The Carbon Recycling Network Conference 4

25 – 27 March 2024

Shrigley Hall
Shrigley Park
Pott Shrigley
Nr Macclesfield
Cheshire
SK10 5SB

Programme and Abstracts

THE CARBON RECYCLING NETWORK

UKRI Biotechnology and Biological Sciences Research Council

Contents

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Welcome

The Management Board welcomes you to the fourth annual CarbonRecycling Network conference. With the news that global warming exceeded 1.5C across an entire year (Feb 2023 to Jan 2024), the importance of gas fermentation as a tool to mitigate fossil carbon footprints becomes ever more apparent. Its exploitation in sustainable chemical production can form part of a net-zero carbon future, so it is encouraging to see this conference so well-supported.

The Network continues to attract new members, currently a community totalling 863 individuals. Industrial membership accounts for 175 of that total and over 200 members come from beyond the UK. This diversity is reflected in the conference presentations where only 8 of the 26 talks are from UK-based researchers, so it is truly an international community.

We are pleased to report that we have committed the full £1m (100%FEC) budget available for 'Proof of Concept' awards with 14 projects either completed or underway. Encouragingly, some of these projects are already leading onto more substantial programmes of applied work. A commissioned report on CO_2 Biomethanation (jointly with EBNet) and a manual on the Safe Handling of C1 Gases have recently been completed and will add significant value to the field. There is still budget available for several Business Interaction Vouchers, so if you have an interesting project to explore with industry, this funding could be a useful resource.

We still have more to do throughout 2024, exploring ideas for a further workshop and an international mission to increase the Network's links and influence.

I am looking forward to meeting you all and hearing about your latest advances in the field. I hope that you find the conference to be a very worthwhile event.

Nigel P Minton
On behalf of the Management Board

CONFERENCE VENUE AND ACCOMMODATION
Shrigley Hall Hotel, Shrigley Park, Pott Shrigley, Nr Macclesfield, Cheshire SK10 5SB.
Two nights' accommodation is provided with breakfast and evening meal. Check-in from **3pm Mon 25 March**; check-out by **11am Wed 27 March**

ORAL PRESENTATIONS
Oral presentations will be in the Hotel's **Tilden Suite**.

The length of oral presentations varies between 15-40 mins as indicated in the Programme. Within that time, presenters should allow 5 min for discussion. All presentations should be prepared in the form of an MS PowerPoint slide show and submitted to the admin team **prior to the conference** but also on a USB stick if needed. The use of a personal computer or Mac is not possible.

POSTER PRESENTATIONS
Poster presentations will be in the Tilden Suite Lounge. The maximum recommended poster size is A0 portrait (90 cm × 120 cm). Velcro tabs will be provided. The presenting author should stand by their poster for the duration of the session.

SOCIAL MEDIA
Our handle on X *(formerly Twitter)* is @CRecycle_Net. We encourage all our delegates to share their attendance at the conference with their networks.
Presenters: please let your audience know if your work is confidential and should NOT be tweeted or recorded.

SOCIAL EVENTS

- **Welcome Dinner**
  Monday 25 March 19:30h - William Turner Suite
- **Drinks Reception* & Conference Dinner**
  Tuesday 26 March 19:00h - William Turner Suite

**The organisers are very grateful to PotterClarkson, IBioIC and NCIMB for their sponsorship of the Drinks reception.*

SHRIGLEY HALL HOTEL

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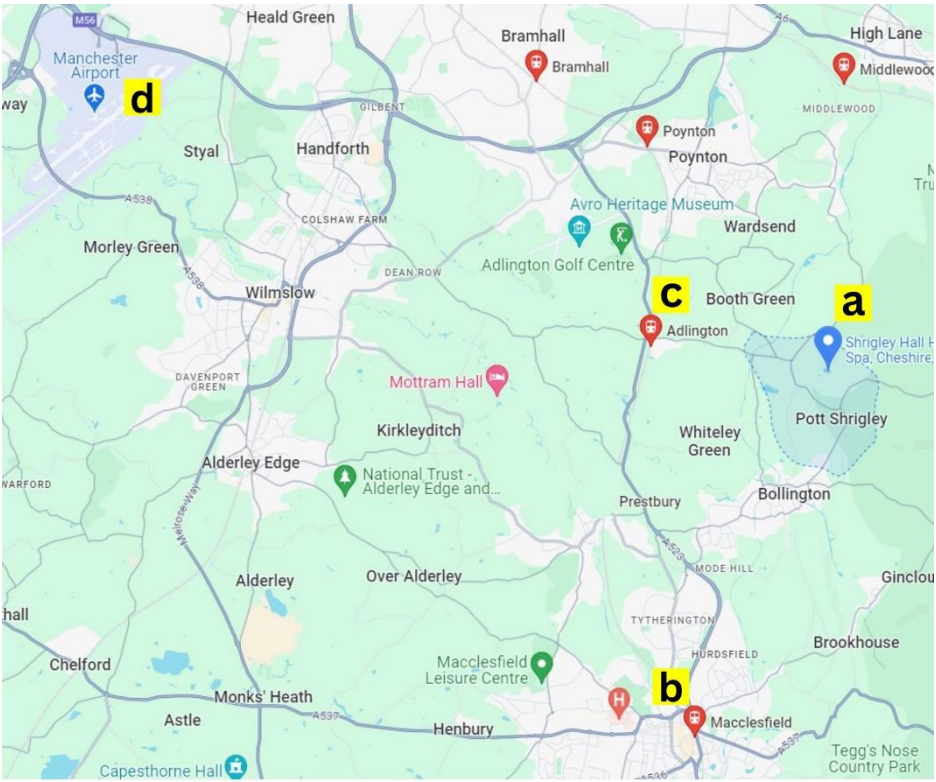
Cheshire SK10 5SB

Located within easy reach of the Peak District National Park.

Travel

- Manchester Airport is 11 miles from the hotel
- There are frequent trains from Manchester Piccadilly to Macclesfield (journey time 20mins).
- The railway station Macclesfield to the hotel is a distance of 6miles (20minutes by taxi)

Map of Local Area



a = Shrigley Hall
b = Macclesfield Train Station
c = Adlington Train Station
d = Manchester Airport

MONDAY 25 MARCH 2024 The Shrigley Hall, Tilden Suite		DAY 1
11:00	**REGISTRATION**	
12:30-13:30	**LUNCH**	
SESSION 1	**CARBON CAPTURE UTILISATION AND STORAGE** **Chair: Prof Nigel Minton (SBRC-Nottingham, UK)**	
13:50-14:00	**Prof Nigel Minton** The Carbon Recycling Network	Welcome & Opening Words
14:00-14:25	**INVITED TALK:** **Dr Qiang Li** Centre for Process Innovation	An Introduction to the Flue2Chem Project - Converting Industrial CO_2 Emissions to Chemicals Using Industrial Biotechnology
14:25-14:50	**INVITED TALK:** **Dr Reuben Carr** Ingenza Ltd	Progress towards a Biocatalytic technology platform for industrial carbon capture utilisation
14:50-15:10	**Dr David Keating** Synata Bio	Efficient Carbon Capture via Advanced Biocatalysts
15:10-15:30	**Dr Tatiana Spatola Rossi** University of Padua	Valorisation of CO_2-rich Waste Gas into Polyhydroxyalkanoates (PHAs) by *Cupriavidus necator*
15:30-16:00	**COFFEE/TEA BREAK**	
SESSION 2	**CARBON CAPTURE UTILISATION AND STORAGE Cont.** **Chair: Dr Stephen Poulston (Johnson Matthey)**	
16:00-16:30	**INVITED TALK:** **Dr Catherine Boccadoro** NORCE: Norwegian Research Centre	$PYROCO_2$ – Demonstrating sustainable value creation from industrial CO_2 by its thermophilic microbial conversion into acetone.
16:30-16:50	**Dr Antti Nyyssölä** VTT Technical Research Centre of Finland	Strain Engineering of Knallgas Bacteria, and Gas Fermentation at VTT Technical Research Centre of Finland
16:50-17:10	**Dr Charles Wickham-Smith** The University of Nottingham	Engineering *Cupriavidus necator* H16 for optimal growth on syngas
17:10-17:30	**Dr Stefan Pflügl** Institute of Chemical, Environmental & Bioscience Engineering, Technische Universität Wien	Continuous Gas Fermentation with *Thermoanaerobacter kivui* Adapted to Carbon Monoxide
17:30	**CLOSE DAY 1**	

TUESDAY 26 MARCH 2024
The Shrigley Hall, Tilden Suite

DAY 2

SESSION 3	C1 UTILISATION Chair: Dr Reuben Carr (Ingenza)	
09:00-09:40	INVITED TALK: **Dr Frank Kensy** b.fab GmbH	Arran Bar-Even Memorial Lecture: Formate Bioeconomy – from its beginnings towards industrial implementation
09:40-10:00	**William Newell** Dept of Bioengineering Imperial College London	Towards C1 Assimilation in *Y. lipolytica*
10:00-10:20	**Tong Wu** Max Planck Institute of Molecular Plant Physiology	EuMP Cycle Enable New Gateway for C1 Assimilation
10:20-10:40	**Dr Marco Garavaglia** The University of Nottingham	Stable Platform for Mevalonate Bioproduction from CO_2
10:40-11:10	**COFFEE/TEA BREAK**	
SESSION 4	**POSTER SESSION**	
11:10-12:30	An opportunity to see the breadth of research activity on C1 feedstocks	
12:30-13:30	**LUNCH**	
SESSION 5	**METHANOL** **Chair: Dr Tithira Wimalasena (Corbion)**	
14:00-14:30	INVITED TALK: **Prof Philippe Soucaille** University of Toulouse	From Systems Biology to Metabolic Engineering of *Eubacterium limosum* B2 for the Conversion of Methanol and CO_2 to C4 Chemicals
14:30-15:00	INVITED TALK: **Dr Nico Claassens** Wageningen University	A systems-view and optimization of bacteria with synthetic reductive glycine pathway for C1-assimilation
15:00-15:30	**Dr Bashir Rumah** The University of Nottingham	Identifying gene essentiality in methanotrophs using TraDIS and future biotechnological implications
15:30-16:00	**COFFEE/TEA BREAK**	

TUESDAY 26 MARCH 2024 The Shrigley Hall, Tilden Suite		DAY 2 cont.
SESSION 6	**MIXED COMMUNITIES** **Chair: Prof Charles Banks (CJC Labs Ltd)**	
16:00-16:30	**INVITED TALK:** **Dr Frank Bengelsdorf** University of Ulm	Lactate-mediated mixotrophic co-cultivation of *Clostridium drakei* and recombinant *Acetobacterium woodii* for autotrophic production of volatile fatty acids
16:30-17:00	**INVITED TALK:** **Dr Mark Walker** University of Hull	Modeling the decarbonisation of carbon-intensive industries through the integration of CO_2 biomethanation: assessment of carbon savings and production costs.
17:00-17:20	**Ameya Pankaj Gupte** Department of Agronomy Food Natural resources Animals and Environment (DAFNAE), Università di Padova	Autotrophic Polyhydroxyalkanoates Production Using Gaseous Streams Produced During Acidogenesis of Fruit Waste
17:20-17:40	**Dr Alberto Robazza** Karlsruhe Institute of Technology	Hybrid Thermochemical-biological Processes for Enhanced Energy Recovery from Waste
17:40	**CLOSE DAY 2**	

WEDNESDAY 27 MARCH 2024 The Shrigley Hall, Tilden Suite		DAY 3
SESSION 7	**GAS FERMENTATION AND SCP** **Chair: Dr Bart Pander (University of Edinburgh)**	
09:00-09:30	**INVITED TALK:** **Dr Marilene Pavan** LanzaTech	Re-Imagining Biomanufacturing to Replace Fossil Sources - Building a circular gas fermentation industry
09:30-09:50	**Harry Newton** Synthetic Biology Research Centre, University of Nottingham	From Genes to Proteins: An Omics Analysis of *Cupriavidus necator* as an Alternative Protein Source
09:50-10:10	**Elodie Vlaeminck** Centre for Industrial Biotechnology and Biocatalysis (InBio.be), Dept of Biotechnology, Ghent University	Valorising Syngas In a Coupled Fermentation via Acetate: Techno-economic Analysis for SCP Production and Pilot-scale Implementation
10:10-10:30	**Dr Christian Fink** Arkeon GmbH	Carbon Recycling The Arkeon Way: We Turn CO_2 Into Functional, Climate Positive Ingredients For Food
10:30-11:00	**COFFEE/TEA BREAK**	
SESSION 8	**THE ROLE OF HYDROGEN** **Chair: Prof Saul Purton (University College London)**	
11:00-11:30	**INVITED TALK:** **Prof Rachael Rothman** University of Sheffield	Developments in Green Hydrogen
11:30-11:50	**Laura Munoz** Biological and Chemical Engineering Dept, Aarhus University	H_2 Consumption Rates by Acetogens Follow First-Order Kinetics in a Wide Range of H_2 Initial Concentrations.
11:50-12:10	**Magda Ardila** Karlsruhe Institute of Technology	Use of Gas Mixtures as a Substrate for H_2 Production with *Parageobacillus thermoglucosidasius* DSM 6285
12:10-12:25	**Sara Holland** Potter Clarkson	The Importance of Intellectual Property
12:25-12:30	**Prof Nigel Minton** The Carbon Recycling Network	Closing Remarks
12:30-14:00	**LUNCH**	
14:00	**CLOSE OF MEETING**	

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Industrial Biotechnology startups are often based around very advanced and technical subject matter. You need to know that your IP advisors are commercially focussed and understand what investors are looking for, and are sensitive to the steep learning curve you are embarking on, but also that they "get" the science your business is founded on.

We are patent attorneys, trade mark attorneys and IP solicitors who work closely together in small, bespoke teams formed around each client. We are over 200 strong (with many of us having spent a long time working in academia or industry) so chances are we have a team with just the right mix of technical background and commercial experience for you.

Most new startups don't necessarily need a new patent application, but do need advice and help around collaboration agreements and in-licensing deals (though we want to review your data regularly!). This is why our blend of patent attorneys and IP solicitors is known for packing a particularly powerful punch in the world of start- ups and spin outs.

We are here to guide you through the process of identifying, protecting and exploiting your IP. The decisions are yours, but you are not alone.

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INVITED SPEAKERS

Dr Qiang Li
CPI

Dr Qiang Li is a Principal Scientist at CPI. He has 16 years of experience working in industrial biotechnology. At CPI he is responsible for delivering projects and acquiring and developing new capabilities for the business. His research interests and expertise span a wide range of topics in industrial biotechnology including upstream and downstream process development and scale-up, with a particular interest in C1 gas fermentations. Previously he has been PDRA at University of Oxford and University College London, before joining INVISTA's bio-derived intermediate business. He has a bachelor's degree in chemical engineering and PhD in fermentation. He is a chartered chemical engineer by IChemE.

Dr Reuben Carr
Ingenza Ltd

Since joining Ingenza in 2004, Reuben has spearheaded the company's core technology development delivering numerous proprietary bioprocesses which has underpinned growth of the company to date.

By utilising Ingenza's world class biotechnology expertise, Reuben has delivered, on time and on budget, commercial projects spanning carbon capture utilisation, biobased chemical production, small molecule API production, medical diagnostics and many others.

Reuben has a strong portfolio in securing investment in mixed private/public and commercial partnerships over many years which has enabled in house and customer technology development.

Reuben plays an integral role in the mentorship of staff at Ingenza and his leadership is founded on a true passion for chemical, life and biomedical sciences which drives his lateral thinking and innovative approach to resolving the needs of Ingenza's customers.

Dr Catherine Boccadoro
NORCE: Norwegian Research Centre

Catherine (Kate) Boccadoro was awarded a PhD in genetic engineering and biodegradation from the University of Cambridge in 2005.

She is Chief Scientist for the Industrial Biotechnology group at The Norwegian Research Centre (NORCE), where she develops and manages projects developing and optimising fermentation processes for industry and academia.

Her research interest are in feedstock diversification, focusing on the use of industrial side streams and byproducts, gas fermentation, and process scale-up.

She will otherwise be found in the mountains climbing, skiing or making up new exciting outdoor hybrid sports.

Dr Frank Kensy
b.fab GmbH

Frank Kensy studied bioprocess engineering at RWTH Aachen University. There, during his doctoral studies with Prof. Jochen Büchs, he developed the BioLector technology, which is now used worldwide for early bioprocess development.

Frank gained his first professional experience at Rhein Biotech GmbH in Düsseldorf in the field of fermentation development for recombinant proteins. From RWTH Aachen University, he and colleagues founded m2p-labs GmbH, which he grew to a leading manufacturer of microbioreactors and led as managing director for almost 10 years. Afterwards, he advised start-ups and biotechnology companies in the field of innovation management and bioprocess development.

Since 2018, he is co-founder and managing director of b.fab GmbH, which specializes in the utilization of CO_2 using electrochemistry and biotechnology. Frank has 25+ years of experience in the Biotech industry leading several industry and publicly funded R&D projects at national and European level.

Prof Philippe Soucaille

University of Toulouse

After an academic career at the University of Toulouse, Philippe Soucaille moved in 1999 to industry, at Genencor International Inc, Palo Alto with the ambition to industrialize some of his findings in the field of sustainable routes to platform chemicals. There, he led the 1, 3 propanediol project that developed the strain and the process used for the last ten years in a facility producing 80,000 tons/year of this chemical.

After coming back to his academic position, he co-founded Metabolic Explorer (now a publically traded company) and became the CSO of the company where he leads a team of 45 researchers that has developed the first industrial process for the biological production of L-methionine. He has an h-index of 55 (Google Scholar), has filed 54 patent families, secured in excess of €10M in funding at the University of Toulouse.

He has served on many national/international committees and as SAB members of several international companies. He holds a Professor position at the University of Toulouse and a chair in synthetic biology at the University of Nottingham.

Dr Nico Claassens
Wageningen University

Dr. Nico J. Claassens is an Associate Professor at the Laboratory of Microbiology, The Netherlands.

A core activity of his Microbial Synthetic Metabolism group is to develop new more rational ways of engineering metabolism and synthetic cells using techniques such as targeted, multiplex genetic engineering and quantitative proteomics. A lot of the research in his group focuses on designing and engineering synthetic pathways to support the efficient use of next-generation, sustainable feedstocks, including CO_2 and one-carbon-substrates, such as formate, methanol and hydrogen. With his research he also wants to contribute to the development of biotechnology to produce carbon-based products more sustainably using CO_2 and green electricity as abundant feedstocks. A specific product class he is interested in is the production of food proteins and other food substances.

After obtaining his PhD at Wageningen University in 2017, Nico worked as postdoc from 2017 till 2019 at the Max Planck Institute Potsdam, Germany with Dr. Arren Bar-Even. In 2019 he obtained a NWO-Veni grant for talented junior researchers and in 2022 he was awarded the NVBMB Prize by the Dutch Society for Biochemistry and Molecular Biology.

In 2021 he co-founded SynBioNL, the Dutch Synthetic Biology Association, to which he acts as advisory board member. Nico is a board member Microbial Biotechnology sections of KNVM (Royal Dutch Academy of Microbiology) and NBV (Dutch Biotechnology Association). Nico serves as Associate Editor for the journal Microbial Cell Factories. Since 2020 Nico has been a member of Wageningen Young Academy, a platform for early-career researchers.

Dr Frank Bengelsdorf
University of Ulm

I work as a senior scientist at the Institute for Molecular Biology and Biotechnology of Prokaryotes at the University of Ulm. In 2022, I have completed my habilitation thesis under guidance of Prof. Dr. Peter Dürre. My group has extensive knowledge of physiology and metabolic engineering in obligate anaerobic bacteria. Comparative physiological and genomic analyzes of anaerobic bacteria are often my methods of choice because they allow the derivation of new hypotheses regarding their metabolism. These hypotheses are then tested by the construction of respective recombinant strains to finally gain new insights. Currently I am working on the following two lines of research. (1) “Metabolic engineering of the acetogenic bacteria” and (2) “Synthetic co-cultures of anaerobic bacteria”

(1) Acetogenic bacteria use the so-called Wood-Ljungdahl pathway to ferment gaseous substrate (CO + CO_2 or H_2 + CO_2) to form e.g. acetate, butyrate, butanol, ethanol, hexanoate, hexanol, lactate or 2,3- Butanediol as metabolic products. *Acetobacterium woodii* and *Clostridium ljungdahlii* are important model organisms.

(2) In nature microorganisms live in complex communities, and their members often engage in metabolic symbioses that can be mutualistic, commensalistic, or parasitic. We focus on the cell-cell interaction of two anaerobic bacteria of an synthetic co-culture.

My research projects have been and are funded, respectively, by grants of the BMBF (German Ministry of Education and Research), FNR (Fachagentur Nachwachsende Rohstoffe e.V.), and DFG (German Research Council).

Dr Mark Walker
University of Hull

Mark graduated with a MEng in Mechanical Engineering from the University of Durham in 2004 and went on to study for his PhD at the University of Southampton, working on the application of membrane bioreactors to the anaerobic digestion (AD) of municipal solid wastes. In his subsequent career as a PDRA at Leeds, and later Sheffield he has continued to work on projects focussing on applications of anaerobic digestion.

Currently Mark is lecturer in Mechanical Engineering at the University of Hull. His research has broadened to incorporate more general issues surrounding energy system integration and decarbonisation, use of biomass resources, industrial/environmental biotechnology and the circular economy. With a focus on biochemical systems and AD, he applies process modelling and simulation to develop integrated models that can be used to design, analyse and optimise engineering systems that involve combinations of processes and technologies, and assess them against sustainability and economic criteria.

Marilene Pavan
LanzaTech

Marilene Pavan is currently working as an Innovation Manager at LanzaTech Inc., a carbon recycling technology company that specializes in converting waste carbon oxides into biofuels and chemicals. She is a Biologist with 15+ years of experience in the fields of synthetic biology, metabolic engineering, and biomanufacturing, with previous experience working for chemical industries like Braskem S/A and leading biotech companies such as Monsanto S/A. She moved to the US in 2016 to work as Research Fellow at Boston University, later joining Lanzatech in 2019.

Expertise also includes tech monitoring, partnerships (prospection and management), community and team building, people management and mentorship, fundraising, business development, writing of grants, patents, and scientific articles, project evaluation and management, budget management, scientific consulting, planning of scientific conferences, and speaker. She holds a Master's degree in Molecular Biology and is also a Specialization in Strategic Management of Technological Innovation. She is currently a Ph.D. Candidate in the Bioenergy Program of the University of Campinas, UNICAMP – Brazil.

Prof Rachael Rothman

University of Sheffield

Rachael is Professor of Sustainable Chemical Engineering, Director of the South Yorkshire Sustainability Centre, Co-Director of the Grantham Centre for Sustainable Futures, Co-Director of the UK Hub for Research Challenges in Hydrogen and Alternative Liquid Fuels and Academic Lead for Sustainability at the University of Sheffield.

Rachael is a thought leader in sustainability, regularly engaging with policy makers, the media and the public. Her research expertise lies in development and analysis of sustainable processes and systems. She has a background in large scale hydrogen production and carbon dioxide utilisation and has more recently worked on the sustainability of Foundation Industry processes, plastics and packaging. Rachael leads projects that take an interdisciplinary, whole systems approach to developing routes to net zero and zero waste, combining insights from engineering, psychology, linguistics, sociology and the physical sciences.

ABSTRACTS OF ORAL PRESENTATIONS

DR QIANG LI
Centre for Process Innovation (CPI)

An Introduction to the Flue2Chem Project - Converting Industrial CO_2 Emissions to Chemicals Using Industrial Biotechnology

The Flue2Chem project is a two-year programme funded by Innovate UK via the UK Research and Innovation (UKRI) Transforming Foundation Industries Challenge. The consortium is made of multiple partners including those from academia, industry, RTOs, media etc, aiming to tackle the waste gas from foundation industries and generate an alternative source of carbon for UK consumers. The role of CPI in this consortium is to develop and demonstrate the technology of converting captured CO_2 from industrial emitters to an intermediate product, fatty acid methyl ester (FAME). In this project, naturally occurring autotrophic species were genetically modified to increase the product titre and to shift the carbon chain length towards medium chain fatty acid. Lab scale gas fermentations were carried out to optimise the fermentation performance, and to generate material for the studies on downstream processing development. Product (triacylglycerol, TAG) recovery and purification from cells was first developed in the lab and then scaled up to pilot plant. Finally process chemistry was applied to convert TAG to FAME using homogeneous catalyst.

DR REUBEN CARR
Ingenza

Progress towards a Biocatalytic technology platform for industrial carbon capture utilisation

Through support from UK government BEIS (now DESNZ) investments Ingenza Ltd and Johnson Matthey Plc are collaborating in the development of a novel CCUS technology. The project builds on the success of former BBSRC NIBB Proof of Concept investigations and is now aiming to take this process to reduce the cost of deployment at industrial scale. This presentation will highlight the progress to date and explore the potential industrial application of this CCUS technology platform to deliver negative Green House Gas footprinted products and processes.

DR DAVID KEATING
Synata Bio, USA

Synata Bio: Efficient Carbon Capture Via Advanced Biocatalysts

Synata Bio is a US-based company focusing on the conversion of waste CO_2 into bio-based fuels and chemicals. Work over the past several years has optimized our gas-fermentation technology and construction of a 50KTA plant is underway in China, with mechanical completion set for the third quarter of 2024. A key advantage of our CO_2 capture technology is our proprietary biocatalyst. This strain, referred to as *Clostridium palustris*, displays elevated hydrogen and CO_2 conversion and greater selectivity towards ethanol, compared to related syngas consuming strains. Furthermore, Synata Bio has leveraged technology advanced by the Clostridium community for development of a series of molecular tools in *C. palustris*, enabling construction of genome modifications via CRISPR and allelic exchange, as well as the use of multicopy plasmids.

Comparison of the genome of *C. palustris* with related strains suggested metabolic explanations for its unusual characteristics. *C. palustris* was found to display elevated expression of the primary bifurcating hydrogenase with respect to *C. autoethanogenum*. This comparative analysis identified an unusual region upstream of the primary bifurcating hydrogenase of *C. palustris* not observed in *C. autoethanogenum* or related syngas-consuming Clostridia. Strain engineering studies have demonstrated that this altered upstream region is sufficient to alter gene expression. Additional genome differences have been observed between *C. palustris* and *C. autoethanogenum*, the role of which are currently being investigated.

DR TATIANA SPATOLA ROSSI
University of Padua, Italy

Valorisation of CO_2-rich Waste Gas into Polyhydroxyalkanoates (PHAs) by *Cupriavidus necator*

TATIANA SPATOLA ROSSI, AMEYA PANKAJ GUPTE, ANNA SANTIN, MARIA SILVIA MORLINO, LORENZO FAVARO, LAURA TREU, STEFANO CAMPANARO

Department of Biology (DiBio), University of Padua, Padua, 35131, Italy

Polyhydroxyalkanoates (PHAs) are a family of biodegradable and biocompatible polymers that comprise sustainable alternatives to petroleum-based plastics. PHAs are produced by some microorganisms as carbon storage granules under nutrient limiting conditions. In particular, *Cupriavidus necator* has been widely studied as a promising platform for PHA production due to its ability to accumulate high levels of PHAs. The bacterium is able to grow both heterotrophically and autotrophically by consuming CO_2. The latter is especially interesting as it presents a way to fix CO_2 and convert it into useful products such as PHAs, a bioprocess that fits within the context of a circular economy.

The production of PHAs, and especially polyhydroxybutyrate (PHB) - the main type of biopolymer produced - in *C. necator* has been widely studied. However, the application of this carbon capture method to real-life waste gasses is scarce. Moreover, the process has a high hydrogen and oxygen requirement, posing the risk of explosiveness, and a relatively low carbon fixation rate. Thus, further need for optimization of the process is required.

In this work, we used *C. necator* for the valorisation of CO_2-rich gas derived from the fermentation of grape must during wine production, a process which emits thousands of tonnes of CO_2 per year. We fed fermentation gas to *C. necator* under phosphate limiting conditions obtaining 25% PHB (w/w). In order to increase this PHB content, we carried out a systematic comparison of several other nutrient stress conditions, obtaining an improved PHB content of 55%, and assessed growth under low H_2 conditions. Finally, we are investigating the optimisation of the CO_2 uptake rate of *C. necator* via genetic engineering, by developing mutant *C. necator* strains which overexpress key genes or regulatory elements of the main metabolic pathways involved in the autotrophic metabolism. These findings aim to better adapt the process for real CO_2 capture applications.

DR CATHERINE BOCCADORO
NORCE

PYROCO$_2$ – Demonstrating sustainable value creation from industrial CO$_2$ by its thermophilic microbial conversion into acetone.

The conversion of gases such as CO_2, CO, CH_4 and H_2 into high value biomass and chemical compounds through microbial fermentation is a promising technology that has gained focus in recent years. PYROCO$_2$ is a Horizon 2020 Green Deal European project initiated in 2021. It aims to demonstrate the scalability, practicality and economic viability of carbon capture and utilization (CCU) to make climate-positive acetone out of industrial CO_2 and renewable electricity-derived hydrogen. The project will build a demonstrator plant strategically placed at Herøya Industry Park, Norway, in close proximity to industries and access to green energy sources. The ambitious goal is to demonstrate the production of 4000 tonnes of acetone from 9100 tonnes of industrial CO_2 by 2026. Acetone is a key commodity in the chemical industry, and the process will not only reduce emissions but also valorize CO_2 through the production of commercially viable products, thereby contributing to the sustainability of Europe's chemical industry.

A 2-step fermentation process is being developed using robust cell factories that have been designed and improved for the thermophilic microbial conversion of CO_2 to acetone. The process is currently already being optimized and scaled up to 160L gas fermentation in a uniquely designed combined stirred tank and air-lift reactor. Process modules for further chemical processing of the acetone are being developed to produce other chemicals, materials and synthetic biofuels.

DR ANTTI NYYSSÖLÄ
VTT Technical Research Centre of Finland

Strain Engineering of Knallgas Bacteria, and Gas Fermentation at VTT Technical Research Centre of Finland

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In AEROCOW VTT is studying the production of secreted edible proteins by a Knallgas bacterium. We are screening different secretion signals and promoters, and their combinations for production of edible proteins from carbon dioxide, hydrogen, and oxygen. Target proteins of the project are various milk, egg, plant, and sweet proteins.

CARBONCHAIN, coordinated by Natural Resources Institute Finland, is a collaborative project between Finnish research partners, businesses, and cities. The project explores the potential of biogas derived carbon dioxide as a resource for various industries and applications. VTT analyses biogenic carbon dioxide fractions from different sources and the effects of various isolation methods on gas compositions. Furthermore, the project evaluates the impact of impurities and gas components on the growth of Knallgas bacteria for their utilization as hosts for single cell protein, chemical and material production.

KNALLRED studies the use of engineered Knallgas bacteria as catalysts for whole-cell reductive biotransformations. The soluble hydrogenase-catalyzed oxidation of hydrogen is employed for regeneration of NAD(P)H required for the reductions. The project has demonstrated the quantitative conversion of xylose to the sweetener and platform chemical xylitol with resting cells of *Cupriavidus necator*, expressing a yeast xylose reductase gene. This work was carried out as collaboration with Wageningen University. The development of the platform for more efficient conversions is underway.

DR CHARLES WICKHAM-SMITH
The University of Nottingham

Engineering *Cupriavidus necator* H16 for optimal growth on syngas

Synthesis gas (syngas) is a promising feedstock for microbial fermentation, to produce industrially relevant chemicals and fuels. Syngas mixtures are energy rich, typically containing large volumes of hydrogen (H_2), as well as carbon dioxide (CO_2) and methane (CH_4) but is often largely comprised of the toxic gas carbon monoxide (CO). Therefore to allow efficient bacterial growth to occur, for increased productivity and titres, a high resistance to the gas is required. The aerobic bacterium *Cupriavidus necator* H16 can grow on CO_2 + H_2 and is able to oxidise CO to CO_2 following genetic engineering, thus has the potential to utilise syngas efficiently. This work aimed to increase CO resistance through adaptive laboratory evolution by continually subculturing the organism in the presence of CO both heterotrophically and autotrophically. Heterotrophic growth with fructose produced isolates that displayed a clear growth advantage over the wild type strain. Whole genome sequencing revealed various mutations, including a single point mutation upstream of a cytochrome *bd* ubiquinol oxidase operon (*cydA2B2*), which was present in all evolved isolates. When a subset of these mutations was engineered into the parental H16 strain, only the *cydA2B2* upstream mutation enabled faster growth in the presence of CO. Subsequent expression analysis, mutation and overexpression suggested that *cydA2B2* transcription is upregulated in the evolved isolates, resulting in increased CO tolerance only under heterotrophic conditions. Through subculturing on a syngas-like mixture with increasing CO concentrations, *C. necator* could also be evolved to tolerate high CO concentrations under autotrophic conditions, whilst utilising CO_2 as a carbon source. A mutation in the gene for the soluble [NiFe]-hydrogenase subunit hoxH was identified in the evolved isolates. When this amino acid-changing mutation was engineered into the parental strain, autotrophic CO resistance was exhibited. A strain constitutively expressing *cydA2B2* and the mutated hoxH gene displayed high CO tolerance under both heterotrophic and autotrophic conditions. This strain offers a promising chassis for syngas-based bioproduction processes.

DR STEFAN PFLÜGL
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Continuous Gas Fermentation with *Thermoanaerobacter kivui* Adapted to Carbon Monoxide

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Conversion of gaseous one carbon substrates (CO, CO_2) and H_2 by acetogenic bacteria is a promising technology to establish sustainable bioproduction scenarios. The thermophilic acetogen *Thermoanaerobacter kivui* (Topt = 66 °C) grows on H_2/CO_2 in chemically defined mineral medium with growth rates (doubling time: 2 h), exceeding those of mesophilic acetogens. Nevertheless, there is currently no bioprocessing system available for quantitative characterization of *T. kivui* as a model thermophilic acetogen under well-defined bioreactor conditions. In this study, we established a continuous high-temperature gas fermentation system and characterized *T. kivui* wildtype as well as a strain adapted to grow on CO (referred to as CO-1). *T. kivui* CO-1 grew in a 100 % CO gas phase in chemically defined mineral medium with a growth rate of up to 0.25 h-1 (doubling time: 2.8 h) and adaptation occurred in as little as 30 generations, similar to previous work by Weghoff and Müller (2016) using rich medium [1]. Genomic analysis of the clonal strain CO-1 using short and long read sequencing technologies indicated that a handful of SNPs and large-scale genomic rearrangements might be responsible for the successful adaptation of *T. kivui* to CO. To characterize the physiology of strain CO-1 in more detail, steady state chemostat cultures operated at specific growth rates of 0.10-0.20 h-1 were used to quantify growth, gas consumption, and acetate production on H_2/CO_2, syngas and pure CO. Transcriptomic analysis gave further clues how the physiology of strain CO-1 is adapted to growth on CO. Furthermore, synthesis gas generated from biomass gasification was successfully evaluated as a feedstock for gas fermentation with *T. kivui* in continuous mode in 200 mL stirred tank bioreactors as well as a 20 L bubble column bioreactor. Collectively, the knowledge gained in this study represents a first step toward establishing high-temperature gas fermentation processes.

1. Weghoff, M. C. & Müller, V. CO Metabolism in the Thermophilic Acetogen Thermoanaerobacter kivui. Appl. Environ. Microbiol. 82, 2312–2319 (2016). https://doi.org/10.1128/AEM.00122-16

DR FRANK KENSY
b.fab GmbH

Formate Bioeconomy – from its beginnings towards industrial implementation

Arren Bar-Even coined the term Formate Bioeconomy and laid the foundations for the utilisation of formic acid in biotechnological value chains with the discovery and establishment of the reductive glycine pathway. By using formic acid as the sole substrate in bioprocesses, the efficient utilisation of CO_2 is made possible by combining electrochemistry with biotechnology. The liquid substrate formic acid produced in an electrolyser can be pumped into the bioprocess instead of sugar substrates. Unlike gas fermentations, the process can be carried out in standard bioreactors and at atmospheric pressures. b.fab has set itself the task of taking up the early research of the Bar-Even Lab and transferring the new synthetic metabolic pathways into industrial practice. As co-founder of b.fab, Arren Bar-Even had a lasting influence on the process and was closely involved until his early death. The presentation will provide insights on the further development of the reductive glycine pathway, bioprocess development, system integration and the status of industrial implementation.

WILLIAM NEWELL
Imperial College London

Towards C1 Assimilation in *Y. lipolytica*

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Soluble C1 compounds like formate can be produced directly from greenhouse gases via electrical reduction, providing a genuinely scalable alternative to current feedstocks. While several prokaryotes have been converted to formatotrophy, no formatotrophic eukaryotes exist. *Y. lipolytica* is a non-model, oleaginous yeast with considerable metabolic flexibility and great potential as a platform for bioproduction. Here, we present a strain of *Y. lipolytica* evolved to grow on formate as a sole carbon and energy source. First, using GSMs, we predict that *Y. lipolytica* hosts several cryptic formatotrophy pathways. We also show that *Y. lipolytica* is able to use formate as a sole energy source during specific culture conditions via endogenous formate dehydrogenases. Then, modelling and C13 tracer experiments show that formate can be used as a supplementary carbon source via endogenous metabolism. We then use this to inform directed evolution experiments which lead to a *Y. lipolytica* strain able to grow on formate. Mutations in mitochondrial biosynthesis, central carbon metabolism regulation and ROS resistance appear to support formatotrophic growth in the evolved strain. We then engineer cofactor metabolism and endogenous ROS resistance mechanisms to improve final biomass titre. We also demonstrate that carotenoids can be produced from formate in *Y. lipolytica*, the first example of formate-derived terpenoid bioproduction. Our work shows how novel formatotrophy can arise from evolution of native metabolic systems alone and could support future engineering efforts in eukaryotic hosts.

TONG WU
Charité – University Medicine Berlin

EuMP Cycle Enable New Gateway for C1 Assimilation

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One-carbon (C1) substrates, such as methanol or formate, are attractive feedstocks for circular bioeconomy. C1 substrates are typically converted into formaldehyde, which represents the entry point into metabolism. Here we designed a synthetic erythrulose monophosphate (EuMP) cycle for formaldehyde assimilation that is based on a promiscuous dihydroxyacetone phosphate dependent aldolase as key enzyme. *In silico* modeling reveals that the cycle is highly energy efficient and has the potential to allow fast growth and to generate high bioproduct yields. Dissecting the EuMP into four modules, we used a stepwise strategy to demonstrate *in vivo* feasibility of the modules in *E. coli* metabolite sensor strains. Adaptive laboratory evolution has been performed for further integration of the modules and identified key mutations enabling the accommodation of the EuMP reactions with endogenous metabolism. Overall, our study demonstrates the proof-of-concept for a highly efficient, new-to-nature formaldehyde assimilation pathway, opening the way for the development of a methylotrophic platform for a C1 fueled bioeconomy in the future.

DR MARCO GARAVAGLIA
The University of Nottingham

Stable Platform for Mevalonate Bioproduction from CO_2

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Stable production of value-added products using microbial chassis is pivotal for determining the industrial suitability of the engineered biocatalyst. Microbial cells often become degenerate of multi-copy expression plasmids during long term cultivations. Owing to the advantages related to titres, yields and productivities when using a multi-copy expression system compared with genomic integrations, plasmid stability is essential for industrially relevant bio-based processes. *Cupriavidus necator* H16, a facultative chemolithoautotrophic bacterium has been successfully engineered to convert inorganic carbon obtained from CO_2 fixation into value-added products. The application of this unique capability in the biotech industry has, however, been hindered by *C. necator* H16 inability to stably maintain multi-copy plasmids. In this study, we designed and tested plasmid-addiction systems based on complementation of essential genes. Among these, implementation of a plasmid-addiction tool based on the complementation of mutants lacking RubisCO, which is essential for CO_2 fixation, successfully stabilized a multi-copy plasmid. Expressing the mevalonate pathway operon (MvaES) using this addiction system resulted in the production of ~10 g/L mevalonate, with carbon yields of ~25%. The mevalonate titres and yields obtained here using CO_2 are the highest achieved to date for production of C6 compounds from C1 feedstocks.

PROF PHILIPPE SOUCAILLE
INSA Toulouse

From Systems Biology to Metabolic Engineering of *Eubacterium limosum* B2 for the Conversion of Methanol and CO_2 to C4 Chemicals

Eubacterium limosum B2 is a strict anaerobic bacterium, belonging to the group of acetogens. Its interest lies in its ability to convert methanol and other one carbon (C1) feedstocks into butyrate, a C4 molecule using the Wood-Ljungdahl Pathway (WLP).

Using an adaptive laboratory evolution process an evolved strain growing on synthetic methanol medium without yeast extract was isolated. Sequencing of the mutant revealed an homologous recombination event in the genes encoding the type I restriction-modification system between and a different methylome between the two strains. Exploration of the total proteomes of the native and an evolved clone revealed significant differences in proteins involved in gluconeogenesis, anaplerotic reactions, and sulphate metabolism. Taken together, the genomic, proteomic and methylomic data suggest a possible epigenetic mechanism of metabolic regulation

A systems biology approach was developed to fully characterized the central metabolism of the evolved *E. limosum* B2 growing on a mineral media, in chemostat cultures, with methanol or glucose as a carbon source. The physiological parameters were determined as well as all the input and output fluxes. These values were integrated into an *in silico* genome scale model to estimate the specific fluxes of each enzyme of the central metabolism. From these estimations, energy conversation models were developed for the two conditions studied. The absolute number of mRNA and protein molecules per cell were then determined. These data allowed the determination of the strength of the promoters and ribosome binding sites as well as the estimation of the *in vivo* turnover rates for each enzyme. This parameter was useful to compare enzyme activities and identify the most limiting ones.

Based on this systems biology characterization, a rational metabolic engineering approach was applied to *E. limosum* B2 to develop recombinant strains producing different C4 compounds from methanol at very high carbon yield.

DR NICO CLAASSENS
Wageningen University, The Netherlands

A systems-view and optimization of bacteria with synthetic reductive glycine pathway for C1-assimilation

In recent years several synthetic C1-fixation pathways, including the reductive glycine pathway for the energy-efficient assimilation of formate and other C1-feedstocks has been demonstrated. However, the strains of the bacteria *Escherichia coli* and *Cupriavidus necator* that were initially equipped with this full pathway had still relatively slow growth rates and yields, which limits their potential for sustainable industrial bioproduction. In our recent work we study the engineered C1-utilizers at a system-level to understand potential bottlenecks, especially by quantitative proteomic analysis and metabolic modelling. We have studied the original formatotrophic *E. coli* strain utilizing the reductive glycine pathway, as well as two laboratory evolved strains, investigating their quantitative proteome allocation. For the natural formatotroph *Cupriavidus necator*, in which we implemented the rGlyP previously, we have further engineered a library of genome-engineered strains, selected better performing strains and also studied their proteome allocation. The best performing strain of *C. necator* has the now highest reported yield on formate for this synthetic pathway, by now beating the yield of its natural counterpart, the less energy-efficient Calvin cycle during. Overall, these studies provide us clues to get closer to industrially viable C1-substrate utilizers.

DR BASHIR RUMAH
The University of Nottingham

Identifying Gene Essentiality in Methanotrophs using TraDIS and Future Biotechnological Implications

Methanotrophic bacteria are Gram-negative, aerobic organisms that use methane as sole source of carbon and energy. Methanotrophs can be used to produce metabolites like poly-3-hydroxybutyrate (PHB) and single cell proteins for animal feed. Here, we constructed and exemplified a CRISPR/Cas9 genome editing system and used it to successfully make gene deletions and insertions in the type I *Methylococcus capsulatus* Bath and the type II *Methylocystis parvus* OBBP. To determine suitable gene targets for deletion in these methanotrophs, Transposon Directed Insertion Sequencing (TraDIS), a powerful sequencing tool for determining essential and non-essential genes in bacteria was used. A Tn5 transposon plasmid was used to make a library of approximately 1,000,000 transposon mutants. Using the TraDIS pipeline for downstream processing of these transposon mutant libraries, essential and non-essential genes were determined. In *Methylocystis parvus*, CRISPR/Cas9 was used to validate predictions from TraDIS by attempting to delete 11 genes related to PHB metabolism. PHB metabolism genes predicted to be essential could not be deleted with CRISPR/Cas9, whereas all non-essential genes were deleted demonstrating the reliability of our TraDIS data. The outcome provided a reliable guide for understanding genes important in PHB metabolism and how biosynthesis of this important biopolymer can be enhanced.

DR FRANK BENGELSDORF
Ulm University

Lactate-mediated Mixotrophic Co-cultivation of *Clostridium drakei* and Recombinant *Acetobacterium woodii* for Autotrophic Production of Volatile Fatty Acids

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Background
Anaerobic chemolitoautotrophic bacteria, also known as acetogens, are promising whole cell biocatalysts that fixate CO_2 during their growth. However, because of energetic constraints, acetogens exhibit low growth and a narrow product spectrum often limited to acetate. Enabling acetogens to form more valuable products such as volatile fatty acids during autotrophic growth is imperative for strengthening their place in a future carbon neutral industry. Co-cultivation of strains with different capabilities has the potential to ease the limiting energetic constraints. The lactate-mediated co-culture of an *Acetobacteriun woodii* mutant strain, capable of lactate production, with the *Clostridium drakei* SL1 type strain can produce butyrate and hexanoate. In this study the preceding co-culture is characterized by comparison of monocultures and different co-culture approaches.

Results
C. drakei shows limited growth using H_2 + CO_2 as singular substrate and is prospered when further supplemented with D-lactate. Gases and lactate were consumed in a mixotrophic manner with acetate and butyrate as main products and slight accumulation of hexanoate. A lactate-mediated co-culture of the engineered *A. woodii* [P_{bgaL}_ldhD_NFP] strain and *C. drakei* produced up to 4 mM hexanoate and 18.5 mM butyrate, quadrupling and doubling the respective titers compared to a non-lactate-mediated co-culture. Further co-cultivation experiments revealed the possible advantage of sequential co-culture over concurrent approaches. Formate was periodically produced and eventually consumed and probably serves as a second intermediate next to lactate. Electron microscopy of the strains revealed cell-to-cell contact between the co-culture partners. Finally, a combined pathway of *A. woodii* [P_{bgaL}_ldhD_NFP] and *C. drakei* for chain-elongation with positive ATP yield is proposed.

Conclusion
Lactate was proven to be a well-suited intermediate to combine the high gas uptake capabilities of *A. woodii* with the chain elongation potential of *C. drakei*. The involved metabolic pathways of both strains were elaborate, but for *C. drakei* it is still puzzling to fully explain the consumption of formate while H_2 + CO_2 is also available. The cell-to-cell contact observed here remains to be further characterized in its nature but hints at diffusive processes being involved in the co-culture.

DR MARK WALKER
University of Hull

Modeling the Decarbonisation of Carbon-intensive Industries Through the Integration of CO_2 Biomethanation: Assessment of Carbon Savings and Production Costs

CO_2 Biomethanation is the biological conversion of carbon dioxide and hydrogen to biomethane via the action of hydrogenotrophic methanogens. In this modeling study, we investigated the integration of biomethanation technology with carbon intensive industries (Cement, steel, distillery, pulp and paper, and ammonia), applying carbon capture technologies to isolate carbon dioxide in from industrial flue- and off-gasses, with the hydrogen supplied through the electrolysis of water. The produced biomethane was used as a drop-in fuel to replace natural gas use within the processes, while excess production was exported for downstream use.

Integration scenarios were designed whilst making minimal change to the baseline industrial process such that it could be considered a potential retrofit upgrade to existing industrial infrastructure. Process modelling was used to define relevant mass and energy flows which were quantified based on reaction stoichiometry and literature data, which was subsequently used to calculate updated GHG (Greenhouse Gas) emissions and production costs for the integrated systems.

Results of the modelling suggested that integration of CO_2 biomethanation could result in substantial decarbonisation across all industries studied, ranging from 0.70-2.87 $tCO_{2e}/t_{product}$. The decarbonisation was mainly driven by the substitution of natural gas with biomethane, both in the industrial process and exported, and furthermore, results showed that meaningful decarbonisation was only achieved where renewable electricity with the lowest lifecycle GHG emissions was used e.g. wind, solar. Where the carbon source was biogenic (pulp and paper, distillery) the integration meant all direct fossil emissions were replaced with equivalent biogenic emissions, and it was found that the decarbonisation effect was more pronounced such that decarbonisation via biomethanation could be more effective than direct electrification in these cases.

Economic assessment revealed large increases in production cost for the integrated processes relative to the baseline industrial process, mainly driven by the high costs of hydrogen production. It is considered in this work whether the production of higher value products (other than biomethane) could offset these additional costs.

For the integrated processes, two metrics were proposed for comparison with other decarbonisation options. The ‘decarbonisation intensity’, defined as the fossil-GHG avoided per unit of renewable energy consumed, was calculated as 0.084-0.096 tCO_{2e}/MWh, and ‘cost of decarbonisation’, defined as the additional costs of avoiding GHG emissions, was estimated as £773-837/tCO_{2e}.

AMEYA PANKAJ GUPTE
Università di Padova

Autotrophic Polyhydroxyalkanoates Production Using Gaseous Streams Produced During Acidogenesis of Fruit Waste

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Environmental concerns related to fossil plastics necessitate the exploration of alternative biopolymers like polyhydroxyalkanoates (PHAs). However, the current manufacturing costs of PHAs remain prohibitively high. One promising solution involves autotrophic microbes, such as *Cupriavidus necator*, capable of transforming CO_2 and H_2 into PHAs. Classically, the most well-known sources for CO_2 and H_2 are expensive pure gases or syngas, that comprises toxic compounds for most PHAs-accumulating strains. In this study an innovative approach was adopted where, for the first time, H_2 and CO_2 produced using an acidogenic reactor were converted into poly(3-hydroxybutyrate). In the initial phase, a mixed microbial community was adopted to process melon waste into H_2 (26.7%) and CO_2 (49.2%). These byproducts were then utilized in a second bioreactor by *C. necator* DSM 545 to accumulate 1.7 g/L P(3HB). Furthermore, the volatile fatty acids (VFAs) produced during acidogenesis (13 gCOD/L) were processed into 2.7 g/L of P(3HB-co-3HV). This marks the first proof-of-concept for utilizing acidogenic-derived H_2 and CO_2 from fruit waste for PHAs production. This proof of concept is currently being up-scaled considering also process automation. As such, continuous gas flow can be optimized according to *C. necator* DSM 545 requirements to boost PHAs production.

DR ALBERTO ROBAZZA
Karlsruhe Institute of Technology

Hybrid Thermochemical-biological Processes for Enhanced Energy Recovery from Waste

ALBERTO ROBAZZA, ANKE NEUMANN

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The pyrolysis of waste is an important technology for resource recovery and waste management. The process generates two by-products, namely, pyrolysis syngas (PS) and an aqueous condensate (PAC). They can contain up to 60% of the carbon of the waste. The PS is a mixture of mainly CO, CO_2, CH_4, and H_2. The PAC is toxic and can contain organic acids, phenolics, furans and N-heterocycles, depending on waste type and process conditions. PS and PAC have been used separately as substrates for biological processes but limited information is available about their co-fermentation.

This study investigates the co-fermentation of PS and various PACs (originated from the pyrolysis of lignocellulose, sewage sludge, and PE plastics) with anaerobic mixed cultures to enhance carbon and energy recovery.

Kinetic studies (37/55°C, 250mL bottles, 20kPa CO, 25kPa CO_2, 6kPa H_2 and increasing PACs loadings) illustrated the impact of PACs on microbial metabolism and chemicals degradation. Reactor enrichments (37/55°C, pH 5.5, HRT 20 days, PS and increasing lignocellulose PAC loads) showed the effects of process parameters on metabolites and microbial composition. The conversion of carboxylates in the effluent from the bioreactors to L-malate with *Aspergillus oryzae* was tested in a second-stage aerobic process.

The mesophilic and thermophilic mixed cultures performed multiple functions. During the kinetic experiments, the mixed cultures degraded PAC components while fixing C1 compounds. *Clostridium* sensu stricto 12 and *Caproiciproducens* were enriched during co-fermentation in the mesophilic bioreactor. At 55°C, *Morella thermoacetica* and *Methanothermobacter marburgensis* were the main carboxydotrophic microorganisms. Both processes recovered about 50% of the energy from syngas and PAC. *A. oryzae* converted all carboxylates into L-malate up to a yield of 25 mol%.

Integrating pyrolysis and biological processes can improve the recovery of carbon and energy, leading to the production of high-value chemicals.

MARILENE PAVAN
LanzaTech

Re-Imagining Biomanufacturing to Replace Fossil Sources - Building a Circular Gas Fermentation Industry

Climate crisis and rapid population growth are posing some of the most urgent challenges to mankind and have intensified the need for the deployment of carbon recycling and carbon capture and utilization (CCU) technologies. Gas fermentation offers a solution using carbon-fixing chemolithoautotrophic microorganisms.

After a decade of scale up, the technology has recently been commercialized with the first plant operating successfully since 2018 and additional units under construction. Gas fermentation offers unique feedstock and product flexibility when compared to other available gas-to-liquid technologies. Advancements in process technology and synthetic biology enable a broad range of feedstocks including emissions from industry or syngas generated from any biomass resource to be converted into a wide range of molecules realizing a circular economy.

HARRY NEWTON
The University of Nottingham

From Genes to Proteins: An Omics Analysis of *Cupriavidus necator* as an Alternative Protein Source

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This talk dives into the metabolism of *Cupriavidus necator*, a carbon fixing bacteria that can use hydrogen as its sole energy source. Working alongside industrial partner Deep Branch at the University of Nottingham, this study aims to optimise the natural biological mechanics of capturing and upcycling CO_2 into protein rich food.

First, we explore the use of elemental analysis to identify nutrient limitation, we then detail the running 24 autotrophic fermentations under 8 different limitations. Finally, we show the wealth of proteomic and metabolomic data gathered and measure the impact of the cell's natural regulatory mechanisms.

These experiments demonstrate the robustness of the amino acid profile, highlight the scale to which cell content can be manipulated in continuous cultures and show the conditions under which certain high value metabolites are produced.

We also speculatively explore using these results in conjunction with a published genome scale model. In this way highly expressed but under-utilised proteins can be identified as targets for future efforts in streamlining the metabolism.

ELODIE VLAEMINCK
Ghent University

Valorising Syngas in a Coupled Fermentation via Acetate: Techno-economic Analysis for SCP Production and Pilot-scale Implementation

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Third-generation biorefineries using CO_2 as their feedstock have garnered considerable interest for the carbon-neutral biological production of fuels and chemicals. To make this technology economically competitive, energy-efficient fixation of the gaseous carbon is crucial. In this respect, syngas fermentation with acetogenic bacteria appears to be a propitious route, albeit with a limited product spectrum of mainly small organic acids and alcohols. To enhance the product portfolio, we focus on a coupled fermentation approach with acetate, the natural product of the acetogens, serving as the intermediate.

More specifically, we have been investigating the techno-economic feasibility of this intricate process for the production of single-cell protein (SCP) from steel-mill off gas. Experimental data from lab and pilot-scale fermentations were used to build a model and conduct economic analyses. Significant cost reductions could be achieved through optimization of the gas-to-acetate fermentation process, for which a target concentration (45 g/L) and productivity (4 g/L/h) were identified, laying the foundation for further development of the acetate platform.

Another key aspect in this research field is the utilization of real industrial syngas as fermentation feedstock since the varying gas composition and presence of contaminants influence the acetate production. To assess the performance directly at the emission source, a mobile pilot plant for gas fermentation (the Bio Base Mobile Pilot Plant) was utilized to convert biomass-derived syngas into acetate. Conclusively, our work sheds light on practical strategies to advance toward the sustainable and economically viable implementation of third-generation biorefineries.

DR CHRISTIAN FINK
Arkeon GmbH

Carbon Recycling The Arkeon Way: We turn CO_2 into Functional, Climate Positive Ingredients for Food

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We are a Vienna based, innovation-focused biotechnology company. With the help of a pure strain of methanogenic archaea, we convert environmental or industrial CO_2 into blends of amino acids. To date, we are the only start-up company with a continuous gas fermentation process to produce ingredients for food and other applications through methanogenic archaea. The strain is capable of producing all twenty proteinogenic amino acids in a single process, something that currently existing amino acid production methods cannot deliver. By leveraging CO_2 from the industry that would otherwise be released into the atmosphere, our process significantly contributes to tackling climate change through carbon capture & utilization (CCU).

Currently scaling up the infrastructure, we operate our bioprocess at scales from 2 L up to even 150 L in anaerobic, continuous stirred tank reactors (CSTRs) with continuous gas feed, liquid feed and harvesting. For the continuously ongoing production of an amino acid mixture, we use Arkeon's proprietary, wild type strain. At the same time we focus on the generation of genetically engineered strains to create microbial cell factories for overproduction of amino acid mixtures. To achieve this, we utilize state of the art methods for genetic engineering such as markerless mutagenesis, shuttle- and integration vector systems and interspecies DNA transfer. Moreover, the development of innovative methods for genetic and metabolic modifications of methanogenic archaea is carried out. With the aforementioned toolset and a systems biology approach we can address the market's growing demand for sustainably produced amino acids while recycling the CO_2 of heavy emitters.

PROF RACHAEL ROTHMAN
University of Sheffield

Developments in Green Hydrogen

A clean, plentiful and sustainable supply of hydrogen is key for gas fermentation. This talk will give an overview of the national hydrogen landscape, focussing on green hydrogen production technologies and their challenges and opportunities, looking to the future and potential scale up as well as what is available today. In particular, electrolysis of water to form hydrogen and co-electrolysis of water and carbon dioxide to form syngas will be discussed. The integration of electrolyser technologies with waste heat sources is key for high efficiency and economic and environmental sustainability.

The research programme of UK-HyRES, the UK Hub for research challenges in hydrogen and alternative liquid fuels, will be presented, focussing on the production and storage technical themes and the environmental cross-cutting theme. The potential connections of these with the Carbon Recycling Network will be discussed.

LAURA MUNOZ
Aarhus University

H_2 Consumption Rates by Acetogens Follow First-Order Kinetics in a Wide Range of H_2 Initial Concentrations

LAURA MUNOZ[a]*; SUSMIT CHAKRABORTY[a]; JACOPO CATALANO[a]; JO PHILIPS[a].

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Acetogenic bacteria convert carbon dioxide and H_2 into acetate and ethanol to obtain energy. This autotrophic metabolism is of interest for biotechnological CO_2 valorization such as in gas fermentation and microbial electrosynthesis. Diverse acetogenic strains are available for these applications. Our goal is to characterize the differences between the strains and thereby aid optimal strain selection. Here, we focus on the differences in H_2 consumption characteristics and their relevance for the applications.

First, we determined the H_2 threshold, i.e. the H_2 partial pressure at which acetogenesis halts, for eight different acetogenic strains and observed strong differences *(1)*. The observed H_2 thresholds suggest significant variations in the bioenergetics of acetogens, potentially influencing their growth yields and kinetics as well.

Secondly, we determined the H_2 consumption kinetics of different acetogens over a wide range of H_2 initial concentrations. Interestingly, we observed that their H_2 consumption followed first-order kinetics at under-saturated H_2 levels. This is in contrast to the Monod kinetics with a maximum H_2 consumption rate reached at low dissolved H_2 concentrations, which is often assumed from growth rate data. Our results thus suggest that acetogenic conversion rates can be increased by increasing the H_2 partial pressures. In addition, we found that acetogens strongly differ in their first-order H_2 consumption rate. S. ovata had the highest rate constant, while the lowest was measured for *C. ljungdahlii*, whereas *A. woodii* had an intermediate rate constant in our experimental conditions. These strains have gene clusters encoding different types of hydrogenases, which possibly explain their diverse H_2 kinetics.

Overall, we have observed important differences between common acetogenic strains. Knowledge of these differences represents valuable information for selecting the most optimal biocatalysts for specific applications.

References

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MAGDA ARDILA
Karlsruhe Institute of Technology

Use of Gas Mixtures as a Substrate for H_2 Production with *Parageobacillus thermoglucosidasius* DSM 6285

MAGDA ARDILA, ANKE NEUMANN
Karlsruhe Institute of Technology, Karlsruhe/Germany

The use of hydrogen as an energy carrier promises a carbon-neutral solution to replace fossil fuels [1,2]. Biological production of hydrogen occurs with the help of hydrogenogenic carboxydotrophs, able to perform the water-gas shift (WGS) reaction, where CO reacts with water to produce H_2 and CO_2 [3,4].

Parageobacillus thermoglucosidasius produces hydrogen, performing WGS [5,6]. Gas mixtures that contain CO, CO_2, H_2 and other gases, including O_2 are often used for syngas fermentation, however many microorganisms are sensitive to oxygen [7]. The use of microorganisms that can consume oxygen, such as *P. thermoglucosidasius* is beneficial for O_2-containing gas mixtures. However, it has yet to be defined whether the presence of CO_2 and H_2 can inhibit H_2 production through the WGS reaction. Six fermentations on 2.5 L stirred tank reactors were performed in mASM media-containing 5.5 mM glucose. The gas mixtures contained on one hand, H_2 at 5, 12, and 20%, with constant CO (20%) and CO_2 (15%) and on the other hand, CO increasing mixtures with 10, 30, and 50% with no change in CO_2 (15%) and H_2 (15%) amounts, setting the rest of the gas with N_2.

Overall, the presence of H_2 in different compositions had no inhibitory effect on hydrogen production rate, and the maximum H_2 production rate corresponded to 13.65 L H2/L/d, for fermentations containing 30% CO. Gas mixture containing 50% CO inhibited H_2 production, decreasing productivity. An electron balance showed that 88-91% of electrons coming from CO were converted into H_2.

A continuous process providing fresh media could achieve higher H_2 production rate, as it has been observed before with only CO, and it helps to understand the flux of electrons when providing glucose or other carbon source. This will also help elucidate whether *P. thermoglucosidasius* can route electrons from CO into H_2 and from glucose to organic acids simultaneously, which remains unsolved.

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ABSTRACTS OF POSTER PRESENTATIONS

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POSTER #11 **Corinna Hartinger** University of Oxford	Metabolic Modelling Of Synthetic CO_2-Fixation Pathways to Improve Carbon Use Efficiency In Plants At Night
POSTER #12 **Dr Robin Hoeven** University of Manchester	Cultivation Of Chemoautotrophs On Electricity and Air
POSTER #13 **Dr David Keating** University of Birmingham	Synata Bio: Efficient Carbon Capture Via Advanced Biocatalysts
POSTER #14 **Dr Matt Keith** University of Birmingham	Chemical Depolymerisation of PHA for Recycling Applications
POSTER #15 **Dr Rolf Kraehenbuehl/Dr Daniel Chaplin** Bangor University	Comparison Of Extraction And Quenching Methods For The Analysis Of Intracellular Metabolites In Microbial Cells By UPLC-MS/MS
POSTER #16 **Dr Ewa Marek** University of Cambridge	Understanding the Effect of CO_2 Concentrations on Microbially Induced Carbonate Precipitation (MICP) and Bacteria- and pH-Induced Trapping of CO_2
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POSTER #18 **Dr Laura Munoz** Aarhus University	H_2 Consumption Rates By Acetogens Follow First-Order Kinetics In A Wide Range Of H_2 Initial Concentrations.
POSTER #19 **Rosa Anna Nastro** University of Naples Parthenope	CO_2 Conversion Efficiency in Microbial Fuel Cells (MFCs) and Microbial Electrolysis Cells (MECs): Energy Balance And Production of Value Added Compounds
POSTER #20 **William Newell** Imperial College London	Towards C1 Assimilation in *Y. lipolytica*

POSTER #21 **Harry Newton** SBRC, The University of Nottingham	From Genes to Proteins: An Omics Analysis of *Cupriavidus necator* as an Alternative Protein Source
POSTER #22 **Antti Nyyssölä** VTT Technical Research Centre of Finland	Strain Engineering Of Knallgas Bacteria, And Gas Fermentation At VTT Technical Research Centre Of Finland
POSTER #23 **Ivette Parera Olm** Wageningen University and Research	Beating The Odds: Expanding The Product Spectrum Of Syngas Fermentation With Synthetic Microbial Communities
POSTER #24 **Stefan Pflügl** Technische Universität Wien	Continuous Gas Fermentation With *Thermoanaerobacter kivui* Adapted To Carbon Monoxide
POSTER #25 **Dr Margaux Poulalier Delavelle** SBRC, The University of Nottingham	Use of the endogenous CRISPR/Cas system of the acetogen *Acetobacterium woodii* for genome engineering
POSTER #26 **Alberto Robazza** Karlsruhe Institute of Technology	Hybrid Thermochemical-biological Processes for Enhanced Energy Recovery from Waste
POSTER #27 **Bashir Rumah** SBRC, The University of Nottingham	Identifying gene essentiality in methanotrophs using TraDIS and future biotechnological implications
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POSTER #29 **Tatiana Spatola Rossi** University of Padua	Valorisation of CO_2-rich Waste Gas into Polyhydroxyalkanoates (PHAs) by *Cupriavidus necator*
POSTER #30 **Adrie Straathof** Delft University of Technology	Integration Of Syngas Fermentation And Ethanol Recovery

POSTER #31 **Elodie Vlaeminck** Ghent University and Bio Base Europe Pilot Plant	Valorising Syngas In A Coupled Fermentation Via Acetate: Techno-economic Analysis For SCP Production And Pilot-scale Implementation
POSTER #32 **Dr Charles Wickham-Smith** SBRC, The University of Nottingham	Engineering *Cupriavidus necator* H16 for optimal growth on syngas
POSTER #33 **Joe Windo** University of Manchester	Engineering Halomonas Bluephagenesis For Biomanufacturing From Waste Feedstocks
POSTER #34 **Tong Wu** Charité - Universitätsmedizin Berlin	EuMP Cycle Enable New Gateway for C1 Assimilation
POSTER #35 **Ravineet Yadav** Indian Institute of Science Education and Research Mohali	pyMES: A Set of Python Tools for Mathematical Modelling, Prediction of Rational Experiment Design and Scale-up of Microbial Electrosynthesis from CO_2

Poster #01

PEDRO ACUNA LOPEZ
Ghent University & Bio Base Europe Pilot Plant

Demonstrating Pilot Gas Fermentation for Acetate Production from Biomass-derived Syngas Streams

PEDRO ACUÑA LÓPEZ [1,2]*, STEFANO REBECCHI [2], EVELIEN UITTERHAEGEN [2], ELODIE VLAEMINCK [1,2], KOEN QUATAERT [2], CHRISTIAN FRILUND [3], JAANA LAATIKAINEN-LUNTAMA [3], ILKKA HILTUNEN [3], KAREL DE WINTER [2], WIM K. SOETAERT [1]

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The ever-growing atmospheric CO_2 concentration and its climate consequences are driving research efforts towards the development of advanced gas fermentation technologies. Nonetheless, these innovative processes have been investigated at lab scale with synthetic gas mixtures mimicking industrial off-gases, while evaluations of the technologies' robustness in relevant industrial environments are lacking to date.

We have investigated the fermentative production of acetate, an intermediate energy-carrier, from biomass-derived syngas streams with a varying purity level with the acetogenic bacterium *Moorella thermoacetica*. A mobile gas fermentation pilot plant was coupled to a bubbling fluidized-bed gasification facility with a syngas purification process for *in situ* conversion of real biomass-derived syngas from crushed bark. Units of the purification system were modified (acid- or alkaline-water scrubber) or bypassed (adsorption unit and guard beds) to obtain syngas streams with varying concentrations of the main impurities (NH_3, H_2S, HCN, COS, and benzene) and to assess the performance of the syngas fermentation process in the presence of these contaminants.

Fermentation tests using ultra-cleaned syngas showed comparable microbial growth (1.3 g CDW/L) and acetate production (21.8 g/L) to the benchmark fermentation on synthetic gases (1.2 g CDW/L and 23.3 g acetate/L). Additional fermentation trials on partially purified syngas streams suggest only the caustic scrubbing mode is capable of reducing the contaminants concentration below the microbial inhibition threshold.

Conclusively, our work shows the potential of gas fermentation to valorize crude industrial off-gases into platform chemicals, boosting the transition towards a sustainable circular economy.

Poster #02

FRASER ANDREWS

Manchester Institute of Biotechnology, University of Manchester

Upgrading a Cyanobacterial Genome-Scale Model to Guide High-Throughput Strain Design for the Production of Biochemicals

Fraser Andrews, Matthew Faulkner, Harshwardhan Poddar, and Prof. Nigel Scrutton
Manchester Institute of Biotechnology. University of Manchester

Genome sequences can be used to assemble the genes, enzymes, reactions and metabolites of well characterised organisms into a Genome Scale Metabolic Model (GSMM). GSMMs of the cyanobacteria, Synechocystis 6803, can accurately predict metabolic fluxes and genetically engineered strain designs that improve biochemical production. In this study, we upgrade the stoichiometric model, iSynCJ816, with additional enzyme kinetic and enzyme mass constraints using the AUOTPACKMEN module. We validate our new model, iSynCJ816*, against experimental data. iSynCJ816* demonstrates a reduction in solution space size allowing for greater resolution in metabolic flux prediction. We also produce a large library of strain designs where gene knockouts are predicted to increase biochemical yield. Particularly promising strain designs are earmarked for implemented *in vivo* to produce a new generation of complex design.

Poster #03

MAGDA ARDILA
Karlsruhe Institute of Technology

Use of Gas Mixtures as a Substrate for H_2 Production with *Parageobacillus thermoglucosidasius* DSM 6285

MAGDA ARDILA, ANKE NEUMANN
Karlsruhe Institute of Technology, Karlsruhe/Germany

The use of hydrogen as an energy carrier promises a carbon-neutral solution to replace fossil fuels [1,2]. Biological production of hydrogen occurs with the help of hydrogenogenic carboxydotrophs, able to perform the water-gas shift (WGS) reaction, where CO reacts with water to produce H_2 and CO_2 [3,4].

Parageobacillus thermoglucosidasius produces hydrogen, performing WGS [5,6]. Gas mixtures that contain CO, CO_2, H_2 and other gases, including O_2 are often used for syngas fermentation, however many microorganisms are sensitive to oxygen [7]. The use of microorganisms that can consume oxygen, such as *P. thermoglucosidasius* is beneficial for O_2-containing gas mixtures. However, it has yet to be defined whether the presence of CO_2 and H_2 can inhibit H_2 production through the WGS reaction. Six fermentations on 2.5 L stirred tank reactors were performed in mASM media-containing 5.5 mM glucose. The gas mixtures contained on one hand, H_2 at 5, 12, and 20%, with constant CO (20%) and CO_2 (15%) and on the other hand, CO increasing mixtures with 10, 30, and 50% with no change in CO_2 (15%) and H_2 (15%) amounts, setting the rest of the gas with N_2.

Overall, the presence of H_2 in different compositions had no inhibitory effect on hydrogen production rate, and the maximum H_2 production rate corresponded to 13.65 L H2/L/d, for fermentations containing 30% CO. Gas mixture containing 50% CO inhibited H_2 production, decreasing productivity. An electron balance showed that 88-91% of electrons coming from CO were converted into H_2.

A continuous process providing fresh media could achieve higher H_2 production rate, as it has been observed before with only CO, and it helps to understand the flux of electrons when providing glucose or other carbon source. This will also help elucidate whether *P. thermoglucosidasius* can route electrons from CO into H_2 and from glucose to organic acids simultaneously, which remains unsolved.

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DR HEMAKA BANDULASENA
Loughborough University

Poster #04

Intensification of Gas Fermentation by Hot Microbubble Stripping

MATTHIEU FAUVEL[1], DAVID ORTEGA[2], HEMAKA BANDULASENA[1]

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[2] Phase Biolabs Ltd, Biodiscovery Institute, University of Nottingham, University Park, Nottingham, NG7 2RD.

Gas fermentation is a commercially attractive approach in converting greenhouse gas emissions to sustainable chemicals. Phase Biolabs have developed an anaerobic process that uses acetogens as the microorganism to catalyse the conversion of a gas mixture consisting of CO_2 and H_2 into ethanol and other useful chemicals. However, product (ethanol) inhibition at relatively moderate product concentrations (> 2% (v/v) limit the fermentation process and increase downstream separation costs. If ethanol can be continuously extracted from the fermentation broth without any adverse effect on the microbial culture, ethanol productivity and process economics can be dramatically improved. Furthermore, this could facilitate operation in a continuous culture.

Continuous extraction of ethanol from fermentation broth and ethanol-water mixtures was investigated using hot microbubble gas stripping. A custom-made microbubble stripping unit, which produces a cloud of hot microbubbles using a porous nickel membrane and a fluidic oscillator, was used for this purpose. Batch stripping of the fermentation broth and ethanol-water mixtures demonstrated reduction of ethanol concentration from 5% (v/v) to approximately 1.5% (v/v) within 1 h. Addition of antifoam to the liquid phase noticeably increased the stripping rate. Another set of experiments were carried out to mimic ethanol generation in a fermenter by adding ethanol to fermentation media containing dead microbial culture. These experiments demonstrated ethanol removal rate of 5 g L-1 h-1 from the broth while maintaining the residual ethanol concentration at ~0.5% (v/v). Overall, this study demonstrates the capability of hot microbubble stripping to maintain low ethanol concentration in the fermentation broth while removing products continuously at relatively high concentration. The future work will focus on stripping ethanol from a live fermenter.

Poster #05

KIRA BAUR
Institute of Molecular Biology and Biotechnology of Prokaryotes, University of Ulm

CO_2-based Production of Lactic Acid by an Engineered *Acetobacterium woodii* Strain

BAUR, K.S., MOOK A., BENGELSDORF, F. R.

Institute of Molecular Biology and Biotechnology of Prokaryotes, University of Ulm, Ulm, Germany; contact: kira.baur@uni-ulm.de

Climate change is caused by increasing amount of greenhouse gases such as CO_2 into our atmosphere. Acetogens such as *A. woodii* are capable of autotrophic growth using CO_2 and H_2, producing acetate. By knocking out the native genes encoding the bifurcating lactate dehydrogenase complex and introducing plasmid-borne expression of a D-lactate dehydrogenase (*ldhD*) from *Leuconostoc mesenteriodes*, a new recombinant *A. woodii* strain emerged that can produce additionally lactate using H_2 + CO_2 (Mook et al., 2022, doi: 10.1007/s00253-022-11770-z). Lactate can be used as a renewable resource to produce biodegradable plastics (Vaidya et al., 2005, doi: 10.1080/10643380590966181).

To increase the lactate production with *A. woodii*, the methyl branch of the Wood-Ljungdahl pathway will be bypassed using the enzymatic reaction of the pyruvate-formate-lyase (PFL).

PFL functions in a reversable manner in many anaerobic bacteria (e.g. *Clostridium pasteurianum*) and converts acetyl-CoA together with formate into CoA and pyruvate. It depends on a PFL-activating enzyme (ACT). ACT activates PFL by introducing a glycyl radical in its glycine radical domain.

A plasmid containing the *pfl* as well as the *act* gene from *C. pasteurianum* was constructed. Thereby *act* is controlled by the constitutive $P_{pta\text{-}ack}$ promoter from *C. ljungdahlii* and *pfl* by the theophylline-inducible $P_{ackA\text{-}theo}$ promoter (Beck et al., 2020, doi: 10.1007/s00253-019-10248-9).

The *ldhD* gene is encoded on the plasmids pMTL83251_P_{lctA}_NFP or pMTL83251_P_{bgaL}_NFP and is regulated either by the selfinducing promoter system P_{lctA} from *A. woodii* or the lactose inducible promoter system P_{bgaL} from *C. perfringens* strain 13. The *pfl* as well as the *act* gene are encoded on the plasmid pMTL871X_$P_{pta\text{-}ack}$_act_$P_{ackA\text{-}theo}$_*pfl*. Thus, a two-plasmid system is used for gene expression in the recombinant *A. woodii ΔpyrE ΔlctBCD* strain. Growth characteristics and product formation under autotrophic conditions of the respective strains will be analyzed.

DR TINA BAUR
University of Tübingen

Poster #06

Expanding the Genetic Toolbox of *Methanothermobacter thermautotrophicus* ΔH: Investigating Different Promoter and Reporter Systems

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Methanothermobacter thermautotrophicus is a potent methane producer and is used in industrial power-to-gas applications. Despite this, the first genetic system was only reported in 2021, thereby opening the door to fully exploit its metabolic potential, such as producing high-value chemicals from CO_2. To perform metabolic engineering, different genetic tools, including shuttle vectors as well as promoter and reporter systems are essential. One obstacle is the thermophilic nature of *M. thermautotrophicus*. Thus, any heterologous element should be thermostable.

One example is the thermostable β-galactosidase from *Geobacillus stearothermophilus*. In this study, a codon-optimized *bgaB* gene was used to characterize the expression profile of the anhydrotetracycline (aTc)-inducible P_{tet} promoter from *Escherichia coli* in comparison to the native P_{hmtB} promoter in *M. thermautotrophicus*. The β-galactosidase activity was found to be dependent on the inducer concentration with an optimum at 1 µg/mL aTc. Under these conditions, the enzyme activity reached a 1.3-times higher maximum than measured for the strain with P_{hmtB} controlling bgaB expression.

Another reporter system is the fluorescence-activating and absorption-shifting tag (FAST), which relies on the interaction of the FAST protein with a fluorogenic ligand. An engineered version of FAST (pFAST) was put under control of the P_{hmtB} promoter and introduced into *M. thermautotrophicus*. The fluorescence intensity (FLU) of *M. thermautotrophicus* [pMVS1111A_P_{hmtB}_pFAST] and a strain harboring the empty vector was investigated at 50 and 60 °C. A bright fluorescence of the pFAST-producing strain was detected after 6 h under both conditions, although the maximum FLU at 50 °C was twice as high as at 60 °C. Furthermore, the fluorescence of the 60 °C-culture was completely gone after 24 h, whereas at 50 °C, roughly one-fifth of the initial FLU could still be detected indicating that the thermostability of pFAST can still be improved.

Poster #07

DR CHRISTIAN FINK / MAX KLEIN
Arkeon GmbH

Carbon Recycling the Arkeon Way: We turn CO_2 into Functional, Climate Positive Ingredients for Food.

FINK, C.[1], KLEIN, M.[1], HILTS, A.[1], FENNESSY, R.[1], RITTMANN, S. K.-M. R.[1]

[1] *Arkeon GmbH, Tulln an der Donau, Austria.*

We are a Vienna based, innovation-focused biotechnology company. With the help of a pure strain of methanogenic archaea, we convert environmental or industrial CO_2 into blends of amino acids. To date, we are the only start-up company with a continuous gas fermentation process to produce ingredients for food and other applications through methanogenic archaea. The strain is capable of producing all twenty proteinogenic amino acids in a single process, something that currently existing amino acid production methods cannot deliver. By leveraging CO_2 from the industry that would otherwise be released into the atmosphere, our process significantly contributes to tackling climate change through carbon capture & utilization (CCU).

Currently scaling up the infrastructure, we operate our bioprocess at scales from 2 L up to even 150 L in anaerobic, continuous stirred tank reactors (CSTRs) with continuous gas feed, liquid feed and harvesting. For the continuously ongoing production of an amino acid mixture, we use Arkeon's proprietary, wild type strain. At the same time we focus on the generation of genetically engineered strains to create microbial cell factories for overproduction of amino acid mixtures. To achieve this, we utilize state of the art methods for genetic engineering such as markerless mutagenesis, shuttle- and integration vector systems and interspecies DNA transfer. Moreover, the development of innovative methods for genetic and metabolic modifications of methanogenic archaea is carried out. With the aforementioned toolset and a systems biology approach we can address the market's growing demand for sustainably produced amino acids while recycling the CO_2 of heavy emitters.

Poster #08

DR MARCO GARAVAGLIA
The University of Nottingham

Stable Platform for Mevalonate Bioproduction from CO_2

MARCO GARAVAGLIA[a], CALLUM MCGREGOR[a b], RAJESH REDDY BOMMAREDDY[a c], VICTOR IRORERE[a d], CHRISTIAN ARENAS[a b], ALBERTO ROBAZZA[a e], NIGEL P. MINTON[a] and KATALIN KOVACS[a f]

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Stable production of value-added products using microbial chassis is pivotal for determining the industrial suitability of the engineered biocatalyst. Microbial cells often become degenerate of multi-copy expression plasmids during long term cultivations. Owing to the advantages related to titres, yields and productivities when using a multi-copy expression system compared with genomic integrations, plasmid stability is essential for industrially relevant bio-based processes. *Cupriavidus necator* H16, a facultative chemolithoautotrophic bacterium has been successfully engineered to convert inorganic carbon obtained from CO_2 fixation into value-added products. The application of this unique capability in the biotech industry has, however, been hindered by *C. necator* H16 inability to stably maintain multi-copy plasmids. In this study, we designed and tested plasmid-addiction systems based on complementation of essential genes. Among these, implementation of a plasmid-addiction tool based on the complementation of mutants lacking RubisCO, which is essential for CO_2 fixation, successfully stabilized a multi-copy plasmid. Expressing the mevalonate pathway operon (MvaES) using this addiction system resulted in the production of ~10 g/L mevalonate, with carbon yields of ~25%. The mevalonate titres and yields obtained here using CO_2 are the highest achieved to date for production of C6 compounds from C1 feedstocks.

JAKUB GIZEWSKI
Karlsruhe Institute of Technology

Poster #09

Enhanced Biomass and Trehalose Production by *Cupriavidus necator* Through Hydrogen and Formate Utilization - Supported by a Techno-Energetic Analysis

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The chemolithoautotrophic bacterium *C. necator* presents itself as an ideal candidate for sustainable process development. It addresses significant challenges in our economy by utilizing hydrogen as an electron donor and CO_2 as a carbon source, as opposed to the conventional practice of burning fossil fuels and emitting CO_2. However, a critical impediment lies in the efficiency of the Calvin cycle, wherein 18 ATP and 12 NAD^+ are required to convert 6 CO_2 into fructose [1]. Various strategies can be employed to overcome this challenge, including genetic engineering and optimizing the metabolism. This study focuses on a co-feeding strategy supplying hydrogen and formate (Figure 1). The aim was to increase the efficiency of the Calvin cycle by introducing additional formate as a supplementary electron donor. This approach resulted in elevated biomass growth and increased product formation.

Figure 1: Scheme of hydrogen and formate metabolism and carbon dioxide utilization by *C. necator.*

Experimental results confirmed lower rates of biomass growth and product formation when hydrogen serves as the exclusive energy and oxidant supply. In contrast, co-feeding hydrogen and formate led to a doubling of biomass growth and product formation rates.

These could represent a step towards a more sustainable production. A techno-energetic analysis is necessary to assess the potential utility of these findings in an exemplary process design. The selected design incorporates fermentation and electrochemical steps for the production of hydrogen and formate (Figure 2). The objective is to compare the advantageous co-feeding strategy, which involves the electrochemical generation of both substrates, with the less efficient feed variant solely relying on hydrogen and omitting formate synthesis.

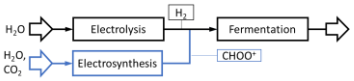

Figure 1: Example of a sustainable process design with electrochemical feed production.

The language of this text was improved by AI

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Poster #10

DR AMEYA PANKAJ
The University of Padova, Italy

Autotrophic Polyhydroxyalkanoates Production Using Gaseous Streams Produced During Acidogenesis of Fruit Waste

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Environmental concerns related to fossil plastics necessitate the exploration of alternative biopolymers like polyhydroxyalkanoates (PHAs). However, the current manufacturing costs of PHAs remain prohibitively high. One promising solution involves autotrophic microbes, such as *Cupriavidus necator*, capable of transforming CO_2 and H_2 into PHAs. Classically, the most well-known sources for CO_2 and H_2 are expensive pure gases or syngas, that comprises toxic compounds for most PHAs-accumulating strains. In this study an innovative approach was adopted where, for the first time, H_2 and CO_2 produced using an acidogenic reactor were converted into poly(3-hydroxybutyrate). In the initial phase, a mixed microbial community was adopted to process melon waste into H_2 (26.7%) and CO_2 (49.2%). These byproducts were then utilized in a second bioreactor by *C. necator* DSM 545 to accumulate 1.7 g/L P(3HB). Furthermore, the volatile fatty acids (VFAs) produced during acidogenesis (13 gCOD/L) were processed into 2.7 g/L of P(3HB-co-3HV). This marks the first proof-of-concept for utilizing acidogenic-derived H2 and CO_2 from fruit waste for PHAs production. This proof of concept is currently being up-scaled considering also process automation. As such, continuous gas flow can be optimized according to *C. necator* DSM 545 requirements to boost PHAs production.

CORINNA HARTINGER
University of Oxford

Poster #11

Metabolic Modelling of Synthetic CO_2-Fixation Pathways to Improve Carbon Use Efficiency in Plants at Night

CORINNA HARTINGER, EDWARD N. SMITH, LEE J. SWEETLOVE

Department of Biology, South Parks Road, University of Oxford

Plant growth in agricultural settings is constrained by limited carbon availability. This is in part due to nocturnal carbon losses from cellular respiration, which increase under stress conditions. We used constraint-based metabolic modelling to identify and evaluate potential strategies to refix nocturnal, respiratory CO_2 to improve carbon use efficiency.

We first explored the nocturnal carbon-recycling capabilities of the endogenous leaf metabolic network and identified a metabolic behaviour known as CAM cycling. This was an efficient solution but may be difficult to engineer due to the use of endogenous enzymes of central carbon metabolism. Therefore, CAM cycling was constrained to create a carbon-limited model, which would be amenable to using exogenous metabolic pathways to refix respiratory CO_2 at night.

We then added previously published, synthetic CO_2-fixing pathways in the model and evaluated their impact on the predicted carbon use efficiency and growth rate. The activity of the pathway and the rest of the plant metabolic network was assessed to determine the feasibility of operating the pathway and any additional metabolic engineering efforts that may be required.

As an initial example, we describe the results from integration of the crotonyl-CoA/ethylmalonyl-CoA/hydroxybutyryl-CoA (CETCH) cycle. While the CETCH cycle is used in the model to refix most nocturnal, respiratory CO_2, this comes at a substantial energetic cost. Respiration increases to generate the additional ATP and NADPH required to run the cycle, resulting in further losses of CO_2. The efficiency of a nocturnal CETCH cycle depends crucially on which redox cofactors are used and how they are regenerated.

Poster #12

DR ROBIN HOEVEN
University of Manchester

Cultivation of Chemoautotrophs on Electricity and Air

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This is a proof of concept study that looks at ways to grow chemoautotrophic bacteria on electricity, either with a mediator molecule or via direct electron uptake from an electrode. We solely conduct experiments under aerobic conditions, as the main aim is to use CO_2 from air as the carbon source.

The first step is using axenic strains of chemoautotrophs that will be tested for growth with reduced versions of a mediator molecule. To find the optimal voltage for reduction of the mediator molecule we do cyclic voltametry (CV). This is followed by chronoamperometry to fully reduce all of the mediator compound in solution before it is mixed into the medium.

Futhermore, we will also look at environmental samples to conduct an enrichment experiment to select for organisms that can accept electrons from an electrode and use CO_2 from air.

Any help or suggestions are greatly appreciated, particularly on the electrochemistry.

DR DAVID KEATING
Synata Bio, USA

Poster #13

Synata Bio: Efficient Carbon Capture via Advanced Biocatalysts

Synata Bio is a US-based company focusing on the conversion of waste CO_2 into bio-based fuels and chemicals. Work over the past several years has optimized our gas-fermentation technology and construction of a 50KTA plant is underway in China, with mechanical completion set for the third quarter of 2024. A key advantage of our CO_2 capture technology is our proprietary biocatalyst. This strain, referred to as *Clostridium palustris*, displays elevated hydrogen and CO_2 conversion and greater selectivity towards ethanol, compared to related syngas consuming strains. Furthermore, Synata Bio has leveraged technology advanced by the Clostridium community for development of a series of molecular tools in *C. palustris*, enabling construction of genome modifications via CRISPR and allelic exchange, as well as the use of multicopy plasmids.

Comparison of the genome of *C. palustris* with related strains suggested metabolic explanations for its unusual characteristics. *C. palustris* was found to display elevated expression of the primary bifurcating hydrogenase with respect to *C. autoethanogenum*. This comparative analysis identified an unusual region upstream of the primary bifurcating hydrogenase of *C. palustris* not observed in *C. autoethanogenum* or related syngas-consuming Clostridia. Strain engineering studies have demonstrated that this altered upstream region is sufficient to alter gene expression. Additional genome differences have been observed between *C. palustris* and *C. autoethanogenum*, the role of which are currently being investigated.

Poster #14

DR MATT KEITH
University of Birmingham

Chemical Depolymerisation of PHA for Recycling Applications

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Poly(hydroxy alkanoates) (PHAs) are a bio-derived and biodegradable alternative to conventional plastics [1] with production expected to grow by a factor of ten by 2028, resulting in large quantities of waste [2]. To strive for true sustainability, the circularity of these materials must be maximised. Through depolymerisation, we can effectively recycle the carbon before it's released into the environment. Hence, this research investigated and modelled the depolymerisation of a common PHA; poly(3-hydroxy) butyrate (P3HB).

6 g of P3HB were hydrolysed in 100 mL of water. Acetic acid, zinc acetate, urea, and sodium hydroxide (NaOH) were all screened as potential catalysts. To minimise the energy requirement, a low temperature range of 50 to 80°C was selected, along with reaction times of 10 mins to 4 h. The hydrophobic P3HB surface may hinder the mass transfer of the reactants. Therefore, some samples were chemically modified by soaking them overnight in different solvents.

Of the catalysts investigated, only NaOH showed potential under the conditions investigated. Further experiments demonstrated an optimum concentration of 2 M NaOH. Contact angle measurements showed that soaking the samples in methanol increased the hydrophilicity. This significantly accelerated the depolymerisation reaction with 98% conversion achieved in 90 min at 70°C, compared to 210 min for the non-soaked samples. Through the application of shrinking core and pseudo-first order models, the activation energy was calculated to be 47 and 59 kJ/mol respectively. This is slightly lower than the 76 kJ/mol which has been previously calculated for the alkaline hydrolysis of PLA [3].

This work has demonstrated possible conditions for the alkaline hydrolysis of P3HB. Further downstream processing could enable the recovery of the monomer, or even create value added chemicals. Through technologies such as this, carbon can be effectively recycled, its value retained, and potential environment harm avoided.

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DR ROLF KRAEHENBUEHL & DR DANIEL CHAPLIN
Centre for Environmental Biotechnology, Bangor University

Poster #15

Comparison of Extraction and Quenching Methods for the Analysis of Intracellular Metabolites in Microbial Cells by UPLC-MS/MS

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A fast, sensitive and robust method for extraction and quantification of intracellular metabolites in microbial cells is important for the analysis of their metabolic activity and product formation. The data inform synthetic biology approaches and the metabolic modelling of microbial responses to genetic modifications and environmental changes like different feedstocks and the availability of nutrients.

Using ultra-performance liquid chromatography (UPLC) coupled to a triple quadrupole mass spectrometer (TQMS), we compared different extraction and quenching methods for the quantification of eight intracellular metabolites (ATP, ADP, AMP, Acetyl-CoA, NAD+, NADH, NADP+ and NADPH) in *E. coli* cells.

The extraction and quenching methods can be applied to a range of microorganisms like bacteria and yeasts sampled from a variety of environments including fermentation cultures grown under different growth conditions. The proposed fast UPLC-MS/MS method increases sample throughput, while fast polarity switching and improved chromatographic separation maximise analytical sensitivity.

Poster #16

DR EWA MAREK
University of Cambridge

Understanding the Effect of CO_2 Concentrations on Microbially Induced Carbonate Precipitation (MICP) and Bacteria- and pH-Induced Trapping of CO_2

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Trapping of CO_2 into liquid solutions, so-called solubility trapping, involves an impractically slow step of CO_2 hydration, which can be accelerated with commonly occurring carbonic anhydrases (CAs) enzymes. Those enzymes accelerate the rates of CO_2 hydration by 6 orders of magnitude, leading to real-time effective CO_2 fixation. Ureolitic bacteria are known for their capability to transform carbon from urea into a solid mineral, calcium carbonate $CaCO_3$, in a process called Microbially Induced Carbonate Precipitation (MICP). While MICP requires urease enzyme (Ur) to drive the ureolitic reactions, most MICP bacteria also use CAs, but their role and potential symbiotic effects with Ur remain little understood. Since the activities of CA and Ur result in carbon trapping, either as soluble carbonate ions or as $CaCO_3$, MICP can offer enhanced, dual-enzyme-driven trapping.

Here, through the use of the ureolytic bacterium *Sporosarcina pasteurii*, we investigate the role of Ur and CA in ureolysis, CO_2 hydration, and $CaCO_3$ precipitation – all as a function of CO_2(g) concentrations. We show that Ur activity increases when the bacterial solution is exposed to high CO_2 concentrations, coinciding with higher concentrations of bicarbonate ions, HCO_3- produced via the simultaneously enhanced CO_2 hydration driven by CA. We conclude that CA promotes buffering, which enhances solubility trapping. Additionally, we show that the mineral form of the produced $CaCO_3$ also correlates with CA activities – as expected for pH variations from the Bjerrum plot. Thus, the CA-driven hydration of CO_2 affects the core MICP process, ureolysis, and its final result - $CaCO_3$ precipitation. This indicates potential optimisation strategies to maximise CO_2 trapping in mineral and soluble forms, opening new avenues for biologically enhanced CO_2 drawdown approaches.

JOSHUA MCCLUSKEY
University of Tübingen

Poster #17

Enhanced Ethanol Production from CO and Acetate in *Clostridium ljungdahlii*

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Rising atmospheric carbon levels pose a challenge and highlight the need for new technologies to enable circular industrial practices. Organic molecules of various chain lengths have been produced from CO_2 and CO present in carbon-rich waste gases using bioreactors, with autotrophic microbes that fix C1 carbon into more complex molecules. The state-of-the-art in such gas fermentation strategies is the identification and optimisation of the most efficient organisms to function as a biocatalyst.

Acetogens have been identified as useful biocatalysts for their ability to fix CO_2 and CO to acetate during anaerobic growth. In addition to acetate, certain acetogens produce more industrially relevant products. *Clostridium ljungdahlii* produces ethanol from surplus acetate. The use of acetogens for ethanol production through waste-gas fermentation has reached commercialisation, yet there remains room to increase the efficiency and yields of the process. The current challenges in the field are increasing the total ethanol yield and improving the ethanol-to-acetate production ratio. The identification of genetic variations which enhance ethanol production efficiency would be valuable to the field.

We have grown an engineered strain of *C. ljungdahlii* on pure CO in continuous bioreactors with minimal media and acetate as a supplement. As surplus acetate is converted to ethanol, it was posited that *C. ljungdahlii* may accumulate mutations to enhance the conversion efficiency with increasing acetate concentrations. The addition of acetate of up to 100 mM increased the growth rate and cell density within the reactors, while higher concentrations were inhibitory to growth. Throughout the operating period, ethanol production and the ethanol-to-acetate ratio increased, suggesting physiological changes occurred within *C. ljungdahlii* to enhance the conversion. The maximum ethanol production rate was 0.461 $g.l^{-1}.h^{-1}$ without cell retention and the maximum ethanol-to-acetate ratio was 22:1.

Poster #18

DR LAURA MUNOZ
Aarhus University

H_2 Consumption Rates by Acetogens Follow First-Order Kinetics in a Wide Range of H_2 Initial Concentrations

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Acetogenic bacteria convert carbon dioxide and H_2 into acetate and ethanol to obtain energy. This autotrophic metabolism is of interest for biotechnological CO_2 valorization such as in gas fermentation and microbial electrosynthesis. Diverse acetogenic strains are available for these applications. Our goal is to characterize the differences between the strains and thereby aid optimal strain selection. Here, we focus on the differences in H_2 consumption characteristics and their relevance for the applications.

First, we determined the H_2 threshold, i.e. the H_2 partial pressure at which acetogenesis halts, for eight different acetogenic strains and observed strong differences *(1)*. The observed H_2 thresholds suggest significant variations in the bioenergetics of acetogens, potentially influencing their growth yields and kinetics as well.

Secondly, we determined the H_2 consumption kinetics of different acetogens over a wide range of H_2 initial concentrations. Interestingly, we observed that their H_2 consumption followed first-order kinetics at under-saturated H_2 levels. This is in contrast to the Monod kinetics with a maximum H_2 consumption rate reached at low dissolved H_2 concentrations, which is often assumed from growth rate data. Our results thus suggest that acetogenic conversion rates can be increased by increasing the H_2 partial pressures. In addition, we found that acetogens strongly differ in their first-order H_2 consumption rate. *S. ovata* had the highest rate constant, while the lowest was measured for *C. ljungdahlii*, whereas *A. woodii* had an intermediate rate constant in our experimental conditions. These strains have gene clusters encoding different types of hydrogenases, which possibly explain their diverse H_2 kinetics.

Overall, we have observed important differences between common acetogenic strains. Knowledge of these differences represents valuable information for selecting the most optimal biocatalysts for specific applications.

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Poster #19

ROSA ANNA NASTRO
University of Naples “Parthenope”

CO_2 Conversion Efficiency in Microbial Fuel Cells (MFCs) and Microbial Electrolysis Cells (MECs): Energy Balance and Production of Value Added Compounds

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In this work, we aimed at maximizing the efficiency of CO_2 capture at the cathodes of our Bioelectrochemical Systems (BESs) by minimizing any potential factors preventing or hampering the formation of a cathodic biofilm in Microbial Electrochemical Cells (MECs) and Microbial Fuel Cells. Therefore, we prepared biocathodes under conditions facilitating biofilm formation and used culture media to support a gradual metabolic adaptation in *Clostridium saccharoperbutylacetonicum* NT-1 and *Ralstonia eutropha* towards the utilization of inorganic carbon. The overall effect was the constitution of an electroactive biofilm, with an activated Wood–Ljungdahl (in *C. saccharoperbutylacetonicum*) and Calvin Benson Bessam (*R. eutropha*) pathways ready to use CO_2 at the cathode of BESs, with high capture yield in MFCs and significant reduction in the overall start-up phase. We demonstrated the possibility to set up MFCs able to capture CO_2, with prevalent biosynthesis of formate (30 mg/L*d) in *C. saccharoperbutylacetonicum* (Nastro et al., 2023) and of POLYHYDROXYBUTYRATE (PHBs) (74±2% $V_{granules}$/Vcell) in *R. euthopha*. In both cases, the energy needed to promote CO_2 organication at the cathode was provided by the degradation of glycerol (0.4% in M9 medium) by a *Pseudomonas aeruginosa* PA/031 and *Shewanella oneidensis* MR-1 at the anode with production of anionic and non- ionic surfactants. The application of an external potential led to the production mainly of acetate and formate in MEC inoculated with *Clostridium* while a reduction in PHBs biosynthesis was detected in *R. eutropha* cells. We also show for the first time that *C. saccharoperbutylacetonicum* NT-1, a strain used in conventional industrial fermentation processes, can also be used in BESs for the electrosynthesis of organic compounds from CO_2. Under an energy balance perspective, the calculations indicate that the amount of energy spent to capture one mole of CO_2 by MFCs was 86.4 J mol m−2 in *C. saccharoperbutylacetonicum* and 2.95 x 10-2 W h per mole of CO_2 captured from the gas mix by *R. eutropha*. We also observed a change in the pattern of utilization of carbon sources caused by the prolonged utilization of the strains at the cathode of MFCs.

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WILLIAM NEWELL
Imperial College London

Poster #20

Towards C1 Assimilation in *Y. lipolytica*

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Soluble C1 compounds like formate can be produced directly from greenhouse gases via electrical reduction, providing a genuinely scalable alternative to current feedstocks. While several prokaryotes have been converted to formatotrophy, no formatotrophic eukaryotes exist. *Y. lipolytica* is a non-model, oleaginous yeast with considerable metabolic flexibility and great potential as a platform for bioproduction. Here, we present a strain of *Y. lipolytica* evolved to grow on formate as a sole carbon and energy source. First, using GSMs, we predict that *Y. lipolytica* hosts several cryptic formatotrophy pathways. We also show that *Y. lipolytica* is able to use formate as a sole energy source during specific culture conditions via endogenous formate dehydrogenases. Then, modelling and C13 tracer experiments show that formate can be used as a supplementary carbon source via endogenous metabolism. We then use this to inform directed evolution experiments which lead to a *Y. lipolytica* strain able to grow on formate. Mutations in mitochondrial biosynthesis, central carbon metabolism regulation and ROS resistance appear to support formatotrophic growth in the evolved strain. We then engineer cofactor metabolism and endogenous ROS resistance mechanisms to improve final biomass titre. We also demonstrate that carotenoids can be produced from formate in *Y. lipolytica*, the first example of formate-derived terpenoid bioproduction. Our work shows how novel formatotrophy can arise from evolution of native metabolic systems alone and could support future engineering efforts in eukaryotic hosts.

Poster #21

HARRY NEWTON
The University of Nottingham

From Genes to Proteins: An Omics Analysis of *Cupriavidus necator* as an Alternative Protein Source

HARRY NEWTON, KLAUS WINZER[1], PREBEN KRABEN[2], BART PANDER[3], YING ZHANG[1]

[1] University of Nottingham, [2] Deep Branch, [3] University of Edinburgh

This talk dives into the metabolism of *Cupriavidus necator*, a carbon fixing bacteria that can use hydrogen as its sole energy source. Working alongside industrial partner Deep Branch at the University of Nottingham, this study aims to optimise the natural biological mechanics of capturing and upcycling CO_2 into protein rich food.

First, we explore the use of elemental analysis to identify nutrient limitation, we then detail the running 24 autotrophic fermentations under 8 different limitations. Finally, we show the wealth of proteomic and metabolomic data gathered and measure the impact of the cell's natural regulatory mechanisms.

These experiments demonstrate the robustness of the amino acid profile, highlight the scale to which cell content can be manipulated in continuous cultures and show the conditions under which certain high value metabolites are produced.

We also speculatively explore using these results in conjunction with a published genome scale model. In this way highly expressed but under-utilised proteins can be identified as targets for future efforts in streamlining the metabolism.

DR ANTTI NYYSSÖLÄ
VTT Technical Research Centre of Finland Ltd

Poster #22

Strain Engineering of Knallgas Bacteria, and Gas Fermentation at VTT Technical Research Centre of Finland

TYTTI JÄMSÄ, LAURA SALUSJÄRVI, NICO CLAASSENS, NORMAN ADLUND, ANTTI AALTO, TUULA KAJOLINNA, OUTI KOIVISTOINEN, MARKKU SALOHEIMO, ANTTI NYYSSÖLÄ

In AEROCOW VTT is studying the production of secreted edible proteins by a Knallgas bacterium. We are screening different secretion signals and promoters, and their combinations for production of edible proteins from carbon dioxide, hydrogen, and oxygen. Target proteins of the project are various milk, egg, plant, and sweet proteins.

CARBONCHAIN, coordinated by Natural Resources Institute Finland, is a collaborative project between Finnish research partners, businesses, and cities. The project explores the potential of biogas derived carbon dioxide as a resource for various industries and applications. VTT analyses biogenic carbon dioxide fractions from different sources and the effects of various isolation methods on gas compositions. Furthermore, the project evaluates the impact of impurities and gas components on the growth of Knallgas bacteria for their utilization as hosts for single cell protein, chemical and material production.

KNALLRED studies the use of engineered Knallgas bacteria as catalysts for whole-cell reductive biotransformations. The soluble hydrogenase-catalyzed oxidation of hydrogen is employed for regeneration of NAD(P)H required for the reductions. The project has demonstrated the quantitative conversion of xylose to the sweetener and platform chemical xylitol with resting cells of *Cupriavidus necator*, expressing a yeast xylose reductase gene. This work was carried out as collaboration with Wageningen University. The development of the platform for more efficient conversions is underway.

Poster #23

IVETTE PARERA OLM
Wageningen University and Research Netherlands

Beating the Odds: Expanding the Product Spectrum of Syngas Fermentation with Synthetic Microbial Communities

IVETTE PARERA OLM[1,2], SARA BENITO-VAQUERIZO[3], SIEBE PEEK[1], MARTIJN DIENDER[1,2], CHARLES DUBAERE[1], VITOR A.P. MARTINS DOS SANTOS[3], MARIA SUAREZ-DIEZ[3], DIANA Z. SOUSA[1,2]

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The growing demand for sustainably produced chemicals underscores the need for innovative technologies addressing carbon recycling from recalcitrant wastes and biomass. A promising strategy is the gasification of these feedstocks into syngas (CO, H_2, CO_2) followed by microbial fermentation. However, the capacity to diversify product range from syngas using pure cultures of acetogens remains limiting. An alternative strategy involves the use of synthetic microbial communities, where microbial strains are selected to construct desired metabolic networks. For example, the co-cultivation of an acetogen with the chain-elongating bacterium *Clostridium kluyveri* enables the production of medium-chain carboxylic acids (MCCAs) of even number of carbon (i.e., butyrate, caproate) from syngas. Here, we compared the performance of various acetogenic strains in co-culture with *C. kluyveri* at different pH values, revealing significative differences in the growth and product spectrum of the CO/H_2-fed cultures. Additionally, we describe a synthetic microbial community capable of producing odd-chain products solely from CO. The consortium comprises three Clostridia species: the acetogen *Acetobacterium wieringae*, the propionigenic bacterium *Anaerotignum neopropionicum* and *C. kluyveri*. While even-chain products remained dominant, metabolic cross-feeding within this consortium during fed-batch fermentation enabled the formation of valerate (1 g L^{-1}) and pentanol (0.4 g L^{-1}), unusual products in CO-fermenting systems. Furthermore, we demonstrate the practical utility of (multi-species) genome-scale metabolic models (GEMs) in studying and optimising microbial processes, using the co-cultures we established as case study.

STEFAN PFLÜGL
Technische Universität Wien

Poster #24

Continuous Gas Fermentation with *Thermoanaerobacter kivui* Adapted to Carbon Monoxide

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Conversion of gaseous one carbon substrates (CO, CO_2) and H_2 by acetogenic bacteria is a promising technology to establish sustainable bioproduction scenarios. The thermophilic acetogen *Thermoanaerobacter kivui* (T_{opt} = 66 °C) grows on H_2/CO_2 in chemically defined mineral medium with growth rates (doubling time: 2 h), exceeding those of mesophilic acetogens. Nevertheless, there is currently no bioprocessing system available for quantitative characterization of *T. kivui* as a model thermophilic acetogen under well-defined bioreactor conditions. In this study, we established a continuous high-temperature gas fermentation system and characterized *T. kivui* wildtype as well as a strain adapted to grow on CO (referred to as CO-1). *T. kivui* CO-1 grew in a 100 % CO gas phase in chemically defined mineral medium with a growth rate of up to 0.25 h^{-1} (doubling time: 2.8 h) and adaptation occurred in as little as 30 generations, similar to previous work by Weghoff and Müller (2016) using rich medium [1]. Genomic analysis of the clonal strain CO-1 using short and long read sequencing technologies indicated that a handful of SNPs and large-scale genomic rearrangements might be responsible for the successful adaptation of *T. kivui* to CO. To characterize the physiology of strain CO-1 in more detail, steady state chemostat cultures operated at specific growth rates of 0.10-0.20 h^{-1} were used to quantify growth, gas consumption, and acetate production on H_2/CO_2, syngas and pure CO. Transcriptomic analysis gave further clues how the physiology of strain CO-1 is adapted to growth on CO. Furthermore, synthesis gas generated from biomass gasification was successfully evaluated as a feedstock for gas fermentation with *T. kivui* in continuous mode in 200 mL stirred tank bioreactors as well as a 20 L bubble column bioreactor. Collectively, the knowledge gained in this study represents a first step toward establishing high-temperature gas fermentation processes.

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Poster #25

DR MARGAUX POULALIER DELAVELLE
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Use of the Endogenous CRISPR/Cas System of the Acetogen *Acetobacterium woodii* for Genome Engineering

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Klaus Winzer and Nigel P. Minton

Acetogenic bacteria represent an innovative alternative for the sustainable production of chemicals and fuels. They are capable of producing acetyl-CoA from CO and/or CO_2 coupled to energy conservation.

In *Acetobacterium woodii*, attempts to implement the heterologous CRISPR/Cas9 system from *Streptococcus pyogenes* resulted in poor plasmid transfer. Preliminary experiments showed that poor transformation efficiency could be attributed to both the nuclease toxicity and a Restriction-Modification (R-M) site in its encoding sequence.

This study aims to facilitate exploitation of CRISPR/Cas endogenous systems as genome engineering tools, for easier, faster and more streamlined improvements of yield in industrially relevant acetogens.

A Python script to automate endogenous CRISPR/Cas systems PAM prediction was developed and validated with available literature. As a proof-of-concept, the Type I B CRISPR/Cas system of *A. woodii* was analysed *in silico* and Protospacer Adjacent Motif candidates were characterised *in vivo*. Expression of synthetic CRISPR arrays consisting of the native leader sequence, direct repeats, and adequate spacer along with homology arms for homologous recombination successfully led to *pyrE* and *pheA* knockouts of 300 bp and 354 bp respectively. Homology arms lengths longer than 1 kb were found to significantly increase editing efficiency. To validate the method, a 3.2 kb bp knockout of *hsdR1* and a reporter gene KI were successfully obtained in *A. woodii*. This is the first report of genome engineering of *Acetobacterium woodii* using its endogenous CRISPR/Cas system.

Poster #26

DR ALBERTO ROBAZZA
Karlsruhe Institute of Technology

Hybrid Thermochemical-biological Processes for Enhanced Energy Recovery from Waste

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The pyrolysis of waste is an important technology for resource recovery and waste management. The process generates two by-products, namely, pyrolysis syngas (PS) and an aqueous condensate (PAC). They can contain up to 60% of the carbon of the waste. The PS is a mixture of mainly CO, CO_2, CH_4, and H_2. The PAC is toxic and can contain organic acids, phenolics, furans and N-heterocycles, depending on waste type and process conditions. PS and PAC have been used separately as substrates for biological processes but limited information is available about their co-fermentation.

This study investigates the co-fermentation of PS and various PACs (originated from the pyrolysis of lignocellulose, sewage sludge, and PE plastics) with anaerobic mixed cultures to enhance carbon and energy recovery.

Kinetic studies (37/55°C, 250mL bottles, 20kPa CO, 25kPa CO_2, 6kPa H_2 and increasing PACs loadings) illustrated the impact of PACs on microbial metabolism and chemicals degradation. Reactor enrichments (37/55°C, pH 5.5, HRT 20 days, PS and increasing lignocellulose PAC loads) showed the effects of process parameters on metabolites and microbial composition. The conversion of carboxylates in the effluent from the bioreactors to L-malate with *Aspergillus oryzae* was tested in a second-stage aerobic process.

The mesophilic and thermophilic mixed cultures performed multiple functions. During the kinetic experiments, the mixed cultures degraded PAC components while fixing C1 compounds. *Clostridium* sensu stricto 12 and *Caproiciproducens* were enriched during co-fermentation in the mesophilic bioreactor. At 55°C, *Morella thermoacetica* and *Methanothermobacter marburgensis* were the main carboxydotrophic microorganisms. Both processes recovered about 50% of the energy from syngas and PAC. *A. oryzae* converted all carboxylates into L-malate up to a yield of 25 mol%.

Integrating pyrolysis and biological processes can improve the recovery of carbon and energy, leading to the production of high-value chemicals.

DR BASHIR RUMAH
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Poster #27

Identifying Gene Essentiality in Methanotrophs Using TraDIS and future Biotechnological Implications

Methanotrophic bacteria are Gram-negative, aerobic organisms that use methane as sole source of carbon and energy. Methanotrophs can be used to produce metabolites like poly-3-hydroxybutyrate (PHB) and single cell proteins for animal feed. Here, we constructed and exemplified a CRISPR/Cas9 genome editing system and used it to successfully make gene deletions and insertions in the type I *Methylococcus capsulatus* Bath and the type II *Methylocystis parvus* OBBP. To determine suitable gene targets for deletion in these methanotrophs, Transposon Directed Insertion Sequencing (TraDIS), a powerful sequencing tool for determining essential and non-essential genes in bacteria was used. A Tn5 transposon plasmid was used to make a library of approximately 1,000,000 transposon mutants. Using the TraDIS pipeline for downstream processing of these transposon mutant libraries, essential and non-essential genes were determined. In *Methylocystis parvus*, CRISPR/Cas9 was used to validate predictions from TraDIS by attempting to delete 11 genes related to PHB metabolism. PHB metabolism genes predicted to be essential could not be deleted with CRISPR/Cas9, whereas all non-essential genes were deleted demonstrating the reliability of our TraDIS data. The outcome provided a reliable guide for understanding genes important in PHB metabolism and how biosynthesis of this important biopolymer can be enhanced.

Poster #28

LISA MARIE SCHMITZ
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Power-to-Protein: Micronutrient-enriched Biomass from Electric Power and Carbon Dioxide

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Protein is the primary nitrogen and amino acid source for humans and animals, forming an essential part of nutrition. Currently, meat, animal products, and plants are the primary protein sources in human nutrition. However, their production by current agricultural practices is not sustainable and is on the brink of collapsing with an increasing world population. Developing and implementing renewable technologies is needed to close the nutrient and carbon cycles and support a circular economy. Producing single-cell protein is a promising alternative to safeguard the Earth's resources. In the Power-to-Protein system, we use acetate as a versatile intermediate molecule because it can be produced continuously from CO_2 and H_2 using various new sustainable technologies; it is safe and non-explosive and a direct intermediate of the central metabolism of many microbes.

In a two-stage bioprocess, CO_2 was reduced to acetate with H_2 as an electron donor by the acetogenic bacterium *Thermoanaerobacter kivui* under strictly anaerobic conditions. Transferring the acetate as a sole carbon source to a second-stage bioreactor under aerobic conditions, *Saccharomyces cerevisiae* was grown with a ~40–50 % protein content. In addition to protein, high intracellular concentrations of folate were enriched. The yeast biomass obtained from Stage B provides the recommended daily allowance of folate (~400 µg d^{-1}) already at a serving size of ~6 g dry mass per day. Enriching essential micronutrients in the yeast biomass makes it a valuable protein source for direct human consumption. By this, our system fulfills two primary goals: (1) protein provision for nutrition security; and (2) guaranteeing adequate folate levels for optimal health.

DR TATIANA SPATOLA ROSSI
University of Padua, Italy

Poster #29

Valorisation of CO_2-rich Waste Gas into Polyhydroxyalkanoates (PHAs) by *Cupriavidus necator*

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Polyhydroxyalkanoates (PHAs) are a family of biodegradable and biocompatible polymers that comprise sustainable alternatives to petroleum-based plastics. PHAs are produced by some microorganisms as carbon storage granules under nutrient limiting conditions. In particular, *Cupriavidus necator* has been widely studied as a promising platform for PHA production due to its ability to accumulate high levels of PHAs. The bacterium is able to grow both heterotrophically and autotrophically by consuming CO_2. The latter is especially interesting as it presents a way to fix CO_2 and convert it into useful products such as PHAs, a bioprocess that fits within the context of a circular economy.

The production of PHAs, and especially polyhydroxybutyrate (PHB) - the main type of biopolymer produced - in *C. necator* has been widely studied. However, the application of this carbon capture method to real-life waste gasses is scarce. Moreover, the process has a high hydrogen and oxygen requirement, posing the risk of explosiveness, and a relatively low carbon fixation rate. Thus, further need for optimization of the process is required.

In this work, we used *C. necator* for the valorisation of CO_2-rich gas derived from the fermentation of grape must during wine production, a process which emits thousands of tonnes of CO_2 per year. We fed fermentation gas to *C. necator* under phosphate limiting conditions obtaining 25% PHB (w/w). In order to increase this PHB content, we carried out a systematic comparison of several other nutrient stress conditions, obtaining an improved PHB content of 55%, and assessed growth under low H_2 conditions. Finally, we are investigating the optimisation of the CO_2 uptake rate of *C. necator* via genetic engineering, by developing mutant *C. necator* strains which overexpress key genes or regulatory elements of the main metabolic pathways involved in the autotrophic metabolism. These findings aim to better adapt the process for real CO_2 capture applications.

Poster #30

DR ADRIE STRAATHOF
Delft University of Technology

Integration of Syngas Fermentation and Ethanol Recovery

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Syngas fermentation to ethanol by *Clostridium autoethanogenum* is an upcoming industrial process. A deep understanding of the metabolism of this microbe and the influence of operational conditions on fermentation performance is required to advance the technology and enhance production. We studied the individual impact of dilution rate, gas transfer rate, and acetic acid addition on metabolic shifts, product titres and rates in continuous CO fermentation by *C. autoethanogenum* [1]. These variables jointly determine ethanol production rates, presumably because ethanol production requires a certain minimum undissociated acetic acid concentration; hence also fermentation pH is a key variable.

Consequently, acetate (partly undissociated) is co-produced, at the expense of the yield of ethanol on syngas. Recovering and valorising this acetate as co-product is complicated. Also biomass is produced is at the expense of the yield of ethanol on syngas. On the other hand, a high biomass concentration is desired to obtain a high ethanol production rate.

To maximize the yield of ethanol on syngas and the ethanol production rate, we propose to recycle acetate and biomass to the fermentation, after ethanol recovery in a separate unit. Therefore, we designed industrial-scale vacuum distillation at fermentation temperature, because microbial viability may be retained at such conditions. Such distillation removes all ethanol and a small part of the water, while keeping the rest of the broth in the system. Upgrading to 99.9% ethanol was designed as well. Heat integration minimized energy use, and costs of downstream processing were calculated to be modest despite the vacuum conditions [2].

The integration of the continuous syngas fermentation with the continuous ethanol recovery, while recycling ethanol-depleted broth to fermentation, is a promising option for further development.

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Poster #31

ELODIE VLAEMINCK
Ghent University and Bio Base Europe Pilot Plant

Valorising Syngas in a Coupled Fermentation via Acetate: Techno-economic Analysis for SCP Production and Pilot-scale Implementation

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Third-generation biorefineries using CO_2 as their feedstock have garnered considerable interest for the carbon-neutral biological production of fuels and chemicals. To make this technology economically competitive, energy-efficient fixation of the gaseous carbon is crucial. In this respect, syngas fermentation with acetogenic bacteria appears to be a propitious route, albeit with a limited product spectrum of mainly small organic acids and alcohols. To enhance the product portfolio, we focus on a coupled fermentation approach with acetate, the natural product of the acetogens, serving as the intermediate.

More specifically, we have been investigating the techno-economic feasibility of this intricate process for the production of single-cell protein (SCP) from steel-mill off gas. Experimental data from lab and pilot-scale fermentations were used to build a model and conduct economic analyses. Significant cost reductions could be achieved through optimization of the gas-to-acetate fermentation process, for which a target concentration (45 g/L) and productivity (4 g/L/h) were identified, laying the foundation for further development of the acetate platform.

Another key aspect in this research field is the utilization of real industrial syngas as fermentation feedstock since the varying gas composition and presence of contaminants influence the acetate production. To assess the performance directly at the emission source, a mobile pilot plant for gas fermentation (the Bio Base Mobile Pilot Plant) was utilized to convert biomass-derived syngas into acetate. Conclusively, our work sheds light on practical strategies to advance toward the sustainable and economically viable implementation of third-generation biorefineries.

DR CHARLES WICKHAM-SMITH
The University of Nottingham

Poster #32

Engineering *Cupriavidus necator* H16 for Optimal Growth on Syngas

Synthesis gas (syngas) is a promising feedstock for microbial fermentation, to produce industrially relevant chemicals and fuels. Syngas mixtures are energy rich, typically containing large volumes of hydrogen (H_2), as well as carbon dioxide (CO_2) and methane (CH_4) but is often largely comprised of the toxic gas carbon monoxide (CO). Therefore to allow efficient bacterial growth to occur, for increased productivity and titres, a high resistance to the gas is required. The aerobic bacterium *Cupriavidus necator* H16 can grow on CO_2 + H_2 and is able to oxidise CO to CO_2 following genetic engineering, thus has the potential to utilise syngas efficiently. This work aimed to increase CO resistance through adaptive laboratory evolution by continually subculturing the organism in the presence of CO both heterotrophically and autotrophically. Heterotrophic growth with fructose produced isolates that displayed a clear growth advantage over the wild type strain. Whole genome sequencing revealed various mutations, including a single point mutation upstream of a cytochrome *bd* ubiquinol oxidase operon (*cydA2B2*), which was present in all evolved isolates. When a subset of these mutations was engineered into the parental H16 strain, only the *cydA2B2* upstream mutation enabled faster growth in the presence of CO. Subsequent expression analysis, mutation and overexpression suggested that *cydA2B2* transcription is upregulated in the evolved isolates, resulting in increased CO tolerance only under heterotrophic conditions. Through subculturing on a syngas-like mixture with increasing CO concentrations, *C. necator* could also be evolved to tolerate high CO concentrations under autotrophic conditions, whilst utilising CO_2 as a carbon source. A mutation in the gene for the soluble [NiFe]-hydrogenase subunit hoxH was identified in the evolved isolates. When this amino acid-changing mutation was engineered into the parental strain, autotrophic CO resistance was exhibited. A strain constitutively expressing *cydA2B2* and the mutated hoxH gene displayed high CO tolerance under both heterotrophic and autotrophic conditions. This strain offers a promising chassis for syngas-based bioproduction processes.

JOE WINDO
University of Manchester

Poster #33

Engineering *Halomonas Bluephagenesis* for Biomanufacturing from Waste Feedstocks

JOE WINDO

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Despite the great potential of biomanufacturing in providing more sustainable alternatives to petrochemical-derivatives, high production costs have so far limited its implementation and scale-up. The employment of extremophilic chassis such as the salt-tolerant alkaliphile *Halomonas bluephagenesis* has the potential to drastically lower these costs by removing the need for sterile conditions and large volumes of fresh water. Despite this, the organism remains poorly understood and the feasibility of using waste such as short chain fatty acids as feedstocks has yet to be well characterised. Our work interrogates the assimilation mechanisms for these carbon sources, with the aim of improving their ability to support growth and product formation through approaches such as adaptive laboratory evolution, multiomics and targeted genome editing. Along with with developing new techniques to assay for necessary cofactors and pathway function, we are introducing carbon fixation pathways into the chassis, to diversify potential feedstocks and enable net zero chemical production.

TONG WU
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Poster #34

EuMP Cycle Enable New Gateway for C1 Assimilation

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One-carbon (C1) substrates, such as methanol or formate, are attractive feedstocks for circular bioeconomy. C1 substrates are typically converted into formaldehyde, which represents the entry point into metabolism. Here we designed a synthetic erythrulose monophosphate (EuMP) cycle for formaldehyde assimilation that is based on a promiscuous dihydroxyacetone phosphate dependent aldolase as key enzyme. *In silico* modeling reveals that the cycle is highly energy efficient and has the potential to allow fast growth and to generate high bioproduct yields. Dissecting the EuMP into four modules, we used a stepwise strategy to demonstrate *in vivo* feasibility of the modules in *E. coli* metabolite sensor strains. Adaptive laboratory evolution has been performed for further integration of the modules and identified key mutations enabling the accommodation of the EuMP reactions with endogenous metabolism. Overall, our study demonstrates the proof-of-concept for a highly efficient, new-to-nature formaldehyde assimilation pathway, opening the way for the development of a methylotrophic platform for a C1 fueled bioeconomy in the future.

Poster #35

RAVINEET YADAV
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pyMES: A Set of Python Tools for Mathematical Modelling, Prediction of Rational Experiment Design and Scale-up of Microbial Electrosynthesis from CO_2

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Microbial Electrosynthesis (MES), a biotechnology that utilizes electrochemical processes to stimulate microbial metabolism for the production of industrially relevant organic products from CO_2, shows promise as a CO_2 utilization technology. However, the realization of commercial viability appears to pose challenges. Until now, efforts have primarily focused on advancing cathode materials, microbial enrichment, diversifying product profile, increasing productivity, and experimenting with diverse operating conditions, with a majority of studies conducted using H-type reactors. As it moves ahead, the field anticipates prioritizing the development of efficient reactors and overall processes with optimized mass and ion transport.

A versatile, robust, and simple mathematical model that can function as a predictive tool for MES is required besides the ongoing fundamental research and materials development to enable scale-up assessment of this CO_2 utilization technology. To this end, we have developed a set of Python tools named pyMES to facilitate the theoretical identification of process-limiting steps, assist in reactor design, and predict process outcomes that can be validated with experimentation. It includes modules for analysing electrochemical, biological, and process parameters. This tool is expected to facilitate systematic experiment design, standardize protocols and process analysis. pyMES addresses a crucial gap in MES technology development, offering a comprehensive set of tools to facilitate further development of computational modelling approaches which remain underutilized thus far.

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