C1net

The Conference

14-16 January 2015
Nottingham, UK

Programme and Abstracts

C1net Management Board

Nigel Minton	The University of Nottingham, UK
David Fell	Oxford Brookes University, UK
Nigel Scrutton	Manchester University, UK
Phillip Wright	Sheffield University
Robin Mitra	Centre for Process Innovation Ltd, UK
Ian Fotheringham	Ingenza Ltd, UK
Michelle Gradley	BioSyntha Technology Ltd, UK
Edward Green	Green Biologics Ltd, UK
Bob Tooze	Sasol UK Ltd, UK
Saun Simpson	Lanzatech, USA
Jacque Minton	The University of Nottingham, UK

Conference Secretariat:

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

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

with the acknowledged help of Janice Sablitzky and Louise Dynes

WELCOME

Much current attention is focused on deriving fuels and chemicals through the establishment of microbial chassis using Synthetic Biology. Until now, the emphasis has been on lignocellulosic fermentative processes that use non-food plant biomass. However, developing economic processes that efficiently convert plant material into the necessary sugar feedstock is proving challenging.

An alternative approach is to 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 (*e.g.,* steel manufacturing, oil refining, coal and natural/shale gas) as well as 'synthesis gas' (CO & H_2) produced from sustainable resources, such as biomass and domestic/ agricultural wastes. This enables low carbon fuels and chemicals to be produced in any industrialized geography without consumption of valuable food or land resources.

C1net is championing the further development of gas fermentation technology through the creation of a cross-disciplinary community of academics and industrialists working together to achieve the networks goals. It is, therefore, with much pleasure that David, Jacque and I welcome you to the first conference of the BBSRC NIBB, C1net. We 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.

Nigel P Minton
On behalf of the C1net management board

GENERAL INFORMATION

CONFERENCE VENUE AND ACCOMMODATION

Hilton Hotel
Milton Street
Nottingham
NG1 3PZ

ORAL PRESENTATIONS

Oral presentations will be in the Wembley Suite. The length of oral presentations is scheduled from 15 to 35 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. All presentations will be uploaded to the computer in the lecture hall. This can be done anytime, but at least 2-3 hours before your session or the evening before for early morning presentations.

POSTER PRESENTATIONS

Poster presentations will be in the Wembley Suite. 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 Reception, Wednesday, 14 January 2015, 19:00, Wembley Suite, Hilton Hotel.

Conference Dinner, Tuesday, 15 January 2015, 19:00, Wembley Suite, Hilton Hotel

TAXIS

DG Taxis 0115 9 607607 or ask the hotel.

PROGRAMME

WEDNESDAY 14th January - ARRIVAL AT HILTON HOTEL (Check-in from 15:00)		
18:00	**Registration**	**Hilton Hotel**
19:00	**Reception**	**Hilton Hotel**
20:30	**Dinner**	**Hilton Hotel**
THURSDAY 15th January 2015		
Chair: David Fell (Oxford Brookes, UK)		
09.00 – 09.05	**Jacque Minton** *The University of Nottingham, UK*	Welcome
09.05 – 09:35	**Lionel Clarke** *UK SBLC*	Synthetic Biology and the Role of SBRCs in the UK
09.35 – 10.00	**Nigel Minton** *The University of Nottingham, UK*	SBRC Nottingham and C1net
10:00 – 10.25	**Nigel Scrutton** *Manchester University, UK*	Manchester Centre for Synthetic Biology of Fine and Speciality Chemicals
10.25 – 10.50	**Stephen Chambers** *CEO-SynbiCITE- Imperial College London, UK*	Closing the Translation Gap in Synthetic Biology Innovation
10.50 - 11.20	**Coffee/Tea Break**	
Chair: Sean Simpson (Lanzatech, USA)		
11.20 – 11.50	**Peter Duerre** *University of Ulm, Germany*	Gas fermentation using autotrophic acetogenic bacteria for production of chemicals and fuel
11.50 – 12.20	**Steve Martin** *BioSyntha Technology Ltd, UK*	Chemicals from C1 Feedstocks - Enabling a Circular Economy
12.20 – 12.50	**Philippe Soucaille** *INSA, Uni. of Toulouse, France*	From C1 to C2 and C4 alcohols by fermentation of anaerobic bacteria
12.50 – 13.10	**Eric Liew** *The University of Nottingham, UK*	Inactivation of Carbon Monoxide Dehydrogenases in C1 Chassis *Clostridium autoethanogenum*
13.10 – 14.30	**Lunch**	**Hilton Hotel**
Chair: Edward Green (Green Biologics Ltd, UK)		
14.30 – 15.00	**Sean Simpson** *Lanzatech, USA*	Commercial-scale production of sustainable fuels and chemicals from gases
15.00 – 15.30	**Auxiliadora Prieto** *CIB-CSIC, Spain*	SYNPOL- A platform for the bioplastics production from complex wastes by syngas fermentation
15.30 – 15.50	**Saskia Vander Meeren** *Biobase Europe Plant, Belgium*	Capture and Utilisation: the role of proper piloting
15.50 – 16.10	**Jagroop Pandhal** *Sheffield University, UK*	Engineering Microbial Communities for Fuel Chemicals
16.10 – 18.00	**POSTERS/ Refreshments**	
19.30	**Dinner**	**Hilton Hotel**

FRIDAY 16th January 2015		
Chair: Bob Tooze (Sasol UK Ltd)		
09.00 – 09.30	**Reuben Carr** *Ingenza, UK*	Optimization of Biocatalytic Activities for Multigenic Bio-Based Chemical Production Processes
09.30 – 09.50	**Neil Swainston** *University of Manchester, UK*	GeneGenie: Optimized Oligomer Design For Directed Evolution
09.50 – 10.05	**Anne M. Henstra** *The University of Nottingham, UK*	GASCHEM: Optimising C1 gas fermentation by the acetogen *Clostridium autoethanogenum*
10.05– 10.20	**Ronja Breitkopf** *The University of Nottingham, UK*	Carbon monoxide based succinate fermentation in *C. autoethanogenum*
10.20 – 10.35	**Craig Woods** *The University of Nottingham, UK*	Transposon mutagenesis of *C. autoethanogenum*
10.35 – 10.50	**Bart Pander** *The University of Nottingham, UK*	Carbonic Anhydrase in Acetogens
10.50 - 11.20	**Coffee/Tea Break**	
Chair: Michelle Gradley (BioSyntha Technology Ltd, UK)		
11.20 – 11.50	**Alex Toftgaard Nielsen** *Biosustain, Denmark*	Engineering of biochemicals production from various carbon sources
11.50 – 12.10	**Bob Lovitt** *Swansea University, UK*	Intensified Bioreactors for C1 Growth and Production
12.10 – 12.30	**Xin Tu** *The University of Liverpool, UK*	Atmospheric pressure non-thermal plasma technology: a solution for gas cleaning and fuel production
12.30 – 12.50	**Barry Azzopardi** *The University of Nottingham, UK*	Multiphase Flow in Industrial Biotechnology
12.50 – 14.30	**Lunch**	**Hilton Hotel**
Chair: Robin Mitra (Centre for Process Innovation, UK)		
14:30 – 15:00	**Josh Silverman** *Calysta, USA*	Food and Energy Security through Methane Technology
15:00 – 15:20	**Ying Jiang** *Cranfield University, UK*	Influence of Ammonia to Methanogenic Pathway from Acetate and Methanogen Composition in Anaerobic Digesters
15:20 – 15.40	**Cecilia Fenech** *Cranfield University, UK*	Bio-Thermal RED: Supporting SMEs In Anaerobic Digestion
15.40 – 16:00	**Peter Licence** *The University of Nottingham, UK*	C1net – How Chemists Can Contribute
16.00	**Refreshments/Depart**	

ABSTRACTS OF ORAL PRESENTATIONS

Synthetic Biology and the Role of SBRCs in the UK

LIONEL CLARKE

UK Synthetic Biology Leadership Council

Synthetic Biology has been identified by the UK government as a field of particular interest and exceptional opportunity, earning its designation as one of the 'Eight Great Technologies'. A UK Roadmap was published in July 2012, recommending a number of initiatives critical to the successful development of the field in the UK within an International context These were fully supported, and are now in the process of being put into action, if not already established. One of the recommendations was to support a small number of multidisciplinary research centres, subsequently termed Synthetic Biology Research Centres (SBRCs). This presentation will describe the framework for the formation and operation of SBRCs, and the role of the UK Synthetic Biology Leadership Council (SBLC) in reviewing progress against the initial Roadmap goals, and in refreshing the Roadmap implementation plan.

SBRC Nottingham and the BBSRC NIBB C1net

NIGEL P. MINTON

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

Current energy and chemical needs are met by the extraction and processing of fossil fuels. Such resources are finite 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. Biological routes represent a promising option, but strategies to date rely on the use of microbes to convert through fermentation the easily accessible carbohydrates (sugar and starch) of plants (such as sugar cane or corn) into chemicals and fuels. This has led to concerns over competition with the use of these carbohydrates as food, and a re-focussing of efforts on non-food, plant cell wall material (lignocellulose). However, lignocellulose, is extremely resistant to being broken down into the sugar needed for fermentation. Overcoming this recalcitrance in a cost effective manner is proving extremely challenging. There is, however, an exciting low-cost alternative, and that is to directly capture carbon, by harnessing the ability of certain bacteria to 'eat' single carbon GHG gases such as carbon monoxide/dioxide (CO/ CO_2) and methane (CH_4) derived from non-food sources. The gas is injected into the liquid medium of fermentation vessels where it is consumed by the bacteria and converted into the chemicals we need. Fortunately, such gases are an abundant resource, and may be derived from non-food sources such as waste gases from industry as well as 'synthesis gas' produced from the gasification (heating) of non-food biomass and domestic/ agricultural wastes. This enables a wide range of valuable advanced fuels and chemicals to be produced in any industrialized geography without consumption of valuable food or land resources. SBRC Nottingham will focus on the development and exploitation of gas fermenting chassis for the production of chemicals and fuels, with an emphasis on platform chemicals. In parallel, the BBSRC NIBB C1net provides both the opportunity for the SBRC to engage with the wider community and is fostering the creation of a national community of academics and industrialists able to exploit gas fermentation technology across the full spectrum of TRLs.

Manchester Centre for Synthetic Biology of Fine and Speciality Chemicals

NIGEL S. SCRUTTON

Centre for Synthetic Biology of Fine and Speciality Chemicals, Manchester Institute of Biotechnology, The University of Manchester, M1 7DN, UK

The Manchester SYNBIOCHEM Centre will be a UK and European Centre of Excellence for Synthetic Biology in relation to fine and speciality chemicals production. It will provide a national focus to spearhead UK academic and industrial research and to accelerate the application of synthetic biology in fine and speciality chemicals production and the generation of new state-of-the-art tools to facilitate this translation. The Centre will be located in the Manchester Institute of Biotechnology, a unique cross-disciplinary research centre at the University of Manchester, bringing together more than 500 researchers with expertise in molecular biology, chemistry, engineering, material and computing science, and medicine at the forefront of international developments in synthetic biology. As part of the MIB, the Centre will build on a long and distinguished track record in spin-off formation and translating innovative research to industrial application, including a substantial portfolio of partnerships, e.g., with Syngenta, GSK, BASF and Shell. The Centre will operate an open and inclusive approach driven by the unique industrial needs of synthetic biology. This will allow it to harness the scientific expertise of the synthetic biology community at Manchester and throughout the country, by facilitating multiple research projects positioned primarily at the Technology-Readiness Levels 1 to 3, but also including industry-driven academic-led proof-of-concept and proof-of-utility projects with partners from industry and academia at the higher Technology-Readiness Levels.

The Centre is structured around the following major objectives: (1) Transform synthetic biology approaches into frontline discovery technology for fine and speciality chemicals synthesis by uniting world-leading expertise, enhanced technology and scientific capabilities, strategic intelligence and new collaboration infrastructures into a single multidisciplinary research centre. (2) Build a suite of state-of-the-art and integrated technology platforms, and implement these platforms into leading research programs that propel fine and speciality chemicals production towards green and more sustainable manufacturing processes by harnessing the power of synthetic biology. (3) Harness the exceptional collaborative culture, external networks and strong foundational sciences within the proposed Centre that underpins fine and speciality chemicals synthesis to build extensive collaborative projects in synthetic biology with academic and industrial stakeholders, boost national/international research capacity and stimulate innovation with industry and other key stakeholders. (4) Foster early dialogue to delineate and stimulate Responsible Innovation in the application of synthetic biology to fine and speciality chemicals synthesis, and to understand and mitigate risks that could potentially reduce technology uptake.

Closing the Translation Gap in Synthetic Biology Innovation

STEPHEN CHAMBERS

CEO-SynbiCITE- Imperial College London, London SW7 2AZ, UK

SynbiCITE is a Synthetic Biology Innovation and Commercialisation Industrial Translation Engine. It is a pan-UK operating facility that engages and interacts with academic, industrial and commercial partners from across the UK. SynbiCITE is part-funded through the UK Research Councils and InnovateUK, and is an Innovation and Knowledge Centre – IKC - that serves the whole of the UK's interests in synthetic biology R&D through to industrialisation. The aim of SynbiCITE is to perform the important function of academic and business integration by creating an effective Industrial Translation Engine - bridging the gap between university-based research and industrial processes to create products and jobs, through industry, for the benefit of the UK economy. The goal is to create in the UK an internationally recognized lead centre in industrializing synthetic biology research.

Gas fermentation using autotrophic acetogenic bacteria for production of chemicals and fuels

PETER DÜRRE

Institut für Mikrobiologie und Biotechnologie, Universität Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany

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

Clostridium ljungdahlii is such an acetogen, able to ferment either organic compounds or CO_2 + H_2 and syngas (CO + H_2). The genome of *C. ljungdahlii* comprises 4,630,065 base pairs. Experimental data and *in silico* comparisons revealed differences in energy metabolism. Unlike *Moorella thermoacetica*, no cytochromes and quinones are involved in energy generation, but instead an H^+-dependent Rnf system is present, analogous to *Acetobacterium woodii* with a Na^+-dependent Rnf system. Electroporation of *C. ljungdahlii* with plasmids bearing heterologous genes for butanol production was successful and formation of the biofuel could be demonstrated. Thus, *C. ljungdahlii* can be used as a novel microbial production platform based on syngas.

As the organism does not grow well on CO_2 + H_2 mixtures, *Clostridium aceticum* was chosen for this type of gaseous substrate. Expression of both, heterologous butanol- and acetone-forming enzymes could be demonstrated. Genome sequencing of this species is currently being performed. *C. aceticum* can also use syngas as a carbon source.

Finally, *A. woodii* was improved for acetate formation from CO_2 + H_2 by introducing and overexpressing clostridial genes encoding Wood-Ljungdahl pathway enzymes.

Chemicals from C1 Feedstocks – Enabling a Circular Bioeconomy

STEVEN M MARTIN

BioSyntha Technology Ltd, BioPark Hertfordshire, Broadwater Road, Welwyn Garden City, Hertfordshire, AL7 3AX

The chemical industry generates more than US$3 trillion per annum and its wide array of products are incorporated into more than 95% of the world's manufactured goods. The largest market segment is the provision of basic and intermediate chemicals such as ethylene, propylene, butanediol, and butadiene. The industry has many challenges however, including rising prices, price volatility, availability and overall sustainability of routes dependent upon petrochemicals. Renewable routes to these important chemical building blocks are being actively pursued. The economics of these routes will ultimately determine their commercial success, and as production costs are largely dominated by feedstock costs, the use of low cost C1 feedstocks, such as syngas and methanol, is expected to deliver overall economic competitiveness and drive market share.

BioSyntha has developed a novel bio-route to 1,3-butanediol (1,3-BDO) which, in turn, can be converted to butadiene by further biological or chemical processing. Butadiene is an important feedstock used in the manufacture of car tyres with a global market estimated to reach US$32 billion by 2018. BioSyntha is using synthetic biology approaches to accelerate the development of its 1,3-BDO process in collaboration with industry partners. An example of how a bio-route to 1,3-BDO can provide an enabling link in the overall lifecycle of car tyres will be presented. The development of a circular economy can deliver multiple economic benefits to industrial partners including reduced costs, reduced waste and significant environmental and sustainability benefits.

From C1 to C2 and C4 alcohols by fermentation of anaerobic bacteria

PHILIPPE SOUCAILLE

LISBP, INSA, University of Toulouse, Pathways Evolution and Engineering in Prokaryotes, 135 avenue de Rangueil, 31077 Toulouse, FRANCE

Anaerobic bacteria are of considerable potential for the biotechnological production of a number of organic acids or alcohols though they remain relatively unexploited due in part to a limited understanding of the physiological concepts regulating their metabolism. Recently detailed genome scale models of the metabolism of the acetogenic bacteria *Eubacterium limosum* and the solventogenic bacteria *Clostridium acetobutylicum* were established with the identification of the key reactions controlling carbon and electron flows. Based on these two models, a two step fermentation strategy was developed to convert C1 to C2 and C4 alcohols.

In a first step, methanol and CO_2 mixtures were continuously converted to butyric acid by *E. limosum*. In a second step the supernatant was complemented with glycerol and used by a mutant of *C. acetobutylicum* for the continuous production of a mixture of butanol and ethanol. The overall carbon yield of this continuous process is the highest ever reported for the production of C2 and C4 alcohols.

Inactivation of Carbon Monoxide Dehydrogenases in C1 Chassis *Clostridium autoethanogenum*

FUNGMIN (ERIC) LIEW[1,2], ANNE M. HENSTRA[1], KLAUS WINZER[1], MICHAEL KÖPKE[2], SEAN D. SIMPSON[2], NIGEL P. MINTON[1]

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

[2]*LanzaTech Inc., 8045 Lamon Avenue, Suite 400, Skokie, IL, USA*

The production of biofuels and platform chemicals via gas fermentation is an emerging technology that does not utilize food-based substrates as feedstocks. Industrial waste gases and syngas generated from municipal solid waste, agricultural and forestry residues comprise mainly of carbon monoxide, carbon dioxide, and hydrogen. *Clostridium autoethanogenum* is a non-pathogenic acetogen that can utilize these gases as the sole source of carbon and energy to synthesize ethanol and 2,3-butanediol. To further enhance the metabolic capability of this acetogen, we report the development of transformation strategies and application of ClosTron (1) and Allele-Coupled Exchange (ACE) (2) mutagenesis.

C. autoethanogenum encodes two putative mono-functional carbon monoxide dehydrogenases CODH1 (CAETHG_3005) and CODH2 (CAETHG_3899), and a bi-functional CODH/ACS complex (CAETHG_1620-1621). Each of these CODHs was individually knocked out using ClosTron and the impact on growth and metabolite profiles were investigated under heterotrophic and autotrophic conditions.

References:
(1) Heap JT, Pennington OJ, Cartman ST, Carter GP, Minton NP. The ClosTron: A universal gene knock-out system for the genus Clostridium. Journal of Microbiological Methods. 2007;70(3):452-64.
(2) Heap JT, Ehsaan M, Cooksley CM, Ng YK, Cartman ST, Winzer K, et al. Integration of DNA into bacterial chromosomes from plasmids without a counter-selection marker. Nucleic Acids Res. 2012;40(8):e59.

Commercial-scale production of sustainable fuels and chemicals from gases

SEAN D SIMPSON

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

World energy demand is expected to increase by up to 40% by 2030. The key challenge facing the global community is to not only increase the sources of energy supply, but to also maximize the use of sustainable forms of energy to safeguard the environment while ensuring that the latter do not detrimentally impact food supplies. In this regard, renewable sources of energy will play an increasing role in the global primary energy supply. Internationally, governments have already been mandating the increased use of renewable fuels in the transport sector. Similarly, as a result of consumer driven demand, the global market for more environmentally sustainable alternatives to today's oil and coal-derived chemicals is anticipated to exceed $100 billion by 2020.

The production of biofuels and platform chemicals via gas fermentation is a rapidly developing emerging technology for high volume, sustainable, production of fuels and chemicals that does not require food-based substrates as a feedstock. LanzaTech has developed and scaled a complete process platform to allow the continuous biological production of fuels and an array of chemicals intermediates from gases at scale. To date, this technology has been successfully demonstrated with such diverse gas streams as by-product gases from steel making, reformed natural gas, and syngas produced from gasified biomass and gasified municipal solid waste.

The company has developed a proprietary strain of an acetogenic clostridium that is used in combination with a novel reactor design, and optimized process chemistry in order to ensure efficient, single-pass gas conversion with a high selectivity to the product off interest. In order to maximise the value that can be added to the array of gas resources that the LanzaTech process can use as an input, the company has developed a robust genetic toolbox to allow the carbon an energy consumed by its proprietary gas fermenting microbe to be channelled in to a spectrum of valuable chemicals. Gas fermentation offers an efficient route to add much greater value to gas streams than established technologies, while also reducing greenhouse emissions and providing a strategically important alternative to food or farmed resources for domestic production of sustainable fuels and chemicals. The company will present data demonstrating stable and continuous production of a sustainable fuel at scale using industrial off gases as a feedstock.

SYNPOL – A Platform For The Bioplastics Production From Complex Wastes By Syngas Fermentation

M. AUXILIADORA PRIETO, OLIVER DRZYZGA, EDUARDO DÍAZ, JOSÉ L. GARCÍA

Dept. of Environmental Biology, Biological Research Center (CIB-CSIC), C/Ramiro de Maeztu 9, 28040 Madrid, Spain

Valorization and utilization of wastes via their bioconversion into valuable products is one of the most distinctive strategies of Bio-economy. Although some wastes might be homogeneous, many others (e.g., municipal waste) present very complex compositions. In this context, one of the processing methods that can be used in biorefineries is the gasification of organic materials to synthesis gas (syngas) followed by microbial fermentation.

Syngas is a gaseous mixture composed mainly of H_2 and CO that can be fermented by specialized microorganisms. The SYNPOL platform is an integrated European project (www.synpol.org) that integrates biopolymer production through modern processing technologies, with bacterial fermentation of syngas, and the pyrolysis of highly complex biowaste (e.g., municipal, commercial, agricultural). The R&D activities focus on the integration of innovative physico-chemical, biochemical, downstream and synthetic technologies to produce a wide range of new biopolymers. The integration will engage novel and mutually synergistic production methods as well as the assessment of environmental benefits and drawbacks.

SYNPOL´s concept shows the implementation of novel microwave-induced pyrolytic treatments of organic waste together with systems biology-defined highly efficient and physiologically balanced recombinant bacteria. The latter will produce biopolymer building blocks and polyhydroxyalkanoates (PHAs) that will serve to synthesize novel bio-based plastic prototypes by chemical and enzymatic catalysis.

Rhodospirillum rubrum is a purple non-sulfur bacterium that naturally produces PHA. It is subject of a substantial amount of physiological and genetic analysis within the SYNPOL platform due to its ability to grow under a broad variety of aerobic and anaerobic conditions using fermentation, respiration or photosynthesis for the production of energy. *R. rubrum* can utilize CO from syngas for the synthesis of PHAs and therefore is one of the model organisms within the SYNPOL project.

Carbon Capture and Utilisation: the role of proper piloting

SASKIA VANDER MEEREN, HENDRIK WAEGEMAN

Bio Base Europe Pilot Plant, Rodenhuizekaai 1, 9042 Ghent

Since the industrial revolution, human activities contributed to the climate change by adding carbon dioxide to the atmosphere faster than natural processes can remove it. Different greenhouse gases exist, like carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and fluorinated gases, but carbon dioxide is by far the most abundant. The main source of this carbon dioxide is the combustion of fossil fuels (coal, natural gas and oil) for energy and transportation.

To lower this amount, logically the amount of fossil fuel consumption needs to be reduced. Other extra measurements are carbon capture and storage (CCS) or carbon capture and utilisation (CCU). The goal of carbon capture is capturing waste carbon dioxide from large point sources, such as fossil fuel power plants. This can be transported to a storage site for a long term, for example in deep geological formations or in the form of mineral carbonates. However, economically it is more interesting to use the waste carbon dioxide to produce valuable products, like bulk chemicals or bio-fuels. The conversion can take place with a chemical catalyst, via electrolysis or with micro-organisms.

Bio Base Europe is mostly focused on the microbiological conversions, to produce bio-chemicals in an efficient way starting from carbon dioxide. This is a rather new technology and brings a lot of problems and questions like: What is the requested purity of the gas? How can the solubility of the gas be increased? What about the safety? Etc. These conversions ask for specialized and expensive equipment, which are typically available in pilot facilities. The role of piloting is diverse in developing such a process from scratch. First, the idea needs to be evaluated on lab scale, tested in a small fermentor and optimized and subsequently thoroughly validated on a pilot and demonstration scale. Furthermore, the isolation and purification of the product out of an aqueous environment such as a fermentation broth is also a challenge that can be tackled by a pilot plant, by using the flexible and large amount of available equipment. In addition, pilot tests will provide accurate date in order to be able to make a techno-economical evaluation of the process.

Although the CO2 accumulation in the atmosphere has been heavily debated in recent years and is considered a being one of the most important environmental threats for the current generation, little efforts have been made so far to bring CCU technologies that are being developed a laboratory scale to a demonstration scale. Action needs to be taken.

Engineering Microbial Communities For Fuel Chemicals

JAGS PANDHAL

Advanced Biomanufacturing Centre, Department of Chemical and Biological Engineering, University of Sheffield, Sheffield, S1 3JD

Most highly controlled and specific applications of microorganisms in biotechnology involve pure cultures. Maintaining single strain cultures is important for industry as contaminants can reduce productivity and lead to longer "down-times" during sterilisation.

However, microbes working together provide distinct advantages over pure cultures. They can undertake more metabolically complex tasks, improve efficiency and even expand applications to open systems. By combining rapidly advancing technologies with ecological theory, the use of microbial ecosystems in biotechnology will inevitably increase.

Once a microbial community or co-culture is identified for a specific production process, the engineering paradigm of measure, model, manipulate and manufacture, can be applied to improve system performance. Systems to improve biofuel production using microalgae (algae to produce aviation fuel chemicals) are described and the potential to improve overall yields in cellulosic degradation processes are discussed.

Optimization of Biocatalytic Activities for Multigenic Bio-Based Chemical Production Processes

REUBEN CARR

Ingenza Ltd., Roslin, United Kingdom

Replacement of petroleum oil-based products and manufacturing processes with competitive bio-based alternatives is attracting increased attention due to concerns around petroleum oil's supply, price and sustainability. The use of affordable C1 gas-derived feedstocks could provide an environmentally benign stepping stone *en route* towards the large-scale utilization of more carbon-neutral feedstock options. Replacement of conventional petroleum oil-based refining processes with biosynthetic routes that use C1 gas-based feedstocks will require the development of genetic modification tools for use in host organisms that either don't typically consume C1 gases or that consume C1 gases but cannot readily be engineered. These tools include methods to improve microbial strain construction, their context-dependent evaluation and the subsequent optimization of multicomponent biosynthetic pathways directed towards the generation of ubiquitous intracellular metabolic intermediates from C1 gas feedstocks to facilitate access to target chemical end products. In this talk we will describe the use of Ingenza's synthetic biology tools and proprietary combinatorial genetics platform (inABLE®) to rapidly clone, express, select and optimize target activities for many separate enzymatic reactions initially identified from metagenomic and phylogenetic discovery approaches. The utility of this approach will be illustrated through the rapid identification and expression of critical pathway enzymes, optimal gene coding sequences and enzyme variants from inABLE®-derived high quality variant libraries for bio-based polymer production from alternative feedstock options. Our approach aims to improve the predictability of bioengineering design to overcome limitations associated with current iterative and empirical processes for recombinant microbial strain improvement.

GeneGenie: Optimized Oligomer Design For Directed Evolution

NEIL SWAINSTON, ANDREW CURRIN, PHILIP J DAY, DOUGLAS B KELL

Manchester Institute of Biotechnology, University of Manchester, Manchester M1 7DN

GeneGenie, a new online tool available at http://www.gene-genie.org, is introduced to support the design and self-assembly of synthetic genes and constructs. GeneGenie allows for the design of oligonucleotide cohorts encoding the gene sequence optimized for expression in any suitable host through an intuitive, easy-to-use web interface. The tool ensures consistent oligomer overlapping melting temperatures, minimizes the likelihood of misannealing, optimizes codon usage for expression in a selected host, allows for specification of forward and reverse cloning sequences (for downstream ligation) and also provides support for mutagenesis or directed evolution studies.

Directed evolution studies are enabled through the construction of variant libraries via the optional specification of 'variant codons', containing mixtures of bases, at any position. For example, specifying the variant codon TNT (where N is any nucleotide) will generate an equimolar mixture of the codons TAT, TCT, TGT and TTT at that position, encoding a mixture of the amino acids Tyr, Ser, Cys and Phe.

This facility is demonstrated through the use of GeneGenie, in conjunction with a novel gene synthesis method, SpeedyGenes, to develop and synthesize a library of enhanced green fluorescent protein variants. The longer-term goal of this work is to apply these novel approaches in directed evolutionary studies in order to improve existing, and to develop entirely novel, biocatalysts.

GASCHEM: Optimising C1 gas fermentation by the acetogen *Clostridium autoethanogenum*

ANNE M. HENSTRA

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

Clostridium ljungdahlii and *Clostridium autoethanogenum* are model organisms for gas fermentation, a rapidly maturing technology for the production of fuels and chemicals from industrial waste gases. A natural process that captures CO, CO_2 and H_2, and converts them to products such as acetate, ethanol and 2,3 butanediol. Further engineering of these strains promises the production of diverse platform chemicals, that can be used as building blocks in the production of synthetic polymers. Despite their strict anaerobic, fastidious, and ‘difficult to work with' nature, we have established our Clostridial 'Road Map' to gene modification in these Clostridia. This includes gene transfer through electroporation and conjugation, gene knock-in through Allele Coupled Exchange (ACE) and gene knock-out through ACE, ClosTron and in-frame deletion methods. These methods serve as foundation for BBSRC's sLoLa GASCHEM that seeks detailed understanding and to engineer *C. autoethanogenum* using a systems and synthetic biology approaches at the new gas fermentation facility at SBRC Nottingham.

Succinate fermentation in *Clostridium autoethanogenum*

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

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

Due to the increased awareness of environmental problems, caused by the continued use of fossil fuels, there is an increased need to generate fuels and platform chemicals in a more sustainable manner. Succinic acid is a chemical that is believed to have great economical potential in a bio-based economy. Already used in food and pharmaceutical market, it also functions as C4 building block and can therefore supply the basis for high value-added derivated with applications in the technical and chemical industry.

Using the homoacetogenic *Clostridium* autoethanogenum as a microbial chassis, the proposed research aims to combine the utilisation of exhaust and waste fumes with the fermentative production of succinic acid. A prerequisite for this is a thorough understanding of the existing native route(s) to succinate, which is produced in low amounts by the organism, as well as interconnecting pathways. This will be achieved through a combination of gene inactivation/overexpression and C13 labelling studies.

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

Transposon Mutagenesis of *C. autoethanogenum*

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

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

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

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

.

Carbonic Anhydrases of *Clostridium autoethanogenum*

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

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

Carbonic anhydrases (CAs) are enzymes that catalyse the reversible conversion of carbon dioxide and water to bicarbonate and protons. Both carbon dioxide and bicarbonate ions are important substrates in multiple reactions in most organisms and both need to be readily available. In *Ralstonia eutropha* and *Clostridium perfringens* CAs are reported to be essential for growth under conditions with low carbon dioxide partial pressures. However, the precise function of CAs in autotrophic acetogens remains unclear. *Acetobacterium woodii* expresses CA activity, but little or no CA activity is detected in *Moorella thermoacetica*.

CA activity in the closely related acetogens *Clostridium autoethanogenum* and *Clostridium ljungdahlii* has not been described in literature. These clostridial acetogenic autotrophs are used in industry to fix carbon monoxide and carbon dioxide into organic matter and valuable products via the Wood-Ljungdahl pathway. For a complete understanding of the carbon metabolism of these cells it is necessary to understand the role CAs play in these microorganisms.

We identified two putative carbonic anhydrase genes in the genomes of *C. autoethanogenum* and *C. ljungdahlii*. One codes for a β- carbonic anhydrase (β-CA) with very little similarity to other known β- CAs but the pattern of amino acid residues essential for β-carbonic anhydrase activity is present. The other is a γ-carbonic anhydrase (γ-CA) with clear homology to other proteins in the γ-CA protein family. However, this family is not well characterised and other proposed functions for genes of this family are: acetyltransferase, phenylacetic acid degradation (PaaY) and Ferripyochelin Binding.

Both putative carbonic anhydrase genes of *C. autoethanogenum* were cloned, heterologously expressed and purified. ClosTron knock-out mutants were generated. Further characterisation of enzyme activity and gene knock-out mutants is ongoing.

Engineering of biochemicals production from various carbon sources

ALEX TOFTGAARD NIELSEN

Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Hoersholm, Denmark

There is an urgent need for transforming the chemical industry towards using more sustainable and renewable raw materials. It is furthermore becoming increasingly clear that novel cheap carbon sources are needed in order to lower the production cost of bulk biochemicals. The Novo Nordisk Foundation Center for Biosustainability at the Technical University of Denmark aims at determining and expanding the spectrum of chemicals that can be produced biologically and to shorten the time of production strain development.

Activities focusing on utilization of C1 carbon sources using both thermophilic gas fermentation as well as electrosynthesis will be reviewed. Part of the work focuses on engineering of thermophilic *Moorella* species for production of biochemicals. Here, significant differences in carbon utilization have been found between various strains, which have a direct impact of strain selection for metabolic engineering purposes. The presentation will additionally review advances within the production of both known and novel biochemicals, including coumaric acid, sulphated phenolic compounds, and amino acids using heterotrophic production organisms.

Novel tools for improving the engineering and performance of production strains are also required. Expression of heterologous proteins often results in misfolding and loss of activity, which hinders the generation of functional cell factories. A novel reporter system that enables simultaneous monitoring of protein production and protein translation at the single cell level in *E. coli* will be presented. Such reporter systems are being used to optimize expression of challenging proteins as well as generating protein wide information regarding amino acid residues that are important for protein folding and expression.

Intensified Bioreactors for C1 Growth and Production

BOB LOVITT*†and MYRTO ZACHAROFF†, DARREN OATLEY-RADCLIFFE*†

**Membranology Ltd and †Centre for Complex Fluid Processing,*
School of Engineering, Swansea University, Singleton Park, Swansea SA2 8PP

One of the challenges for growing CI anaerobes is their growth and production of chemical with high specific and volumetric productivity. This presentation will review the possible reactor designs that can be employed to give high productivity systems and the possible implications of such design and operation.

One of the most promising approaches is to use membrane bioreactor systems (MBR) that incorporate enhanced gas mass transfer and in-situ product recovery. By combining these features, MBR systems potentially can become highly productive. Examples will be given of these approaches associated with:

- the design and operation of membrane reactors,
- the limitations of gas mass transfer may be overcome
- the methods of in situ product recovery may be applied to such fermentation processes.

Comparative performance of MBR systems from our work using *E limosum, C butylicum*, Lactic acid bacteria and the published literature will be given of organic acid production from anaerobes that show the potential of these systems in which cells concentrations of 20-100 g/l dry weight cells can be achieved with volumetric reactor productivities of up to 50 times that of conventional batch and continuous cultures.

We also report how such designs may be developed and applied to gaseous fermentations and the implications of such approaches for organic acids production.

Atmospheric pressure non-thermal plasma technology: a solution for gas cleaning and fuel production

YUZUAN ZENG, XINBO ZHU, XIN TU

Department of Electrical Engineering and Electronics, University of Liverpool, Liverpool, L69 3GJ, UK

Atmospheri pressure non-thermal plasma technology provides an attractive alternative to the conventional thermal or catalytic route for the removal of small concentrations of a wide range of pollutants (e.g. PM, SO_x, NO_x, VOCs and PAHs) in waste gas streams at low temperatures. In non-thermal plasmas, the overall gas temperature can be as low as room temperature, while the electrons are highly energetic with a typical electron temperature of 1-10 eV, which is sufficient to break down most chemical bonds of environmental pollutants and produce chemically reactive species: free radicals (e.g. OH, O), excited atoms, ions and molecules for the initiation of both physical and chemical reactions. The non-equilibrium character of such plasma could overcome thermodynamic barriers in chemical reactions and enable thermodynamically unfavourable reactions (e.g. methane reforming) to occur under ambient conditions. High reaction rate and fast attainment of steady state in plasma processing allows rapid start-up and shutdown of the process compared to other thermal treatment technologies, which significantly reduces the overall energy cost and offers a promising route for industrial applications. A particular advantage is the flexibility of plasma processing and its ability to be combined with other technologies such as catalysis and electrochemistry. The integration of plasma and solid catalysts, known as plasma-catalysis, has the great potential to generate a synergistic effect, which can activate catalysts at low temperatures and improve the activity and stability of the catalysts, resulting in the remarkable enhancement of reactant conversion, selectivity and yield of desirable end-products, as well as the energy efficiency of the plasma process. Recently, plasma technology has been applied for the preparation and modification of catalyst materials at low temperatures (<100 ^{o}C), which can significantly reduce metal particle size and enhance the metal dispersion on the catalyst surface, resutling in the enhancement of catalyst activity and stability (e.g. reduce coke deposition).

In this presentation, we will focus on the use of non-thermal plasma and the advantages that this can bring for environment pollution clean-up and fuel production i) plasma removal of low concentration of VOCs (e.g. formaldehyde) over Cu-Ce catalysts; ii) plasma gas cleaning process for the removal of tar from biomass gasification; iii) plasma-catalytic process for the conversion of biogas into value-added fuels and chemicals

Multiphase Flow in Industrial Biotechnology

BARRY AZZOPARDI and BUDDHIKA HEWAKANDAMBY

Manufacturing and Process Technologies Research Division
Faculty of Engineering, University Of Nottingham
University Park, Nottingham, NG7 2RD, UK

The behaviour of the simultaneous flow of gas and liquid has been studied for many years at Nottingham. These investigations are now extended to simultaneous flow of two immiscible liquids as well as liquid-solid enabling us to understand various flow configurations leading towards better control. We apply advanced instrumentation to these flows. We have studied flow in pipe as well as in chemical reactor types: bubble columns (with and without external downcomers); plunging jet units. We present the result obtained show the measured values of bubble size and specific interfacial area obtained and how it varies with liquid physical properties and gas distributor design. We then identify how the expertise should be extended to industrial biotechnology – particularly to the scale up of fermenter units where gas-liquid and solids flow takes place.

Food and Energy Security through Methane Biotechnology

JOSH SILVERMAN

Calysta, Inc, USA

The recent rise in domestic production of methane has driven the cost of natural gas to record lows. Calysta Energy has developed a genetic engineering platform for host organisms (methanotrophs) capable of metabolizing this abundant domestic feedstock to a variety of biofuels and biochemicals. The genetic tools, together with innovative fermentation and bioprocess approaches, enable the rapid implementation of well-characterized pathways to utilize natural gas as a biological feedstock instead of sugar. Methane's 34x higher greenhouse gas contribution relative to CO2 implies that capturing these sources will have a significant environmental benefit. Longer term, biomass-to-methane strategies may eventually enable a fully renewable carbon cycle if 'green' methane-based technologies are developed.

Calysta's proprietary biological GTL platform utilizes genetically engineered methanotrophs. Methanotrops are prokaryotes that utilize methane as their sole source of carbon and energy. Methanotrophs have been observed in a wide range of environments, including both aerobic and anaerobic, typically in association with natural methane sources such as degrading biomass or petroleum offgas. While methanotrophs are a logical starting point for the development of a biological methane conversion platform, a critical requirement for the development of a biotechnology platform is the availability of tools for the directed manipulation and modification of the host cell's metabolism. Although such tools are commonplace for model organisms (e.g. Escherichia coli or Saccharomyces cerevisiae), relatively little effort has been expended to develop similar capabilities in methanotrophs. Calysta Energy has therefore developed a suite of tools for the expression of heterologous proteins in methanotrophs, as well as tools for the efficient targeted manipulation of the methanotroph genome. As Calysta continues to improve the technical capability to modify methanotrophic organisms, we are also making the tools available at no cost to the academic research community to help build interest and critical mass in the field.

It is important to note that the central metabolism of methanotrophs is comparable to that of most model organisms, in that it proceeds through typical metabolites such as pyruvate and acetyl-coA. This means that metabolic pathways which have been developed, characterized, and validated in other host organisms can be readily adapted for use in methanotrophs. Using this approach, Calysta has successfully demonstrated production of a variety of chemicals from methane via engineered metabolic pathways.

Influence of Ammonia to Methanogenic Pathway from Acetate and Methanogen Composition in Anaerobic Digesters

YING JIANG[1], YUE ZHANG[2], SONIA HEAVEN[2] AND CHARLES BANKS[2]

1.Centre for Bioenergy & Resource Management, School of Energy, Environment & Agrifood, Cranfield University, Cranfield, MK43 0AL, UK

2. Faculty of Engineering and the Environment, University of Southampton, Southampton SO17 1BJ, UK

Due to the selective inhibition of ammonia to acetoclastic methanogens, it is postulated that the syntrophic hydrogenotrophic methanogenesis is the dominant route for methane formation in food waste anaerobic digesters with high total ammonia nitrogen (TAN). A radioactive labelling technique was adopted using radiolabelled isotope [2-14C] sodium acetate as a tracer to study the methanogenic pathway in ten mesophilic food waste digesters (TAN within 3.5- 11.1 g), a municipal waste water digester (TAN= 1.58 g) and four mesophilic CSTR digesters fed on low nitrogen artificial food waste (TAN within 0.2-0.3 g). $^{14}CO_2$ and $^{14}CH_4$ produced from each sample was quantified using a scintillation counter. $^{14}CO_2/^{14}CH_4$ ratio, which indicates the syntrophic acetate oxidation intensity in each sample, was calculated based on scintillation counting results.

For the ten high ammonia food waste digester, $^{14}CO_2/^{14}CH_4$ ratios were in the range of 2.1-3, indicating 68-75% of the methane was produced via hydrogenotrophic route; whereas in low ammonia samples the ratio was in the range of 0.06-0.3, indicating 6-22% of the methane was produced via hydrogenotrophic route. In addition, phylogenetic study suggested in low ammonia digesters, the majority of the methanogen population belongs to the taxa of *methanosaeta* which use acetate exclusively as energy source, whereas in high nitrogen food waste digesters *methanosaeta* was not presented but strictly hydrogenotrophic *methanobacterium kanagiense* was found in large quantity.

This conclusively proved ammonia concentration in anaerobic digesters is a key environmental factor that influences the diversity of methanogen population and the metabolic pathway for methane formation from acetate.

Bio-Thermal RED: Supporting SMEs In Anaerobic Digestion

CECILIA FENECH, RAFFAELLA VILLA

Centre for Bioenergy and Resource Management, Cranfield University, Cranfield, MK43 0AL, UK

The Anaerobic Digestion (AD) industry is one of the fastest growing within the UK, with the number of UK AD plants outside of the water sector having more than doubled within the past three years. As the industry grows and matures, it provides an unprecedented opportunity to integrate opportunities for commercial exploitation of the products of this industry, a key product of which is methane.

The Bio-Thermal RED (Biological and Thermal Renewable Energy Demonstrator) project at Cranfield University has set-up a knowledge and networking hub for SMEs in the East of England region involved in bioenergy. This ERDF (European Regional Development) funded project has involved working with a number of SMEs involved or interested in anaerobic digestion and thermal technologies, to provide free project-based support and the organisation of a number of topical workshops relevant to the renewable energy SME sector. The project-based support is available to SMEs to work with Cranfield University researchers and gain access to the university's facilities to de-risk key technologies and facilitate innovation in design, integration, operation and maintenance of AD and thermal technologies. The project also involves the commissioning of a new large pilot-scale food-waste AD, which will treat food-waste arising from the Cranfield University campus and be available for large-scale R&D projects.

During this presentation, a number of case-studies resulting from work by Cranfield University and SMEs as part of the Bio-Thermal RED project will be presented. Projects carried out to date include work on utilisation of new feedstocks for AD, feasibility analysis for small scale energy from waste, development of a best practice guide for energy crop storage, engineering optimisation and technology analyses. The specific focus will be on delivered projects related to process optimisation and their impact on the AD process.

ABSTRACTS OF POSTER PRESENTATIONS

Making Jet Fuel from Greenhouse Gases.
(or The Production of 2,3-butanediol in *Clostridium autoethanogenum*.)

FLORENCE J. ANNAN

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

Recently, there has been a clamouring amongst scientists, politicians, activist groups and random celebrities to "sort out the oil problem". Use of fossil fuels pollutes every environment, and our usage is threatening huge climate change consequences, however, we cannot stop using fossil fuels until alternatives to all of the derivatives have been found. 2,3-butanediol is an important bulk chemical for the production of a wide range of products such as solvents, food additives, synthetic rubbers and as a precursor for polyurethane foams and pharmaceuticals. Traditionally derived from petrochemical routes, dwindling fossil fuels make the microbial production of the diol an increasingly attractive prospect. Over 30 species of microorganisms are able to produce 2,3-butanediol, however, most of these, such as *Klebsiella*, utilize primary stage "food" feedstocks. Syngas is a third generation feedstock which is a mixture of hydrogen, CO, and CO_2 generated from hydrocarbons. These can be obtained from any type of biomass and most importantly municipal waste or industrial waste gases. One organism able to grow on syngas is *Clostridium autoethanogenum*. It is an acetogen and can produce acetate, ethanol, butanol and butyrate from acetyl-coA as well as a range of other chemicals such as 2,3-butanediol. Using *C. autoethanogenum* offers the benefit of using a non-pathogenic strain and also uses syngas fermentation, decoupling the production of 2,3-butanediol from the usage of farmed or food sugars, but instead can be used to convert anthropogenic wastes into useful clean products. *Clostridium autoethanogenum* uses the Wood-Ljungdahl pathway to generate acetyl-CoA from syngas. Acetyl-CoA can be used by the bacterium as a precursor to the diol. This poster attempts to describe the process of the production of the diol in the bacteria, explain the impact of 2,3-butanediol and shed further light on the role of two key enzymes in the production of the diol – 2,3-butanediol dehydrogenase and an alcohol dehydrogenase, by genetically engineering strains which have these genes deleted and overexpressed, using In Frame Deletion, allelic coupled exchange (ACE) with a *pyrE* selection system. Further developments in the process and in the organism will provide a bright new future for the production of 2,3-butanediol by *C. autoethanogenum.*

Cupriavidus necator: A New Approach to the Sustainable Production of Biofuels and Chemical Commodities

CHRISTIAN ARENAS, NIGEL P. MINTON, KLAUS WINZER

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

Easily accessible sources of fossil fuels are depleting at an alarming rate. However, fossil fuel demand is unrelenting, and its effects are apparent on a global scale. Besides being the largest contributor to greenhouse gas (GHG) emissions, increasing energy demands are leading to a rise in crude oil costs, thus affecting economic markets and societies worldwide. There is therefore an urgent need to develop more environmentally friendly and sustainable routes for fuel and chemical production.

The sustainable production of advanced biofuels and chemical commodities requires efficient release of sugars and other fermentable compounds from lignocellulosic biomass. However, the recalcitrance of this material to deconstruction poses a major problem. In particular the lignin component remains inaccessible, as it cannot be efficiently degraded by physical, chemical, or enzymatic treatment.

A possible alternative is therefore the gasification of biomass to yield syngas, a gas mixture consisting of carbon monoxide and hydrogen. In the absence of oxygen, syngas can be utilised by several strictly anaerobic bacteria as the sole source of carbon and energy and this ability is currently being commercially exploited for the production of ethanol from industrial off- gases. Unfortunately, however, the described anaerobic fermentation process cannot drive the production of more complex and energy-demanding compounds. We will therefore investigate whether aerobic gas-fermenting organisms, in particular the bacteria *Cupriavidus necator*, can be engineered to produce desirable chemicals and fuels. Initially, the resistance of the organism to a range of commercially interesting chemicals will be assessed. Metabolic routes to those tolerated at high concentrations will be evaluated and one chosen for implementation using state-of-the-art metabolic engineering and synthetic biology approaches.

The Biological Conversion of Methane to Methanol Using Fine Bubble Aeration

CHARLOTTE E. BJORCK

Department of Chemical and Biological Engineering, Sir Robert Hadfield Building, University of Sheffield, Sheffield, S1 3JD, UK

This project is concerned with the conversion of methane to methanol using biocatalytic methods. There are multiple drivers for interest in this transformation: the highly energy intensive commercial synthesis of methanol, the vast quantity of methane wasted globally, greenhouse gas mitigation strategies, and interest in the selective activation of the notoriously stable C-H bond in methane.

A class of bacteria known as methanotrophs, that are unique in their ability to utilise methane as a sole carbon and energy source, catalyse the transformation to methanol under ambient conditions using the methane monooxygenase (MMO) enzyme. This reaction is followed by the sequential oxidation of methanol to formaldehyde, formate and carbon dioxide producing reducing equivalents for the cell. Previous studies have successfully isolated methanol from methanotroph suspensions, however the key challenges are to stop cellular reactions after the first step and overcome the inhibitory effects of methanol accumulation. This is in addition to the low solubility of methane in water being a growth limiting factor.

This project will investigate use of an airlift bioreactor with fine bubble aeration to maximise methanol production from a methanotrophic culture. Fluidic oscillator generated microbubbles offer enhanced mass transfer of methane from the gas phase to the aqueous phase, and also in the reverse direction in use of the sparging gas for stripping of the volatile methanol product as it forms. In addition to an efficient method for product separation and concentration, product removal will prevent reaction inhibition due to methanol accumulation, while also avoiding over oxidation to formaldehyde.

Novel Chassis For Industrial Biotechnology

BEN BRADLEY

Chain Biotechnology, Imperial College Incubator, Level 1 Bessemer Building, Imperial College, London, SW7 2AZ

CHAIN Biotechnology Ltd. is a UK based Industrial Biotechnology (IB) that provides synthetic biology tools and underpinning services for the bioscience community. The Company focuses on the development of non-conventional microbial hosts (chassis) for a wide range of IB products and applications including biofuels, renewable chemicals, fine chemicals, enzymes, secondary metabolites and nutraceuticals.

CHAIN exploits recent advances in molecular biology for a wide range of industrial microbes. This platform (plasmid vectors, methods for gene knockout and integration, gene expression and transposon mutagenesis) supports the development of non-conventional microbial species including Clostridium, Bacillus, Acetogens and Methanotrophs. The platform technology facilitates metabolic pathway engineering and synthetic biology allowing for higher yields of natural products and/or the production of novel products from a wide range of sustainable feedstocks. These solutions deliver superior and sustainable bio-products resulting in increased profitability.

We report on recent work and plans for improving the performance of gas fermenting microbes.

Integrated approach for optimal design and evaluation of bioprocesses

MLADEN CRNOMARKOVIC, MARCEL EIJKENBOOM

Maturus Optimi Industrial Consulting, London, UK

Bioprocesses are regularly considering new and unproven technologies which impact on process feasibility, energy consumption and economics is often not well understood or properly evaluated. Due to the nature of material streams in bioprocesses, downstream processing can be complex and it requires thorough evaluation and optimization of a single or combined separation technologies. Furthermore, energy requirements of bioprocesses are often too large to make them economically feasible. Therefore it is important to consider and optimize process energy consumption from the very beginning of the design.

Integrated approach for optimal design and evaluation of bioprocesses aims to reduce process development costs and deliver cost effective processes by:

- process optimization with respect to economic and environmental impact
- considering technology, energy efficiency and economics as key process evaluation factors
- minimizing waste and valorisation of waste streams
- enabling fast and reliable evaluation of many different process option and alternatives
- early on identifying and mitigating risks related to process scale-up (technology, energy & economics)
- supporting and guiding process scale-up activities
- guiding R&D activities and setting targets for techno-economically optimal design (yields, temperatures, pressure, etc.)
- supporting process development activities through the whole project life cycle and at each TRL stage.

Converting Organic Waste Into Bioproducts – The European SYNPOL project

OLIVER DRZYZGA, M. AUXILIADORA PRIETO, EDUARDO DÍAZ, JOSÉ L. GARCÍA

Dept. of Environmental Biology, Biological Research Center (CIB-CSIC), C/Ramiro de Maeztu 9, 28040 Madrid, Spain

"Don´t waste waste!" – The SYNPOL project will convert different organic waste streams into bioplastics and building block chemicals.

Waste conversion is going to be a massive growth industry worldwide in the coming years. And there are many waste resources hidden in our communities. For example, municipal solid waste (MSW), agricultural residues and sewage sludge from water treatment plants contains lots of reusable carbon fractions. To recover those means recovering a valuable product as well as preserving the environment.

The EU-funded SYNPOL ("*Biopolymers from syngas fermentation"*) project aims at converting waste into high-value added bioproducts. This will be done by project partners from 8 European countries by coupling waste pretreatment, the subsequent pyrolysis of the waste to produce the so-called syngas (= synthesis gas made of CO, H_2 and CO_2) with bacterial fermentation using the produced syngas as carbon and energy source for bacterial growth. The bacteria will produce building block chemicals and polyhydroxyalkanoates (PHA) that will serve to synthesize novel bio-based plastic prototypes by chemical and enzymatic catalysis. The knowledge generated through this innovative biotechnological approach will not only benefit the environmental management of terrestrial wastes, but also reduce the harmful environmental impact of petroleum-based plastics.

Until September 2016, the R&D activities of the 14 participating partners from academia and industry of the SYNPOL project will be revolutionary in its implementation of novel microwave induced waste pyrolysis coupled to systems-biology defined highly efficient and physiologically balanced recombinant bacteria.

The SYNPOL project (www.synpol.org) presented at the C1net – CHEMICALS FROM C1 GAS CONFERENCE offers a timely strategic action that will enable the EU to lead worldwide the syngas fermentation technology for waste revalorisation and sustainable biopolymer production.

Synthetic Biology Approach For The Production Of Valuable Chemicals

MUHAMMAD EHSAAN, VINOD KUMAR, KLAUS WINZER, NIGEL MINTON

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

The extensive use of fossil based resources for energy generation and petro based chemicals has raised a number of concerns over the sustainability and release of toxic gases into the environment. The recent advances in biotechnology and synthetic biology have the potential to generate biofuels and industrially important chemicals from the resources available abundantly in nature using recombinant bacterial strains. The most commonly used resources are C5/C6 sugars as well as glycerol which is a C3 based substrate. The aim of this project is to engineer the metabolic pathway of *Ralstonia eutropha* using synthetic biology approaches for the production of 3-Hydroxypropionic acid (3-HP) using CO_2 as the carbon source. 3-HP is an important organic chemical with three-carbon used for the production of a number of other chemicals and as a cross-linking agent for polymer coatings, metal lubricants and antistatic agents for textiles. Several pathways are known for the 3-HP production in various microorganisms which will be engineered for *Ralstonia eutropha* using CO_2 as the carbon source.

Hypertransform the Ineffective Transformable

ALEXANDER GROSSE-HONEBRINK, YING ZHANG, NIGEL P. MINTON

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

Efficient transformation of biotechnologically important microorganisms builds the foundation of any serious genetic manipulation. The fact that many organisms are not readily transformable or transformable only with very low efficiencies might therefore present a major barrier for genetic engineering of otherwise highly useful organisms.

One such organism is *Clostridium pasteurianum* with its ability to ferment the biodiesel derived waste product glycerol to the higher value products 1,3-propanediol, ethanol and butanol. A recently published transformation protocol using methylases protecting plasmids against predicted restriction systems could not be reproduced in the wild-type strain by several independent researchers in our laboratory which led us to explore new ways of transforming this species.

In this work, we present a novel technique for the selection of hyper-transformable *C. pasteurianum* mutants. Next generation sequencing allowed us to pinpoint the underlying genotype and efforts are made to prove the SNPs involvement in the hypertransformable phenotype.

A mutant found with the method and labelled *C. pasteurianum* H0D0R1 was shown to be transformable with all gram-positive replicons in the pMTL80000 series. Also, the ClosTron, Allelic Exchange and Allelic Coupled Exchange (ACE) were shown to work in this mutant. Transformation efficiency has further been improved by applying a square wave electro-pulse as opposed to the established exponential decay pulse. Furthermore, the mutant was subjected to phenotypic assays and no difference to the *C. pasteurianum* DSM 525 wild type strain was found. This mutant is thus a suitable laboratory strain for metabolic engineering of this promising biofuel producing species.

We believe our method of finding hypertransformable mutants is applicable in other microorganisms with very low transformation efficiencies.

Chimeric Protein Complex Engineering

KATALIN KOVÁCS, KLAUS WINZER AND NIGEL P. MINTON

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

Natural cellular metabolism relies on highly coordinated and simultaneously processing enzymatic pathways to catabolize the available substrates and synthesise essential biomolecules. The metabolic flux balance in each of these pathways is achieved by channelling substrates between its enzymes. In many metabolic pathways where substrate channelling is crucial, these cellular reactions are catalysed by multi-enzyme complexes or metabolons. Engineered metabolic pathways employing synthetic or heterologous genes often lack the regulatory mechanisms and the structural complexity necessary to substrate channelling. This also results in metabolic flux imbalances and low product yield.

Several studies have shown that synthetic nanostructures can provide modular control over substrate channelling in both bacteria and yeast. In these systems, the presence of a protein scaffold allowed for enhanced cascade catalysis. Here, we aim is to build synthetic protein scaffolds that spatially convert the metabolic enzymes in the synthetic solvent production pathways into designable nanostructures that can modulate the reaction kinetics and substrate channelling and ultimately increase productivity.

We have previously successfully used standardized biological parts, in BioBrick 2 (BB2) format, to assemble multi-functional cellulosomes in *Clostridium acetobutylicum.* Making use of our existing library, the proposed engineered nanostructure assemblies will be built according to the design principles of the cellulosomal systems of cellulolytic bacteria, making use of their well characterized cohesion-dockerin interaction. Accordingly, we aim to build a series of scaffoldin proteins of various complexities to serve as docking modules for the catalytic modules in a programmable manner. In parallel, the enzymes of the targeted pathways will be converted to dockerin bearing enzymes in order to facilitate complex formation. Complex formation and structural complexity will be assessed by native-PAGE and super-resolution microscopy.

Using Synthetic Biology responsibly for the production of sustainable chemicals

GARETH LITTLE, KLAUS WINZER AND NIGEL P MINTON

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

Traditional feedstocks such as crude oil, used for the production of industrial chemicals, result in compounds with an environmentally damaging footprint with low sustainability. Often the production of the required compound necessitates the use of harsh reaction conditions. Polymers made in this way are not typically biodegradable. Using microbes as factories for the production of these chemicals can allow for sustainable manufacture. Sustainable feedstocks such as sugars, waste biomass, or even waste gases such as CO_2 from carbon capture and storage (CCS) can be employed. A wider range of potential synthesis pathways are available using enzymes, with milder reaction conditions, less toxic by-products, and the products themselves can be biodegradable. Examples of industrially useful products that could be created using microorganisms include 3-hydroxypropionic acid and its associated polymers, adipic acid, indigo, artemisinin, shikimate and 1,3-propanediol. Synthetic biology is an industry expanding each year and the renewable chemicals market as a whole is likely to be worth $80bn by 2020. Synthetic biology uses concepts such as standardisation and interoperability pioneered by the engineering disciplines, to create useful compounds from living organisms. Increases in product yield are enabled by the use of custom metabolic pathways, the fine tuning of the expression of these, enzyme design and engineering for new synthesis routes and the control potential offered by genetic circuits. Scientists are able to utilise new technological developments in wet-lab automation, DNA synthesis and sequencing and genetic engineering to rapidly develop microbes capable of production of desired compounds. An example of an early success with synthetic biology is the biosynthesis of artemisinin, an anti-malarial compound.

At Nottingham all of our research is progressing under the framework of Responsible Research and Innovation (RRI). The four principles of RRI are anticipation, reflexivity, inclusion and responsiveness. At all stages of the research, the ethical concerns of interested stakeholders are considered; including the end users, the general public and the researchers themselves. Openness in the decision making of the project is coupled with public education and discussion, shaping the progress and end-goals of the research.

Exploring Regulations in Gas fermentation of *C. autoethanogenum*

THOMAS MILLAT

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

The world's current economy and our current way of life heavily depend on natural gas and petrol. The two fossil-based substances form the basis for the synthesis of a large range of value-added chemicals and products thereof, but additionally provide more than the half of the energy consumed worldwide. However, growing environmental concerns and limited resources of natural gas and petrol raise renewed efforts to establish an ecologic and sustainable production of biochemical and bioenergy. For that purpose, several microbial systems are currently subject of increased considerations.

In microbial gas fermentation, hydrogen, carbon monoxide, and carbon dioxide are used as substrates for a C1-based metabolism providing energy and carbon for the growth of bacterial communities. *Clostridium autoethanogenum* is one of the strict anaerobic bacteria supporting this mode of life. A central role in this special metabolism plays the reductive acetyl-CoA (Wood-Ljungdahl) pathway that combines a molecule of carbon dioxide (carbonyl branch) and a molecule of carbon monoxide or dioxide (methyl branch) to acetyl-CoA. The resulting acetyl-CoA molecule is then either converted into the metabolic end products acetate, ethanol, or 2,3-butanediol or used as building block for more complex molecules required in diverse cellular processes. Interestingly, the formation of the industrial-relevant chemicals ethanol and butanediol can be externally controlled by varying the gas supply, i.e. the gas composition and the gas flow rate. However, the cellular processes regulating the product ratios are not well understood.

This projects aims to unveil the cellular regulations that directs the metabolic flow towards a specific product with a focus on the industrial-relevant chemicals ethanol and butanediol. Towards this end, data from continuous culture experiments applying different gas supply rates will be used as input for mathematical models to investigate the caused changes in the metabolic flow. In addition to cellular regulations, the role of thermodynamic and kinetic regulations is considered to elucidate the complex metabolic network of gas fermentation in *C. autoethanogenum*. This information provide the basis for improvements on industrial application of gas fermentation using deliberated mutations and attuned environmental conditions.

- .

Constructing a Metabolic Network for *C. autoethanogenum*

RUPERT NORMAN

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

Clostridium autoethanogenum is able to convert mono-carbon molecules into valuable chemicals. Flux Balance Analysis will be used as part of an iterative research program designed to optimise this process. A comprehensive metabolic model and its subsequent stoichiometric matrix are required to enable constraint based modelling. Plans to develop software tools enabling accessible understanding of modelling results and relevant data integration are underway.

Fermentation of crude syngas from the bioliq® plant Karlsruhe

FLORIAN OSWALD & ANKE NEUMANN

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

The new bioliq® pilot plant at the Karlsruhe Institute of Technology covers the complete process chain required for producing customized fuels from dry lignocellulosic biomass. For energy densification of the biomass, fast pyrolysis is applied. The liquid pyrolysis oil and solid char obtained can be processed further in the entrained flow-gasifier to tar-free, low-methane raw synthesis gas. Prior to chemically catalyzed fuel synthesis a multistep cleaning of raw synthesis gases is performed: Particles, alkaline salts, HCl, H_2S, COS, CS2, NH_3, and HCN are removed to avoid catalyst poisoning during fuel synthesis. The pilot plant is equipped with an innovative hot-gas cleaning system for particle filtration, pollutant decomposition and adsorption at 500 °C.

Acetogenic bacteria are able to ferment syngas to a variety of organic acids and alcohols, e.g. ethanol, butanol and acetate. In contrast to the catalysts used in the Fischer-Tropsch (FT) process, these biological catalysts can process a broad range of syngas compositions and deal with impurities like sulphur compounds or CO_2. At the moment intense efforts are made to genetically modify *C. ljungdahlii* to optimize product yields, product spectrum and establish synthesis routes to new products. To assess industrial large scale applicability of these strains it will be necessary to determine their performances with crude syngas, as each purifying step will decrease the economy of the process.

Our aim is to establish a platform process for evaluating the use of different qualities of raw syngas obtained from the different steps of Karlsruhe bioliq® plant. Therefore, a setup of multiple 2 L bioreactors with online gas and product analytic is currently developed in our lab.

Quorum sensing in industrial fermentation: elucidating sporulation habits in *C. autoethanogenum*

PAWEL PIATEK, SAMANTHA MCLEAN, NIGEL P. MINTON, KLAUS WINZER

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

Clostridium autoethanogenum is a Gram-positive, motile and anaerobic bacterium. The rod-shaped organism was first discovered and isolated from rabbit faeces and is capable of autotrophic growth by fixing carbon in the form CO and CO_2. Acetogenic organisms such as *C. autoethanogenum* share the Wood-Ljungdahl pathway, which is integral to carbon fixation, and producing a variety of fuel-viable solvents. Within these organisms, there exist forms of cell to cell communication, termed “quorum sensing”, which are responsible for concerted, population-wide changes in gene expression and behavior in response to cell population density.

Quorum sensing is not fully understood in *Clostridium autoethanogenum*, and efforts are underway to delete components of several putative *agr* signalling systems. It is hypothesised that quorum sensing might be responsible for sporulation initiation, as shown for other clostridial species. It may also play a role in regulating fermentation metabolism as shown for the related but non-acetogenic *Clostridium acetobuytlicum.*

Current experiments have shown that sporulation is an intermittent event occurring in *C. autoethanogenum*. From preliminary tests, heat-resistant spores were not observed, but exposure of cells to a 1% oxygen atmosphere appeared to trigger sporulation. However, repeat experiments contradicted previous results as low level sporulation appeared to occur under both strictly anaerobic and hypoxic conditions. Interestingly, genome analysis of the organism revealed a SNP in a conserved region of the *spo0A* gene, encoding the master regulator of sporulation, and further sequence screening in cell populations revealed a majority ratio of mutated to non-mutated variants. This observation may explain the irregular sporulation characteristics seen in the sporulation experiments described above. As laboratory strains of *C. autoethanogenum* appear to have a tendency to lose sporulation, identifying the causes for this could help circumvent further problems when seeking phenotypes in regards to its quorum sensing systems.

Metabolic Analysis of Solventogenic *Clostridium saccharoperbutylacetonicum* N1-4 (HMT)

ELIZABETH SAUNDERS

Microbial and Cellular Sciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, UK.

The market for solvent production is predicted to reach $43.4 billion by 2018 with n-butanol having over 20% market share value. Its main use is for the production of biofuel, butyl-acetate, butyl-acrylate, glycol-ethers and plasticisers.

This project focuses on the metabolic and physiologic characterisation of the acetone-butanol producing model strain N1-4. We will use a systems biology approach involving the construction of a genome scale metabolic model of the microorganism, which will be experimentally validated and informed, through the calculation of parameters associated to growth, maintenance, and solvent biosynthesis.

Chemostat cultures will be used to determine the effects of cell stress caused by acid production in solventogenic clostridia, on bacterial growth and energy metabolism. Organic acids are initial products formed during early stages of growth, while solvent production is produced later on, possibly triggered by changes in pH.

Solventogenesis in N1-4 is under control of the *sol* operon, which contains genes for solvent production, including *bld* *ctfA, ctfB*, *adc*, and *adh*. However, the solventogenic genes contained within the *sol* operon vary between solvent producing species. The saccharolytic species *C. beijerinckii* and N1-4 have an operon structure identical to the amylolytic *C. acetobutylicum.* It was found that a N1-4 degenerate strain carrying the wild-type genotype of *sol* operon, failed to fully induce butanol production. This clearly indicates that other factors, regulatory proteins, 4RNA, and/or metabolites are important for the induction of solvent production. This highlights the need to understand how different operons and Induction mechanisms are connected in order to overcome such problems.

Linking metabolic network analysis, genome analysis and metabolic and physiological observations will help to elucidate the metabolic limitations in solvent production and to design metabolic engineering strategies to overcome those limitations.

Metabolic Pathway Profiling of *Clostridium autoethanogenum* for Sustainable Bioenergy Production

SARAH SCHATSCHNEIDER, LAUDINA SAFO, KLAUS WINZER, NIGEL P. MINTON, DAVID A. BARRETT

Centre for Analytical Bioscience, Boots Science Building, University Park, University of Nottingham, Nottingham, NG7 2RD, UK

Acetogenic *Clostridium* species, such as *C. autoethanogenum*, are able to capture carbon in the form of CO or CO_2 through anaerobic gas fermentation via operation of the Wood-Ljungdahl pathway. Carbon monoxide or CO_2 and hydrogen are converted via acetyl-CoA into ethanol and other products. Gas fermentation represents an extremely promising platform for the sustainable production of biofuels and bulk chemicals.

To understand key metabolic pathways in *C. autoethanogenum* and to overcome bottle-necks for conversion of gases to industrially useful chemical intermediates we are investigating the metabolic pathways of *C. autoethanogenum*. For the quantification of metabolites of the important Wood-Ljungdahl pathway and central carbohydrate metabolism targeted metabolite analysis was performed with measurements of standard compounds in comparison to bacterial extracts via LC-MS/MS analysis. In addition, a global metabolic pathway profiling methodology (metabolomics) was established to detect any wider effects on metabolic pathways as a result of metabolic engineering. For this metabolomics method we use ultrahigh performance liquid chromatography (UPLC) coupled to Thermo Exactive (Orbitrap) high accurate mass MS. Volatile fermentation products and intermediates (acetic acid, ethanol, 2,3-butanediol and isobutanol) were measured using gas chromatography-mass spectrometry (GC-MS).

Carbon capture by non-photosynthetic autotrophs

DEEPTANSHU PANDEY AND SEETHARAMAN VAIDYANATHAN

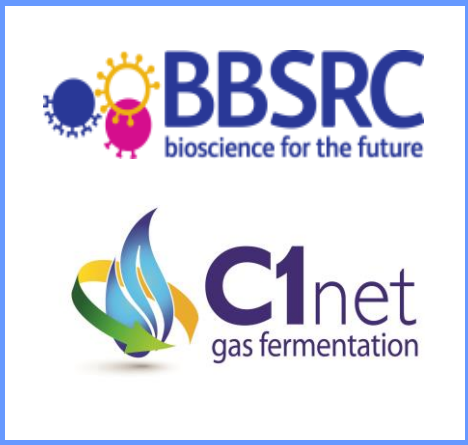
Department of Chemical & Biological Engineering, The University of Sheffield, Sheffield S1 3JD

Carbon dioxide emission into the atmosphere is a major environmental concern, as it is a greenhouse gas. Burning of fossil fuels for the generation of energy is resulting in excess carbon dioxide in the atmosphere. The capture of this C1 carbon and its conversion to products of value is a significant research motivation globally. Carbon dioxide is fixed naturally by photoautotrophs that rely on photosynthesis or light driven processes. Biological carbon fixation using non-photosynthetic autotrophic microorganisms is an alternative route that depends on the provision of reducing equivalents in the form of inorganic compounds. This is predominantly carried out by bacteria that can grow fast. Our interest is in studying the carbon dioxide uptake by these bacteria in a reactor environment and understanding the metabolic versatility of these organisms in utilising different sources of reducing equivalents. An overview of some of the challenges and approaches taken to meet these challenges will be discussed.

PARTICIPANTS

LAST NAME	FIRST NAME	AFFILIATION	EMAIL ADDRESS
Anderson	Edward	The University of Nottingham, UK	pcxeca@nottingham.ac.uk
Annan	Florence	The University of Nottingham, UK	mrxfja@nottingham.ac.uk
Azzopardi	Barry	The University of Nottingham, UK	barry.azzopardi@nottingham.ac.uk
Bashir	Zeenat	The University of Nottingham, UK	zeenatbio@gmail.com
Bjorck	Charlotte	University of Sheffield, UK	c.bjorck@sheffield.ac.uk
Bradley	Ben	Chain Biotechnology, UK	Ben.bradley@chainbiotech.com
Breitkopf	Ronja	The University of Nottingham, UK	mrxrb14@nottingham.ac.uk
Burbidge	Alan	The University of Nottingham, UK	alan.burbidge@nottingham.ac.uk
Carr	Reuben	Ingenza, UK	r.carr@ingenza.com
Chambers	Steve	SynbiCITE- ICL, UK	s.chambers@imperial.ac.uk
Clarke	Lionel	UK SBLC	lionel.clarke.uk@gmail.com
Conde	Sara	Centre for Process Innovation Ltd, UK	sara.conde@uk-cpi.com
Crnomarkovic	Mladen	Maturus Optimi Industrial Consulting, UK	mladen@maturusoptimi.com
Dias	Tatiana	The University of Nottingham, UK	pcxtd@nottingham.ac.uk
Doherty	Tosin	Centre for Process Innovation, UK	Tosin.Doherty@uk-cpi.com
Drzyzga	Oliver	CIB-CSIC, Spain	drzyzga@cib.csic.es
Duerre	Peter	University of Ulm, Germany	peter.duerre@uni-ulm.de
Dynes	Louise	The University of Nottingham, UK	louise.dynes@nottingham.ac.uk
Ehsaan	Muhammad	The University of Nottingham, UK	muhammad.ehsaan@nottingham.ac.uk
Fell	David	Oxford Brookes University, UK	dfell@brookes.ac.uk
Fenech	Cecilia	Cranfield University, UK	c.fenech@cranfield.ac.uk
Finney	Laura	The University of Nottingham, UK	pcxlcf@nottingham.ac.uk
Gabrielli	William	Sasol Technology (UK) Ltd	William.Gabrielli@eu.sasol.com
Gameiro	Mariana	The University of Nottingham, UK	pcxmg4@nottingham.ac.uk
Gemmell	Renia	Green Biologics Ltd, UK	reniag@greenbiologics.com
Goddard	Andrew	Viridor Waste Management Ltd, UK	agoddard@viridor.co.uk
Goodwin	Matt	Centre for Process Innovation Ltd, UK	matt.goodwin@uk-cpi.com
Gradley	Michelle	BioSyntha Technology Ltd, UK	michelle.gradley@biosyntha.com
Green	Edward	Green Biologics Ltd, UK	EdwardG@greenbiologics.com
Grosse-Honebrink	Alex	The University of Nottingham, UK	mrxag11@nottingham.ac.uk
Habgood	Robert	The University of Nottingham, UK	stxrhh@exmail.nottingham.ac.uk
Harrison	Peter	Ecotricity, UK	Peter@weberdee.com
Henry	Paul	The University of Nottingham, UK	pcxph1@nottingham.ac.uk
Henstra	Anne	The University of Nottingham, UK	anne.henstra@nottingham.ac.uk
Higson	Adrian	National Non-Food Crops Centre, UK	A.Higson@nnfcc.co.uk
Ibenegbu	Christopher	University of Bath, UK	chrisibenegbu@yahoo.com
Jiang	Ying	Cranfield University, UK	y.jiang81@gmail.com
Johannessen	Arild	Calysta, Norway	ajo@calysta.com
Juna	Azhar	Centre for Process Innovation Ltd, UK	azhar.juna@uk-cpi.com
Kovacs	Katalin	The University of Nottingham, UK	Katalin.Kovacs@nottingham.ac.uk
Kuehne	Sarah	The University of Nottingham, UK	sarah.kuehne@nottingham.ac.uk
Kumar	Vinod	The University of Nottingham, UK	Vinod.Kumar@nottingham.ac.uk
Lau	Matthew	The University of Nottingham, UK	mbxmsl@nottingham.ac.uk
Licence	Peter	The University of Nottingham, UK	peter.licence@nottingham.ac.uk
Liew	Eric	The University of Nottingham, UK	mrxfml@nottingham.ac.uk
Little	Gareth	The University of Nottingham, UK	gareth.little@nottingham.ac.uk
Lopez	Christian Arenas	The University of Nottingham, UK	mrxca1@nottingham.ac.uk
Lovitt	Bob	Swansea University, UK	r.w.lovitt@swansea.ac.uk

Mansfield	Robert	The University of Nottingham, UK	stxrpm@nottingham.ac.uk
Martin	Steve	BioSyntha Technology Ltd, UK	Steven.Martin@biosyntha.com
Mascia	Rossella	The University of Nottingham, UK	pcxrm4@nottingham.ac.uk
Mather	Richard	Carbon Clean Solutions, UK	richard@richardmather.co.uk
Maxwell-Hogg	Steven	The University of Nottingham, UK	pcxsm16@nottingham.ac.uk
McElroy	David	Ingenza, UK	david@ingenza.com
Miles	Colin	BBSRC, UK	colin.miles@bbsrc.ac.uk
Millard	James	The University of Nottingham, UK	stxcjmi@nottingham.ac.uk
Millat	Thomas	The University of Nottingham, UK	thomas.millat@nottingham.ac.uk
Minton	Jacque	The University of Nottingham, UK	jacqueline.minton@nottingham.ac.uk
Minton	Nigel	The University of Nottingham, UK	nigel.minton@nottingham.ac.uk
Mitra	Robin	Centre for Process Innovation, UK	robin.mitra@uk-cpi.com
Nerlich	Brigitte	The University of Nottingham, UK	brigitte.nerlich@nottingham.ac.uk
Neumann	Anke	Karlsruhe Institute for Technology, Germany	anke.neumann@kit.edu
Norman	Rupert	The University of Nottingham, UK	stxron@nottingham.ac.uk
Omar	Basil	Chain Biotechnology, UK	basil@chainbiotech.com
Oswald	Florian	Karlsruhe Institute for Technology, Germany	florian.oswald@kit.edu
Pandal	Jagroop	Sheffield University, UK	j.pandhal@sheffield.ac.uk
Pander	Bart	The University of Nottingham, UK	mrxbp@nottingham.ac.uk
Piatek	Pawel	The University of Nottingham, UK	mrxpp2@nottingham.ac.uk
Prieto	Auxiliadora	CIB-CSIC, Spain	auxi@cib.csic.es
Roeschlaub	Carl	Synthomer Ltd, UK	carl.roeschlaub@synthomer.com
Ruijssenaars	Harald	Corbion, The Netherlands	Harald.Ruijssenaars@corbion.com
Rumah	Bashir	The University of Nottingham, UK	msxbr@nottingham.ac.uk
Sablitzky	Janice	The University of Nottingham, UK	Janice.Sablitzky@nottingham.ac.uk
Santt	Olivier	BioSyntha Technology Ltd, UK	olivier.santt@biosyntha.com
Saunders	Elizabeth	University of Surrey, UK	elizabeth.saunders@surrey.ac.uk
Schatschneider	Sarah	The University of Nottingham, UK	pazss1@nottingham.ac.uk
Scrutton	Nigel	Manchester University, UK	Nigel.Scrutton@manchester.ac.uk
Silverman	Josh	Calysta, USA	jsilverman@calysta.com
Simpson	Sean	Lanzatech, USA	sean@lanzatech.com
Soucaille	Philippe	INSA, France	soucaill@insa-toulouse.fr
Spencer	Jennifer	The University of Nottingham, UK	stxjs15@nottingham.ac.uk
Stead	Christopher	The University of Nottingham, UK	chris.e.stead@gmail.com
Swainston	Neil	University of Manchester, UK	neil.swainston@manchester.ac.uk
Taylor	Fiona	University Of York, UK	fiona.taylor@york.ac.uk
Thomsett	Megan	The University of Nottingham, UK	mthomsett@live.co.uk
Toftgaard Nielsen	Alex	DTU Biosustain, Denmark	atn@biosustain.dtu.dk
Tolhurst	Lucinda	Lucid Insight Ltd, UK	lucinda@lucid-insight.com
Tooze	Bob	Sasol UK Ltd, UK	Bob.Tooze@eu.sasol.com
Tu	Xin	The University of Liverpool, UK	xin.tu@liv.ac.uk
Vaidyanathan	Raman	University of Sheffield, UK	s.vaidyanathan@sheffield.ac.uk
Vander Meeren	Saskia	Bio Base Europe Pilot Plant, Belgium	saskia.vandermeeren@bbeu.org
Wang	Hengzheng	The University of Nottingham, UK	Hengzheng.Wang@nottingham.ac.uk
Winzer	Klaus	The University of Nottingham, UK	Klaus.winzer@nottingham.ac.uk
Woods	Craig	The University of Nottingham, UK	mrxcw4@nottingham.ac.uk
Zhang	Ying	The University of Nottingham, UK	ying.zhang@nottingham.ac.uk



Acknowledgement

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