes in *C. autoethanogenum*, greatly accelerating the genome editing effort still necessary to improve the potential of this organism for C1 gas fermentation.

Bio-conversion of Methane to Transportation Fuel

CHRISTOPHER STEAD

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

Methane is a potent greenhouse gas with a warming potential up to 72 times greater than carbon dioxide (over a 20 year period). Methane is also a low cost feedstock with high potential for upgrading to higher value liquid fuel, though due to ineffective conversion is released into the atmosphere. Effective bio-conversion and utilisation of methane through methane oxidation is seen in a specialised group of gram negative bacteria called methanotrophs. It is envisaged that this group of organisms can be used as a microbial chassis for upgrading methane to drop-in biofuel.

The aim is to create a bioconversion technology for upgrading methane to drop-in liquid biofuel which will mitigate the global warming potential of these emissions and reduce the UK dependence on imported petroleum.

A range of methanotrophs spanning three genera (*Methylocystis, Methylocaldum and Methylococcus*) were isolated from methane rich environments using enrichment and serial dilution techniques. Two organisms with robust growth characteristics, putatively identified as *Methylocystis rosea* and *Methylococcus capsulatus,* were chosen as potential microbial chassis. Essential characteristics such as genetic tractability were demonstrated via conjugation using pMTL9 series plasmid. Building on this, reporters, promoters, replicons and selection markers were used to establish an expression vector in addition to the development of suicide plasmid based gene knockout. This work provides initial steps in expressing a recombinant metabolism towards methane bioconversion to liquid transportation fuel.

Enzyme Engineering to Improve Ethylene Production in *Cupriavidus necator*

ALEXANDER M. W. VAN HAGEN, SAMANTHA BRYAN, JOSPEH WEBB, CARRIE ECKERT, NIGEL MINTON

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

Currently we are dependent on non-renewable fossil fuels for energy and to produce chemicals and plastics, which has resulted in the release of large amounts of greenhouse gases, significantly increasing global temperatures. We urgently need to find sustainable alternatives.

Ethylene is an industrially significant platform chemical used to produce a wide variety of plastics and chemicals. It is responsible for 1.5% of the USA's carbon footprint. There are a diverse number of Ethylene-forming enzymes (EFE), predominantly found in pathovars of *Pseudomonas syringae*. EFE from *P. syringae* pv. phaseolicola PK2 (EFEP) produces ethylene in a variety of heterologous hosts.

Enzyme engineering techniques are required to improve the performance of the enzyme for ethylene production. We have also demonstrated that co-expression of protein chaperones with EFEP improves ethylene production in *Cupriavidus necator*. The effect on EFEP solubility will also be assessed using these co-expressed chaperones as the solubility of EFEP in heterologous host is a critical factor.

Redox Homeostasis in Acetogens

ANJA WIECHMANN and VOLKER MÜLLER

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

We present a modular view on acetogenesis from various substrates. The common module is the CO_2-reduction pathway, the Wood-Ljungdahl pathway (WLP). Electrons are gained in an oxidation module from organic substrates such as sugars or alcohols or from CO or H_2. Depending on the redox potential of the electron donor, different electron acceptors are used in the oxidative module. Thus, a redox homeostasis module is required which connects the oxidation and reductive module. This module must be able to convert NADH, reduced ferredoxin, NADPH and hydrogen. Three proteins are believed to be necessary for these conversions: the electron-bifurcating hydrogenase, the Nfn-complex and the Rnf-/Ech-complex[1,2].

We will present different scenarios to illustrate the variability and the concerted action of the redox homeostasis module. This modular organisation makes acetogenesis a prime candidate for a synthetic biology approach to change the substrate and product portfolio of acetogens to be used as industrial production platforms.

1. Schuchmann K, Müller V. 2014. Autotrophy at the thermodynamic limit of life: A model for energy conswervation in acetogenic bacteria. Nat Rev Microbiol **12:**809-821

2. Bertsch J, Müller V. 2015. Bioenergetic constraints for conversion of syngas to biofuels in acetogenic bacteria. Biotechnol Biofuels **8:**(210)1-12

Novel Aerobic Chassis for the Conversion of Mixed CO/CO2 Feedstocks

CAROLINE KEMP and KLAUS WINZER

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

The aim of this project was to isolate and characterise novel bacterial strains capable of aerobic growth at 30°C and/or 45°C with CO and CO/H2 as the sole sources of carbon and energy. Samples were obtained from seven different locations within the UK and Germany, and up to six different sites within each location. These included natural soil and water habitats located within nature reserves as well as CO polluted locations, with soil sampling sites located on top of underground coal seam fires.

Over the course of the project, different enrichment and isolation regimes were used, but all involved multiple transfers in semi-defined media containing CO as the sole source of carbon and energy. Growth under these conditions was generally very slow, requiring initial incubations for several weeks before being observable macroscopically.

Several hundred separate enrichments were carried out, yielding a total of 161 putative carboxydothrophic isolates, 118 and 43 from the 30°C and 45°C isolation regimes, respectively. However, growth of most of these isolates was very poor. Furthermore, those that grew reasonably well only did so in the form of distinct pellicles, which made direct comparisons to a control strain (*Oligotropha carboxidovorans*) difficult and also prevented the use of a higher throughput, automated micro-fermentation platform (BioLector Pro). From these, 15 isolates were chosen for 16S rDNA sequencing and analysed for accumulation of intracellular storage compound (4 and 11 strains from the 30°C and 45°C regimes, respectively). Thermophilic isolates appeared to be related to the genera *Steptomyces, Bacillus, Brevibacillus* and *Nomomuraea*. The 16S rDNA data suggested the presence of 6 unique species, as several isolates yielded identical sequences. The mesophilic isolates seemed to belong to the β- and γ-proteobacteria, including the previously described genus *Hydrogenophaga*. Only three isolates related to *Streptomyces* and *Hydrogenophaga* produced storage compounds under the conditions tested, most likely polyhydroxyalkanoates.

This is a POC POSTER PRESENTATION - by the recipient of BBSRC-NIBB C1net "Proof of Concept" funding.

Transposon Mutagenesis of *C. autoethanogenum*

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

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

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

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

Clostridium autoethanogenum is able to utilise CO_2 + H_2 or CO or as a sole carbon and energy source and produce valuable fuel and chemical compounds. TraDIS can be used to identify the underlying genetics of this important biological phenomenon. One challenge to the microbial production of fuels and chemicals is that of product toxicity. TraDIS can identify differential gene fitness values with or without the presence of toxic products to provide targets for the directed strain engineering of a more robust production organism.

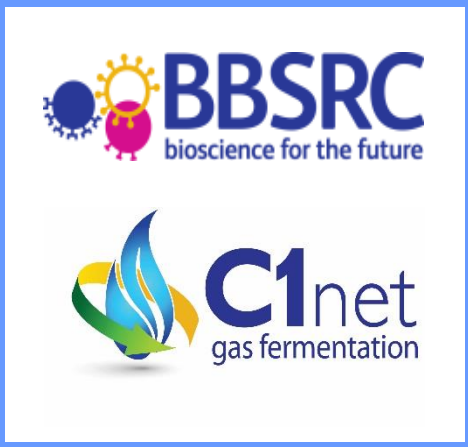
PARTICIPANTS

SURNAME	FIRST NAME	INSTITUTE	ROLE	EXPERTISE	EMAIL ADDRESS
Angenent	Lars	University of Tübingen, Germany	Professor	Syngas fermentation	l.angenent@uni-tuebingen.de
Anyanwu	Valentine	University of Nottingham, UK	PhD student	Metabolic Engineering	enxvea@nottingham.ac.uk
Arenas	Christian	University of Nottingham, UK	PhD student	Gas fermentation	mrxca1@nottingham.ac.uk
Arnold	Alison	Ingenza, UK	Team leader	Gas fermentation	alison.arnold@ingenza.com
Asimakopoulos	Konstantinos	Technical University Denmark	PhD student	Mixed microbial consortia	kona@kt.dtu.dk
Azbar	Nuri	Ege University, Turkey	Professor	Microbiological CO2 capture	nuri.azbar@ege.edu.tr
Baker	Jonathan	University of Nottingham, UK	PDRA	Synthetic biology	jonathan.baker@nottingham.ac.uk
Beck	Matthias	University of Ulm, Germany	PhD student	Acetogenic bacteria	matthias.beck@uni-ulm.de
Bjorck	Charlotte	University of Sheffield, UK	PhD student	Methanotrophs	c.bjorck@sheffield.ac.uk
Bommareddy	Rajesh	University of Nottingham, UK	PDRA	BioProcess Development	rajesh.bommareddy@nottingham.ac.uk
Breitkopf	Ronja	University of Nottingham, UK	PhD student	Synthetic Microbiology	mrxrb14@nottingham.ac.uk
Brigham	Christopher	University of Massachusetts, USA	Assistant Professor	Bioengineering	cbrigham@umassd.edu
Bryan	Samantha	University of Nottingham, UK	PDRA	Microbiology, Biochemistry	samantha.bryan@nottingham.ac.uk
Burbidge	Alan	University of Nottingham, UK	Centre Manager	IB commercialisation	alan.burbidge@nottingham.ac.uk
Chalmers-Brown	Rhiannon	University of South Wales, UK	PhD student	Carbon Capture	rhiannon.chalmers-brown@southwales.ac.uk
Conradie	Alex	University of Nottingham, UK	Professor	Process control	alex.conradie@nottingham.ac.uk
Cornack	Ruth	University of Nottingham, UK	Research Technician	Molecular Biology	ruth.cornock@nottingham.ac.uk
Crombie	Andrew	University of East Anglia, UK	PDRA	Molecular Biology, Microbial Genetics	a.crombie@uea.ac.uk
Cudzich-Madry	Alexander	University of Nottingham, UK	PhD student	Synthetic Biology	Alexander.Cudzich-Madry@nottingham.ac.uk
Daglioglu	Tugce	Ege University, Turkey	Research Assistant	Anaerobic Bioprocess	kalkantugce@gmail.com
Dalwadi	Mohit	The University of Nottingham, UK	PDRA	Mathematical modelling	mohit.dalwadi@nottingham.ac.uk
De Winter	Karel	Bio Base Europe Pilot Plant, Belgium	Business Developer	Scale-up fermentation	karel.de.winter@bbeu.org
Dietrich	Helge	Goethe University, Germany	PhD student	Biochemistry	dietrich@bio.uni-frankfurt.de
Duerre	Peter	University of Ulm, Germany	Professor	Anaerobes	peter.duerre@uni-ulm.de
Dynes	Louise	University of Nottingham, UK	Outreach Officer	Outreach	louise.dynes@nottingham.ac.uk
Erz	Catarina	University of Ulm, Germany	PhD student	Syngas fermentation	catarina.erz@uni-ulm.de
Fell	David	Oxford Brookes University, UK	Professor	Modeller	dfell@brookes.ac.uk
Finney	Alex	University of Dundee, UK	PhD student	Bacterial physiology	a.j.finney@dundee.ac.uk
Garcia-Gonzalez	Linsey	VITO NV, Belgium	Senior Scientist	Unit Separation, Conversion Tech.	linsey.garcia-gonzalez@vito.be

Gardner	Gerard	HEL Ltd, UK	Sales director	Gas fermentation	gerardgardner@helgroup.com
Gascoyne	Joshua	University of Nottingham, UK	PhD student	Microbiology	msxjlg@nottingham.ac.uk
Gilbert	James	University of Nottingham, UK	Research Associate	Modelling, Transcriptomics	james.gilbert@nottingham.ac.uk
Goddard	Andrew	Freeland Horticulture Ltd, UK	R & D manager	Waste management, AD	andrew@freelandhorticulture.co.uk
Gradley	Michelle	ZuvaSyntha, UK	Director	Microbiology, Genetics	michelle.gradley@zuvasyntha.com
Green	Edward	CHAIN Biotechnolgy, UK	Chief Scientific Advisor	Industrial Biotech	edward@chainbiotech.com
Grob	Ralph	Centre for Process Innovation, UK	Group Manager	Process Engineering	grobralph@gmail.com
Grosse-Honebrink	Alex	University of Nottingham, UK	Postdoc	Microbiology	mbzag2@nottingham.ac.uk
Hanko	Erik	University of Nottingham, UK	PhD student	Synthetic biology	Erik.Hanko@nottingham.ac.uk
Hay	Rosalind	Centre for Process Innovation, UK	Programme Lead	Gas fermentation	rosalind.hay@uk-cpi.com
Henstra	Anne	University of Nottingham, UK	Research Fellow	Gas fermentation	anne.henstra@nottingham.ac.uk
Holtman	Dirk	Dechema - Forschungs Institut	Group leader	CO2 Conversion, Biotechnology	holtmann@dechema.de
Humphreys	Christopher	University of Nottingham, UK	PDRA	Synthetic Biology	c.humphreys@nottingham.ac.uk
Infants	Alba	Karlsruhe Institute for Technology, Germany	PhD student	Gas fermentation	alba.infantes@kit.edu
Johannessen	Arild	Biosentrum AS, Norway	Managing Director	Fermentation, Process development	ajo@iris.no
Kapoore	Rahul	The University of Sheffield, UK	PDRA	Systems Biology, Bioenergy	rahulkapoore@gmail.com
Karl	Michael Karl	University of Ulm, Germany	PhD student	Moleculat Microbiology	michael-1.karl@uni-ulm.de
Katsyv	Alexander	Goethe University, Germany	Masters Student	C1 metabolism	katsyv@yahoo.de
Klask	Christian-Marco	University of Tübingen, Germany	PhD student	Syngas fermentation	christian-marco.klask@uni-tuebingen.de
Kottenhahn	Patrick	Goethe University, Germany	Masters Student	Acetobacterium woodii	p.kottenhahn@lucky-men.com
Kovacs	Katalin	University of Nottingham, UK	PDRA	Microbiolgy, Synthetic Biology	Katalin.Kovacs@nottingham.ac.uk
Krabben	Preben	Centre for Process Innovation, UK	Senior Research Scientist	Biology	preben.krabben@gmail.com
Kremp	Florian	Goethe University, Germany	Masters Student	C1 metabolism	flokremp@yahoo.de
Li	Qi	Shanghai Insititutes of Biological Sciences, China	PhD student	CRISPR gene-eiditing	liqi@sibs.ac.cn
Liang	Zhixuan	University of Chester, UK	PhD student	AD, Modelling	z.liang@chester.ac.uk
Locker	Jessica	University of Nottingham, UK	PhD student	Synthetic biology	jessica.locker@nottingham.ac.uk
Malys	Naglis	University of Nottingham, UK	Senior Research Fellow	Biochemistry, Synthetic Biology	Naglis.Malys@nottingham.ac.uk
McGregor	Callum	University of Nottingham, UK	PhD student	Synthetic biology	stxcm19@exmail.nottingham.ac.uk
Mesfin	Noah	Oxford Brookes University, UK	PhD student	Metabolic modelling	14102280@brookes.ac.uk

Miles	Colin	BBSRC, UK	Head of Strategy	IBB	colin.miles@bbsrc.ac.uk
Millat	Thomas	University of Nottingham, UK	PDRA	Metabolic modelling	thomas.millat@nottingham.ac.uk
Minton	Nigel	University of Nottingham, UK	Professor	Gas fermentation	nigel.minton@nottingham.ac.uk
Minton	Jacque	University of Nottingham, UK	Network Manager	Project management	jacqueline.minton@nottingham.ac.uk
Mitra	Robin	Centre for Process Innovation, UK	Senior Program Manager	BioProcess Development	robin.mitra@uk-cpi.com
Molitor	Bastian	University of Tübingen, Germany	Postdoc	Syngas fermentation	bastian.molitor@googlemail.com
Montgomery	Lucy	National Non-Food Crops Centre, UK	Bioeconomy Consultant	Start ups	l.montgomery@nnfcc.co.uk
Müller	Volker	Goethe-University Frankfurt, Germany	Professor	Acetogenesis, syngas fermentation	vmueller@bio.uni-frankfurt.de
Neary	Joanne	Centre for Process Innovation, UK	Senior Technical Lead	Process development	joanne.neary@uk-cpi.com
Neumann	Anke	Karlsruhe Institute for Technology, Germany	PDRA & Lecturer	Syngas fermentation	anke.neumann@kit.edu
Norman	Rupert	University of Nottingham, UK	PhD student	Synthetic Biology	stxron@nottingham.ac.uk
Öppinger	Christian	Goethe University, Germany	PhD student	Molecular Biology	oeppinger@bio.uni-frankfurt.de
Oswald	Florian	Karlsruhe Institute for Technology, Germany	PhD student	Anaerobic fermentation of syngas	florian.oswald@kit.edu
Pander	Bart	The University of Nottingham, UK	PhD student	Gas fermentation	bartpander@gmail.com
Patel	Vanisha	University of Nottingham, UK	PhD student	Mircobiology	mbxvp@nottingham.ac.uk
Patel	Rajan	University of Nottingham, UK	PhD student	Industrial Biotechnology	stxrp11@nottingham.ac.uk
Pentassuglia	Simona	Italian Institute of Technology, Italy	PhD student	Microbiology, Genetic tools	mbasp@nottingham.ac.uk
Poehlein	Anja	University of Goettingen, Germany	PDRA	Genomics, Transcriptomics	apoehle3@gwdg.de
Quataert	Koen	Bio Base Europe Pilot Plant, Belgium	Team leader	Syngas fermentation	koen.quataert@bbeu.org
Raut	Mahendra	University of Sheffield, UK	PDRA	Proteomics, Metabolomics	m.raut@sheffield.ac.uk
Reitzer	Pierre	University of Nottingham, UK	PhD student	Biotechnology	stxpbr@nottingham.ac.uk
Roger	Magli	University of Dundee, UK	PDRA	Molecular Biology	m.roger@dundee.ac.uk
Rossi	Jose	Calysta, UK	Research Assistant	Molecular Biology	ricardorossi85@hotmail.com
Rumah	Bashir	University of Nottingham, UK	PhD student	Biochemistry	msxbr@nottingham.ac.uk
Salgado	Vera	University of Nottingham, UK	PhD student	Biotech, Molecular Biology	pcxvs1@nottingham.ac.uk
Saracco	Guido	Italian Institute of Technology, Italy	Centre Coordinator	Sustainable Future Technologies	guido.saracco@iit.it
Sargent	Frank	University of Dundee, UK	Professor	Bacterial metabolism	f.sargent@dundee.ac.uk
Schwarz	Fabian	Goethe University, Germany	Masters Student	C1 metabolism	numerofabian@gmx.de
Seys	Francois	University of Nottingham, UK	PhD student	Metabolic Engineering	francois.seys@nottingham.ac.uk
Simpson	Sean	Lanzatech, USA	Founder & MD	Gas Fermentation	sean@lanzatech.com

Singh	Jasbir	HEL Ltd, UK	Director	Gas Fermentation	jasbirsingh@helgroup.com
Skill	Steve	Greenskill Ltd, UK	Director	Photobiotechnology	Steve@greenskill.co.uk
Smith	Tom	Sheffield Hallam University, UK	Professor	Methanothrophs	t.j.smith@shu.ac.uk
Stead	Christopher	University of Nottingham, UK	PhD student	Microbiology	msxcs3@nottingham.ac.uk
Steinbüchel	Alexander	University of Münster, Germany	Professor	Metabolic pathways	steinbu@uni-muenster.de
Thauer	Rolf	Max Planck Institute, Germany	Professor Emeritus	Methanogens	thauer@mpi-marburg.mpg.de
Tibaldero	Giogia	University of Nottingham, UK	PhD student	Sythetic Biology	stxgt5@nottingham.ac.uk
Van Hagen	Alexander	University of Nottingham, UK	PhD student	Microbiology, Synthetic Biology	stxamv@nottingham.ac.uk
Vaud	Sophie	University of Nottingham, UK	PhD student	Cloning, CRISPR, Recombination	mbxsv@nottingham.ac.uk
Wang	Yanming	University of Nottingham, UK	PDRA	Bioprocess development	yanming.wang@nottingham.ac.uk
Warren	Martin	Kent University, UK	Professor	Biochemistry	M.J.Warren@kent.ac.uk
Wickham-Smith	Charlie	University of Nottingham, UK	PhD student	Microbiology	Charliewickham87@hotmail.com
Wiechmann	Anja	Goethe University, Germany	Research Technician	Molecular Biology, Biotech	A.Wiechmann@em.uni-frankfurt.de
Wilkinson	Stephen	University of Chester, UK	Professor	Fermentation, Modelling	s.j.wilkinson@chester.ac.uk
Wimalasena	Tithira	Calysta, UK	Senoir Scientist	Fermentation, Synthetic biology	tithiraw@calysta.com
Winzer	Klaus	University of Nottingham, UK	Associate Professor	Bacterial metabolism	Klaus.winzer@nottingham.ac.uk
Woods	Craig	University of Nottingham, UK	PhD student	Clostridia Synthetic Biology	mrxcw4@nottingham.ac.uk
Wright	Phillip	Newcastle University, UK	Pro-Vice-Chancellor	Chemical Engineering	Phillip.wright@ncl.ac.uk
Zhang	Ying	University of Nottingham, UK	Senior Research Fellow	Metabolic engineering	ying.zhang@nottingham.ac.uk
Zhuang	Shiwen	Calysta, UK	Fermentation Engineer	Methane fermentation	szhuang@calysta.com
Zimmerman	William	University of Sheffield, UK	Professor	Fluid & microbubble dynamics	w.zimmerman@shef.ac.uk



Acknowledgement

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