The Carbon Recycling Network **Conference 5**

9 - 12 March 2025

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

Programme and Abstracts









Biotechnology and Biological Sciences Research Council

Contents

| Management Board | 3 |
|-----------------------------------|-----------|
| Welcome | 4 |
| General Information | 5 |
| Venue and Surrounding Areas | 6 |
| Map of Local Area | 7 |
| Programme | 8 - 12 |
| Sponsors | 13 - 18 |
| Invited Speakers | 19 - 31 |
| Abstracts of Oral Presentations | 32 - 63 |
| Abstracts of Poster Presentations | 64 - 98 |
| Participant List | 99 - 103 |
| Notes | 104 - 108 |

Management Board

| Nigel Minton | SBRC Nottingham, UK |
|----------------------|---------------------------|
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| Mark Poolman (Col) | Oxford Brookes University |
| Saul Purton (Col) | UCL |
| Will Zimmerman (Col) | University of Sheffield |
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| Geoff Bryant | Calysta |
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| Adrian Higson | NNFCC |
| Stephen Poulston | Johnson Matthey |
| Sean Simpson | Lanzatech |
| Kris Wadrop | CPI |
| Tithira Wimalasena | Corbion |

Conference Secretariat: Wendy Solis The University of Nottingham Biodiscovery Institute Clifton Boulevard University Park Nottingham NG7 2RD wendy.solis@nottingham.ac.uk

Welcome







The Management Board welcomes you to the fifth annual CarbonRecycling Network conference. This is the last in the series as the funding underpinning the NIBB's activities ceases at the end of March this year. Given the increasing interest in gas fermentation and the ever-urgent need to mitigate climate change, we hope that this final conference will be a key platform highlighting and celebrating the advances made in our field of sustainable C1 molecule usage and their industrial application.

Despite coming to the end of its funding, the Network's membership continues to grow, primarily through early career researcher membership. It is good to note, given C1 gas fermentation is a niche but important field of endeavour, that the community has grown to a total of 882 individuals. Industrial membership accounts for 200 of that total and the community is truly international with over 212 members coming from 35 countries. At the 2024 conference only 8 of the 26 talks were from UK-based researchers. Once again the diversity of talks at this year's conference demonstrates a truly international community.

All of the Proof-of-Concept and Business Interaction Voucher funding which could be committed has been invested in important projects. All of these have been completed and final reports submitted to BBSRC. Encouragingly, some of these projects are already leading onto more substantial programmes of applied work. Within the last year two commissioned reports were finalised. A report on CO₂ Biomethanation (jointly with EBNet) was submitted to BBSRC and forms the basis of a forthcoming academic publication. A guidance manual on the Safe Handling of C1 Gases was greeted with enthusiasm by the NIBB's membership and has been published on the CarbonRecycling website.

There is much more to do, but I am confident that the presentations will indicate advances in the field and the real utility of gas fermentation in the evolving landscape of sustainable industry.

We look 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.

Three nights' accommodation is provided with breakfast and evening meal. Check-in from **3pm Sun 9 March**; check-out by **11am Wed 12 March**

ORAL PRESENTATIONS

Oral presentations will be in the Hotel's William Turner Suite.

The length of oral presentations varies between 20-50 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 Drawing Room/Board Room. The maximum recommended poster size is A0 portrait (90 cm \times 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.

<u>**Presenters**</u>: please let your audience know if your work is confidential and should NOT be tweeted or recorded.

SOCIAL EVENTS

- Welcome Dinner
 Sunday 9 March 19:30h Tilden Suite
- Drinks Reception* & Conference Dinner Monday 10 March 19:00h – Tilden Suite

*The organisers are very grateful to Aerbio, IBioIC Inovo, Forge Genetics, NCIMB Ltd and Photanol for their sponsorship of the Drinks reception.

The Venue and Surrounding Area

SHRIGLEY HALL HOTEL

https://www.shrigleyhallhotelandspa.co.uk

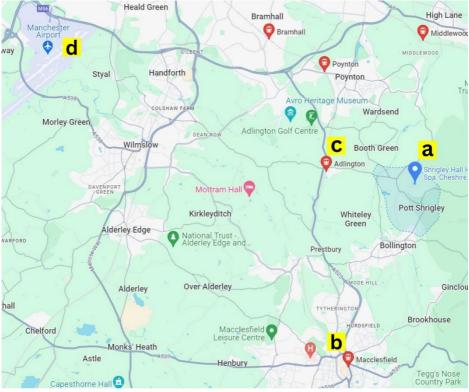


Shrigley Park, Pott Shrigley, Nr Macclesfield, Cheshire SK10 5SB

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)



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

Programme

SUNDAY 9 MARCH 2025 The Shrigley Hall, Reception 15:00 REGISTRATION 19:30 WELCOME DINNER – TILDEN SUITE

MONDAY 10 MARCH 2025 The Shrigley Hall, William Turner Suite

DAY 1

| SESSION 1 | Acetogens Chair: Klaus Winzer (SBRC-Nottingham, UK) | |
|--------------|--|--|
| 09:00 -09:10 | Klaus Winzer The Carbon Recycling Network | Welcome & Opening Words |
| 09:10-10:00 | INVITED TALK: Volker Müller University of Frankfurt | Arren Bar-Even Lecture: Many Ways Lead to Rome: Fundamental Principles and Differences in Acetogenesis among Species |
| 10:00-10:20 | INVITED TALK: Marilene Pavan LanzaTech Inc | Ethanol, SAF, and Nutritional Protein Production via Microbial Gas Fermentation |
| 10:20-10:40 | Diana Machado de Sousa Wageningen University & Research | A Synthetic Co-Culture Approach for the Conversion of Carbon Monoxide to Polyhydroxyalkanoates |

10:40-11:00 COFFE

COFFEE/TEA BREAK – ELLEN SUITE

| SESSION 2 | Acetogens continued Chair: Diana Machado de Sousa | |
|-------------|--|---|
| 11:00-11:30 | James Heffernan The University of Queensland | CO(2)-Metabolism: Quantifying Mixed Carbon Oxide and Hydrogen Gas Fermentation by <i>Clostridium autoethanogenum</i> |
| 11:30-11:50 | Anna Stock Technical University of Munich | CO-Addition Enhances Autotrophic D-Lactate Formation with Engineered Acetobacterium woodii |
| 11:50-12:10 | Jose Antonio Velazquez Gomez University of Tübingen | Enhancing Autotrophic Acetate Production by <i>Thermoanaerobacter kivui:</i> Combining Biomass Retention, High Dilution Rates, and Moderate Overpressure in Continuous Bioreactors |
| 12:10-12:30 | Frank Bengelsdorf University of Ulm | Reclassification, Metabolism and Genetic Engineering of Acetogens in the <i>Eubacterium</i> genus |

| 12:40-13:40 | Ľ |
|-------------|---|
|-------------|---|

UNCH – OAKRIDGE RESTAURANT

MONDAY 10 MARCH 2025 The Shrigley Hall, William Turner Suite

| SESSION 3 | Aerobic C1 organisms Chair: Frank Sargent | |
|-------------|--|--|
| 14:00-14:30 | INVITED TALK: Sebastian Wenk University of Groningen | Engineering Microbial Growth on CO ₂ Derived Feedstocks – a Feasible Solution for a Sustainable Bioeconomy |
| 14:30-14:50 | INVITED TALK: Katalin Kovacs University of Nottingham | Bioproduction of Chemicals from CO ₂ |
| 14:50-15:10 | Eun Yeol Lee Kyung Hee University | Metabolic Engineering of Methanotrophs and Its Application to Methane Bioconversion |
| 15:10-15:30 | Ari Satanowski Max Planck Institute for Terrestrial Microbiology | Design and In Vivo implementation of Aerobic, Ambient CO ₂ -reduction as an Entrypoint for Enhanced Carbon Fixation |

| 15:30-16:00 | COFFEE/TEA BREAK – ELLEN SUITE |
|-------------|---|
| | Poster session Drawing Room/Board Room |

| 17:30 | CLOSE DAY 1 |
|-------|---|
| | |
| 19.00 | DRINKS RECEPTION AND CONFERENCE DINNER – TILDEN SUITE |

TUESDAY 11 MARCH 2025 The Shrigley Hall, William Turner Suite

| SESSION 5 | NIBB Funded exemplar projects Chair: Katalin Kovacs | |
|--------------|---|--|
| 09:00 -09:30 | INVITED TALK: Claudio Avignone Rossa University of Surrey | Microbial Electrochemical Systems for the Capture and Transformation of CO ₂ into Multicarbon Organic Compounds |
| 09:30-09:50 | INVITED TALK: Frank Sargent Newcastle University | Harnessing Bacterial Hydrogen Metabolism (with the help of the BBSRC NIBBs) |
| 09:50-10:10 | INVITED TALK: Rajesh Bommareddy University of Northumbria | Cupriavidus Species as Potential Chassis for Chemical Production from CO_2 |
| 10:10-10:30 | INVITED TALK: Ying Zhang University of Nottingham | Genetic Tools Development in Novel Methylotrophs |

10:30-11:00

COFFEE/TEA BREAK – ELLEN SUITE

| SESSION 6 | Feedstocks Chair: Charles Banks | |
|-------------|--|--|
| 11:00-11:30 | INVITED TALK: Sara Walker University of Birmingham | Hydrogen Integration into Energy Systems: What, Where and When |
| 11:30-11:50 | INVITED TALK: Selina Ambrose Promethean Particles | Project MONET: Demonstrating the Industrial-Scale Viability of MOF-Based Carbon Capture |
| 11:50-12:10 | INVITED TALK: Yue Zhang University of Southampton | Predicting pH Rise as a Control Measure for Integration of CO ₂ Biomethanisation with Anaerobic Digestion |
| 12:10-12:30 | Qiang Li Centre for Process Innovation Ltd | A Feasibility Study on the Use of Hydrogen as a Feedstock to Platform Chemicals |

| 12:40-13:40 | LUNCH – COURTYARD BAR |
|-------------|-----------------------|
|-------------|-----------------------|

TUESDAY 11 MARCH 2025 The Shrigley Hall, William Turner Suite

DAY 2

| SESSION 7 | The Start-up Journey Chair: Tithira Wimalasena | |
|-------------|---|---|
| 14:00-14:20 | INVITED TALK: David Ortega Phase Biolabs | Commercialisation of CO ₂ -Based Gas Fermentation Processes Using Engineered Acetogens |
| 14:20-14:40 | INVITED TALK: Robert Mansfield AerBio | From lab to industry: experience in biotech scale-up and commercialisation |
| 14:40-15:00 | David Keating Synata Bio | Synata Bio: CO ₂ Valorization via Advanced Biocatalysts |
| 15:00-15:30 | PANEL DISCUSSION | |

| 15:30-16:00 | COFFEE/TEA BREAK – ELLEN SUITE |
|-------------|--------------------------------|
|-------------|--------------------------------|

| SESSION 8 | Scaling-up Chair: David Keating | |
|-------------|--|---|
| 16:00-16:20 | INVITED TALK: Koen Quataert Bio Base Europe | The Importance of Open-Access Piloting for Microbial CCU Technologies Using Real Industrial C1-Gasses |
| 16:20-16:40 | INVITED TALK: Rosalind Hay Centre for Process Innovation (CPI) | The Reality of Technical Transfer: Derisking the Unknown |
| 16:40-17:00 | INVITED TALK: Geoff Bryant Calysta | Insights from the Top of the Fermenter |
| 17:00-17:30 | PANEL DISCUSSION | |

| 17:30 | CLOSE DAY 2 |
|-------|-----------------------|
| | |
| 19.30 | DINNER – TILDEN SUITE |

WEDNESDAY 12 MARCH 2025 The Shrigley Hall, William Turner Suite

| SESSION 9 | Biorefining - Bioconversion Chair: Ying Zhang | |
|--------------|---|---|
| 09:00 -09:30 | INVITED TALK: Ivan Ilic Electroarchaea GmBH | Electrocatalysis Meets Biocatalysis: Bioelectrochemical Methanation System |
| 09:30-09:50 | INVITED TALK: Kees Kwant Integrated Biorefineries | Mission Innovation: Integrated Biorefineries Programme |
| 09:50-10:10 | Ayodeji Aluko University of Huddersfield | Optimizing Anaerobic Digestion for Domestic Applications: Challenges and Opportunities |
| 10:10-10:30 | Ludovic Jourdin Delft University of Technology | Microbial Electrosynthesis from CO ₂ Reaches Productivity of Syngas and Chain Elongation Fermentations |

10:30-11:10 **COFFEE/TEA BREAK – ELLEN SUITE**

| SESSION 10 | The Funders' pitch Chair: Sonia Heaven | |
|-------------|---|--|
| 11:10-11:30 | INVITED TALK: Dana Galili DSIT | UK Government Approach to Engineering Biology |
| 11:30-11:50 | INVITED TALK: Oliver Sexton Future Planet Capital | Future Planet Capital |
| 11:50-12:10 | INVITED TALK: Steve Chambers SOCV | From Lab to Market: Lost in Translation |
| 12:10-12:15 | Klaus Winzer The Carbon Recycling Network | Closing Remarks |

| 12:30-13:30 | LUNCH – OAKRIDGE RESTAURANT |
|-------------|-----------------------------|
| | |
| 13:45 | CLOSE OF MEETING |

SPONSORS



Aerbio is scaling aerobic gas fermentation to produce alternative proteins from hydrogen and carbon dioxide, tackling global food security challenges while reducing the environmental impact of protein production for animal feed.

The company has successfully validated its technology at pilot scale, demonstrating both process feasibility and product performance in application trials.

Now advancing to demonstration-scale deployment alongside its new headquarters in Copenhagen, Denmark, Aerbio is finalizing commercial and technical de-risking to enable industrial-scale production. As the company expands, it is building a worldclass team and seeking talented individuals and strategic partners to drive the growth of its carbon recycling and gas fermentation platform, shaping the future of alternative proteins and sustainable biomanufacturing through precision fermentation.

Name: Aerbio A/S Address: Produktionsvej 12, 2600 Glostrup, Denmark Email: <u>info@aer.bio</u> Web: <u>https://aer.bio</u>



IBioIC is a networking and support organisation that connects industry, academia and government to bring biotechnology processes and products to the global market.

Name: IBioIC Inovo Address: IBioIC Inovo, 121 George Street, Glasgow, UK G1 1RD Email: <u>info@ibioic.com</u> Web: <u>https://www.ibioic.com</u>



Forge Genetics is a University of Nottingham spin-out contract research organisation offering strain development services.

We specialise in creating modified strains in difficult to work with species using our alternative to CRISPR — "Forge Editing". Forge Editing works where other geneediting methods have failed, and allows users to avoid the patent thicket associated with CRISPR-based techniques.

Based at the Biodiscovery Institute in Nottingham, UK we have access to specialist facilities for manipulation of diverse species including:

Gas fermentation suite (H_2 , O_2 , CH_4 , CO_2 , N_2 , CO) for acetogens, methanotrophs, hydrogen oxidising bacteria, and others.

Analytics suite (HPLC, GC, MS) for product profile characterisation of strains.

Robotics suite for high throughput screening, directed evolution, and large scale experiments.

Containment level 2 laboratory for pathogen work.

Anaerobic workstations for anaerobic and microaerophilic cultures.

Sequencing and bioinformatics standard and custom services using Oxford Nanopore and Illumina systems.

Forge works with large companies, SMEs, and research institutes on both fee for service arrangements and research collaborations. If you are curious about working with us we'd be delighted to talk. Get in touch via <u>enquiries@forgegenetics.com</u> or our website <u>www.forgegenetics.com</u>



NCIMB is an innovation-focussed biotechnology company offering a range of microbiology products and services to support your process development.

- **Culture collection**: We hold the UK's largest collection of industrially useful bacteria including robust candidates for gas fermentation and single cell protein. We offer commercial licences on request.
- **Microbial identification**: fast, accurate identification of bacterial and fungal isolates.
- Whole genome sequencing including screens for antimicrobial resistance genes, virulence factors and toxins.
- 16S metagenomics: to understand the composition of mixed communities in complex samples.
- qPCR services: to detect, quantify and monitor microorganisms. We offer commonly requested tests and can develop customised assays for organisms or functional genes of interest.
- **Strain storage**: NCIMB is an Internationally recognised as a Depositary Authority for patent deposits and our storage services provide a safe and easy offsite backup, ensuring you always have access to your important biological resource.

Our customers include household names in food, drink, energy, and pharmaceutical production as well as universities, research institutes and SMEs. Whoever we are working with we pride ourselves on delivering high levels of customer service.

Name: NCIMB Ltd Address: NCIMB Ltd, Wellheads Place, Dyce, Aberdeen, AB21 7GB, UK Email: enquiries@ncimb.com Web: https://www.ncimb.com



Photanol has developed a breakthrough biotechnology platform that converts CO_2 directly into valuable chemicals using only sunlight as an energy source. The company's proprietary cyanobacteria-based technology uses minimal amounts of water and nutrients while producing renewable chemicals that can replace fossil-based alternatives across multiple chemical markets.

Through continuous innovation in strain engineering and process development, Photanol aims to make its sustainable production platform cost-competitive with conventional fossil-based chemical manufacturing while helping to decarbonize the chemical industry.

Name: Photanol B.V Address: Photanol B.V, Science Park 406, 1098 XH Amsterdam, The Netherlands Email: info@photanol.com Web: https://photanol.com/

INVITED SPEAKERS



Volker Müller Goethe University

Professor Müller is Head of the Department of Molecular Microbiology and Bioenergetics at Goethe University, Frankfurt. His research interest is the metabolism and biochemistry of anaerobic microorganisms with a focus on acetogenic bacteria. His group discovered how these bacteria make a living during autotrophic and heterotrophic growth, characterized the enzymes involved in bioenergetics, carbon and electron flow and redox homeostasis. His group established the use of acetogens to capture and store hydrogen as well as carbon dioxide. The lab also uses archaea to study the metabolic processes that allow microbial life under extreme energy limitation and that couple CO₂ fixation to ATP synthesis. He has directed an European-wide ERA-IB Network on industrial applications of acetogenic bacteria. He has co-authored more than 300 papers and was awarded in 2016 one of the prestigious Advanced Investigator Grants of the European Research Council and in 2022 with a Reinhart-Koselleck project of the German Research Foundation (DFG) for his work on the role of cytochromes in actetogens. In 2023 he was elected as fellow of the American Academy of Microbiology and in 2024 as member of the German National Academy of Sciences Leopoldina. For further information: www.mikrobiologie-frankfurt.de.



Marilene Parvan 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.



Sebastian Wenk University of Groningen

Sebastian studied molecular biology at the University of Potsdam (Germany) and synthetic and structural biology at the University of Strasbourg (France). After shifting from plant science to immunology, he discovered his true passion, microbiocidal biotechnology. This led him to pursue a PhD under the supervision of Arren Bar-Even, focusing on engineering synthetic pathways for one-carbon (C1) assimilation in *E. coli*. He then continued in the same group as a postdoc, engineering new-to-nature enzymes to expand the potential of formate assimilation. As a Marie Curie Fellow, he joined Victor de Lorenzo's lab, developing molecular tools for *in vivo* enzyme evolution.

In January 2025, Sebastian launched his own group at the Groningen Biomolecular Sciences and Biotechnology Institute, where he combines *in vivo* enzyme evolution with synthetic C1 metabolism. His goal? To engineer the next generation of microbial cell factories – turning microbes into living machines that power a sustainable bioeconomy



Katalin Kovács University of Nottingham

Dr. Katalin Kovács, Associate Professor at the University of Nottingham, School of Pharmacy, UK.

Katalin has more than 10 years of research experience in synthetic biology and biological (plant and bacterial) engineering, and she is one of only a few UK based scientist with previous experience in the generation and characterisation of transplastomic plants. She has played a leading role in the development of synthetic biology tools and methodologies for non-model, aerobic and anaerobic organisms, as well as novel gene-drive systems for advanced chloroplast editing in plants. Her current research activities include studying and exploiting the potential of autotrophic microorganisms and plants for the sustainable production of on site, on-demand molecules including pharmaceuticals from CO_2 and renewable energy.



Claudio Avignone Rossa University of Surrey

Claudio Avignone Rossa (CAR) is a Professor of Systems Microbiology at the School of Biosciences of the University of Surrey, with research interests focusing on the metabolic and ecological mechanisms involved in the development and evolution of microbial consortia in natural or artificial biological processes, including microbial electrochemical systems for bioremediation, wastewater treatment and for CO₂ capture and conversion. He was awarded the 2018 Newton Prize for his design of low-cost microbial fuel cells for treatment of wastewater from primary coffee production. He is currently involved in projects related to the bioremediation of water and soil and the recovery of strategic metals from batteries and electronic waste. His research has been supported by BBSRC, EPSRC, EU, Newton Fund, BEIS, GCRF and by biotech and pharma industries (GSK, BASF, Eli Lilly/Elanco, Green Biologics, SymBio, Abbott, Avecia, etc).

CAR has been a member of BBSRC and GCRF committee panels and advisor and evaluator of research proposals for national and international research funding institutions. CAR has participated in the creation of various national and international consortia related to microbial biotechnology, Europe, Africa, Asia and LatinAmerica. CAR has been a contributor to the Policy Briefing report "Supporting microbiology to prevent the next global catastrophe" commissioned by the Society for Applied Microbiology (now Applied Microbiology International). He is a Fellow of the Surrey Institute for Sustainability and the Royal Society of Biology, and is a Member of the Network of Argentine Scientists in the United Kingdom.

CAR has organized and participated in workshops and events for the dissemination of microbial biotechnology for bioenergy and sustainability and has been keynote speaker in various international meetings for stakeholders, industrialists, and policymakers. He has been interviewed by TV and radio broadcasters, newspapers, trade magazines, and social media channels in the UK, EU, Latin-America, and Asia. He has been invited speaker at various dissemination events such as "Doing Science with Colombia 2022", COP26, EDD, and Science Museum Lates. His research has been showcased by BBSRC in its "Biotechnology impacting everyday life" booklet and in the Delivery Plan 2019.



Selina Ambrose Promethean Particles

Selina currently holds the position of product manager at Promethean Particles ("Promethean"), an independent venture-capital backed spin-out from the University of Nottingham. Promethean is a UK-based, global pioneer, leading the industrial-scale manufacture of metal-organic frameworks (MOFs) – a class of advanced materials. The Company is primarily focused on developing and manufacturing MOFs for the energy transition, particularly for carbon capture and storage ("CCS") and water harvesting applications.

Selina has more than 15 years of experience in the development and commercialisation of advanced material technologies. After being promoted into her current role in 2022, Selina now leads the product management function at Promethean, acting as the critical interface between the external needs of customers and collaboration partners, and the Company's internal capabilities to deliver them. Prior to this, Selina held the position of Promethean's technical manager, where she played a key role in successfully winning over £3.5 million in non-dilutive, grant funding for the company to deliver a range of innovative R&D projects.

Selina holds a PhD in Chemical Engineering from the University of Nottingham, where her thesis focused on the continuous-flow hydrothermal synthesis of functional nanomaterials. She is currently an advisory board member of the University of Nottingham Centre for Doctoral Training in Sustainable Chemistry.



Yue Zhang University of Southampton

Yue Zhang is an Associate Professor in Environmental Engineering at University of Southampton, working on bioprocesses for resource recovery from organic waste and wastewater including anaerobic digestion, mixed-culture fermentation with integrated product recovery, and CO₂ biomethanisation. During her postdoctoral career she undertook two Defrafunded research contracts on anaerobic digestion, the results of which have already been successfully applied in the commercial sector in the UK. Her current research focuses on microbial aspects of bioprocesses, aiming to link these to practical benefits that can be achieved through engineering control of these systems to maximise resource recovery and at the same time minimise the use of external inputs.



David Ortega Phase Biolabs

David completed his undergraduate degree in biochemistry where he developed an interest in metabolism. He was fortunate to secure a PhD position under the supervision of Professor Nigel Minton at the Synthetic Biology Research Centre. His research focused on metabolic engineering of *Clostridium* to produce sustainable chemicals and fuels from waste. In 2020 David founded Phase Biolabs, a startup commercialising gas fermentation technology to produce ethanol directly from CO₂. David is an alumnus of Y Combinator and a Breakthrough Energy Fellow.



Robert Mansfield Co-founder & CTO, Aerbio A/S

Rob has been developing aerobic gas fermentation technology and products thereof for commercialization since 2018. As CTO at Aerbio, he leads the company's technology scale-up strategy and is responsible for executing it's commercialization roadmap. This includes overseeing the development of Products, Processes, and Hardware, from lab-scale research to future industrial deployment.

Following the successful pilot-scale deployment in the Netherlands and validation of product performance in aquaculture trials, his current focus is on delivering Aerbio's first fully-integrated demonstration-scale facility - the next critical milestone in de-risking the path to commercial deployments."



Koen Quataert Bio Base Europe Pilot Plant

Koen Quataert is a bio-engineer from Ghent University currently working as innovation manager bio-CCU at Bio Base Europe Pilot Plant, where he leads a team of R&D engineers and PhD researchers to steer research activities and technology development a.o in the framework of several regional and EU-funded projects



Rosalind Hay CPI

After gaining over 25 years' experience in the Biotechnology Industry, including years at Quorn and Fujifilm, Rosalind Hay leads a team of process managers, scientists and operations as Head of Biomanufacturing at CPI.

Rosalind has experienced a broad range of processes in fermentation, primary separation and extensive years of large-scale processing at an open-access facility, resulting in her vast knowledge of technical transfer and programme management. Facing challenges from such a diverse landscape has created an opportunity for Rosalind to gain a unique skill set and knowledge base.

Rosalind was fundamental in the commissioning of CPI's pilot and demonstration plants; from collaborating on the plant design to transferring in the first process, Rosalind added value to each phase of the process and built a team from the ground up. Following this, she led the build and commission of a bespoke large-scale customer plant, managed customer relations and led the transfer of the development process into the manufacturing scale bioreactors.

Rosalind is a pragmatic leader who excels in seeing through the details to find the route of a problem and is solution focussed to ensure constant progression and improvements for her teams and the company.



Geoff Bryant Calysta

Geoff joined Calysta in 2020 as Chief Technology Officer, and has 25 years' experience leading R&D and technology teams in food and petfood. Based at Calysta's UK Technology Hub in Teesside, he oversees global product development, quality and innovation activities and is supporting the delivery of the world's first FeedKind® production facility, Calysseo, Calysta's 50/50 joint venture with Adisseo in China.

Prior to joining Calysta, Geoff led Quorn's R&D and technical teams in fermentation science, "meat -free" food product development, innovation, quality, regulatory and process technology. He also spent 20 years with Mars Inc in several global innovation and R&D roles across Snackfood, Chocolate and Petfood categories. Geoff holds Ph.D. and BSc degrees in Chemistry from The University of East Anglia.



Ivan Ilic Electroarchaea

Ivan Ilic studied chemistry in Zagreb and Berlin. During his Ph.D. at Max Planck Institute of Colloids and Interfaces in the department of Prof. Markus Antonietti he developed expertise in electrochemistry and biomaterials, defending his thesis with the title: "Design of sustainable cathodes for Li-ion batteries" in 2020. Afterwards, he joined the group of Prof. Martin Oschatz, where he worked on the development of hard carbon materials as anodes for Na-ion batteries. In 2021 he joined Dr. Mario Caironi's group at the Istituto Italiano di Tecnologia where he worked on the Electronic Food project. Using the common biomolecules, riboflavin and quercetin, he developed the first rechargeable edible battery, an invention that got a Special Mention on TIME Magazine's list: "The Best Inventions of 2023".

In 2023 he joined Electrochaea, where he is currently working as a Team Leader Bio-Electrochemistry, leading the efforts to develop an electrochemical biomethanation one-pot reactor. This reactor has methanogenic microbes, Electrochaea's proprietary strain of archaea, living in direct contact with the cathode of the electrolyzer, ensuring hydrogen is consumed at its source. Furthermore, he is active in the efforts of commercialising Electrochaea's core technology, supporting the team with his expertise in chemistry, materials, and electrochemistry.



Kees W Kwant Netherlands Enterprise Agency (RVO)

Kees W. Kwant is a Senior Expert on Bioenergy and Circular Biobased Economy at the Netherlands Enterprise Agency (RVO), a department of the Ministry of Climate and Green Growth in the Netherlands. He primarily links researchers and industry to help develop the circular biobased economy and bioenergy in the Netherlands and abroad. He is Member CEM Biofuture Initiative and leads the Mission Innovation Mission Integrated Biorefineries as Mission Director.





Dana is a Policy Advisor at the Department for Science Innovation and Technology (DSIT), working in the Engineering Biology (EB) Growth team. Prior to joining the EB team, she worked at the Government Office of Science in the Technology and Science Insights group, where she assessed the challenges in scaling up EB applications in the UK. Prior to that, she was a research associate at the MRC Laboratory of Molecular Biology where she studied how differences in brain anatomy and wiring between male and female *Drosophila* flies control sexspecific behaviour. Dana obtained a PhD in Neurobiology from the Max Planck of Neurobiology in Munich, Germany, an MSc in Neurobiology from the Weizmann Institute of Science, Israel, and a BSc in Life Sciences and Psychology from the Hebrew University of Jerusalem, Israel.



Oliver Sexton Future Planet Capital

Oliver is an Investment Director at UKI2S. Specialising in high impact biology based investments he has managed the engineering biology fund since its inception in 2014. He has invested in a broad range of companies in sectors as diverse as therapeutic, agritech and clean biotech, all of which are using biology to make sustainable and highly novel products.

He sits on multiple boards and has overseen exits including the £85m sale of Quethera to Astellas. He is also a member of the Future Planet Capital ESG steering group.

Prior to joining UKI2S, Oliver worked at the venture fund Imperial Innovations as well as other roles in the industry. He has a background in strategy analysis and consultancy including roles in New York and has a masters from the University of Bristol and commenced a PhD in bioremediation at the University of Nottingham.



Steve Chambers SoSV

Stephen is a General Partner at SOSV and the Managing Director at IndieBio in New York. He has invested in over 100 life science startups in the therapeutic and industrial biotechnology sectors. As a founding scientist at Vertex Pharmaceuticals (NASDAQ: VRTX), he was instrumental in discoveries that led to the FDA approval of multiple drugs. He went on to co-found Abpro Therapeutics (NASDAQ: ABP) advancing antibody discovery and development. As CEO of SynbiCITE, he propelled the UK's synthetic biology sector to new heights, achieving record growth and investment. With over 30 peer-reviewed publications and numerous patents, Stephen is recognized for his expertise in cultivating innovative life science companies and ecosystems around the globe. Recently, recognized as one of City & State New York's 2024 Trailblazers in Economic Development. Previously held the prestigious role of Royal Society of Biology.

Abstracts of Oral Presentations

ABSTRACTS OF ORAL PRESENTATIONS

VOLKER MÜLLER

Goethe University Frankfurt

Many ways lead to Rome: fundamental principles and differences in acetogenesis among species.

Microbial production of acetate from CO_2 is catalyzed by the ecophysiologically as well as biotechnologically important group of acetogenic bacteria. CO₂ is reduced by the Wood-Ljungdahl pathway (WLP), a two branched, linear pathway in which one CO₂ is reduced to a methyl group and another to a carbonyl group that are combined by the key enzyme, the CODH/ACS to acetyl-CoA, the precursor of acetate. The carbon reduction pathway to acetate is not coupled to net ATP formation but nevertheless it allows acetogens to make a living. Net ATP synthesis is catalyzed by a respiratory chain that is hooked up to the carbon reduction pathway. The respiratory chains are different in different acetogens and are either of the Rnf- or Ech-type that are going to be discussed. Both respiratory chains use reduced ferredoxin as electron donor that is reduced with hydrogen as reductant by electron bifurcating hydrogenases. The concept in electron bifurcation in saving cellular energy will be discussed. Of critical importance is the nature of the electron carriers involved in the carbon reduction pathway: any use of reduced ferredoxin will reduce the amount of ATP synthesized. I will describe how different acetogens solved the problem of reducing CO₂ to formic acid, the first step in the methyl branch of the WLP, without using reduced ferredoxin as reductant. Some use a hydrogendependent CO₂ reductase, others an electron-bifurcating formate dehydrogenase. Acetogens are phylogenetically very different but have in common the basic chemistry of acetogenesis. However, they differ in (i) the respiratory chain used for ATP synthesis, (ii) the nature of the electron carriers used in the WLP and finally (iii) the amount of ATP synthesized. This is the more important since acetogens operate at the thermodynamic limit of life and the production of many value-added compounds from $H_2 + CO_2$ is thermodynamically restricted. At the end, I will present the entire biochemistry and bioenergetics of acetogenesis from $H_2 + CO_2$ or CO in model acetogens such as Acetobacterium woodii, Thermoanaerobacter kivui, Eubacterium limosum, Sporomusa ovata, Clostridium aceticum and Clostridium autoethanogenum and outline ways to improve the energetics for producing ATPintensive valued-added products from $H_2 + CO_2$.

MARILENE PAVAN

LanzaTech, Inc.

Ethanol, SAF, and Nutritional Protein Production via Microbial Gas Fermentation.

LanzaTech is pioneering a circular economy approach to carbon recycling using gas fermentation technology. Our process captures waste carbon sources and converts them into valuable products, ethanol being the primary output. The company's proprietary gas fermentation process uses anaerobic acetogen bacteria to convert CO, CO2, and H2 into ethanol. A key advantage is the flexibility to use diverse carbon sources including industrial off-gases, municipal and agricultural solid waste, and fermentation and atmospheric CO2. LanzaTech has commercial-scale plants operating globally, producing 46,000-64,000 metric tons of ethanol annually. This ethanol serves as a feedstock for sustainable aviation fuel (SAF), chemicals, materials, and consumer products. We are also expanding into direct production of other chemicals and nutritional protein from CO2 via gas fermentation. Our technology platform includes advanced synthetic biology, including high throughput genetic modification and screening of anaerobic, gas fermenting microorganisms, and AI capabilities to engineer new microbial strains and optimize processes. Our approach offers several benefits: it recycles waste carbon that would otherwise be emitted. production is independent of agricultural and climate factors, and it accesses large addressable markets across fuels, chemicals, and materials while maintaining drop-in compatibility with existing infrastructure and supply chains. With operational commercial plants and an expanding product portfolio, LanzaTech aims to demonstrate that recycling carbon waste via microbial gas fermentation can be a scalable, economically viable approach to reducing emissions and producing sustainable materials.

DIANA MACHADO DE SOUSA

Wageningen University & Research

A Synthetic Co-Culture Approach for the Conversion of Carbon Monoxide to Polyhydroxyalkanoates.

TIMON M. TORRES RUANO, MARTIJN DIENDER AND DIANA Z. SOUSA

Polyhydroxyalkanoates (PHAs) have been proposed as a potential alternative to petrochemical plastics, however the high cost of substrates used for PHA production has limited large-scale implementation. C1 substrates are a cheap, more sustainable alternative for PHA production, but efficient biocatalysts for this process are still lacking.

In this work we describe a novel method for the anaerobic production of PHA from carbon monoxide (CO) as sole substrate, by using a synthetic coculture of *Rhodosprillum rubrum* and *Acetobacterium woodii*. In this system, *R. rubrum* utilizes CO to drive the water gas shift reaction, yielding H₂ and CO₂. These products are subsequently converted to acetate by *A. woodii*, which is used by *R. rubrum* as an organic carbon source for PHA accumulation. While co-cultures were unstable during chemostat cultivation, fed-batch cultivation yielded a PHA production rate of 58 ± 11 mg/L/day with a final PHA content of 38 ± 5% (dry weight). A mono-culture of R. rubrum was not able to produce PHB when fed solely with CO, showing the significance of the co-cultivation approach.

Overall the synthetic co-culture of *R. rubrum* and *A. woodii* demonstrated in this work is a promising and exciting new approach for the production of PHB from waste resources. This highlights the potential of co-cultivation strategies in unlocking new pathways for sustainable and efficient bioproduction processes.

Acknowledgements: Research was funded by the Netherlands Science Foundation (NWO) domain Applied and Engineering Sciences (AGS) (Perspectief Programma P16-10/P2), and by the Centre for Living Technologies from the EWUU Alliance.

JAMES K. HEFFERNAN

The University of Queensland

CO₍₂₎-Metabolism: Quantifying Mixed Carbon Oxide And Hydrogen Gas Fermentation By *Clostridium Autoethanogenum.*

R AXAYACATL GONZALEZ-GARCIA Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD, Australia TIMOTHY MCCUBBIN Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD, Australia MICHAEL KÖPKE LanzaTech, Inc., Skokie, IL, USA KASPAR VALGEPEA ERA Chair in Gas Fermentation Technologies, Institute of Technology, University of Tartu, Tartu, Estonia LARS NIELSEN Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD, Australia ESTEBAN MARCELLIN Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD, Australia

Industrial-scale gas fermentation of CO-to-ethanol is a commercial reality now, producing a more sustainable fuel than traditional, fossil-derived sources. *Clostridium autoethanogenum* is a model industrial acetogen strain with a rapidly expanding product spectrum for commodity chemicals due to its genetic engineering toolbox. Expanding this process from CO-based gases to mixtures that facilitate co-metabolism of CO, CO2 and H₂ shows strong possibility for enabling broader application of C1 recycling. Quantifying CO fermentation in the absence of H2 results in an entirely different metabolism to CO2/H2 fermentation, where differences between carbon source (CO2 or CO) may be confounded with available energy source (H2 or CO).

To accommodate these potential variables into a comparison of CO2 and CO metabolism, here we quantify chemostat steady-states (CSSs) under constant H2 supply with a gradual, stepwise switch from CO2-dominant to CO-dominant feed. A multi-omics quantification of these CSSs forms a comprehensive characterization of the differences between *C. autoethanogenum*'s CO and CO2 metabolism. We demonstrate that metabolism of CO2/CO by *C. autoethanogenum* is highly flexible in the presence of H2, maintaining co-utilization of CO2 and CO over a wide range of gas compositions. Further, a multi-omics analysis elucidates novel mechanisms of C1 metabolism and redox homeostasis. Surprisingly, we also find conditions with high 2,3-butanediol flux, a feature thought to be driven by CO-based metabolism. Use of these findings could be influential to broad C1 gas-to-liquid processes, providing a novel avenue for the circular carbon economy and sustainable production platforms.

ANNA STOCK

Technical University of Munich

CO-Addition Enhances Autotrophic D-Lactate Formation with Engineered Acetobacterium woodii.

<u>A. STOCK</u>^a, I. SOTZECK^a, K. S. BAUR^b, F. R. BENGELSDORF^b, D. WEUSTER-BOTZ^a

^a Chair of Biochemical Engineering, Technical University of Munich, Boltzmannstr. 15, 85478, Garching, Germany

^b Institute of Microbiology and Biotechnology of Prokaryotes, University of Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany

One approach to reduce greenhouse gas emissions is to reuse CO_2 as a carbon source to produce organic matter. The Wood-Ljungdahl pathway (WLP) enables acetogenic bacteria to fix carbon from CO_2 . The main product of the WLP is acetate, because its formation enables ATP regeneration. The WLP depends on hydrogen (H₂), and/or carbon monoxide (CO) as electron donors. Since CO enables an increased provision of reducing equivalents, the presence of CO in the gas mixture yields more ATP compared to processes providing only H₂ and CO₂.

Acetobacterium woodii is a genetically accessible and robust acetogen, commonly used for H₂ + CO₂ fermentation. High CO partial pressures inhibit growth of *A. woodii* due to the inhibition of the hydrogen-dependent CO₂ reductase (HDCR). This study aims to investigate the impact of the addition of small amounts of CO on the recombinant production of D-lactate using an engineered *A. woodii* ΔpyrE ΔlctBCD [pMTL83251_PlctA_NFP] can be redirected towards D-lactate formation, rather than acetate or biomass production. Thus, autotrophic batch processes were performed with engineered *A. woodii* cells in fully controlled stirred-tank bioreactors with continuous gassing with 70% H₂, \geq 27% CO₂, and varying amounts of CO.

The addition of 3% CO led to strongly enhanced biomass formation (2.9 g L⁻¹ after 69 h) at reduced product concentrations compared to autotrophic, CO-free processes. With 0.8% CO, D-lactate formation strongly increased. Simultaneously, the lag phase was prolonged compared to cultivations with no and 3% CO in the gas mixture. D-lactate concentrations started to rise strongly, as soon as exponential growth was established. After 129 h, D-Lactate concentrations reached 6.2 g L⁻¹. That represents an increase of 189% compared to the autotrophic reference process without CO. Simultaneously, acetate formation could be significantly reduced with 0.8% CO.

JOSE ANTONIO VELAZQUEZ GOMEZ

University of Tübingen

Enhancing Autotrophic Acetate Production By *Thermoanaerobacter Kivui*: Combining Biomass Retention, High Dilution Rates, And Moderate Overpressure In Continuous Bioreactors.

JOSE ANTONIO VELAZQUEZ GOMEZ, LARGUS T. ANGENENT

Thermoanaerobacter kivui is a promising candidate for industrial acetic acid production due to the ability to metabolise various C1 compounds, the lack of vitamin requirements, and the production of high titres of acetate at thermophilic conditions. However, it exhibits production inhibition due to the accumulation of acetic acid in the media. Meanwhile, previous studies in our lab achieved a production rate of 0.63 g/L-h in 1.5-L laboratory-scale bioreactors in continuous mode, and 3.5 g/L_{catholyte} h in small-scale (40mL) microbial electrosynthesis systems (MESs). Our study aimed to enhance autotrophic acetate production by coupling biomass retention with acetate removal at high dilution rates and moderate overpressure. We used 1.5-L bioreactors that were fed with an 80:20 mixture of hydrogen and carbon dioxide and inoculated with a preadapted strain. Biomass was retained with a membrane module, allowing the removal of media without washing out the cells. The acetate concentration in the system was monitored and maintained in a range of around 250 mM or lower by modifying the dilution rate to avoid inhibition by the product. Media filtration was used to circumvent autoclaving large volumes of liquid. To enhance the gas-toliquid mass transfer by increasing the solubility of hydrogen and carbon dioxide, we pressurised moderately the reactor. After pressurisation to 0.4-0.5 bar and increasing the dilution rate to 0.233 h⁻¹, the performance of the reactor improved, and the OD and production rate spiked to 14.7 and 3.7 g/L·h, respectively. These results show that combining biomass accumulation, high dilution rate, and overpressure helps increase the acetate production rate. Further research is ongoing to optimise these conditions and the minimal media composition and assess repeatability and scalability for industrial settings.

FRANK R. BENGESLDORF

University of Ulm

Reclassification, Metabolism and Genetic Engineering of Acetogens in the *Eubacterium* genus

MAXIMILIAN FLAIZ, FRANZIKA HÖFELE, FRANK R. BENGESLDORF*

Institute of Molecular Biology and Biotechnology of Prokaryotes, University of Ulm, Ulm, Germany

* Corresponding author: frank.bengelsdorf@uni-ulm.de

Acetogens in the genus *Eubacterium* are known for their ability to produce butyrate and have attracted attention due their ability to reduce greenhouse gases (CO and CO₂). Their broad metabolic potential and genetic accessibility brands them as interesting new biocatalyst for gas fermentation.

We investigated the genomic, phylogenetic, physiological, and genetic characteristics of 11 Eubacterium strains, categorizing them into three distinct clades dominated by the type strains of E. limosum, E. callanderi, and E. maltosivorans. Additionally, we analysed the gene clusters in the respective genomes that encode gene products facilitating methanol utilization and reverse β-oxidation. Growth experiments demonstrated that strains from all clades can convert $H_2 + CO_2$, CO and methanol either as single substrate or in combination and produce acetate, butyrate, and hexanoate through chain elongation. Furthermore, sugar rich heterotrophic growth experiments showed and heterofermentative lactate and acetate production. Additionally, we established an efficient electroporation protocol suitable for constructing recombinant cells in eight different Eubacterium strains. A plasmid encoding the fluorescence-activating and absorptionshifting tag (FAST) was used as a fluorescence reporter to guickly verify successful recombinant strain construction at the single-cell level using flow cytometry. FAST expression, controlled by the strong constitutive ferredoxin promoter (Pfd) from Clostridium ljungdahlii, resulted in a homogeneous population of 98.2% fluorescent cells in 6 of the 8 tested Eubacterium strains.

In conclusion, a detailed analysis of acetogens belonging to the species *E. limosum, E. callanderi*, and *E. maltosivorans* revealed high overall similarities in metabolic features and potential for future metabolic engineering approaches.

SEBASTIAN WENK

University of Groningen

Engineering microbial growth on CO₂ derived feedstocks – a feasible solution for a sustainable bioeconomy.

Atmospheric CO_2 is a significant contributor to global warming and climate change, posing a major threat to life on Earth. However, it also represents a scalable carbon source that can be harnessed to establish a circular carbon economy. To achieve this, technologies for capturing and converting CO_2 into reduced one-carbon (C1) compounds like formate, are rapidly advancing. By integrating both natural and synthetic C1-assimilation pathways into industrially relevant microbes, we can create sustainable bioproduction platforms.

In this talk, I will discuss my work on engineering synthetic C1 assimilation pathways in *Escherichia coli*. I will first introduce the establishment of the Serine Threonine Cycle (STC), a synthetic formate assimilation pathway. Through a combination of rational engineering and adaptive laboratory evolution, we successfully established the STC in *E. coli*, enabling growth on formate under ambient CO₂ conditions.

Given that only a few enzymes can assimilate formate into biomass, the metabolic solution space for synthetic formate assimilation pathways is currently limited. To overcome this challenge, enzyme design and engineering are crucial. In the second part of my talk, I will present recent advancements in engineering new-to-nature formate assimilation reactions that broaden the potential of formate-based biotechnological applications. By employing targeted enzyme engineering, we developed a metabolic route from formate to formaldehyde, creating opportunities for methylotrophs to use formate as a substrate for bioproduction.

KATALIN KOVÁCS

The University of Nottingham

Bioproduction Of Chemicals From CO₂.

<u>KATALIN KOVÁCS</u>^{1,2}, CALLUM McGREGOR¹, ALEJANDRO SALINAS¹, MARCO GARAVAGLIA¹, RAJESH BOMMAREDDY¹, KLAUS WINZER¹, PHILIPPE SOUCAILLE¹ AND NIGEL P. MINTON¹

¹BBSRC/EPSRC Synthetic Biology Research Centre (SBRC), Biodiscovery Institute, School of Life Sciences, The University of Nottingham, Nottingham NG7 2RD, U.K.; ²School of Pharmacy, University Park, The University of Nottingham, Nottingham NG7 2RD, U.K

Fermentation-based biological processes utilizing one-carbon (C1) feedstocks, such as carbon dioxide (CO₂) and methane, hold significant potential for advancing the sustainable production of chemicals, fuels and pharmaceuticals from renewable resources. These processes offer the added benefit of reducing greenhouse gas (GHG) emissions by converting industrial waste gases - such as those from steel manufacturing, oil refining, anaerobic digesters, and coal or natural/shale gas - into valuable products. *Cupriavidus necator* H16 (formerly *Ralstonia eutropha*), a Gram-negative, non-pathogenic, facultatively chemolithoautotrophic bacterium, can grow on organic substrates or hydrogen (H₂) and carbon dioxide (CO₂) under aerobic conditions. Its ability to use CO₂ as its sole carbon source makes it an ideal chassis organism for the sustainable production of chemicals ranging from polyhydroxybutyrate (PHB, a biopolymer) to alcohols, alkanes, alkenes, biopolymer- and pharmaceutical precursors.

We have successfully engineered *Cupriavidus necator* H16 to produce 3hydroxypropionic acid (3-HP), 3-HP-co-PHB copolymers and mevalonate from CO₂. Starin engineering strategies, titers and yields obtained will be discussed.

EUN YEOL LEE

Kyung Hee University

Metabolic Engineering of Methanotrophs and Its Application to Methane Bioconversion.

Department of Chemical Engineering, Kyung Hee University, Gyeonggi-do 446-701, Republic of Korea e-mail: eunylee@khu.ac.kr

Methane is a potent carbon substrate because it is the main component of natural gas, biogas and industrial off-gas. Thus, methane bioconversion can offer a sustainable approach to convert it into chemicals and fuels. Methanotrophs are bacteria that solely utilize methane for carbon and energy sources. Methanotrophs can act as promising biocatalysts to convert methane into valuable products including biodegradable polyhydroxybutyrate. However, the biosynthetic capability of wild-type methanotrophs are limited to produce non-natural metabolic products. In this presentation, metabolic engineering of methanotrophs to produce various methane-derived products will be presented. Development of methane gas fermentation will be also discussed.

ARI SATANOWSKI

Max Planck Institute for Terrestrial Microbiology

Design and *in vivo* implementation of aerobic, ambient CO₂-reduction as an entry-point for enhanced carbon fixation.

<u>ARI SATANOWSKI</u>^{1,2,†}, DANIEL G. MARCHAL^{1,†}, ALAIN PERRET³, JEAN-LOUIS PETIT³, MADELEINE BOUZON³, VOLKER DÖRING³, IVAN DUBOIS³, HAI HE¹, EDWARD N. SMITH⁴, VIRGINIE PELLOUIN³, HENRIK M. PETRI¹, VITTORIO RAINALDI², MAREN NATTERMANN¹, SIMON BURGENER¹, NICOLE PACZIA¹, JAN ZARZYCKI¹, MATTHIAS HEINEMANN⁴, ARREN BAR-EVEN², TOBIAS J. ERB^{1,5}

[†] equal contributions

- ¹ Max Planck Institute for Terrestrial Microbiology, Marburg, Germany
- ² Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany
- ³ Génomique Métabolique, Genoscope, Institut François Jacob, Evry, France
- ⁴ Molecular Systems Biology, University of Groningen, Groningen, Netherlands

⁵ Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany

The direct reduction of CO_2 into one-carbon molecules is key to highly efficient biological CO_2 fixation routes like the Wood-Ljungdahl pathway or the O_2 -tolerant reductive glycine pathway. However, in nature, direct conversion of CO_2 to reduced one-carbon intermediates such as formate is restricted to anaerobic organisms with low-redox potential electron donors. To enable this energy-efficient carbon fixation strategy in O_2 -tolerant organisms, we designed the CORE cycle, a new-to-nature metabolic pathway that converts CO_2 to formate under aerobic conditions and with atmospheric CO_2 levels, using only NADPH as a reductant.

We combined theoretical pathway design, *in vitro* enzyme screening, *in vivo* pathway assembly and adaptive laboratory evolution to realize the CORE cycle in *E. coli*. We demonstrated that the cycle supports growth of engineered selection strains, supplying their serine biosynthesis and demand for one-carbon units from CO₂. Additionally, we show that the CORE cycle can serve as a new entry-point for CO₂ in synthetic autotrophy or engineered plant photorespiration.

Overall, our work expands the solution space for biological carbon reduction, offering a promising approach to enhance CO₂ fixation processes such as photosynthesis, and opening new avenues for engineered microbial autotrophy.

CLAUDIO AVIGNONE ROSSO

University of Surrey

Microbial Electrochemical Systems for the Capture and Transformation of CO₂ into Multicarbon Organic Compounds

Microbial electrosynthesis (MES) or Electrofermentation (EF) can overcome some of the issues associated to conventional fermentation, such as stoichiometric and energy limitations, low yields, nutrient demands, etc. In MES, the extracellular redox potential is modified by supplying external electrons through the cathode, therefore displacing the intracellular redox balance required to obtain the desired fermentation products. Therefore, it is possible to utilize compounds of low degree of reduction (e.g. CO or CO₂) as carbon sources to synthesize reduced molecules by supplying exogenous electrons to specific microorganisms.

A limited number of microbial species are able to utilize C1 substrates for the synthesis of metabolic intermediates or precursors. However, the assimilation of CO₂ can be achieved in species able to utilize electrons supplied exogenously. Recently, we have shown the assimilation of inorganic carbon for electrosynthesis of platform chemicals, polyhydroxybutyrate and surfactants in BES inoculated with *Clostridium saccharoperbutylacetonicum*. The electrical power required can be offset by the electricity generated by an electrogenic microbial community colonizing the anode of the BES, able to oxidize organic matter as the anodic feedstock. The net outcome of this process is the storage of energy in the covalent bonds of organic compounds synthesized from captured CO2 with the reduction of pollutant levels in water.

Electrofermentation of CO_2 to produce valuable chemicals is a sustainable strategy that provides a viable alternative to current methods of chemical synthesis, contributing to the reduction of greenhouse gas emissions.

FRANK SARGENT

Newcastle University

Harnessing Bacterial Hydrogen Metabolism (with the help of the BBSRC NIBBs).

The bacterium Escherichia coli has long been a workhorse of Microbial Biotechnology, Synthetic Biology and now Engineering Biology projects. Its natural metabolic flexibility and tolerance of foreign or synthetic DNA make it a go-to chassis for bioengineering projects. The bacterium has a elaborate hydrogen metabolism where the genome encodes at least four nickel-dependent hydrogenases. One of these is called formate hydrogenlyase (FHL-1), which is a formate dehydrogenase module attached to a hydrogenase module. Under fermentative conditions FHL-1 oxidises formic acid and produces CO₂ and H₂ as products. The enzyme is also reversible – thus, in the presence of CO_2 and H_2 gas mixtures (especially when under controlled pressure) E. coli will generate formic acid as a result. This inspired us to harness that activity and generate cell factories that could capture carbon and convert it to formic acid, and then use the formic acid as sole fixed carbon source for growth and for building secondary metabolites. This has required us to delve in to metabolic engineering and directed evolution experiments and to collaborate with industry to help meet NetZero targets and make compounds that industry wants and needs.

RAJESH BOMMAREDDY

University of Northumbria

Cupriavidus species as potential chassis for chemical production from CO₂.

Technologies that enable carbon capture and conversion of greenhouse gases into useful products are pivotal to mitigate climate change and establish true circular economies. Industrial decarbonisation is one of the core areas of UN sustainable development goals that needs to be addressed. Microbial cell factories used in fermentation provides path for sustainable manufacturing of biochemicals. Gas fermentation offers a unique flexibility in terms of feedstock by using waste gases such as CO₂, CO and CH₄. These gaseous feedstocks may be an industrial waste gas or synthesis gas produced from gasification of waste biomass. Current commercial production of chemicals via gas fermentation is limited towards producing ethanol using acetogenic anaerobic bacteria. Expanding the product portfolio that can be produced via gas fermentation is pivotal for creating economically viable and environmentally benign bioprocesses. Moreover, current research on using Bio-electrochemical systems (BES) is only limited to produce acetate and ethanol as main products from CO₂.

The facultative chemolithoautotrophic *Cupriavidus sp.* is engineered to produce nonnative value-added platform chemicals from CO₂. Metabolic engineering and a safe, efficient gas fermentation platform has been established for bench scale testing the potential of stable, continuous production of these chemicals. Value-added chemicals, such as 2,3-butanediol, Isopropanol, 1,3-butanediol etc. were demonstrated from engineered metabolic pathways. Chromosomal integration of the optimised metabolic pathways enabled continuous stable production of these chemicals from CO₂. Productivities between 0.35-0.50 g/L.h were observed for above mentioned products. Technoeconomic analysis for Isopropanol production using black liquor as the feedstock showcased the feasibility potential of producing bulk chemicals valued at 1000\$/tonne using heat integrated aerobic gas fermentation.

Electro-active *Cupriavidus* is also studied for its capacity to uptake or donate electrons from and to an electrode respectively. The electron transfer mechanisms are being elucidated using systems biology approaches establishing processes towards producing value-added chemicals from CO_2 using electrons derived from external sources.

Cupriavidus sp. as a cell factory takes the advantage of fixing CO_2 using the Calvin cycle and being strictly respiratory. Compared to anaerobic cell factories, *Cupriavidus* does not produce low value fermentative by-products. Selectivity of more than 85% (CO_2 conversion efficiency) of products are observed in the cultures. Given aerobic cell factories can target a wider product spectrum, the heat integrated aerobic gas fermentation has promise as a best-in-class technology for renewable commodity chemical production.

YING ZHANG

The University of Nottingham

Genetic Tools Development in Novel Methylotrophs.

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

Methylotrophs are a diverse group of microorganisms that can use reduced one-carbon compounds, such as methanol or methane. Methane (CH₄) is an important greenhouse gas and a source of energy for heating, lighting and generation of electricity. CH₄ also serves as a source of carbon and energy for methane-oxidizing bacteria - methanotrophs. In addition to bioremediation properties and balancing CH₄ levels in the environment, methanotrophs can be used to produce platform chemicals like lactate; biopolymers such as poly-3-hydroxybutyrate (PHB) and animal feed in the form of single cell protein. To harness their industrial and environmental potential, it is important to establish methods for genetic engineering of this group of organisms. We have developed an armoury of forward and reverse genetic tools to facilitate gene function studies, identify essential genes, and engineer the organisms for desired traits.

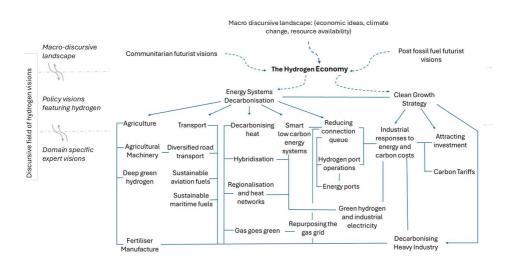
- Tn5 based transposon random mutagenesis system was implemented and used for TraDIS (transposon-directed insertion site sequencing) to identify essential genes and genes crucial for niche specific conditions.
- CRISPR technology for rapid generation of clean gene deletions and DNA cargo insertions in methanotrophs, demonstrating CRISPR-Cas9 genome editing in industrially relevant *Methylococcus capsulatus* and *Methylocystis* parvus species.

SARA WALKER

University of Birmingham

Hydrogen integration into energy systems: what, where and when

Various sections of Government, and related agencies such as NESO and Climate Change Committee, see a role for hydrogen in the future energy system. The exact details of this role are contested on many levels. Visions for hydrogen, from a policy perspective, relate to: industrial decarbonisation and hard to abate sectors, enabling inter-seasonal storage on the electricity grid, repurposing of the natural gas network, enabling smart low carbon regional systems, transport, agriculture, and international trade. In HI-ACT, we are working to explore these visions, in order to better understand the system implications of what, where and when for hydrogen.



SELINA AMBROSE

Promethean Particles Ltd

Project MONET: Demonstrating The Industrial-Scale Viability Of MOF-Based Carbon Capture.

<u>SELINA AMBROSE¹</u>, LEAH MATSINHA¹, REBECCA RYDER¹, SCOTT PRIEST¹, CHITRAKSHI GOEL¹

¹Promethean Particles Ltd., 1-3 Genesis Park, Midland Way, Nottingham NG7 3EF, United Kingdom, <u>selina.ambrose@proparticles.co.uk</u>

Promethean Particles ("Promethean") is a UK-based global pioneer, leading the industrial-scale manufacture of metal-organic frameworks (MOFs) – an exciting class of advanced materials showing significant potential for use in carbon capture, water harvesting, and gas storage applications. However this potential has never been realised due to low manufacturing volumes and high costs. Promethean is changing this paradigm by utilising its proprietary continuous flow manufacturing technology to produce MOFs at a scale and price point that enables the deployment of MOFs at industrial scale, and spearheading application development projects to demonstrate the viability of MOFs for industrial use.

This oral presentation will introduce one such project: MONET (\underline{MO} F-based \underline{N} egative \underline{E} missions \underline{T} echnology), led by Promethean.¹ This Department of Energy Security and Net Zero funded project supports the development of next generation carbon capture, utilisation, and storage technology within the UK. The project aims to develop a prototype MOF-based carbon capture and storage (CCS) system, which will be deployed at a UK power station, and its efficacy to selectively adsorb CO₂ will be evaluated. Specifically, this presentation will detail Promethean's pilot-scale MOF production (>100kg) as part of Project MONET, and the company's journey to industrial scale MOF manufacture, including the associated considerations and challenges when working at such scales. The presentation will also highlight the relevance and need for MOF-based carbon capture to meet global decarbonisation goals, and summarise the importance of demonstration projects such as MONET in advancing novel carbon capture technologies towards commercial deployment.

[1] Carbon Capture, Usage and Storage (CCUS) Innovation 2.0 competition: Call 2 successful projects - GOV.UK (www.gov.uk)

YUE ZHANG

University of Southampton

Predicting pH Rise as a Control Measure for Integration of CO₂ Biomethanisation with Anaerobic Digestion.

YUE ZHANG, SONIA HEAVEN, CHARLES BANKS, BING TAO

Water and Environmental Engineering Group, Faculty of Engineering and Physical Sciences, University of Southampton, Southampton, SO16 7QF, UK

The biological methanisation of CO_2 is a promising process for a range of applications, from biogas upgrading to increase the efficiency of carbon utilisation in waste feedstocks, to helping balance the electricity grid by providing a means to convert surplus energy from intermittent renewables into a storable biofuel which is compatible with current infrastructure.

 CO_2 biomethanisation by adding H_2 directly to an anaerobic digester fed on organic waste is of interest to the anaerobic digestion industry, which already has many digesters and even upgrades biogas for grid injection at some UK sites. In a conventional digester with H_2 addition, a methane production rate of 4 m³ m⁻³ digester day⁻¹ is a good improvement on typical rates in commercial plant. The major technical limitation for this process is that the reaction causes CO_2 depletion which drives up pH, potentially leading to instability and even digestion failure.

The study validates fundamentally derived predictive equations as tools to manage H₂ addition to anaerobic digesters. This allows estimation of the maximum achievable biogas methane content compatible with stable operation. The methodology used data from the literature and from experimental digesters operated with excess H_2 to a point of failure and subsequent recovery. Two equations were tested: the first relating pH to CO_2 partial pressure, and the second extending this to include the influence of volatile fatty acids and ammonia. The first equation gave good agreement for data from studies covering a wide range of operating conditions and digester types. Where agreement was not good, this could usually be explained, and in some cases improved, using the second equation, which also showed excellent predictive performance in the experimental study. Both equations could provide a basis for process control of CO₂ biomethanisation without the risk of exceeding critical values using routine monitoring of pH or CO₂ partial pressure with additional analysis for volatile fatty acids and total ammonia nitrogen when required. The research thus further strengthens the case for promoting CO₂ biomethanisation as a means of maximising the value of existing infrastructure and resources in the waste and agri-food sectors.

QIANG LI Centre for Process Innovation Ltd

A Feasibility Study On The Use Of Hydrogen As A Feedstock To Platform Chemicals.

QIANG LI, Mona Gayle-Jinadu, Charanjeet Singh, Deepan Shah

Address: CPI, Wilton centre, Redcar TS10 4RF, United Kingdom

giang.li@uk-cpi.com

Although the role of low carbon hydrogen in the post decarbonisation world is still not clear and debated, it is undeniable that it will play an indispensable role in chemical industry which is widely recognised as impossible to complete decarbonise by electrification. A joint project between CPI and AMRC was set up to study the role of low carbon hydrogen as a feedstock to platform chemicals in the context of net zero. This project was funded by the High Value Manufacturing Centre as a part of a wider Hydrogen Strategic Project in FY23/24. CPI studied different manufacturing routes for the "Magic 7" base chemicals and the cases if and how they could be replaced with carbon dioxide as the carbon source and hydrogen as energy source, paving the way to circular and low carbon economy in the UK. In this talk we will present the findings of the study and from which make prediction as to where technology breakthrough and industrial adaptation of low carbon hydrogen are likely to come forward in the near future.

DAVID ORTEGA

Phase Biolabs

Commercialisation of CO2-based Gas Fermentation Processes Using Engineered Acetogens.

Acetogens hold great promise for the valorisation of C1 waste gases. With advancements in our understanding of acetogenic metabolism, a growing number of genetically tractable strains with ever unique abilities and with increasing analytic and genetic tools available, it has never been a better time to be a synthetic biologist, microbiologist or metabolic engineer working with acetogens. To date, more than 50 different compounds have been produced from gas fermentation using wildtype and engineered acetogens, but why has only one process been commercialised?

The ancient and seemingly simple Wood-Ljungdahl pathway lies at the heart of an extremely efficient acetogenic metabolism, however, its efficiency is a double-edged sword, it requires less energy for carbon fixation but also generates less energy for growth and biomass formation. What is the best approach to optimise this pathway? Can thermodynamic limitations be overcome? Can engineers override and overcome native regulation of cells for a commercial purpose? What is needed to engineer an acetogen with commercially relevant levels of production?

Phase's objective is to develop one biocatalyst to produce one product from one feedstock, specifically, ethanol from CO_2 and H_2 . The challenges, successes and general learnings accumulated over the past two years of this project will be shared from a team who has applied metabolic engineering principles and approaches to improving performance of ethanol production.

ROBERT MANSFIELD

AerBio

Scaling novel technology from concept to commercialization. Opportunities, prioritization, and people

Some lessons from the journey so far, and a look ahead at navigating the scale-up 'valley of death' to de-risk technology readiness and commercial viability.

This talk explores the practical challenges of scaling breakthrough technologies, balancing innovation with execution to build a commercially viable solution for alternative protein production from aerobic gas fermentation."

DAVID KEATING

Synata Bio

Synata Bio: CO₂ Valorization Via Advanced Biocatalysts

Synata Bio is a company based in Warrenville, Illinois, focused on the bioconversion of CO₂ into single-cell protein, fuels, and chemicals. A key advantage of our CO₂ capture technology is our proprietary biocatalyst, which displays elevated hydrogen and CO₂ conversion with respect to related gas fermenting strains. We will describe studies leveraging these improved capabilities for growth in syngas mixtures containing reduced amounts of CO. Furthermore, we will discuss unusual characteristics of our strain, particularly with respect to vitamin metabolism and carbohydrate utilization. Finally, we will provide an update on our commercialization journey, which culminated in the construction of a 50 KTA commercial plant focused on the production of ethanol and single-cell protein. In summary, the scalability and robustness of the Synata Bio technology enables carbon capture in diverse industrial sectors, allowing the manufacture of low-cost and low-carbon products.

KOEN QUATAERT

Bio Base Europe

The Importance Of Open-access Piloting For Microbial CCU Technologies Using Real Industrial C1-gasses.

KOEN QUATAERT*, ELODIE VLAEMINCK, KAREL DE WINTER, HENDRIK WAEGEMAN,

Bio Base Europe Pilot Plant (BBEPP), Rodenhuizekaai 1, 9042 Ghent, Belgium

*koen.quataert@bbeu.org

Bio Base Europe Pilot Plant (BBEPP) is an open-access facility for developing, scaling up, and custom manufacturing bio-based processes. Equipped with state-of-the-art lab and industrial infrastructure, BBEPP covers the full value chain from biomass to refined products, including pretreatment, fermentation, downstream purification, bio-catalysis, and green chemistry.

Beyond biomass-derived feedstocks, CO₂ is emerging as a sustainable carbon source for fermentation, converting industrial emissions into chemicals, plastics, fuels, and alternative proteins. Valorizing CO₂ depends on its origin—industrial point sources (e.g., steel mills, power plants), biogenic sources (e.g., biorefineries, breweries), or atmospheric CO₂—each requiring tailored fermentation approaches. Despite advancements in strain engineering and reactor design, many gas fermentation processes face challenges in reaching pilot or commercial scale.

Scale-up hurdles include technical complexities, lack of infrastructure, skilled personnel, and safety permits. To accelerate development, BBEPP is constantly expanding its gas fermentation capabilities, offering access and support to research institutes, start-ups, SME, and large companies. A key innovation is BBEPP's mobile gas fermentation pilot unit, designed for on-site validation at industrial CO_2 emitters. This enables real-environment testing, bridging the gap from lab to demonstration scale.

This presentation showcases case studies where BBEPP's containerized unit validated biological CCU processes using real flue gases, such as blast furnace gas from steel production and syngas from biomass gasification.

ROSALIND HAY

Centre for Process Innovation (CPI)

The Reality of Technical Transfer: Derisking The Unknown.

Progressing from a laboratory-scale development fermentation towards commercialisation is accomplished by successful technical transfer. Having a solid understanding of the organism behaviours and how differing parameters effect product quality and yield is essential for defining the process envelope; this knowledge reduces the technical transfer risks and contributes to large-scale success. A process gap analysis will identify the 'known unknowns' and experiments can fill those knowledge gaps, but only technical transfer experience can troubleshoot the inevitable 'unknown unknowns' whilst operating at a larger scale.

An integrated approach to technical transfer will significantly reduce the timescale from TRL 1 to 7. Utilising experts across disciplinaries to work together in a cyclic route, rather than a linear format, will provide a pragmatic and efficient scale-up that leads to commercialisation. Considering the commercial target as early into the process as practicable provides a focussed development route to suit the commercial scale bioreactor geometry and capabilities; resulting in a technical transfer that provides an operating envelope suitable for available manufacturing options.

Technical transfer comes with its challenges, most of which are common and can be avoided with the right advice in the early stages of the process. This presentation will provide real life examples and insights into those challenges and successes of technical transfer.

GEOFF BRYANT

Calysta

Insights from the Top of the Fermenter.

Scaling microbial fermentation from laboratory proof of concept to full-scale industrial production is a complex and iterative process that presents significant scientific, engineering, and operational challenges. While small-scale fermenters provide crucial insights into strain optimization and process parameters, they often fail to capture the complexities introduced at pilot and commercial production scales. The transition from laboratory precision to industrial robustness requires a deep understanding of biological variability, mass transfer limitations, oxygen uptake dynamics, and heat control—factors that become exponentially more challenging as scale increases.

This talk provides a technical overview of Calysta's decade-long journey in scaling up fermentation, highlighting key learnings from each stage. Beginning with foundational research at lab scale, moving through the critical scale-up phase at the pilot plant, and culminating in the construction and commissioning of one of the world's largest feed fermentation facilities, this presentation explores the real-world obstacles that arise and the strategies required to overcome them.

The talk will examine how initial confidence in lab-scale results often gives way to managing challenges and failures at pilot scale before reaching a deeper, more nuanced understanding at full-scale production. The discussion will cover:

- Challenges in translating laboratory success to commercial viability
- The critical role of pilot-scale fermentation in identifying and mitigating unforeseen biological and engineering challenges.
- Scaling to full production—why process efficiencies, downstream processing, and operational realities must be integrated from the outset.
- Beyond technical challenges—the impact of remote operations, crossfunctional team alignment, and managing investor expectations in industrial fermentation.

Scaling fermentation is not a linear process—it is an ongoing cycle of learning, adaptation, and refinement. This session offers a technical and strategic perspective on navigating this journey between innovation and large-scale commercial production.

IVAN ILIC Electroarchaea GmBH

Electrocatalysis Meets Biocatalysis: Bioelectrochemical Methanation System.

IVAN ILIC, MAREN LANG, AKSHAY SUDHAKARAN, JOHANNES ERBEN, NITANT PATEL, JOSE RODRIGO

Electrochaea GmbH, Semmelweisstr. 3, 82152 Planegg, Germany

The switch from fossil-based to renewable energy sources, primarily intermittent solar and wind, comes with the challenge of energy storage. Power-to-Gas has been recognized globally as one of the key elements for the transition towards a sustainable energy system. Power-to-Methane (PtG-CH₄) is of special interest since it can be easily integrated into the existing gas infrastructure and into well-established industrial and mobility applications.

Electrochaea's BioCat Process converts renewable energy and greenhouse carbon dioxide into grid-quality BioCat Methane, a renewable synthetic methane that is reliable, practical, and safe for storage and distribution in the existing infrastructure. With the BioCat Process Electrochaea has reached industrial pilot scale and near-term commercial application and provides the core for a commercially viable, grid-scale energy storage technology.

Here we introduce Electrochaea's compact technology, where we combine an electrolyzer with biocatalyst, allowing for the biocatalyst to consume hydrogen as it is being produced at the cathode. This novel technology, called bioelectrochemical methanation system (BEMS), will allow us to eliminate the cost associated with having a separate electrolyzer and bioreactor. This capital and operational expenditure savings will enable Electrochaea to develop smaller ready-to-deploy bio-methanation plants, expanding the market by including small CO₂ emitting plants as users of PtG-BEMS'. The challenge is to create a system that allows the electrocatalyst to operate in the immediate vicinity of the biocatalyst, as their optimum performance is observed in vastly different conditions. While the biocatalyst thrives in near-neutral pH, the optimal conditions for electrocatalysts are at the extremes, either when exposed to very acidic or basic conditions. By carefully choosing the electrolyzer materials and engineering the liquid flow system, we were able to form a BEMS that already exceeds the efficiency of 30 g of methane per kWh of input of electricity at productivities higher than 100 mA cm⁻².

KEES KWANT Integrated Biorefineries

Mission Innovation: Integrated Biorefineries Mission for Fuels and Chemicals.

The Integrated Biorefineries Mission offers the opportunity to support the development of bio-based Sustainable Fuels and Chemicals. It is part of Part of Mission Innovation, an international initiative to support innovation to a net-zero economy.

Participants in this mission are Brazil, Canada, India, European Commission, the Netherlands, the UK and Switzerland, but the mission is open to new members. The co-leads of this mission are India and the Netherlands.

Mission Innovation is a governmental collaboration, but acts in close collaboration with industry, research institutes and other international bodies.

This collaboration has led to the formulation of a roadmap and organizing national consultations and webinars. There is ongoing collaboration on innovation in Sustainable Aviation Fuels, Biopolymers, Financing opportunities, Standards, Sustainability and improved Market conditions.

In 2024 a start was made with matchmaking researchers and industries from the participating countries through the digital tool B2Match. International collaboration around research, development, and demonstration (RD&D) for fuels and chemicals will help to accelerate their production at commercial scale. This will be expanded in 2025.

AYODEJI ALUKO

University of Huddersfield

Optimizing Anaerobic Digestion for Domestic Applications: Challenges and Opportunities.

AYODEJI ALUKO, GINA JAVANBAKHT, JOHN ALLPORT School of Computing and Engineering, University of Huddersfield

Municipal solid waste generation has risen significantly in recent years, with food waste constituting approximately one-third of the total. Anaerobic digestion (AD) provides an efficient and sustainable approach to managing food waste while addressing energy sustainability challenges. This research investigates the potential of a domestic-scale anaerobic digester (AD) to transform food waste from a university kitchen into biogas and digestate. The study highlights the benefits of AD, including reduced waste disposal costs, decreased reliance on conventional cooking fuels, and the production of biofertilizer. It evaluates biogas yield and methane content under varying feedstock conditions, with findings indicating that mixed food waste yields higher methane concentrations compared to starchy substrates. Methane content increased from 26.55% to 48.35% when switching from starchy to mixed food waste, emphasizing the critical role of substrate composition in optimizing biogas production. Additionally, the digestate demonstrated comparable nutrient quality to commercial fertilizers, supporting its application as a biofertilizer.

This research underscores the importance of scaling AD technology to a domestic level, providing households with an economical and environmentally friendly solution for waste management and renewable energy generation. Despite the high energy costs observed in biogas production at this scale, the environmental and socio-economic benefits, including reduced greenhouse gas emissions, minimized landfill dependency, and improved public health, validate its significance. The findings advocate for further development of domestic AD systems, optimizing feedstock, design, and operational parameters to enhance economic feasibility and accessibility. The study establishes domestic-scale AD as a promising technology for sustainable waste management and renewable energy production at the source.

LUDOVIC JOURDIN

Delft University of Technology

Microbial Electrosynthesis from CO₂ reaches Productivity of Syngas and Chain Elongation Fermentations.

Carbon-based products are essential to society, yet producing them from fossil fuels is unsustainable. Microorganisms have the ability to take up electrons from solid electrodes and convert CO₂ to valuable carbon-based chemicals. However, higher productivities and energy efficiencies are needed to reach a viability that can make the technology transformative. Here we show how a biofilm-based microbial porous cathode in a directed flow-through electrochemical system can continuously reduce CO₂ to evenchain C2-C6 carboxylic acids over 248 days. Robustness and stability of the biocatalysts are major advantages for industrial application. Our novel reactor mitigated mass transport limitations. We demonstrate a 3-fold higher biofilm concentration, volumetric current density, and productivity than the state of the art. Most notably, the volumetric productivity resembles those achieved in lab-scale and industrial syngas (CO-H₂-CO₂) fermentation and chain elongation fermentation. This work highlights key design parameters for efficient electricity-driven microbial CO₂ reduction. There is need and room to improve the rates of electrode colonization and microbe-specific kinetics to scale-up the technology.

DANA GALILI

DSIT

UK Government Approach To Engineering Biology.

Department for Science, Innovation and Technology

The government recognises the transformative potential of Engineering Biology (EB) for carbon recycling, environmental solutions and clean growth. Since 2007, UKRI has invested over £700m in EB in the UK. Activities include £115m investment into the Synthetic Biology for Growth Programme, between 2014-2022. In 2023, EB was identified as a Critical Technology under the Science & Technology Framework, and DSIT continues to prioritise EB. We are building on an area of strength; the UK is one of the global leaders in EB, but we recognise there is more to do to help the sector develop.

To help realise this potential, the government published the National Vision for Engineering Biology in December 2023 following extensive consultation with key stakeholders. The Vision identified the need for government intervention in six areas to maintain and enhance UK competitiveness: World class Research and Development; Infrastructure; Talent and skills; Regulations and standards; Adoption in the economy; and Responsible and trustworthy innovation.

To start delivering on these commitments, UKRI invested £100m in EB Missions Hubs and Awards as part of the Technology Missions Fund. The Hubs will bolster the UK's EB research capability and help drive forward technology development and innovation. Many of the funded projects support environmental monitoring and remediation via carbon recycling and delivering less carbon-intensive and more environmentally sustainable manufacturing processes and supply chains.

The UK has the highest number of biotech start-ups in Europe, and UK firms lead for VC investment in EB globally. UK firms innovate to decarbonise industrial sectors, for example by creating new sustainable products to replace existing unsustainable ones, towards a circular bioeconomy. But challenges exist. The government is developing cross-governmental interventions, using a systems approach to support EB research and industry for driving growth and improving the lives of citizens.

Abstracts of Oral Presentations

Steve Chambers

From Lab to Market: Lost in Translation.

The UK boasts world-class universities producing groundbreaking research, yet it chronically underperforms in spinning out high-growth startups compared to the U.S. The problem isn't a lack of innovation-it's a failure in translation. Academic cultures in the UK remain risk-averse, with university tech transfer offices often acting as gatekeepers rather than enablers. Excessive equity demands, bureaucratic delays, and a lack of founderfriendly structures stall commercialization. Unlike the U.S., where top institutions foster entrepreneurial mindsets and deep VC networks, in contrast UK spinouts struggle with undercapitalization, rigid licensing terms, and limited access to experienced operators. If the UK wants to compete globally, it must start to think globally, and rethink how it incentivizes academic entrepreneurship. This means reforming academic policies, increasing access to early-stage capital, and building a stronger founderfriendly ecosystem. Otherwise, transformative research will remain trapped in the lab-lost in translation. That said, there are companies breaking the mould and successfully bridging this gap. In my presentation, I'll highlight standout UK startups from my portfolio that have navigated these barriers and made it from lab to market

Abstracts of Poster Presentations

ABSTRACTS OF POSTER PRESENTATIONS

| POSTER #01 Christian Ebel Karlsruhe Institute of Technology (KIT) | Adaptation of a Model from Literature to Represent Experiments in a Continuous Fermentation System. |
|--|---|
| POSTER #02 Pawel Piatek RISE Research Institutes of Sweden | Valorising and Upgrading C1 Gases Towards Chemicals, Fuels and Animal feed. |
| POSTER #03 Thomas Reed Newcastle University | Characterisation Of The Formate Hydrogenlysae 2 From <i>Pectobacterium atrosepticum</i> In An <i>Escherichia coli</i> Host. |
| POSTER #04 Sergio Blanco-Rosete Mono Carbon Limited | Production Of Renewable Chemicals Utilising Anaerobic Digestion CO ₂ . |
| POSTER #05 Timothé Philippon Aarhus University | Insights into the variations in H ₂ thresholds and growth yields among hydrogenotrophic methanogens. |
| POSTER #06 Robin Hoeven University of Manchester | Cultivation Of Chemoautotrophs On Electricity and Air. |
| POSTER #07 Kim E Rennhack University of Tübingen | Nitrate Availability Mediates The Particular Rearrangement Of Protein Interactions At The Rnf Complex And Energy Metabolism In <i>Clostridium ljungdahlii.</i> |
| POSTER #08 Tina Baur University of Tübingen | Characterization Of Novel Tools To Unveil Gene Expression Profiles In <i>Methanothermobacter thermautotrophicus</i> ΔH For Use In Metabolic Engineering. |
| POSTER #09 Antonia Ebert The University of Queensland | Assessing Multi One-Carbon Conversion in Hydrogenophaga pseudoflava. |
| POSTER #10 Zu Thane Mwae Khin University of Huddersfield | Heating And Electricity Production Via Domestic- Scale Bio-Digester to Drive Micro Gas Turbine. |

| POSTER #11 Rohit Murali University of Surrey | From Mechanistic Models to Data-Driven Models for Predicting Biogas Production in Anaerobic Digestion Plants. |
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| POSTER #12 Elodie Vlaeminck Bio Base Europe Pilot Plant | Demonstration Of Advanced Biofuels: Production of Triacylglycerols From CO ₂ in A Coupled Fermentation Via Acetic Acid. |
| POSTER #13 Jennifer Roth Goethe University Frankfurt | Purification and Characterization of an Electron- Bifurcating Formate Dehydrogenase/Hydrogenase Complex from Sporomusa ovata. |
| POSTER #14 Lara Marie Waschinger Goethe University Frankfurt | A Cytochrome <i>c</i> -containing Periplasmic Nitrate Reductase in the Acetogen <i>Sporomusa ovata</i> . |
| POSTER #15 Yvonne Burger Goethe University Frankfurt | A Formate Transporter and Sulfur Transferase in the Acetogenic Bacterium <i>Thermoanaerobacter kivui</i> are Important for Hydrogen and Formate Interconversion. |
| POSTER #16 Florian Rosenbaum Goethe University Frankfurt | Energy Conservation by Using Dimethyl Sulfoxide as Alternative Electron Acceptor in the Acetogen <i>Moorella thermoacetica</i> . |
| POSTER #17 Raphael Trischler Gothe University of Frankfurt | Formate as Electron Carrier in the Gut Acetogen Blautia luti. |
| POSTER #18 Abdullah Labbo University of Nottingham | Optimizing Ectoine Production Using Methanol- Consuming Halotolerant Methanotrophs. |
| POSTER #19 Christian Kröly Wageningen University & Research | C1-Compounds For Sustainable Bioplastics: Engineering Acetogen-PHA Co-cultures. |
| POSTER #20 Mungyu Lee Delft University of Technology | Targeted Recovery of C6 Carboxylate from CO ₂ - based Microbial Electrosynthesis. |

| POSTER #21 Derya Acar KU Leuven, Belgium | Microwave-assisted hydrothermal carbonization (MW-HTC) of anaerobic digested sludge for resource recovery. |
|---|--|
| POSTER #22 Victoria Chinonyerem Udemezue University of Tartu | Optimization Of Plasmid Curing From Genetically-Engineered <i>Clostridium</i> <i>autoethanogenum.</i> |
| POSTER #23 Ali Nawaz & Etini Etuk University of Huddersfield | Eco-friendly Biosynthesis of Cellulase Using Post-Consumer Textile Waste. |
| POSTER #24 Ashraf Hauwa Nyako Loughborough University | Continuous Recovery of Fermentation Products using Novel Ho23t Microbubble Gas Stripping. |
| POSTER #25 Jo Philips Aarhus University | Selecting The Best Acetogen for Fast and Efficient Microbial CO ₂ Recycling into Acetate. |
| POSTER #26 Kira Baur University of Ulm | Improving Lactate Production using Genetically Modified Acetobacterium woodii Strains. |
| POSTER #27 Maximilian Flaiz Wageningen University & Research | Expanding the Genetic Toolbox for <i>Acetobacterium wieringae</i> . |
| POSTER #28 Diego Orol-Gŏmez National Renewable Energy Centre (CENER) | Gas fermentation. A promising CO ₂ conversion technology for decarbonization challenges. |
| POSTER #29 Dominic Clyde-Smith University College London | Urban Carbon Capture: Harnessing Hydroponic Green Walls. |
| POSTER #30 Mark Walker University of Hull | Outcomes from the 'New Biomethane' Workshop: Exploring the Future Pathways and Technologies for Biomethane Production Beyond Biogas Upgrading |
| POSTER #31 William Newell Imperial College London | Exploring C1 growth constraints |

CHRISTIAN EBEL

Karlsruhe Institute of Technology (KIT)

Adaptation of a Model from Literature to Represent Experiments in a Continuous Fermentation System.

CHRISTIAN EBEL, JÖRG SAUER

Institute of Catalysis Research and Technology (IKFT), Karlsruhe Institute of Technology, Hermannvon-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen Baden-Wurttemberg, Germany

One novel way to utilize waste gases such as CO_2 is syngas fermentation. In this process, CO, CO_2 and H_2 are used as substrate gases to produce base chemicals and fuels with acetogenic microorganisms. However, the product yield and ratio are highly dependent on the composition of the syngas. To better understand the effect of different syngas compositions, pressure and cell retention on the production of ethanol and acetate by acetogenic organisms, several long-term and continuous fermentation experiments with *Clostridium Ljungdhalii* were performed by Stoll et al. (Stoll, Boukis and Sauer, 2019) and Perret et al. (Perret, Boukis and Sauer, 2023, 2024). The main challenge of these fermentation experiments is the long time required to establish steady-state operation, resulting in a high time investment of up to 3000 h per experiment. Therefore, an accurate model of the reactor system is needed to better plan and design fermentation.

To this end, a kinetic literature model (Medeiros *et al.*, 2019) was used as a basis and fitted to weighted experimental gas, product and biomass data (Perret, Boukis and Sauer, 2023, 2024). The fitted model generally followed the data but was challenged to represent the acetate to ethanol ratio under high gas flow cell retention, as well as under conditions with reactor pressures > 1 bar. To mitigate this, further additions were made to the model such as CO_2 substrate inhibitions and inhibition terms for the acetate to ethanol conversion rates. These changes further improved the model's ability to represent pressurised conditions. The adapted model was able to predict additional similar experiments conducted by Perret et al. as well as being used as a general prediction tool for steady-state conditions of the fermentation setup.

Medeiros, E.M. *et al.* (2019) 'Dynamic modeling of syngas fermentation in a continuous stirred-tank reactor: Multi-response parameter estimation and process optimization', *Biotechnology and bioengineering*, 116(10), pp. 2473–2487. Available at: https://doi.org/10.1002/bit.27108.

Perret, L., Boukis, N. and Sauer, J. (2023) 'Influence of Increased Cell Densities on Product Ratio and Productivity in Syngas Fermentation', *Industrial & Engineering Chemistry Research*, 62(35), pp. 13799–13810. Available at: https://doi.org/10.1021/acs.iecr.3c01911.

Perret, L., Boukis, N. and Sauer, J. (2024) 'Synthesis gas fermentation at high cell density: How pH and hydrogen partial pressure affect productivity and product ratio in continuous fermentation', *Bioresource Technology*, 391, p. 129894. Available at: https://doi.org/10.1016/j.biortech.2023.129894.

Stoll, I.K., Boukis, N. and Sauer, J. (2019) *Syngas Fermentation at Elevated Pressure - Experimental Results*. ETA-Florence Renewable Energies.

PAWEL PIATEK

RISE Research Institutes of Sweden

Valorising and Upgrading C1 Gases Towards Chemicals, Fuels and Animal feed.

Division of Built Environment, RISE Research Institutes of Sweden, Gothenburg, Sweden

Gas fermentation has established itself as a promising technology for capturing and converting C1 waste gas streams into high-value fuels, chemicals and feed. While its commercialization has been proven through its viability as a sustainable solution for energy and chemical production, gas fermentation requires further refinement and adaptation to gain broader acceptance and implementation across industry. RISE Research Institutes of Sweden (RISE) is well-positioned as an international partner for C1 gas valorisation projects and collaborates with industry and academy in furthering C1 gas exploitation alongside developing industrial and urban symbiosis. RISE has developed innovative solutions and infrastructure in gas fermentation project collaborations. Synoprotein (CBE-JU project) aims to develop fish feed using single-cell proteins from C1 gases derived from pyrolyzed sawmill residues, capturing 1.97 t of CO₂ per dry tonne of residue. Bioflex (funded by the Swedish Energy Agency) involved in enhancing energy system's flexibility, redundancy, and robustness by integrating biologically produced energy carriers with electrolytic hydrogen. Bio-OH (funded by FORMAS) develops a biorefinery for production of chemicals, feed and fuels from anaerobic digestate. RISE offers a range of testbed centres (from lab-scale to demo-scale) situated across Sweden that accommodate pyrolysis, gasification, anaerobic digestion, gas fermentation and bioprocess scale-up testing. By partnering with RISE, projects in C1 gas valorisation can benefit from a comprehensive and multidisciplinary approach, potentially accelerating the development and implementation of these crucial technologies for a more sustainable future.

THOMAS REED

Newcastle University

Characterisation Of The Formate Hydrogenlysae 2 From *Pectobacterium atrosepticum* In An *Escherichia coli* Host.

THOMAS REED, FRANK SARGENT

Newcastle University Biosciences Institute, Newcastle University, Newcastle upon Tyne, NE1 7RU, England

Global climate change, resulting from greenhouse gas emissions, is having unprecedented effects around the world. Global efforts are being made to reduce carbon dioxide emissions via carbon capture storage and utilisation technologies to reach NetZero by 2050. *Escherichia coli* expressing the formate hydrogenlyase-1 (FHL-1) complex has already shown promise as a potential biological carbon capture technology. Under experimental conditions, FHL-1 can function as a hydrogen-dependent carbon dioxide reductase (HDCR) capable of producing formic acid at levels comparable to that of organisms that naturally produce formic acid from CO₂. One biotechnological draw-back of utilising FHL-1 is its dependence on a catalytic selenocysteine residue which prevents efficient over expression.

In addition to FHL-1, *E. coli* possesses the genetic information required to produce a second FHL complex, FHL-2. However, literary reports regarding the functionality of *E. coli* FHL-2 are contradictory and confusing. Therefore, the *E. coli* FHL-2 complex is still not greatly understood and cannot be definitively classified as active. Although the production and activity of the *E. coli* FHL-2 complex is still debated, important research has identified unambiguously functional FHL-2 complexes in other organisms including *Pectobacterium atrosepticim*. The FHL-2 complex from P. atrosepticum may prove to be a more appropriate complex for biotechnological applications as it is not reliant on a catalytic selenocysteine. Although the *P. atrosepticum* FHL-2 is functional, *P. atrosepticum* is not a suitable host for biotechnological applications as it grows slowly over 40 hours at 24 °C. Therefore, the genes encoding *P. atrosepticum* FHL-2 have been cloned for expression within an *E. coli* host.

Poster #04

SERGIO BLANCO-ROSETE Mono Carbon Limited

Production Of Renewable Chemicals Utilising Anaerobic Digestion CO₂.

There is growing awareness among scientists, policymakers, and industry leaders about the crucial contribution of negative carbon dioxide (CO_2) emissions to reaching Net Zero. Many low-carbon processes using biomass and organic waste, such as Anaerobic Digestion (AD), still produce substantial CO_2 emissions. Utilizing this CO_2 to create renewable chemicals provides a form of negative carbon emissions by effectively locking carbon into materials for extended periods.

Mono Carbon Limited has identified a business opportunity to reuse waste biogenic CO_2 from UK AD plants for the production of industrial chemicals. These biogenic CO_2 emissions are an inherent component of AD. Utilizing this CO_2 for chemical production presents a sustainable solution with economic and environmental benefits.

Reduction of carbon dioxide (CO_2) using non-thermal plasma (NTP) is an emerging technology with promising potential. Some companies report that its current Technology Readiness Level (TRL) has reached level 7, indicating a near-commercial stage where the system has been demonstrated in an operational environment. This method leverages NTP to activate CO_2 molecules under ambient or low-temperature conditions, enabling their conversion into value-added products or simpler molecules such as carbon monoxide (CO).

From an industrial technology standpoint, producing chemicals using C1 chemistry from CO_2 offers an attractive and environmentally friendly alternative to traditional petrochemical-based production. NTP can also be deployed for the production of syngas which opens the alternatives to produce renewable chemicals.

Mono Carbon Limited is looking for partnerships that will allow to fast-track the valorisation of AD CO_2 .

TIMOTHÉ PHILIPPON

Aarhus University

Insights into the variations in H₂ thresholds and growth yields among hydrogenotrophic methanogens.

PHILIPPON Timothé, PHILIPS Jo

Department of Biological and Chemical Engineering, Aarhus University

Among all biotechnological pathways through which Carbon dioxide (CO₂) can be recycled, methanogenesis is of huge interest for both CO₂ capture and utilization, as well as energy storage. This metabolism, performed exclusively by archaeal microbes, reduces CO₂ with the use of H₂ as electron donor into methane. Despite their importance, many metabolic characteristics of these microbes remain not well characterized. This includes the variations in H₂ thresholds (lowest H₂ concentration at which the conversion of CO₂ into methane occurs), as well as variations in growth yields (amount of biomass produced per the amount of CH₄ produced or per amount of both H₂ and CO₂ consumed) among different methanogens. Insights into these variations are nevertheless important to select the most optimal methanogen for biotechnological applications.

In this study, we measured the threshold for H₂ consumption and the growth yield of various methanogens, as our group previously did for acetogens¹. Nine different hydrogenotrophic methanogens were selected, covering a large spectrum of genera (including *Methanococcus, Methanolacinia, Methanobacterium* or *Methanobrevibacter*), which were all mesophilic, and either originated from freshwater or marine environments.

Our results show strong differences in the H₂ thresholds, which ranged from 1,0 \pm 0,5 Pa (7,0 \pm 3,6 nM) for *Methanobrevibacter arboriphilus* to 120 Pa \pm 10 Pa (870 \pm 72 nM) of H₂ for *Methanosarcina mazei*. When compared with acetogens we observe a lower minimum threshold for methanogens and two order of magnitude variation, instead of three orders of magnitude for acetogens. Our presentation will also show how the variations in growth yields relate to these H₂ thresholds. In addition, calculations of the ATP gains from these two experimental measures will be compared.

Poster #06

ROBIN HOEVEN

University of Manchester

Cultivation Of Chemoautotrophs On Electricity and Air.

ROBIN HOEVEN, JAMES WINTERBURN

University of Manchester, Engineering Building A, Grosvenor street, M1 7HL

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 current approach is doing an enrichment experiment of medium with the inoculum coming from environmental samples that are rich in chemoautotrophs (hot spring in Iceland). We run long (multiple weeks) chronoamperometry experiments either with or without aeration and we look for a downward trend in the current (consumption). Biomass that accumulates is sent for DNA analysis to investigate which species can grow electrotrophically. We aim obtain a single species that we can continue to perform laboratory evolution on to improve growth rate and eventually genetically modify to produce a product.

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

KIM E RENNHACK

University of Tübingen

Nitrate Availability Mediates The Particular Rearrangement Of Protein Interactions At The Rnf Complex And Energy Metabolism In *Clostridium Ijungdahlii.*

KIM E. RENNHACK¹, MIRITA FRANZ-WACHTEL², KATHARINA HIPP³, IRIS KOCH³, BRIGITTE SAILER³, CHRISTIAN-MARCO KLASK¹, BASTIAN MOLITOR^{1,7}, BORIS MACEK², AND LARGUS T. ANGENENT^{1,4,5,6,7}

 ¹Environmental Biotechnology Group, Geo and Environmental Science Center, University of Tübingen, Schnarrenbergstraße 94-96, 72076 Tübingen, Germany
 ²Proteom Center Tübingen, Institute for Cell Biology Tübingen, Auf der Morgenstelle 15, 72076 Tübingen, Germany
 ³Electron Microscopy Facility, Max Planck Institute for Biology Tübingen, Max-Planck-Ring 5, 72076 Tübingen, Germany
 ⁴Max Planck Fellow, Max Planck Institute for Biology Tübingen, Max Planck Ring 5, 72076 Tübingen, Germany
 ⁵Department of Biological and Chemical Engineering, Aarhus University, Gustav Wieds Vej 10D, 8000 Aarhus C, Denmark
 ⁶The Novo Nordisk Foundation CO2 Research Center (CORC), Aarhus University, Gustav Wieds Vej 10C, 8000 Aarhus C, Denmark
 ⁷Cluster of Excellence – Controlling Microbes to Fight Infections, University of Tübingen, Auf der Morgenstelle 28, 72074 Tübingen, Germany

The Rnf complex of the acetogenic bacterium *Clostridium ljungdahlii* plays a crucial role in chemiosmotic ion-gradient formation through its ion-pumping function. The ATP synthase utilizes the elevated ion gradient outside the cell to generate ATP. Previous studies have shown an increased ATP yield under nitrate supplementation. In this study, we focus on the interplay between the Rnf complex and proteins involved in energy conversion in the presence and absence of nitrate.

Therefore, we established an interactomic approach for *C. ljungdahlii*, combining affinity copurification of bait-prey-complexes with mass spectrometry. Here, a strep-tagged RnfC served as a bait to capture interacting partners under heterotrophic conditions. Additionally, we performed a proteomic experiment to visualize all of the produced proteins before purification. Interestingly, proteins involved in nitrogen and alcohol metabolism, and formate production interacted with the Rnf complex. The RnfC interactome showed a rearrangement of the prey proteins in the presence of nitrate. The particular bait-prey complexes were further obtained in a co-localization experiment *via* TEM.

In addition, the physiological role of nitrate was examined in more detail. The investigation of the heterotrophic proteome shows that key enzymes of the WLP are significantly inhibited under the influence of nitrate. Moreover, under heterotrophic conditions, nitrate promotes cell elongation and deformation of the flagella. Finally, our results showed a high-ordered Rnf complex in *C. ljungdahlii*, which provides an energetic benefit for the cell. A rearrangement of the supercomplex demonstrated the change of a dynamic microbial system to the environment. Overall, the elucidation of energy generation processes in *C. ljungdahlii* forms the basis for genetic cell modification of this strain to produce an abundance of industry-relevant products in the future.

TINA BAUR University of Tübingen

Characterization Of Novel Tools To Unveil Gene Expression Profiles In *Methanothermobacter thermautotrophicus* ΔH For Use In Metabolic Engineering.

TINA BAUR¹, LARGUS T. ANGENENT¹, AND BASTIAN MOLITOR^{1,2}

¹Environmental Biotechnology Group, Department of Geosciences, University of Tübingen, Schnarrenbergstraße 94-96, 72076 Tübingen, Germany

²Microbial Metabolic Biochemistry, University of Leipzig, Johannisallee 21-23, 04103 Leipzig, Germany

Methanothermobacter thermautotrophicus is a potent methane producer and is used in industrial power-to-gas applications. With the development of a genetic system, it is now possible to investigate and exploit its metabolic potential. For metabolic engineering, it is crucial to use well-suited promoters to drive heterologous gene expression. To investigate gene expression profiles, reporter proteins are required. One example is the β -galactosidase from *Geobacillus stearothermophilus*. During a previous study, which was performed to characterize an anhydrotetracyclineinducible promoter, it was found that the β -galactosidase activity is dependent on the inducer concentration. However, due to its thermostability, the enzyme activity was detectable throughout the experiment, thus, masking potential regulatory events.

To unveil potential promoter regulations, the fluorescence-activating and absorptionshifting tag (FAST) was used. As this system has a limited thermostability and relies on the interaction of the FAST protein with a fluorogenic ligand, the fluorescence read-out represents a direct reflection of when a promoter is active. An engineered version of FAST (pFAST) was put under the control of different promoters and introduced into *M. thermautotrophicus*. The fluorescence intensity (FLU) of *M. thermautotrophicus* [pMVS1111A_PhmtB_pFAST] and a strain with the empty vector were compared to strains with pFAST expression under the control of other native and synthetic promoters. The FLU of *M. thermautotrophicus* [pMVS1111A_PhmtB_pFAST] turned out to be strongest in the early exponential phase. In contrast, the PmctB promoter was only half as strong at that stage and was upregulated towards the late exponential phase. Other promoters were weakly active before the FLU was gone after 48 h. The characterized promoters can now be used to control heterologous genes to produce value-added chemicals and, thus, expand the product spectrum of *M. thermautotrophicus* beyond methane.

ANTONIA EBERT

The University of Queensland

Assessing Multi One-Carbon Conversion in Hydrogenophaga pseudoflava.

ANTONIA EBERT¹, SIMON GRIESHABER², JAMES HEFFERNAN¹, BASTIAN BLOMBACH², ESTEBAN MARCELLIN¹

1 Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, Australia

2 Microbial Biotechnology, Campus for Biotechnology and Sustainability, Technical University of Munich, Straubing, Germany

Microbial waste gas conversion into valuable products is hoped to be a major gamechanger in mitigating the release of greenhouse gases into the atmosphere. Aerobic gas fermenting microbes can produce energy intensive compounds, e.g., polyhydroxyalkanoates, autotrophically from CO₂, CO, H₂, and CH₄, major components of e.g., syn- and biogas. Based on the utilized substrate, these bacteria are grouped as carboxydotrophs (CO, CO₂, H₂), hydrogenotrophs (CO₂, H₂) or methanotrophs (CH₄).

Here, the carboxydotrophic *Hydrogenophaga pseudoflava* strain DSM 1084 is characterized as an autotrophic host for the conversion of CO, CO_2 , and H_2 .

Our focus lies in understanding the native aerobic carboxydo-hydrogenotrophic metabolism through phenotypical characterization in bioreactors. Particularly the growth regulation and activity of the main autotrophic enzymes – hydrogenase and carbon monoxide dehydrogenase – during the feed of different gas mixes. Maximal growth rates of 0.14 ± 0.008 h⁻¹ were observed during growth with 65 % H₂, 10 % CO₂, 4 % O₂ in argon and nitrogen atmosphere.

We aim to establish novel waste-gas-based bioproduction processes in which feedstocks can be highly flexible. Therefore, we will expand the strains substrate spectrum to formate, methanol and methane through heterologous expression and reactivation of natively harboured enzymes.

Our results demonstrated functionality of the methanol and formaldehyde dehydrogenases, validating the strain as a multi one-carbon assimilation host. As *H. pseudoflava* does not comprise a functional soluble formate dehydrogenase, we heterologously express the soluble *fds* cluster of *Cupriavidus necator* H16.

Combination of both the phenotypical analysis and synthetic biology will aid in the development of *H. pseudoflava* as a host for capturing mix gas streams from industry and waste processing.

University of Huddersfield

Heating And Electricity Production Via Domestic-Scale Bio-Digester to Drive Micro Gas Turbine.

ZU THANE MWAE KHIN, MOHAMMAD ALYASSIN AND GRANT CAMPBELL

Anaerobic digestion (AD) converts organic waste into renewable energy sources, including biogas and digestate, addressing a dual solution for waste management and clean energy generation. Micro gas turbines (MGT) have emerged as a promising technology for distributed energy generation, offering a range of advantages that align with the evolving needs of the energy sector to replace fossil fuels with renewable alternatives.

The objective of the research is to demonstrate the feasibility of utilising biogas in micro gas turbine systems by optimising the combination of these technologies, focusing on their potential to reduce carbon emissions and support the global energy transition.

In the initial phase of the project, the gas composition analysis was performed using an Agilent gas chromatography system. Secondly, the baseline performance of the MGT is established using diesel fuel, creating a benchmark for future comparative studies with biogas. Baseline tests mainly determine turbine efficiency, thermal performance, and emission characteristics. Establishing operation parameters for MGTs by using renewable gaseous fuel presents significant challenges. By addressing these challenges, the project aims to establish operational guidelines for efficient and reliable usage of biogas in MGT systems. Moreover, this research aligns with the global transition toward low-carbon energy systems, contributing to the decarbonisation of waste-to-energy processes and fostering the development of circular economies.

The project demonstrates how biogas, a product of anaerobic digestion, can be used as a renewable fuel in micro gas turbines. The findings from this research will provide a valuable framework for implementing a biogas-driven MGT system and advancing sustainable energy solutions.

ROHIT MURALI

University of Surrey

From Mechanistic Models to Data-Driven Models for Predicting Biogas Production in Anaerobic Digestion Plants.

ROHIT MURALI^a, BENAISSA DEKHICI^a, TAO CHEN^a, DONGDA ZHANG^b, MICHAEL SHORT^{a*}

^aSchool of Chemistry and Chemical Engineering, University of Surrey, Guildford, GU2 7XH, UK

^bSchool of Chemical Engineering and Analytical Science, The University of Manchester, Manchester, M13 9PL, UK

Anaerobic digestion (AD) is essential for renewable energy production, transforming organic waste into biogas without oxygen. However, predicting biogas output for real-time applications in AD plants is challenging due to the complexity and dynamic nature of the process. Despite extensive literature on decision-making in AD, there are few industrial reliable models for operators to accurately predict biogas output. Mechanistic models are useful for controlling systems, estimating states, designing reactors, and optimising operations, reducing reliance on expensive experiments. However, their practical application is limited by their complexity and reliance on numerous parameters rarely measured in AD plants. This study compares mechanistic and data-driven models for predicting biogas production from a labscale anaerobic digester. A simplified mechanistic model, based on biomass and substrate concentrations using Haldane kinetics, was developed and fit to experimental data. While it demonstrated moderate accuracy ($R^2 = 0.82$), it struggled to predict feedstock and biomass concentrations for future scenarios and only performed well in the region after steady state was first observed. In contrast, a Long Short-Term Memory (LSTM), trained on the same lab-scale data, showed superior predictive capabilities ($R^2 = 0.93 - 0.98$) and successfully modelled the temporal dependencies expected in the AD process. The LSTM model was further validated using industrial AD data, achieving R² values of 0.95-0.97, confirming its robustness across scales. Compared to time-intensive experiments and mechanistic models, the LSTM model offers a practical and accurate solution for predicting biogas production. Its strong performance highlights its potential for real-time applications, making it a promising tool for optimising operations in large-scale AD plants.

ELODIE VLAEMINCK

Bio Base Europe Pilot Plant

Demonstration Of Advanced Biofuels: Production of Triacylglycerols From CO₂ in A Coupled Fermentation Via Acetic Acid.

ELODIE VLAEMINCK*, KOEN QUATAERT, STEFANO REBECCHI, KAREL DE WINTER

Bio Base Europe Pilot Plant (BBEPP), Rodenhuizekaai 1, 9042 Ghent, Belgium

*elodie.vlaeminck@bbeu.org

The transport sector accounts for approximately 15% of global greenhouse gas emissions, with heavy-duty transport, particularly aviation, posing significant decarbonization challenges due to its limited electrification potential. To address this challenge, (bio)fuels derived from waste and residue feedstocks offer a promising pathway toward a more sustainable and circular economy.

The FUELPHORIA project aims to establish sustainable, competitive, and secure value chains for advanced biofuels and renewable fuels of non-biological origin through demonstrations across four European sites. In Belgium, the project focuses on producing high-value liquid biofuels from CO₂ captured at a biogas upgrading facility (Aquafin, Antwerp) and renewable hydrogen. This is achieved through an integrated biological and thermo-catalytic conversion process.

BBEPP focuses on the biological part, optimizing and scaling up an innovative twostage fermentation process to transform the biogenic CO₂ into triacylglycerols (TAGs), which serve as precursors for hydrocarbon-based biofuels. In the first stage, CO₂ and H₂ are converted by an acetogenic bacterium into acetic acid in a continuous gas fermentation. To evaluate the efficiency of this conversion at the emission source, the Bio Base Mobile Pilot Plant, a state-of-the-art mobile gas fermentation unit, will be deployed. In the second stage, the CO₂-derived acetic acid is upgraded in another continuous fermentation with an oleaginous organism, which intracellularly accumulates TAGs. Advanced downstream processing technologies are then applied to purify the TAGs, preparing them for hydrotreatment into highperformance biofuels.

By demonstrating this integrated, scalable, and sustainable approach, this work contributes to the advancement of innovative biofuel technologies, supporting the decarbonization of the transport sector and the transition toward a circular future.

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JENNIFER ROTH

Goethe University Frankfurt

Purification and Characterization of an Electron-Bifurcating Formate Dehydrogenase/Hydrogenase Complex from *Sporomusa ovata*.

JENNIFER ROTH and VOLKER MÜLLER

Molecular Microbiology & Bioenergetics, Institute of Molecular Biosciences, Goethe University Frankfurt, 60438 Frankfurt am Main, Germany

Acetogenic bacteria are a group of strictly anaerobic bacteria that fix CO₂ and produce acetate as the main product using the Wood-Ljungdahl pathway (WLP). The first step of the WLP is the reduction of CO₂ to formate, catalysed by formate dehydrogenases, but the involved electron carriers differ among acetogens^[1,2]. The model organism for cytochrome-containing acetogens, Sporomusa ovata, has at least three different formate dehydrogenase genes, but the nature of the enzyme involved in the WLP remained to be identified. We aimed to elucidate the subunit composition, mechanism of electron transport and electron carriers involved in the reduction of CO₂ to formate in S. ovata. The formate dehydrogenase of S. ovata was purified from the cytoplasmic fraction using three different chromatographic steps. After each purification step, the fractions containing formate dehydrogenase also showed hydrogenase activity. The purified protein catalyzed the simultaneous reduction of NAD⁺ and Fd with H₂ as well as with formate as electron donor. The enzyme was able to produce formate from H_2 and CO_2 as well as H_2 (+CO₂) from formate. Biochemical analysis revealed that the formate dehydrogenase (FdhA) of S. ovata forms a complex with the electron bifurcating hydrogenase (HydABCDE) and the complex uses two consecutive electron-bifurcating reactions to reduce CO₂ with electrons derived from molecular hydrogen to formate. Ferredoxin and NAD+ are involved as electron carriers.

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LARA MARIE WASCHINGER

Goethe University Frankfurt

A Cytochrome *c*-containing Periplasmic Nitrate Reductase in the Acetogen Sporomusa ovata.

LARA MARIE WASCHINGER and VOLKER MÜLLER

Molecular Microbiology & Bioenergetics, Institute of Molecular Biosciences, Goethe University Frankfurt, 60438 Frankfurt am Main, Germany

Any acetogenic bacterium known to date generates ATP *via* two ferredoxindependent respiratory chains, containing either the Rnf or the Ech complex^[1]. These two membrane-bound respiratory enzymes do not have cytochromes. However, some species such as *Moorella thermoacetica* or *Sporomusa ovata* contain *b*- and *c*-type cytochromes^[2,3], whose functions remain to be identified.

Addition of nitrate led to higher optical densities and to reduced acetate concentrations in *S. ovata* when grown on fructose, methanol or $H_2 + CO_2$. Nitrate was reduced and nitrate reduction was CO_2 /bicarbonate independent. Inspection of the genome sequence revealed *nap* genes coding for a periplasmic nitrate reductase as well as genes encoding cytochrome *c* and heme biosynthesis and the genes coding for a periplasmic nitrite reductase. Transcriptome analyses revealed induction of expression of these genes by nitrate. Biochemical analyses confirmed the presence of cytochrome *c* in nitrate-grown cells. Cytochrome *c* is apparently involved in the utilization of an alternative electron acceptor in *S. ovata*, nitrate, but not in the primary energetics. Rnf is still active in *S. ovata* under these conditions. Whether nitrate reduction produces additional ATP remains to be identified.

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YVONNE BURGER

Goethe University Frankfurt

A Formate Transporter and Sulfur Transferase in the Acetogenic Bacterium *Thermoanaerobacter kivui* are Important for Hydrogen and Formate Interconversion.

YVONNE BURGER and VOLKER MÜLLER

Molecular Microbiology & Bioenergetics, Institute of Molecular Biosciences, Goethe University Frankfurt, 60438 Frankfurt am Main, Germany

Thermoanaerobacter kivui is an anaerobic, thermophilic acetogen that uses the Wood-Ljungdahl pathway to produce acetate from CO₂ and H₂. The first enzyme in this pathway, the hydrogen-dependent CO₂ reductase (HDCR) ^[1], catalyzes the reduction of CO₂ to formate with the concomitant oxidation of H₂ to protons and *vice versa* ^[2]. In times of climate change, the conversion of H₂ to formate is a promising way for H₂ storage. Furthermore, formate is an important intermediate in the metabolism of different anaerobic bacteria and can be used as feedstock to produce valuable chemicals. Thus, *T. kivui* is an ideal production platform in a formate-based bioeconomy but also in biological hydrogen production and storage ^[3].

Right next to the genes coding for HDCR are two genes, *fdhC* and *fdhD*, that are involved in HDCR activity but are not part of the enzyme complex itself. To investigate the functions of those proteins, we used bioinformatic analyses, generation of deletion mutants, growth experiments, cell suspension experiments and enzyme activity assays.

FdhC is similar to the *Escherichia coli* formate transporter FocA. The deletion mutant had no phenotype at pH 7.0 and 150 mM formate, conditions used to grow the wildtype. However, at high pH and low formate concentrations, formate consumption by resting cells of the mutant was drastically reduced. Also, the mutant was more resistant to hypophosphite than the wildtype.

The deletion of the gene *fdhD* resulted in significantly lower hydrogen production in cell suspension experiments. Furthermore, the formate:MV-oxidoreductase activity in crude extract of the *fdhD* deletion mutant was significantly lower than in the wildtype. In sharp contrast, H₂:MV-oxidoreductase activity in crude extract of the *fdhD* mutant was increased. The deletion of *fdhD* also caused a reduced amount of tungsten in HDCR, showing its role in the maturation or integration of the tungsten-containing cofactor.

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Goethe University Frankfurt

Energy Conservation by Using Dimethyl Sulfoxide as Alternative Electron Acceptor in the Acetogen *Moorella thermoacetica.*

FLORIAN ROSENBAUM and VOLKER MÜLLER

Molecular Microbiology & Bioenergetics, Institute of Molecular Biosciences, Goethe University Frankfurt, 60438 Frankfurt am Main, Germany.

Acetogenic bacteria are an ecophysiologically important group of strictly anaerobic bacteria. Their characteristic feature is to oxidize organic as well as inorganic electron donors, coupled to the reduction of CO₂ via the Wood-Ljungdahl pathway (WLP) as terminal electron acceptor. In addition to CO₂, acetogens can use alternative electron acceptors such as nitrate, fumarate, dimethyl sulfoxide (DMSO) or aromatic acrylates^[1]. Although the WLP has been established in *Moorella* thermoacetica, little is still known about the bioenergetics of CO₂ reduction and the possible use of electron acceptors other than CO₂ in *M. thermoacetica*. By far the best studied alternative terminal electron acceptor is nitrate^[2-4]. Here we have analysed whether or not *M. thermoacetica* can reduce DMSO as final electron acceptor. Growth of *M. thermoacetica* on glucose or $H_2 + CO_2$ was stimulated by DMSO. Membranes had a DMSO reductase activity that was induced by growing cells in presence of DMSO. The enzyme used reduced anthraquinone-2,6disulfonate, benzyl- and methyl viologen as electron donor, but not NAD(P)H. Activity was highest at pH 5 and 60°C, the K_m for DMSO was 2.4 mM. Potential DMSO reductase subunits were identified by peptide mass fingerprinting; they are encoded in a genomic region that contains three potential dmsA genes, three dmsB genes and one *dmsC* gene. Transcriptome analysis revealed that two different dmsAB gene clusters were induced in the presence of DMSO. In sum, the data are in line with the hypothesis that *M. thermoacetica* can use DMSO alongside CO₂ as electron acceptor and DMSO reduction is catalysed by an energy-conserving, membrane-bound electron transport chain with DMSO as final electron acceptor.

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RAPHAEL TRISCHLER

Gothe University of Frankfurt

Formate as Electron Carrier in the Gut Acetogen Blautia luti.

RAPHAEL TRISCHLER¹, MATTHEW SORBARA² and VOLKER MÜLLER¹

¹Molecular Microbiology & Bioenergetics, Institute of Molecular Biosciences, Goethe University Frankfurt, 60438 Frankfurt am Main, Germany

²Department Molecular and Cellular Biology, University of Guelph, Guelph, ON, N1G 2W1, Canada

The gut microbiome is a highly complex community, which is involved in the digestion of nutrients but also affects the development of diseases as well as the human well-being. Gut acetogens such as Blautia strains are often connected to human well-being^[1]. However, only little is known about the physiology of different Blautia species. Bacteria of this genus are often classified as acetogenic bacteria, but the use of a functional Wood-Ljungdahl pathway (WLP) has only been proven and characterized for a few strains. Bacteria of the genus Blautia can be divided into FDH-containing and FDH-lacking strains^[2]. While FDH-containing species such as B. schinkii can produce acetate from $H_2 + CO_2$ via the WLP, FDH-lacking strains such as B. luti cannot. However, analysis of the genome sequence revealed that all genes encoding the WLP with exception of the FDH encoding gene are present in the genome of these strains. Interestingly, these bacteria performed acetogenesis from CO + formate, showing an unusual but functional WLP. In addition, B. luti produced formate during heterotrophic fermentation by pyruvate formate lyase; in the presence of CO or H_2 , formate is reduced to acetate by the WLP. Therefore, we conclude that for these gut acetogens, formate plays an essential role as intraspecies electron carrier. Beyond that, formate might also be commonly used as interspecies electron carrier in the human gut.

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ABDULLAH LABBO

Optimizing Ectoine Production Using Methanol-Consuming Halotolerant Methanotrophs.

^{1,2}ABDULLAHI MUHAMMAD LABBO, ¹BASHIR RUMAH, ¹YING ZHANG, ¹KLAUS WINZER

¹Synthetic biology research center, University of Nottingham, United Kingdom

²Sokoto State University, Sokoto

Methanol is an abundant and cost-effective next-generation feedstock with potential applications in biotechnology. Some halotolerant methanotrophs can utilize methanol as the sole carbon and energy source while thriving in hypersaline environments, producing valuable metabolites such as ectoine. Ectoine, a highvalue compound with a market value exceeding 1000 USD per kilogram, has significant applications in the cosmetic, pharmaceutical, and medical industries. Currently, commercial ectoine production relies on a super-leaky mutant of Halomonas elongata, which utilizes expensive and non-renewable sugars as a carbon source. This study aims to improve ectoine accumulation and enhance excretion by exploiting methanol-consuming bacteria as a more sustainable alternative. Initially, optimal conditions for cell growth and ectoine production were determined by varying methanol concentration, medium salinity, and pH. To further enhance ectoine excretion, a bio-milking strategy was employed, where cells were cultivated under high-salinity conditions to promote ectoine accumulation, followed by a sudden reduction in salinity (NaCl 0%) to assess its impact on ectoine release. Finally, the optimized conditions were applied in a bioreactor to scale up fermentation, aiming to maximize biomass and ectoine production. The findings demonstrate the feasibility of converting methanol into high-value ectoine. the implementation of a biomilking strategy effectively enhanced ectoine excretion, suggesting a promising approach for improving downstream processing efficiency.

CHRISTIAN KRÖLY

Wageningen University & Research

C1-Compounds For Sustainable Bioplastics: Engineering Acetogen-PHA Cocultures.

CHRISTIAN KRÖLY, MAXIMILIAN FLAIZ, DIANA Z. SOUSA

Laboratory of Microbiology, Wageningen University & Research, The Netherlands

Developing efficient biocatalysts and processes for C1-compound utilization remains a key challenge in the field of green chemistry and carbon recycling. One exciting application is the microbial production of 'bioplastics', offering a sustainable alternative to petroleum-based plastics. By harnessing C1-compounds such as CO₂, CO, and methanol, microbes can synthesize biopolymers like polyhydroxyalkanoates (PHAs), which are both biodegradable and versatile for use as plastic surrogate.

Building on this concept, we aim to develop a microbial co-culture system, where acetogens are engineered to produce C4-building blocks such as butyrate, butanol, and 3-hydroxybutyrate to work in tandem with a PHA-accumulating microorganism. Here, we describe the development of the first module of this co-culture: an acetogen producing butanol and butyrate from methanol and CO.

We selected *Eubacterium limosum* DSM 20543^T as the acetogenic microbe, due to its natural butyrate production, genetic accessibility, and available tools for molecular engineering. The correlation of high methanol concentrations and butyrate production was investigated in batch experiments. Results indicate that methanol concentrations of up to 1 M promote butyrate production. At the same time, the acetate concentration does not exceed 53 mM in all tested conditions.

To convert butyrate further to butanol, the genes *aor* and *bdhB* from *C. carboxidivorans* DSM 15243 and *C. acetobutylicum* DSM 792 respectively were codon harmonized, introduced with the pMTL80000 shuttle-vector system, and expressed under control of a weak constitutive promoter. Since no butanol could be observed during the cultivation of the recombinant strain with methanol, the substrates will be replaced with methanol/CO. Moreover, the weak promoter will be exchanged with a strong one, and the 5'-UTRs of the promoters adapted to the respective coding sequences.

After successful prove of plasmid-based production, respective genes will be integrated into the genome of *E. limosum*, circumventing the need for antibiotics, which will be detrimental to the PHA-accumulating microorganism.

MUNGYU LEE

Delft University of Technology

Targeted Recovery of C6 Carboxylate from CO₂-based Microbial Electrosynthesis.

MUNGYU LEE, LUDOVIC JOURDIN* and ADRIE J.J. STRAATHOF*

Delft University of Technology, Department of Biotechnology, Delft, Netherlands

*Email: l.jourdin@tudelft.nl (Ludovic Jourdin) and A.J.J.Straathof@tudelft.nl (Adrie J.J. Straathof)

Faced with global environmental challenges, carbon upcycling has become a key component of the circular carbon economy, focusing on transforming CO₂ into valuable products. This strategy supports environmental sustainability by converting carbon emissions into economically advantageous resources. Microbial Electrosynthesis (MES), an emerging technology in carbon capture and utilization, offers an innovative approach by employing microorganisms to convert CO₂ into useful chemicals. Using open culture MES systems we are able to produce 33.6 g/L of a mixture of carboxylates with varying carbon chain lengths, including C2, C4, and C6 compounds. Among these, C6 carboxylates are particularly interesting due to their higher commercial value compared to shorter chain compounds like acetate (C2 to C4), which are the C6 precursors. However, the simultaneous generation of these diverse compounds presents significant challenges in selectively isolating hexanoate (C6) from the mixture. This study investigates the targeted extraction of hexanoate from carboxylates produced through CO₂-driven MES,. The process utilizes a strong anion exchange resin for adsorption, followed by desorption using CO₂-expanded methanol, resulting in the desired protonated form of the target carboxylate. Key factors influencing the adsorption process in CO₂-driven MES were identified and analysed. The selectivity and concentration of C6 were evaluated based on adsorption and desorption outcomes, revealing a 43% adsorption preference for C6 and a 50% desorption efficiency. The non-adsorbed aqueous C2-C4 carboxylate stream will be returned to MES for chain elongation. This study will serve as a critical foundation for advancing the integrated CO₂-based MES process into a practical and scalable industrial application.

Keywords: Anion exchange chromatography, Hexanoate, Microbial electrosynthesis, CO_2 utilization, Power2X.

DERYA ACAR KU Leuven, Belgium

Microwave-assisted hydrothermal carbonization (MW-HTC) of anaerobic digested sludge for resource recovery.

DERYA ACAR, NICK SWEYGERS, LISE APPELS

Carbon recycling from waste materials is an important aspect of sustainability, aiming to reduce greenhouse gases while ensuring efficient resource utilization¹.

AD sludge, rich in organic material and carbon, represents an underutilized resource within the carbon cycle economy². During hydrothermal carbonization, hydrochar and gases, including CO₂ and CH₄, are generated.^{3,4} These gases can be integrated into microbial gas fermentation systems or reused as feedstocks for carbon recycling.⁵ Unfortunately, AD sludge contains undegraded environmental pollutants such as pharmaceuticals.

Therefore, this study explored a hybrid approach for converting anaerobic digested (AD) sewage sludge into valuable carbon-based products via microwave-assisted hydrothermal carbonization (MW-HTC), with a focus on simultaneous hydrochar production and pharmaceutical degradation.

Compared with conventional hydrothermal carbonization, MW-HTC offers clear advantages such as greater energy efficiency, shorter reaction times, and an optimized hydrochar yield. Several authors have reported that hydrothermal carbonization can produce high-quality hydrochar, which can be used for soil improvement or energy applications while producing gaseous byproducts suitable for further microbial conversion.^{6–9} This combined process not only promotes the closing of the circular carbon loop but also maximizes the recovery of natural resources from waste streams, achieving the overall goal of sustainable greenhouse gas management.

Furthermore, MW-HTC approaches the degradation of micropollutants in AD sludge. In particular, pharmaceuticals contained in sewage sludge have been shown to be degradable during MW-HTC.

This research is in line with the mission of the Carbon Recycling Network and aims to advance the field through novel sludge utilization techniques that support the reuse of greenhouse gases and the sustainable development of raw materials, thus closing the carbon cycle in the bioeconomy.

VICTORIA CHINONYEREM UDEMEZUE

University of Tartu

Optimization Of Plasmid Curing From Genetically-Engineered Clostridium autoethanogenum.

VICTORIA CHINONYEREM UDEMEZUE, KURSHEDAKTAR MAJIBULLAH SHAIKH, KASPAR VALGEPEA

Institute of Bioengineering, University of Tartu, Tartu, Estonia

The accumulation of greenhouse gases (GHGs) released by harmful human activities involving the combustion of fossil-fuels is a driver of climate change that threatens biosustainability on Earth. Microbial gas fermentation provides an attractive option to capture CO₂ towards biomanufacturing of value-added products. Acetogens are the preferred biocatalysts for gas fermentation as they can use CO₂ as their sole carbon source with H₂. Metabolic engineering of novel acetogen cell factories is, however, hampered by the slow and complex genetic engineering workflows. Here, we developed different approaches to optimize plasmid curing from the model-acetogen Clostridium autoethanogenum. Firstly, we used a CRISPR/Cas9-based approach where a curing plasmid (C-plasmid) was constructed to express Cas9 and a gRNA targeting the ColE1 origin of replication in both the C-plasmid and the recombinant plasmid to be cured. Additionally, we tested the effects of making the cells to be cured electrocompetent, electroporation without a plasmid, and buffer-washing of glycerol stocks on plasmid curing. Both Colony counts and growth tests showed ~30-100% plasmid curing across the tested approaches. This work significantly accelerates plasmid curing from C. autoethanogenum and thus contributes towards improving genetic engineering workflows for acetogens.

ALI NAWAZ & ETINI ETUK

University of Huddersfield

Eco-friendly Biosynthesis of Cellulase Using Post-Consumer Textile Waste.

ALI NAWAZ¹, ETINI ETUK¹, ZIYAO LIU², CAROL LIN² AND CHENYU DU¹

¹School of Applied Sciences, University of Huddersfield, HD1 3DH, United Kingdom

² School of Energy and Environment, City University of Hong Kong

The textile industry is recognized as the second most harmful sector to the global environment, with less than 1% of textile waste being repurposed into new clothing. Moreover, it ranks as the fifth-largest contributor to carbon emissions, accounting for nearly 10% of total emissions resulting from incineration. This study aims to valorise textile waste as a substrate for the fungal biosynthesis of cellulase, which is then utilized to hydrolyse the waste textiles into cotton and plastic (PET) fibres, cotton residues, and reducing sugars. Hydrolysis was performed using various methods, incorporating different combinations of pretreatment, cellulolytic degradation, and mechanical force assistance. The results of this study led to the successful separation of cotton and PET fibres, cotton residues, and reducing sugars in the hydrolysate. The extracted cotton, PET fibres, and residues will undergo refabrication to produce recycled fibres for the textile industry. Additionally, the reducing sugars will be biorefined to generate value-added products. This comprehensive approach contributes to the realization of a carbon-neutral circular bioeconomy.

Keywords: Carbon neutral, Recycling, Biorefinery, Cellulase, Hydrolysis

90

ASHRAF HAUWA HYAKO

Loughborough University

Continuous Recovery of Fermentation Products using Novel Ho23t Microbubble Gas Stripping

Researchers Office, S building, Department of Chemical Engineering, Loughborough University, LE11 3TU.

Efficient product recovery from fermentation processes is critical due to the inhibitory effects of product accumulation. This study presents a detailed literature review on in-situ product recovery (ISPR) techniques for biofuel production within the last decade. The review reveals that while each ISPR technique has its trade-offs, gas stripping offers an optimal balance of overall performance. However, traditional gas stripping is often limited by its high energy demand. A hot microbubble gas stripping technique developed by our group demonstrates potential for achieving high productivity at a reduced energy consumption, leveraging enhanced gas-liquid contact and heat efficiency.

This work examines the application of the microbubble stripping unit (MSU) under new operating conditions using an iterative approach combining experimental work and numerical modelling in COMOSL Multiphysics. The MSU's capability to maintain ethanol concentrations below the inhibitory threshold ($\sim 2\%$ v/v) of the Clostridium strain was evaluated. A simulated broth was utilised with ethanol being continuously added at ~5 g/(lh) to simulate the ongoing production rate of the strain. The initial experimental findings revealed a lower-than-expected productivity rates, with the ethanol concentrations exceeding the critical threshold. Further experimental optimisations resulted in similar outcomes, prompting a detailed modelling of the process.

The model, validated by current and previous data, indicated that optimising gas flow rates and liquid volumes improved the performance of the system. Experimental validation of the initial optimised parameters maintained the concentration below inhibitory levels and increased productivity to 5.21 g/(lh), compared to 4.0 g/(lh) in the base case.

Future work will aim on stabling MSU operation by refining gas dynamics and thermal control, extending the modelling scope, and exploring applications for a broader range of bioproducts.

JO PHILIPS Aarhus University

Selecting The Best Acetogen for Fast and Efficient Microbial CO₂ Recycling into Acetate

PHILIPS J., MUÑOZ L., CATALANO, J.

Acetogenic bacteria are of interest for the recycling of CO_2 , as these microbes reduce CO_2 into valuable (mostly C2) products, using H_2 as electron donor. Different acetogenic strains are available for this CO_2 recycling through gas fermentation or microbial electrosynthesis.

Our research group has recently characterized differences in the H₂ consumption thresholds and kinetics of range of mesophilic acetogens. We found that H₂ thresholds differed as much as three orders of magnitude, as *Sporomusa ovata* had a H₂ thresholds of 6 ± 2 Pa, while this was a high as 1990 ± 67 Pa for *Clostridium autoethanogenum* (Munoz & Philips, 2023). Surprisingly, we also found that H₂ consumption by acetogens followed first order kinetics, i.e. the H₂ consumption rate increases linearly with the dissolved H₂ concentration up to saturation. Moreover, first order kinetic rate coefficients differed upto six times and *Acetobacterium wieringae* had the highest dry weight specific rate coefficient.

We have further used these insights to simulate the acetate production rate and H_2 utilization efficiency using different acetogenic strains. Our model described a bioreactor in which gaseous CO_2 is sparged and H_2 is produced *in situ* using an electrode (i.e. microbial electrosynthesis). Our model further assumed that H_2 was the main limiting factor, while incorporating microbial H_2 and CO_2 consumption, growth, acetate production and H_2 and CO_2 mass transfer.

We found that higher input H_2 fluxes led to higher acetate production rates, but lower efficiencies. For a certain input H_2 flux, the highest efficiency was obtained for the acetogen with the highest first order kinetic rate coefficient, while also higher initial cell densities increased the efficiency. We further identified that CO₂ sparging can negatively impact the H_2 utilization efficiency by stripping H_2 , which can in part be overcome by selecting an acetogenic strain with a low H_2 threshold and high first order kinetic coefficient, such as *S. ovata* or *A. wieringae*.

My presentation will detail additional lessons learned from these simulations.

KIRA BAUR University of Ulm

Lactate Production using Genetically Modified Acetobacterium woodii Strains.

K. S. BAUR¹, A. STOCK², D. WEUSTER-BOTZ², F. R. BENGELSDORF¹

¹ Institute of Microbiology and Biotechnology of Prokaryotes, University of Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany

² Chair of Biochemical Engineering, Technical University of Munich, Boltzmannstr. 15, 85478, Garching, Germany

Global warming escalates into a climate disaster. One of the reasons is the steady increase in CO_2 emissions. Gas fermentation is an approach to reuse CO_2 . The acetogen *A. woodii* produces natively acetate using CO_2 and H_2 via the Wood-Ljungdahl pathway (WLP).

A. woodii was genetically modified to produce lactate as a second product. Therefore, the genes encoding the native bifurcating lactate dehydrogenase of *A. woodii* were knocked out and the D-lactate dehydrogenase (LdhD) from *Leuconostoc mesenteroides* was expressed in *A. woodii* (Mook et al., 2022, doi: 10.1007/s00253-022-11770-z). An increased yield of lactate should be achieved if more pyruvate is available. Therefore, a pyruvate-formate lyase (PFL) originating from *Clostridium pasteurianum* was expressed in *A. woodii* LDHD. The PFL can convert a formate excess together with acetyl-CoA in a reverse manner into pyruvate. This reaction branches the methyl branch of the WLP, and should lead to higher pyruvate concentrations.

A. woodii LDHD and *A. woodii* LDHD_PFL have been cultivated in a controlled stirred tank bioreactor with a working volume of 2 L (30° C, pH = 7, P/V = 1.2 W L⁻¹). The batch cultivations were performed under atmospheric pressure with a gas mixture of 67% H₂ and 33% CO₂ (0.083 vvm). Both strains reached a max. OD₆₀₀ of around 1.1 within 72 h. *A. woodii* LDHD_PFL yielded 39 mM lactate and 237 mM acetate, *A. woodii* LDHD 31 mM lactate and 254 mM acetate. This result indicates that PFL is works as expected.

Because industrial processes operate at higher pressures, bottle-scale experiments were performed at 1 bar headspace overpressure. *A. woodii* LDHD produced 17 mM formate. In a batch fermentation process, also with 1 bar overpressure, *A. woodii* LDHD_PFL grew to a 23% lower OD₆₀₀ than under atmospheric pressure and produced 11 mM lactate, 59 mM formate, and 120 mM acetate. 1 bar overpressure caused an overall drop in strain performance and is no sustainable way to improve productivity.

MAXIMILIAN FLAIZ Wageningen University & Research

Expanding the Genetic Toolbox for Acetobacterium wieringae.

MAXIMILIAN FLAIZ AND DIANA Z. SOUSA Laboratory of Microbiology, Wageningen University & Research, The Netherlands

Over the last 25 years, scientists have developed various molecular tools, expressed non-native pathways, and enabled recombinant production in a handful of different gas fermenting acetogenic bacteria. While some acetogens, such as the commercialized *Clostridium autoethanogenum*, possess a well-developed toolbox, others have only poor or no genetic system available. One of such promising but understudied acetogens is *Acetobacterium wieringae* JM, a carboxydotrophic acetogen that efficiently converts CO via the Wood-Ljungdahl pathway to produce acetate and ethanol. Unlike other ethanol-producing carboxydotrophic acetogens, *A. wieringae* operates at pH 7, making it an ideal co-culture partner to produce chain-elongated products.

In the present work, we expanded the genetic toolbox of *A. wieringae* by implementing various promoters, a fluorescent reporter protein, and a potent gene-knockout system. We constructed a small promoter library consisting of seven promoters from different acetogenic and solventogenic *Clostridia*. Promoter activity was assessed using the fluorescence-activating an absorption-shifting tag (FAST) as a reporter protein. All tested promoters exhibited activity at varying strengths determined by FAST mediated fluorescence.

Moreover, we implemented the theophylline-inducible CRISPR-Cas-based genome engineering system SIBR-Cas for targeted gene knockouts in *A. wieringae*. Given that deletion of aldehyde ferredoxin oxidoreductases (AOR) in *C. autoethanogenum* influenced ethanol production, we targeted two homologous AOR genes in *A. wieringae* using SIBR-Cas. While the successful deletion of AOR1 did not impact ethanol production when the strain *A. wieringae* Δ AOR1 was cultivated with CO, knockout of AOR2 is still in progress.

Our findings substantially advance the genetic engineering capabilities of *A. wieringae*, paving the way for its use in co-cultures for the production of valuable industrial platform chemicals.

DIEGO OROL-GŎMEZ

National Renewable Energy Centre (CENER)

Gas fermentation. A promising CO2 conversion technology for decarbonization challenges.

OROL-GÓMEZ, DIEGO. ALEGRÍA, IRANTZU

Centro Nacional de Energías Renovables - CENER, Ciudad de la Innovación 7, Sarriguren, (Navarra), Spain.

Gas fermentation has emerged as a highly promising technology for the sustainable production of industrial chemical precursors and fuels. This innovative approach integrates multiple disciplines, including process engineering, metabolic engineering, directed evolution, synthetic biology, in silico modelling, and multi-omics análisis.

The fixation of waste gases can be efficiently carried out by specialized microorganisms known as acetogens, which utilize the acetyl-coenzyme A (acetyl-CoA) metabolic pathway, also referred to as the Wood-Ljungdahl pathway (WLP)¹². Among these, the thermophilic bacteria *Moorella thermoacetica* stands out as a suitable autotrophic microorganism capable of carbon assimilation and energy conservation³⁴. Its metabolism allows to converts gaseous substrates into acetate as the primary product. While the product range of native acetogens is traditionally limited, advancements in synthetic biology and metabolic engineering provide opportunities to expand this range by introducing synthetic pathways⁵⁶.

At CENER, we focus on advancing the biological conversion of waste gases into industrial strategic compounds. Specifically, we aim to utilize this thermophilic acetogen as fermentation platform, engineering it to produce volatile chemicals utilizing an inhouse designed trickled-bed bioreactor (TBR) for the scale-up.

We are currently validating this approach through Adaptive Laboratory Evolution (ALE), and genetic tool development to generate novel mutant strains.

Our goal is to unravel the remarkable features of these microorganisms and obtain insight that will enable us to improve the process scalability for ultimately, transfer it to the industry. CENER strengthens its commitment to innovation through sustainable technologies, positioning itself as a benchmark in biorefining applied to global challenges.

Keywords: CH₄, CO₂, Syngas fermentation, Acetogens, CENER, Mixed cultures, Wood-Ljungdahl pathway, Metabolic engineering, Synthetic biology.

University College London

Urban Carbon Capture: Harnessing Hydroponic Green Walls.

Dominic Clyde-Smith 1 *

1 Department of Civil, Environmental & Geomatic Engineering

This presentation details a method to enhance climate resilience and carbon storage in buildings through the incorporation of hydroponic phytoremediation facades in urban areas. This approach also provides additional benefits, including mitigating overheating, offering sustainable cooling, providing thermal insulation, and enhancing flood resilience.

The design maximizes carbon uptake and storage efficiency by selecting plant species with high biomass production and root exudation traits, combined with substrates engineered to adsorb and lock carbon through mineralization over time. Beyond carbon abatement, these facades mitigate urban overheating, provide sustainable cooling, and offer potential flood resilience through integrated water management.

Soil is crucial for plant growth, hydroponics mimics soil by enhancing air, water, and bio interfaces through ecological engineering. However, biogenic carbon storage isn't permanent due to potential leaks and disturbances. Green walls also risk releasing stored carbon during biomass removal. Soil sequestration capacity in green walls is limited. In this context, biochar as a grow media serves as an electron shuttle in microbial electrochemical systems, facilitating electron transfer and increasing the availability of organic compounds for microbes. This interaction aids in organic matter decomposition and soil organic carbon stabilization. By integrating biochar and microbial electrochemical technology, hydroponic systems can enhance soil carbon sequestration, thereby improving long-term carbon storage.

The presentation will explore the role of substrate and media selection in optimizing carbon storage, demonstrating how tailored hydroponic systems can replace traditional soil in supporting urban phytoremediation. It will also discuss the potential of combining biotechnological interventions with these systems to enhance carbon sequestration and climate adaptation outcomes.

MARK WALKER University of Hull

Outcomes from the 'New Biomethane' Workshop: Exploring the Future Pathways and Technologies for Biomethane Production Beyond Biogas Upgrading

JAMES CHONG^a, ELIZABETH HEIDRICH^b, MARK WALKER^{c,*}, YUE ZHANG^d a - Department of Biology, University of York, York, UK, YO10 5DD. b - School of Engineering, Newcastle University, Newcastle upon Tyne, UK, NE1

a - Department of Biology, University of York, York, UK, YO10 SDD, b - School of Engineering, Newcastle University, Newcastle upon Tyne, UK, NE1 7RU. c.- School of Engineering, University of Hull, Hull, UK, HU6 7RX. d. - Water and Environmental Engineering Group, University of Southampton, Southampton SO16 7QF, UK. *Corresponding author: mark.walker@hull.ac.uk

In January 2025 the EBNet Working Groups in Bioinformatics, Bioelectrochemical Systems, Anaerobic Digestion and Anaerobic Fermentation co-hosted a workshop bringing together key individuals in the field of novel biological pathways to produce Renewable Natural Gas (RNG), termed 'New Biomethane'. The aim of the workshop was to assess new technologies and sources and put them in the context of national & international decarbonisation of energy and adjacent sectors. The event took place over 3 days and was attended by ~30 individuals with a range of backgrounds and expertise including representation from academia, industry, research funders and government.

The scope of the workshop was defined to include the production of biomethane from novel or emerging processes and/or sources such as conversion of biomass through bioelectrical processes or CO_2 biomethanation. This meant that conventional biogas upgrading (CO_2 scrubbing or removal from biogas), or production of methane/syngas from thermal biomass processing were outside of the scope. For the purpose of the workshop, mainly for convenience in terms of the scope, biomethane included any methane produced through a biological process, independent of the carbon source (i.e. biogenic or fossil).

The workshop was opened with introductions from attendees and a number of invited talks, followed by a series of working sessions where participants worked in smaller groups to complete a variety of (numbered) tasks. In the initial tasks, randomly assigned groups were given paper, pens and colour coded post-it notes to produce graphical representations of (1) a technology ecosystem diagram including technologies, feedstock materials and related industries/industrial sites, which were then appended to include (2) innovation and policy requirements, as well as barriers to development of the identified technologies. Between each graphical development stage, participants were given a limited number of coloured dots to place on other groups' diagrams to indicate most/least important aspects, as well as areas that required greater explanation, and groups were invited to clarify any of these.

During subsequent tasks the participants were allowed to self-allocate into three groups to cover the main technological pathways identified as (A) in situ CO_2 biomethanation in an anaerobic digestion plant, (B) biomethane production from wastewater in a microbial electrolysis cell (MEC), and (C) ex situ biomethanation of industrial (fossil or biogenic) CO_2 containing gasses. The groups were tasked with (3) creating a diagram to illustrate the innovation pathway to TRL 9 for the selected technology and application. In the final main task, the same groups were asked to (4) re- frame the diagram produced in (3) in a 3-circle Venn diagram containing the categories of 'Biology', 'Engineering/Practical' and 'Business Case'.

The workshop closed with a general discussion from participants along with an explanation of the next steps from the organisers, namely that the outputs from the workshop will include the raw data (images of the physical outputs produced and statistics surrounding the votes cast by participants), as well as digitized summaries of these, followed by interpretation by the organisers to be reviewed by attendees. The outputs will be attributed to all participants (with their permission) and these are expected to be developed into a position document which may be of interest to industry, research funders and policy makers.

WILLIAM NEWELL

Imperial College London

Exploring C1 growth constraints

Carbon fixation by microbes is an increasingly viable route for sustainable chemical production. However, solubility constraints require elaborate and expensive culture methods to feed gaseous carbon and energy sources to fermentation systems. Furthermore, coupling electricity generation to microbial CO₂ fixation has failed to yield successes at scale due to the difficulty in obtaining low cost electricity. We therefore present a new approach, which uses soluble electron carriers able to act as energy storage systems during the uptime periods of renewable power. These power microbial carbon fixation which then sustains synthesis of FDCA - a potential replacement for TPA, the principal monomer of PET plastic, which has dramatically shorter-lived microplastics as well as improved thermal and mechanical properties. Technoeconomic analysis shows that this could produce carbon-negative polymers at cost-competitive levels using current-generation renewable energy technologies.

PARTICIPANTS

List of Participants

| Name | Institution |
|------------------------------|--------------------------------|
| Derya Acar | KU Leuven |
| John Allport | University of Huddersfield |
| Ayodeji Aluko | University of Huddersfield |
| Mohammad Alyassin | University of Huddersfield |
| Selina Ambrose | Promethean Particles |
| Claudio Angione | Teesside University |
| Beate Averhoff | Goethe University Frankfurt |
| Claudio Avignone Rossa | University of Surrey |
| Charles Banks | CJC Labs Ltd |
| Kira Baur | University of UIm |
| Tina Baur | University of Tübingen |
| Frank Bengelsdorf | University of UIm |
| Sergio Blanco-Rosete | Mono Carbon |
| Rajesh Bommareddy | Northumbria University |
| Geoff Bryant | Calysta |
| Louise Byfield | EBNet NM |
| Angela Bywater | University of Southampton |
| Yvonne Burger | Goethe University of Frankfurt |
| Steve Chambers | SOSV |
| Brandon Childers | NCIMB |
| Benedict Claxton- Stevens | The University of Nottingham |
| John Cloughley | e4 Structures |
| Dominic Clyde-Smith | UCL |
| Christian Ebel | PhD Student |
| Antonia Ebert | The University of Queensland |
| Etini Etuk | University of Huddersfield |
| | |

List of Participants

| Name | Institution |
|-----------------------------|---|
| Nasim Farahmand | London Metropolitan University |
| Maximilian Flaiz | Wageningen University & Research |
| Linsey Fuller | P&G |
| Dana Galili | DSIT |
| Diego Orol Gomez | National Renewable Energy Cente (CENER) |
| Eleanor Hadley Kershaw | University of Exeter |
| Corinna Hartinger | University of Oxford |
| Rosalind Hay | CPI |
| Ashraf Hauwa Nyako | Postdoctoral Researcher |
| Sonia Heaven | University of Southampton |
| James Heffernan | The University of Queensland |
| Anne Henstra | Aerbio (former DeepBranch) |
| Robin Hoeven | University of Manchester |
| Sonia Holland | Diageo |
| Ivan Ilic | Electroarchaea |
| Gina Javanbakht | University of Huddersfield |
| Adrian Jervis | Unilever |
| Lingwei Jiang | University of Surrey |
| Ludovic Jourdin | Delft University of Technology |
| David Keating | Synata Bio |
| Jonathan Kennedy | CPI |
| Zu Khin | University of Huddersfield |
| Katalin Kovacs | The University of Nottingham |
| Christian Kröly | Wageningen University & Research |
| Kees Kwant | Mission Innovation |
| Abdullahi Muhammad Labbo | The University of Nottingham |

List of Participants

| Name | Institution |
|--------------------------------|------------------------------------|
| Eun Yeol Lee | Kyung Hee University |
| Mungyu Lee | Delft University of Technology |
| Qiang Li | CPI |
| Robert Mansfield | AerBio |
| Eleni Mavrothalassiti | Again Bio |
| Colin Miles | BBSRC |
| Volker Müller | University of Frankfurt |
| Rohit Murali | University of Surrey |
| Kyle Moynahan | TU Berlin |
| Ali Nawaz | University of Huddersfield |
| William Newell | Imperial College London |
| Annalisa Occhipinti | Teesside University |
| David Ortega | Phase Biolabs |
| Marilene Pavan | Lanzatech Inc |
| Timothé Philippon | University of Aarhus |
| Jo Philips | Aarhus University |
| Pawel Piatek | RISE Research Institutes of Sweden |
| Mark Poolman | Oxford Brookes University |
| Margaux Poulalier Delavelle | Phase BioLabs Ltd |
| Koen Quataert | Biobase Europe |
| Thomas Reed | Newcastle University |
| Kim Eileen Rennhack | University of Tübingen |
| Jane Renshaw | The University of Nottingham |
| Florian Rosenbaum | Goethe University of Frankfurt |
| Jennifer Roth | Goethe University of Frankfurt |
| | |

| Name | Institution |
|------------------------------------|-------------------------------------|
| Bashir Rumah | The University of Nottingham |
| Maria Constanza Sadino Riquelme | Modela |
| Frank Sargent | Newcastle University |
| Ari Satanowski | Francis Crick Institute |
| Phoebe Seltzer | Future Plant Capital |
| Oliver Sexton | Future Plant Capital |
| Wendy Solis | The University of Nottingham |
| Diana Machado de Sousa | Wageningen University & Research |
| Anna Stock | University of Munich |
| Raphael Trischler | Goethe University of Frankfurt |
| Victoria Udemezue | University of Tartu |
| Jose Antonio Velazquez Gomez | University of Tübingen |
| Elodie Vlaeminck | Bio Base Europe Pilot Plant |
| Kris Wadrop | CPI |
| Mark Walker | University of Hull |
| Sara Walker | University of Birmingham |
| Lara Marie Waschinger | Goethe University of Frankfurt |
| Sebastian Wenk | University of Groningen |
| Tithira Wimalasena | Corbion Innovation Centre |
| Klaus Winzer | The University of Nottingham |
| Craig Woods | Forge Genetics |
| Minyeong Yoo | The University of Nottingham |
| Andreas Zannetou | PhD student at Newcastle University |
| Ying Zhang | The University of Nottingham |
| Yue Zhang | University of Southampton |

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