Welcome to the Gas Fermentation Conference 2024

Advancing the field for creating a new Industry

Esteban Marcellin	University of Queensland
Lars K. Nielsen	University of Queensland & NNF Center for Biosustainability (CFB)
Diana Sousa	Wageningen University & Research
Michael Köpke	LanzaTech
Byung-Kwan Cho	KAIST

Practical information

Tide times

WED 21 Feb	THU 22 Feb	FRI 23 Feb	SAT 24 Feb	SUN 25 Feb
12:32 am 0.85m	1:15 am 0.75m	1:53 am 0.68m	2:26 am 0.66m	2:56 am 0.67m
7:00 am 3.02m	7:38 am 3.11m	8:13 am 3.15m	8:44 am 3.15m	9:12 am 3.1m
1:41 pm 0.89m	2:12 pm 0.84m	2:40 pm 0.82m	3:05 pm 0.81m	3:29 pm 0.8m
7:23 pm 2.3m	7:58 pm 2.41m	8:29 pm 2.49m	8:58 pm 2.54m	9:24 pm 2.57m

Sunrise: 5:45am; Sunset: 6:30pm

Map

All meals: Shearwater Restaurant; Conference: Wistari Meeting Room

Gas fermentation Conference 2024

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Day 1, 21 February 2024

07:30 to 08:00	Arrival to Gladstone	The recommended latest plane arrival at Gladstone would be 08:00 AM.
08:00 to 09:00 AM	Check-in at the Heron Island Ferry Terminal	Please make your way to the Gladstone Marina to take the Ferry to Heron Island. Ferry departs at 09:30
09:30 to 11:30	Ferry to Heron Island	We recommend bringing seasickness tablets for the ferry ride, as it can be rough at times. Your comfort and safety are our top priority.

Lunch 12:00 to 13:00

Session 1

Chair: Lars Nielsen

AV: Damien Cleary

2:30	Lars Nielsen and Esteban Marcellin	Opening Remarks
2:45	Alfred Spormann	Allocation of Cellular Resources in Thermoanaerobacter kivui under Catabolic and Anabolic Limitation
2:55	Bastian Molitor	Thermophilic methanogens in biotechnology: Implementing a genetic toolbox for biomethanation and beyond
3:05	Surbhi Jain	Genetics and physiology of the thermophilic acetogenic bacterium Thermoanaerobacter kivui lacking key genes coding for electron re-cycling enzymes
3:15	Yutaka Nakashimda	Thermophilic production of bulk chemicals from gaseous substrates by genetically engineered Moorella thermoacetica
3:15-3:35	Questions	
3:35-6:30	Snorkelling Break	

Dinner 18:30 to 20:00

Session 2

Chair: Diana Sousa

AV: Daniel Bergen

8:00	Allison Pieja	Biodegradable Polymers from Methane: An Industrial Fermentation Scale-up Journey
8:10	Christian Kennes	Carbon oxides bioconversion and the carboxylate platform for the production of biofuels and bioproducts
8:20	Mirko Basen	Synthesis gas fermentation at high temperature – Thermoanaerobacter kivui as novel platform organism
8:30	Jorge Carrasco Muriel	A Bayesian kinetic model of the Wood-Ljungdahl pathway in Clostridium autoethanogenum
8:40	Axayacatl Gonzalez	Identification of independent regulated modules (iModulons) in Clostridium autoethanogenum during gas fermentation
8:50 -09:10	Questions	

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Day 2, 22 February 2024

Breakfast from 07:00 to 09:30

Session 3

Chair: Esteban Marcellin

AV: James Heffernan

10:00	Eleftherios (Terry) Papoutsakis	Molecular and Process Engineering of Mixotrophic Cocultures to Enable Complete Sugar-Carbon Utilization and CO2 Fixation for Achieving Transformative Metabolite Yields
10:10	Siebe Peek	Exploring pH effects on co-cultures of acetogens and Clostridium kluyveri for syngas fermentation
10:20	Nigel Minton	Genome Editing in Acetogens: I Can't Believe It's Not CRISPR
10:30	Pablo Nikel	Engineering Synthetic Metabolism in Non-Canonical Bacterial Hosts Towards C1-Based Bioproduction
10:40	Maximilian Flaiz	Developing robust CO-utilizing highly engineered anaerobic acetogens
10:50	Antonia Ebert	Systems Biology Investigation of the Reductive Glycine Pathway in Clostridium autoethanogenum
11:00 -11:20	Questions	

Lunch 12:00 to 13:00

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Session 4

Chair: Michael Köpke

AV: Axa Gonzalez Garcia

3:20	Phillipe Soucaille	From Systems Biology to Metabolic Engineering of Eubacterium limosum B2 for the Conversion of Methanol and CO2 to C4 Chemicals
3:30	Frank Bengelsdorf	Cleaning up and illuminating acetogenic Eubacterium strains for reclassification and metabolic engineering
3:40	Chris Greening	Efficient gas conversion by oxygen-insensitive hydrogenases and carbon monoxide dehydrogenasesEfficient gas conversion by oxygen-insensitive hydrogenases and carbon monoxide dehydrogenases
3:50	Birgitta Ebert	Hydrogen-Powered Biomanufacturing: Enhancing Carbon Efficiency and Sustainability
4:00	Daniel Bergen	Boosting Carbon Efficiency of Bioprocesses Using Hydrogen-Powered Whole Cell Biocatalysts
4:10-4:30	Questions	

Dinner 18:30 to 20:00

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Advancing the field for creating a new Industry

Session 5

Chair: Rajni Hatti-Kaul

AV: Hemanshi Galayia

8:00	Amy Rosenzweig	Methane fermentation at the molecular level
8:10	Jianhua Guo	Methane Bioconversion to Volatile Fatty Acids by Aerobic Methanotrophs via a Novel Fermentation Pathway
8:20	Christopher Johnson	Developing Cupriavidusnecatoras a host for conversion of CO2 to fatty acids
8:30	Bastian Blombach	Aerobic gas fermentation with carboxydotrophic Knallgasbacteria
8:40	Marie Schoelmerich	Extrachromosomal Elements in C1-metabolizing Microbes
8:50 -09:10	Questions	

Day 3, 23 February 2024

Breakfast from 07:00 to 09:30

Session 6

Chair: Jochen Forster

AV: Jorge Carrasco Muriel

10:00	Sean Simpson	Re-imagining manufacturing: Building a gas fermentation industry
10:10	Karen Rodriguez	Microbial gas fermentation as platform to produce isobutanol for sustainable aviation fuels
10:20	Lars Puiman	CFD-CRD predicts metabolic shifts in C. autoethanogenum based on the dissolved gas concentration gradients in industrial-scale syngas fermentation
10:30	Ralf Takors	Scaling up and scaling down gas fermentation: A quantitative view on the large scale performance to be mirrored in the lab
10:40	Catarina Rocha	Understanding C1 assimilating microorganisms through Metabolomics
10:50	Volker Sieber	Artificial enzyme cascades for C1-utilization
11:00 -11:20	Questions	

Lunch 12:00 to 13:00

Session 7

Chair: Paul Webley

AV: Evangeline Leong

3:20	Ramon Gonzalez	Orthogonal biomanufacturing from C1 feedstocks
3:30	Hariklia Gavala	Cases studies and variable mass transfer modeling for Gas Fermentations in Trickle Bed Reactors
3:40	Leonie van 't Hag	Biosynthesis of biodegradable polyhydroxyalkanoates (PHAs) for packaging materials from sustainable carbon sources – solving two problems with one solution
3:50	Michael Resch	Enhancing Value-added Production from Flue Gas-Derived CO2 through Integrated Electrochemical and Gas Fermentation Approaches
4:00	Jitendra Joshi	Living in Space and Lessons for Earth: A systems perspective.
4:10-4:30	Questions	

Dinner 18:30 to 20:00

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Session 8

Chair: Torben Vedel Borchert

AV: Karen Rodriguez Martinez

8:00	Ben Woolston	Exploring and Engineering Microaerobic Co-Culture for Carbon-Negative Bioproduction via Gas Fermentation
8:10	James Heffernan	CO(2)-metabolism: quantifying mixed carbon oxide and hydrogen gas fermentation by Clostridium autoethanogenum.
8:20	Joana Alves	Gas fermentation by novel biocatalysts within pressurized systems as new avenues for the sustainable production of biochemicals
8:30	Evangeline Leong	Integration of Direct Air Carbon Capture (DAC) Technology with Gas Fermentation - Accelerating the Biomanufacturing Industry Decarbonisation Journey
8:40	Volker Müller	Gas fermentation with acetogenic bacteria:Biochemistry, energetics and metabolic engineering
8:50 -09:10	Questions	

Day 4, 24 February 2024

Breakfast from 07:00 to 09:30

Session 9

Chair: Cheryl Kerfeld

AV: Antonia Ebert

9:35	Sabine Kleinsteuber	Merging carboxylate and syngas platforms: The power of mixotrophic communities
09:45	Ana Lopez Contreras	Model-driven design of bacterial communities with solventogenic strains for expanding the product range from gas fermentation
09:55	Jin Wang	Biogas conversion using methanotroph-photoautotroph cocultures: recent developments in coculture characterization, modeling and bioreactor design
10:05	Jens Kromer	Designing Productive Microbial Consortia Living on Water, Light, Electricity, N2, and CO2
10:15	Damien Cleary	An atlas of C1 pathways for X.
10:25	Chris Lawson	Engineering synthetic co-cultures for sustainable oleochemical production from C1 feedstocks
10:35 -10:45	Questions	

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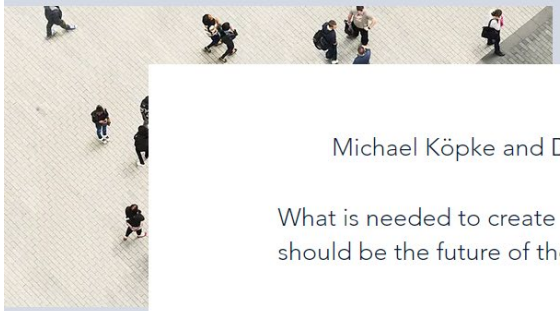

Michael Köpke and Diana Sousa: 11:00 12:00

What is needed to create a new economy, and what should be the future of the conference?

Lunch 12:00 to 13:00

Session 10

Chair: Bernardino Virdis

AV: Catarina Sobral da Rocha

13:05	Cheryl Kerfeld	Natural and Engineered Organelles for Catalysis in Confinement: Carboxysomes and other Bacterial Microcompartments
13:15	Taichen (Julian) Zhu	Exploiting a C1/C5 Bi-Substrate Scenario: Superior Growth Performance and Metabolic Insights in Methylotrophic Yeast Pichia pastoris
13:25	Danielle Tullman-Ercek	Engineering bacterial microcompartments for sustainable production of biochemicals
13:35	Stefano Donati	Understanding the metabolism of C1 assimilating microbes to engineer them for biomanufacturing
13:45	N. Cecilia Martinez-Gomez	TBC
13:55	Aindrila Mukhopadhyay	Strain engineering for conversion of non-canonical carbon sources to valuable products
2:00-2:20	Questions	
2:20-2:30	Esteban Marcellin and Lars Nielsen	Closing Remarks

Drinks on Veranda 18:30 to 19:30

BBQ Dinner 19:30 to 21:30

Day 5, 25 February 2024

Breakfast from 07:00 to 09:30

Checkout before 10:00

Lunch 11:30 to 12:30

Boat to Gladstone
12:45-14:45

Recommended earliest flight out of Gladstone would be 16:45

Session 1

Thermophilic production of bulk chemicals from gaseous substrates by genetically engineered *Moorella thermoacetica*

Yutaka Nakashimada[1], Junya Kato[1,2], Katsuji Murakami[2], Tatsuya Fujii[2], Tomotake Morita[2]

[1]Hiroshima University, Higashi-Hiroshima, Japan. [2]National Institute of Advanced Industrial Science and Technology, Higashi-Hiroshima, Japan

Abstract

The Ministry of Economy, Trade and Industry of Japan has formulated the Roadmap for Carbon Recycling Technologies in June 2019 with the aim of reducing CO_2 emissions. The Government of Japan declared 46% reduction of the greenhouse gases by 2030 compared to 2013, and the zero emissions by 2050. To achieve this goal, Japan has launched “the Green Innovation Fund”, aiming to build a world-class energy and material utilization system that combines economic efficiency and environmental performance.

The demanded technology for carbon recycling in bioindustry is a process that combines "high selectivity" for the target chemicals with "cost-effectiveness" based on "energy saving" of the production process. As such a technology, we have been advancing research and development of the high temperature gas fermentation process. A thermophilic gas fermenting microorganism, Moorella thermoacetica, mainly produces acetic acid using syngas and H_2/CO_2 as substrates. Since it grows vigorously between 50 and 60 °C, it is possible to carry out fermentation with simultaneous distillation in the production of volatile compounds. By combining the production and separation processes, the medium supply is almost unnecessary and the wastewater treatment can be minimized. We, therefore, have developed a genetic engineering technology of this thermophilic acetogen to produce bulk chemicals of low boiling temperatures. Utilizing this technology, we have systematically constructed genetically modified strains that produce C2 and C3 compounds using sugar, syngas, and H_2 / CO_2 as substrates. In the presentation, we will show the strategy and achievement to develop high yield ethanol-producing strain from syngas.

Categories

1. Anaerobes (e.g. acetogens)

Session 1Thermophilic methanogens in biotechnology: Implementing a genetic toolbox for biomethanation and beyond

Bastian Molitor

University of Tübingen, Tübingen, Germany

Abstract

Thermophilic *Methanothermobacter* strains have been used as model microbes to study the physiology and biochemistry of the conversion of molecular hydrogen and carbon dioxide into methane. The microbe *Methanothermobacter thermautotrophicus* is already applied as the industrial biocatalyst for the biological methanation step in large-scale power-to-gas processes. The power-to-gas platform is utilized to store renewable electric power and decarbonize the natural gas grid. To improve the biocatalyst, genetic engineering is required. Yet, a genetic system for these model microbes was missing despite intensive work for four decades. We recently reported the successful implementation of genetic tools for *M. thermautotrophicus* ΔH. We developed shuttle vectors that replicated in *Escherichia coli* and *M. thermautotrophicus* ΔH, and that can be transferred from *E. coli* into *M. thermautotrophicus* ΔH *via* interdomain conjugation. This allowed heterologous production of enzymes, such as a thermostable β-galactosidase and a formate dehydrogenase. Furthermore, we implemented an allelic exchange method to introduce chromosomal mutations, which we applied to delete genes that encode for fimbriae (*i.e.*, cell appendages) and to study the effect of the fimbriae on biotic cell-cell connections. With our genetic tools, we are now targeting the production of chemicals from molecular hydrogen and carbon dioxide with *M. thermautotrophicus* ΔH as a platform host.

Categories

1. Anaerobes (e.g. acetogens)

Session 1

Genetics and physiology of the thermophilic acetogenic bacterium *Thermoanaerobacter kivui* lacking key genes coding for electron re-cycling enzymes

Surbhi Jain[1,2], Volker Müller[2]

[1]Monash University, Clayton, Australia. [2]Johann Wolfgang Goethe University, Frankfurt, Germany

Abstract

Thermoanaerobacter kivui is a thermophilic acetogenic bacterium that fixes atmospheric CO_2 and produces acetate *via* acetyl-coenzyme A in the Wood-Ljungdahl pathway (WLP). The ability to utilise CO_2 or CO or the combination of both with H_2 (also known as syngas) make acetogens ideal candidates for sustainable bio-economy. The metabolism of *T. kivui* under autotrophic and heterotrophic conditions remains largely elusive. Here, we used genetic approaches to understand the role of key genes of WLP such as hydrogen-dependent carbon dioxide reductase (*hdcr*), monofunctional CO-dehydrogenase (*cooS*) and electron-bifurcating hydrogenase (*hydABC*). The HDCR reduces CO_2 to formate in the first step of WLP. The markerless deletion of *hdcr* gene led to no growth of *T. kivui* on H_2 + CO_2 or on sugars. However, addition of formate as electron acceptor restored growth . The molecular basis for the adaptation of *T. kivui* to grow on CO was unclear. The genome of *T. kivui* encodes a putative monofunctional CODH (*cooS*) and a bifunctional CODH (*acsAB*). Deletion of the *cooS* gene led to a complete loss of growth on CO, demonstrating the essentiality of CooS for growth on carbon monoxide. *T. kivui* can oxidize or produce H_2 by the electron-bifurcating hydrogenase. With the markerless deletion of *hydAB* genes, *T. kivui* did not grow autotrophically but no phenotype was observed for growth with sugars, which implies the dispensability of *hydAB* in the heterotrophic metabolism. Altogether, deletion mutations helped in modelling the metabolic schemes of *T. kivui*.

Categories

1. Anaerobes (e.g. acetogens)

Session 1

Allocation of Cellular Resources in *Thermoanaerobacter kivui* under Catabolic and Anabolic Limitation

Alfred Spormann

Novo Nordisk Foundation CO2 Research Center, Stanford University and Aarhus University, Aarhus, Denmark

Abstract

Microorganisms allocate limited cellular resources efficiently to catabolic, anabolic, and maintenance processes. For example, *Escherichia coli,* when growing aerobically using glucose, shifts its metabolism during fast growth to shorter but less efficient pathways to accommodate a larger anabolic proteome (especially higher ribosome content) required for faster growth. In contrast, the hydrogenotrophic methanogen *Methanococcus maripaludis* does not change its proteome composition over a wide range of growth rates under energy-limited conditions, which is presumably more suitable for a niche-specialized organism lacking metabolic versatility. We studied growth rate-dependent resource allocation using anaerobic chemostates in *Thermoanaerobacter kivui* under catabolic and anabolic limitation to gain more insight into the resource allocation strategy of this acetogen with biotechnological relevance. Our results indicate a substantial proteome re-allocation in *T. kivui* in response to growth rate and provide further systems-level insight into bacterial metabolism and ecophysiological strategies.

Categories

1. Anaerobes (e.g. acetogens)

Session 2

Carbon oxides bioconversion and the carboxylate platform for the production of biofuels and bioproducts

Christian Kennes, María C. Veiga

University of La Coruña, La Coruña, Spain

Abstract

Carbon monoxide and carbon dioxide are two major air pollutants of increasing environmental concern, with carbon dioxide playing a key role in climate change. On the other side, it is well known that acetogenic bacteria are able to metabolize either one or both of them. Originally, our first studies, started more than a decade ago, focussed on the acetogenic-solventogenic bioconversion of CO or CO_2 (together with H_2) gases into 2 to 6 carbon volatile fatty acids (acetic, butyric, caproic acids) and alcohols (ethanol, butanol, hexanol). More recently, our research has focused on the opportunities provided by the carboxylate platform, i.e., fermentation of C_1 gases into fatty acids for the subsequent production of biofuels or bioproducts, of potentially higher commercial value, from those acids. This includes chain elongation of short chain fatty acids (acetate and/or butyrate) and ethanol, obtained though gas fermentation, into higher value medium chain fatty acids, i.e., caproate and caprylate. Besides, we have also performed studies on the accumulation of biopolymers (e.g., PHA, PHB) (>40%) by aerobic cultures grown on carboxylic acids resulting from gas fermentation, in sequencing batch reactors. Additionally, different yeast strains able to metabolize C_2-C_6 carboxylic acids and accumulate microbial oils have been used in two stage acetogenic-yeast fermentation processes, accumulating up to more than 40% lipids intracellularly. Methods are also being optimized for the recovery of those intracellular compounds with environmentally-friendly biosolvents. Finally, bioreactors were set-up and optimized for the direct bioconversion of carbon oxides into acetone and iso-propoanol by engineered acetogenic bacteria.

Categories

1. Anaerobes (e.g. acetogens)

Session 2

A Bayesian kinetic model of the Wood-Ljungdahl pathway in *Clostridium autoethanogenum*

Jorge Carrasco Muriel[1], Teddy Groves[1], Lars Nielsen[2]

[1]The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Copenhagen, Denmark. [2]Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland, Brisbane, Australia

Abstract

Clostridium autoethanogenum is an acetogen used commercially to produce ethanol and other byproducts from C^1 sources. This capability of byproduct formation makes it an attractive biotechnological platform to produce carbon negative commodities. However, the link between external conditions and byproduct fraction is still not well understood. Here, we present a Bayesian kinetic model of the carbon intake and byproduct formation of acetate, ethanol and pyruvate derivatives. By careful statistical treatment of multi-omics data, we show how a model of this kind is able to compare the likelihood of different alternative pathways, explain the effect of competitive inhibition of CO across conditions and make suggestions for engineering targets to enhance the production of a particular byproduct.

Categories

1. Anaerobes (e.g. acetogens)

Session 2

Synthesis gas fermentation at high temperature – *Thermoanaerobacter kivui* as novel platform organism

Mirko Basen

University of Rostock, Rostock, Germany

Abstract

Synthesis gas conversion to products ("fermentation") has been established with mesophilic bacteria including *Acetobacterium woodii, Clostridium authoethanogenum* and *Clostridium ljungdahlii*, and commercialized by the company LanzaTech. From the technological point, however, it is advantageous to operate bioreactors at higher temperatures, at foremost due to higher turnover rates and cooling costs, amongst other advantages.

The thermophilic acetogen *Thermoanaerobacter kivui* (Topt 66°C) grows at with H_2+CO_2 or sugars (td <1.5 h), and it has been adapted to thrive on CO as well, with acetate as sole metabolic product. Towards understanding its physiology and the development of an industrially-relevant platform strain, we developed a genetic system that allows for genome integration and plasmid-based protein overproduction. We studied the metabolism of *T. kivui* and its regulation during growth on different substrates, revealing surprises in the redox and energy metabolism. With regards to its bioenergetics, *T. kivui* is now the best understood thermophilic acetogen and serves as a model organism to better understand acetogenesis at high temperatures.

Based on these fundamental findings, we then engineered several strains of *T. kivui* for ethanol production from synthesis gas. These strains have been studied in small-scale batch experiments already, and their biotechnological potential is currently evaluated together with collaboration partners e.g. from the University of Tübingen (Prof. Lars Angenent). Moreover, we developed a reporter-gene assay towards finding inducible promoters that may allow fine tuning of *T. kivui* metabolism towards a faster conversion of synthesis gas to a broader spectrum of industrially-relevant products in the near future.

Categories

1. Anaerobes (e.g. acetogens)

Session 2

Identification of independent regulated modules (iModulons) in Clostridium autoethanogenum during gas fermentation

Axayacatl Gonzalez[1,2], Karen Rodriguez[1], James Heffernan[1], Jorge Carrasco[3], Lars Nielsen[1,3], Esteban Marcellin[1]

[1]The University of Queensland, Brisbane, Australia. [2]Integrated Design Environment for Advanced biomanufacturing (IDEA bio)., Brisbane, Australia. [3]Novo Nordisk Foundation Center for Biosustainability, Copenhagen, Denmark

Abstract

The increasing awareness of the growing carbon dioxide levels in the atmosphere has driven the development of strategies and technologies towards reducing carbon emissions. Now, the utilization of CO2 is becoming a promising field, given the interest in transitioning towards sustainable development and environmental protection. In this regard, autotrophic acetogenic bacteria are the most efficient known microbes for fixing carbon oxides CO2 and CO. Gas fermenting microorganisms such as Clostridium autoethanogenum are strict anaerobes, limiting their handling to close spaces under anoxic conditions. On this regards, we have developed a non-invasive biomass monitoring system to characterize cell growth and implement a phenomics analysis of C. autoethanogenum strains. Here we have evaluated 25 different growth conditions. We recovered the RNA and proteins during the exponential growth phase for transcriptomics and proteomics analysis. We expect that the integrated data will provide new insight in the mechanism that participate in the assimilation of difference carbon sources and the response of C. autoethanogenum to environmental stimuli that can be used to develop rational engineering strategies.

Categories

5. Synthetic Biology tools for gas fermentation

Session 2

Biodegradable Polymers from Methane: An Industrial Fermentation Scale-up Journey

Allison Pieja, Molly Morse

Mango Materials, Oakland, USA

Abstract

Mango Materials is a California-based start-up that uses methane gas to produce biodegradable biopolymers that are economically and functionally competitive with conventional, oil-based plastics. Mango Materials produces powder or pellets of poly-hydroxyalkanoate (PHA), a valuable product that can be converted into a variety of high-margin or high-volume, environmentally friendly goods such as textiles, injection-molded packaging or other products, or films.

Methane is a potent greenhouse gas often produced as a byproduct at sites such as wastewater treatment plants, landfills, and agricultural facilities. In this process, biogas methane is directly utilized as a feedstock for bacteria, which produce PHA intracellularly in a fermentation process. The PHA is then separated from the non-polymer cell mass and used in a variety of downstream applications.

Mango Materials currently operates its Launch facility to produce PHA from biogas methane in the San Francisco Bay Area. The plant produces samples for customer validation and validates the use of equipment that will be used at commercial scale. Mango Materials is currently working on scaling towards its Flagship Plant, a first-of-its-kind commercial plant to produce PHA from methane.

This presentation will discuss Mango Materials' scale-up journey and the path to full-scale commercialization of its technology.

Categories

4. Industrial gas fermentation

Session 3

Genome Editing in Acetogens: I Can't Believe It's Not CRISPR

Nigel P. Minton, Craig Woods, Margaux Delavelle, Francois Seys, James Millard, Christopher Humphreys

University of Nottingham, Nottingham, United Kingdom

Abstract

Nottingham have played a pivotal role in the development of genetic tools for anaerobes. Initially, insertional mutagens for both forward and reverse genetic studies were developed. Whilst ClosTron technology received widespread attention, the use of transposons to generate random mutants also has great potential, ie., TraDIS (Transposon-directed Insertion Sequencing) allows the determination of gene essentiality during autotrophy. Thus, we identified all of those genes essential for growth on CO (*Clostridium autoethanogenum*), CO_2 (*Acetobacterium woodii*) and methanol (*Eubacterium limosum*). Moreover, TraDIS libraries identified mutants with improved autotrophic growth in fermenters.

Post-ClosTron, efforts focussed on creating in-frame deletion mutants by allelic exchange. These were isolated from the background wildtype population using counter selection markers, eg., *codA*, *pyrE/F*, *upp*, toxin/anti-toxin genes and more recently CRISPR/Cas. The latter has favoured the Cas9 of *Streptococcus pyogenes*, eg., RiboCas. More recently we demonstrated the advantages of the endogenous Type I-B CRISPR/Cas systems of *C. autoethanogenum* and *A. woodii* as well as the potential of Target-AID for multiplex genome editing .

CRISPR/Cas9 systems, however, have several disadvantages. Cas9 has off-target effects, distributed license agreements for its use are expensive and the long-lasting patent battle between the two major players has caused uncertainty with potential licensees. We have, therefore, developed an alternative procaryotic engineering biology tool, as effective as CRISPR/Cas9, in some ways better. It has no known off-target effects and has been fully exemplified in several clostridia. As its component parts work in numerous other microbes, the system has wide application.

Categories

5. Synthetic Biology tools for gas fermentation

Session 3

Systems Biology Investigation of the Reductive Glycine Pathway in *Clostridium autoethanogenum*

Antonia Ebert[1,2], James Heffernan[1,2], Tim McCubbin[1,2], Dara Daygon[3], Gabriele Netzel[3], Terra Stark[3], Michael Köpke[4], Esteban Marcellin[1,2,3]

[1]Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, Australia. [2]ARC Centre of Excellence in Synthetic Biology, The University of Queensland, Brisbane, Australia. [3]Metabolomic and Proteomics Australia, Queensland Node, The University of Queensland, Brisbane, Australia. [4]LanzaTech, Skokie, USA

Abstract

The highly efficient Wood-Ljungdahl pathway (WLP) converts CO and CO_2 to chemicals and fuels, a sought-after bioprocess ideal. The WLP is common to acetogens, which includes the model industrial organism *Clostridium autoethanogenum*, utilised in LanzaTech's industrial scale CO-to-ethanol process. However, more carbon fixation pathways exist in nature: for instance, the reductive glycine (RGP) and the glycine synthase reductase pathway (GSRP) have been identified in numerous acetogens. While the WLP is well-characterised in *C. autoethanogenum*, the role of alternative one-carbon assimilation pathways have not been investigated thoroughly yet, and may be a novel avenue for bioprocess optimisation.

A multi-omics analysis of a recently evolved lineage of this strain for enhanced growth on CO_2/H_2 mixtures suggested that the RGP could be active in this acetogen. In this work we phenotypically characterise *C. autoethanogenum* with proteomics and ^{13}C-tracer studies using [U-^{13}C]glycine. We demonstrate activity of the RGP in *C. autoethanogenum* and show that the GSRP is not present in this organism. Consistent with previous work, our study shows that co-utilisation of the WLP and RGP assists metabolic homeostasis under gas fermenting conditions, where redox imbalances could otherwise severely hamper pathway thermodynamics. Further understanding the pathways' synergies under autotrophic conditions (CO_2/H_2) will help promote the development of this industrially relevant host.

Categories

1. Anaerobes (e.g. acetogens)

Session 3

Exploring pH effects on co-cultures of acetogens and *Clostridium kluyveri* for syngas fermentation

Siebe Peek[1], Ivette Parera Olm[1,2], Martijn Diender[1,2], Diana Sousa[1,2]

[1]Laboratory of Microbiology, Wageningen University and Research, Wageningen, Netherlands.
[2]Centre for Living Technologies, Eindhoven-Wageningen-Utrecht Alliance, Utrecht, Netherlands

Abstract

Clostridium autoethanogenum has been co-cultivated with *Clostridium kluyveri* to expand the product spectrum of syngas fermentation via chain elongation. However, a significant challenge observed in this co-culture is the discrepancy between the optimal pH requirements of the two microorganisms. Sustained production of ethanol by the acetogen —optimal at mildly acidic conditions in *C. autoethanogenum* – is key to maintain chain-elongation activity by *C. kluyveri*, which grows best at neutral pH. In this work, we explored the effect of pH on co-cultures using different acetogens with C*. kluyveri*. The selected acetogens, namely *C. autoethanogenum*, *Clostridium carboxidivorans*, *Acetobacterium wieringae* strain JM, *Alkalibaculum bacchi*, *Eubacterium limosum*, and *Clostridium aceticum* were chosen based on their different optimal pH for growth varying in a range from 6 to 8.5. Our goal was to identify the combinations of acetogen-*C. kluyveri* and pH that result in maximum CO and H_2 conversion, and produce the highest titres of medium-chain carboxylic acids (butyrate and caproate).

A. wieringae JM and *C. autoethanogenum* were the best-performing strains in co-cultures with *C. kluyveri* in batch cultivations to produce butyrate and caproate at pH values 7–7.5 and 6, respectively. Moreover, with repeated refilling of the headspace, the *A. wieringae* JM-*C. kluyveri* co-cultures at pH 7.5 almost exclusively produced butanol (17 mM) and hexanol (15 mM), highlighting the solventogenic potential of this co-culture. To further enhance productivity and titres, and explore the solventogenic potential of the co-cultures further, ongoing work involves bioreactor experiments with control of pH and controlled, continuous CO load using the most promising co-cultures.

All in all, this study contributes with the first combined acetogen and pH screening for optimal co-cultivation with *C. kluyveri* for the revalorisation of syngas (CO/H_2). Additionally, the findings of this study contribute with insights into the physiology of different acetogens.

Acknowledgments

S.P. research is funded by a PhD grant of the WIMEK Graduate School of Wageningen University. Furthermore, this research was funded by the NWO domain Applied and Engineering Sciences (AES) (Perspectief Programma P16-10) and the Centre for living technologies (CLT).

Categories

1. Anaerobes (e.g. acetogens)

Session 3

Developing robust CO-utilizing highly engineered anaerobic acetogens

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Abstract

Acetogenic bacteria are potent utilizers of C1-carbon sources, including greenhouse gases like CO_2. However, their narrow natural product range often makes their use on a large scale unappealing. To unlock their industrial potential, it is essential to employ molecular tools for both understanding their physiology but also enhancing their bioproduction. While a variety of tools are available for several acetogens, there is still plenty of room for improvements in genomic engineering, particularly in the realms of gene insertions and knockouts. In our studies, we focused on applying molecular tools for the industrial-relevant acetogen *Clostridium autoethanogenum*, the natural butyrate producer *Eubacterium limosum*, and the narrowly studied CO-tolerant *Acetobacterium wieringae* JM strain.

We applied the CRISPR-Cas-based genome engineering tool 'SIBR-Cas' to integrate the innovative anaerobic reporter protein known as 'fluorescence-activating and absorption shifting tag' (FAST) into the genome of *C. autoethanogenum*. This engineered *C. autoethanogenum*::FAST strain serves a crucial role in synthetic co-cultures by enabling easy differentiation, based on its fluorescence, from the second co-culture partner. Notably, a plasmid-based approach failed to provide the required homogeneity within the FAST-producing *C. autoethanogenum* population. In addition, we targeted genes of the butyrate production operon (*bcs*)-operon of *E. limosum* through homologous recombination using linear DNA, resulting in the creation of knockout mutants. Respective knockout strains, in which *bcs*-gene expression is interrupted, serve as the foundation to investigate butyrate metabolism and to redirect its metabolism to more favorable, non-native products. Moreover, we conducted initial experiments to enable genomic engineering of *A. wieringae* JM strain. These efforts encompassed the establishment of fundamental tools such as reporter proteins and various promoters.

Our results substantially contribute to the development of novel and robust CO-utilizing, highly engineered acetogen, which can be employed in mono- or co-cultures to produce valuable industrial platform chemicals.

Categories

5. Synthetic Biology tools for gas fermentation

Session 3

Engineering Synthetic Metabolism in Non-Canonical Bacterial Hosts Towards C1-Based Bioproduction

Pablo I. Nikel

DTU Biosustain, Kgs Lyngby, Denmark

Abstract

Industrial chemical production largely relies on fossil fuels nowadays, resulting in the unavoidable release of carbon dioxide (CO_2) into the atmosphere. The concept of a circular carbon bioeconomy has been proposed to address this issue, wherein CO2 (and derived molecules) is captured and used as a raw material for manufacturing new chemicals and products. Microbial cell factories, either natural or engineered, emerged as potential catalysts for upcycling CO2 and other one-carbon (C1) substrates to value-added products. Among the range of feedstocks available to this end, formate is a promising, water-soluble C1 substrate for biotechnology that can be efficiently produced from CO_2 by electrochemistry—but formatotrophy has been engineered in just a few industrially-relevant microbial hosts. We addressed the challenge of widening the spectrum of cell factories tailored for C1-based bioproduction by adopting *Pseudomonas putida* as a robust platform to engineer synthetic C1 assimilation. Here, the metabolism of *P. putida* was deeply rewired to establish synthetic auxotrophies that could be functionally complemented by expressing components of synthetic metabolisms designed for C1 assimilation. This general principle will be illustrated with different pathways and synthetic modules, including examples of the reductive glycine pathway and metabolic C1 shunts.

Categories

7. Novel organisms

Session 3

Molecular and Process Engineering of Mixotrophic Cocultures to Enable Complete Sugar-Carbon Utilization and CO2 Fixation for Achieving Transformative Metabolite Yields

Eleftherios Terry Papoutsakis, Hyeongmin Seo, Noah Willis, Sofia Capece, John Hill, Jonathan Otten

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Abstract

Developing a carbon neutral (CNeu) or negative (CNeg) platform for production of chemicals is a compelling approach for sustainable biomanufacturing. Sugar catabolism leads to loss of a third of the carbon as CO2. To overcome this, we needs engineered metabolism and electron sources. An approach is acetogenic mixotrophy with one organism, whereby sugar substrates used together with electron-rich sources (eg, H_2) and additional CO_2 are the basis for CNeu/CNeg manufacturing (*Nature Commun.* 7: 12800 (2016). To attain modularity and increase process intensification, synthetic syntrophic cocultures offer potent opportunities thus extending the potential of acetogenic mixotrophy. Syntrophy is obligately mutualistic metabolism to enable culture stability. Three questions emerge in dissecting such systems. How do cells of different species communicate with each other; how does the metabolism of each affects the other; and how to tune metabolic engineering strategies to attend to partner behavior given the communication complexity.

We address these questions by dissecting the synthetic *Clostridium acetobutylicum* (*Cac*) and *C. ljungdalhii* (*Clj*) syntrophy aiming to produce isopropanol (IPA) and other solvents with no carbon loss plus using exogenously-supplied CO_2 and electrons. We engage both strain engineering and bioreactor- operation strategies working synergistically to achieve targeted performance metrics. We show that metabolic engineering of *Cac* and *Clj* aiming to achieve CNeu and CNeg fermentatiosn poses challenges in electron management never previously encountered. The concept is generalizable for production of other native and non-native metabolites.

Supported by ARPA-E Contract DE-AR0001505.

Categories

5. Synthetic Biology tools for gas fermentation

Moved to Session 5 as per schedule

Aerobic gas fermentation with carboxydotrophic Knallgasbacteria

Bastian Blombach

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Abstract

Industrial waste gases emit large amounts of greenhouse gases into the atmosphere and may contain significant amounts of CO_2, CO, and H_2 but depending on the source also O_2 (e.g. from the cement industry). Such gas steams represent an abundant source for gas fermentation to produce chemicals with microbial catalysts. Anaerobic gas fermentation has become a technology of industrial maturity and first production plants have been successfully installed. However, due to the anaerobic lifestyle, the commonly applied acetogenic bacteria are energy limited and the production of ATP-demanding molecules might become challenging. To overcome this limitation, we recently proposed to utilize carboxydotrophic Knallgasbacteria as cell factories to convert O_2-containing waste gases into valuable chemicals. Aerobic carboxydotrophic Knallgasbacteria are a group of microorganism that possess the unique trait to oxidize CO as a sole energy source with O_2 to produce CO_2 which subsequently is used for biomass formation via the Calvin-Benson-Bassham cycle. Moreover, most of these organisms are also able to oxidize H_2 with hydrogenases to drive the reduction of carbon dioxide in the absence of CO. These properties render this group of bacteria promising biocatalysts for aerobic gas fermentation. One representative is *Hydrogenophaga pseudoflava* which shows comparable high growth rates under autotrophic conditions. We sequenced and annotated the genome of *H. pseudoflava* and established a genetic engineering toolbox, which allows markerless chromosomal modification and heterologous gene expression. As proof of concept, we engineered this bacterium for the aerobic production of the C_{15} sesquiterpene (E)-α-bisabolene from syngas.

Categories

7. Novel organisms

Session 4

Cleaning up and illuminating acetogenic *Eubacterium* strains for reclassification and metabolic engineering

Maximilian Flaiz[1,2], Anja Poehlein[3], Wiebke Wilhelm[1], Rolf Daniel[3], Peter Dürre[1], Frank R. Bengelsdorf[1]

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Abstract

Acetogens in the genus *Eubacterium* are well elucidated and especially known for their ability to produce butyrate from methanol. Recently, some Eubacterium strains have attracted attention due their ability to reduce greenhouse gases (CO and CO_2) and because of their potential in metabolic engineering approaches, which brands them interesting candidates to become biocatalyst in gas fermentation.

We examined genomic, phylogenic, physiologic, and genetic features of 11 Eubacterium strains and assigned all of them into three distinct clades dominated by the type-strains of *E. limosum*, *E. callanderi*, and *E. maltosivorans*. Moreover, in the respective genome sequences we analyzed the gene clusters encoding gene products that facilitate methanol utilization and reverse beta-oxidation. Corresponding growth experiments revealed that strains from all clades are capable of converting methanol and produce acetate, butyrate, and hexanoate via chain elongation. In addition to that an efficient electroporation protocol was established, which is ideally suited to constructed recombinant cells of in total eight different Eubacterium strains. A plasmid encoding the fluorescence-activating and absorption shifting tag (FAST) was used as a fluorescent reporter to rapidly verify successfully constructed recombinant strains on single cell-level using flow cytometry. Moreover, FAST production controlled by the strong constitutive ferredoxin promoter (Pfd) from Clostridium ljungdahlii caused a homogenous population of 98.2 % fluorescent cells in 6 of 8 tested *Eubacterium* strains.

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

Categories

5. Synthetic Biology tools for gas fermentation

Session 4

Hydrogen-Powered Biomanufacturing: Enhancing Carbon Efficiency and Sustainability

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Abstract

Bioproduction using microbial fermentation holds promise for sustainable manufacturing. However, current processes suffer from significant carbon loss, releasing over 30% as CO_2 during fermentation. This not only reduces product yield but also contributes to the emission of valuable feedstock carbon in the form of the potent greenhouse gas CO_2. Enhancing carbon efficiency during bioconversion is crucial to improving the overall efficiency of the bioprocess.

We propose the utilization of hydrogen as an auxiliary, carbon-free energy source to decouple carbon and energy metabolism in heterotrophic microbial fermentations. This strategy aims to suppress the oxidation of the organic carbon source into CO_2 for energy formation, thereby minimizing carbon loss and increasing the efficiency of converting carbon into desired products. By coupling H_2 utilization with any organic carbon source, we can fully leverage the metabolic versatility of microbes. This approach enables the conversion of a wide range of feedstocks, including agricultural and industrial waste streams, while optimizing the atom economy of bioproduction.

The presentation will showcase results from metabolic modelling studies exploring the potential of H_2-co-feeds in various scenarios, converting different substrates derived from abundant renewable feedstocks or waste streams into industrially relevant products. Our investigations assume the utilization of *Pseudomonas putida*, an attractive and widely adopted host for industrial biotechnology. The findings will be discussed regarding the potential of H_2-powered biomanufacturing to drive carbon efficiency, reduce greenhouse gas emissions, and maximize resource utilization.

Categories

3. Aerobic hydrogenotrophs

Session 4

Boosting Carbon Efficiency of Bioprocesses Using Hydrogen-Powered Whole Cell Biocatalysts

Daniel Bergen[1], Pablo Ivan Nikel[2], Esteban Marcellin[1], Birgitta Ebert[1]

[1]Australian Institute for Bioengineering and Nanotechnology, Brisbane, Australia. [2]Technical University of Denmark, Copenhagen, Denmark

Abstract

Current microbial workhorses in biotechnology, such as Escherichia coli, Corynebacterium glutamicum, and Pseudomonas putida, are widely utilised for industrial production of biofuels, nutrients, and pharmaceuticals. Although bioprocesses are often more sustainable than traditional chemical production methods, they still result in significant CO2 emissions. These emissions stem from the requirement of cells to generate reduced redox cofactors (NAD(P)H, PQQH2, FADH2, FMNH2, etc.) for fuelling energy production, growth and biosynthesis. Thereby regeneration of redox cofactors primarily occurs through the oxidation of carbon metabolites, predominantly in the tricarboxylic acid cycle, leading to the release of CO2. However, not only is atmospheric CO2 a major contributor to global climate change, the loss of CO2 also reduces the carbon efficiency of biotechnological processes.

Through computational modelling, we have discovered that 30-50% of the available carbon is not utilised for biomass or product formation but is instead oxidised to CO2 solely for NADH regeneration. To address this limitation, we have investigated alternative electron sources. Previous proposals, such as the auxiliary substrate concept introduced by Babel et al. (2009), suggested the use of supporting carbon substrates like formate or acetate to retain more carbon from the primary carbon source. However, these concepts still resulted in carbon metabolite oxidation and CO2 production. In this study, we suggest employing hydrogen as a carbon-free energy source. Our simulations using hydrogen as an electron source demonstrate complete elimination of carbon oxidation for NADH regeneration, effectively eliminating CO2 emissions, maximising bioprocess efficiency and resource utilization.

Categories

5. Synthetic Biology tools for gas fermentation

Session 4

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

Philippe Soucaille

University of Toulouse, Toulouse, France

Abstract

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

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

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

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

Categories

1. Anaerobes (e.g. acetogens)

Session 4

Efficient gas conversion by oxygen-insensitive hydrogenases and carbon monoxide dehydrogenasesEfficient gas conversion by oxygen-insensitive hydrogenases and carbon monoxide dehydrogenases

Chris Greening

MOnash University, Melbourne, Australia

Abstract

Diverse microorganisms grow and survive through aerobic respiration of molecular hydrogen and carbon monoxide. We've shown that the obligate aerobe Mycobacterium smegmatis survives by consuming these gases at atmospheric concentrations. Here I will describe the functional and structural characterisation of the enzymes responsible, namely the [NiFe]-hydrogenase Huc and a [MoCu]-carbon monoxide dehydrogenase. These enzymes are both remarkable for their high substrate affinities, complete oxygen insensitivity, and high catalytic efficiencies. Structural characterisation through high-resolution cryo-EM microscopy demonstrates the molecular basis of these unusual catalytic features. Moreover, in a new paradigm for bioenergetics, these enzymes depend on long-range quinone transport via the previously uncharacterised proteins HucM and CoxG. These insights provide a foundation to develop oxygen-insensitive enzymes for whole-cell and enzyme-based gas fermentation.

Categories

4. Industrial gas fermentation

Session 5

Methane fermentation at the molecular level

Amy Rosenzweig

Northwestern University, Evanston, USA

Abstract

Under the mounting threat of climate change, increasing atmospheric methane concentrations are a constant source of concern and debate. Conversion of methane to desirable fuels and chemicals could simultaneously mitigate global warming and meet increasing energy demands. Industrial catalysts that can selectively activate the 105 kcal mol^{-1} C-H bond in methane require high temperatures and pressures, along with significant capital expenses. The use of biocatalysts produced by methanotrophic bacteria provides an environmentally friendly alternative. The primary biocatalyst in methanotrophic bacteria is the copper-containing, membrane-bound enzyme particulate methane monooxygenase (pMMO). Any use of methanotrophs for biological gas-to-liquids conversion or for bioremediation requires a detailed understanding of pMMO structure and function. Despite extensive research, the molecular details of the pMMO copper active site remain controversial, in part because the enzyme loses activity upon isolation from methanotroph membranes. Thus, it is critical to structurally characterize pMMO in its native membrane environment. Our quest to achieve molecular characterization of pMMO in situ will be discussed.

Categories

2. Methanotrophs

Session 5

Expansion of the family of Borg archaeal extrachromosomal elements reveals a syntenous core genetic repertoire

Marie Schoelmerich[1], Lynn Ly[2], Rohan Sachdeva[1], Ling-Dong Shi[1], Jacob West-Roberts[3], Simonetta Gribaldo[4], Ben Woodcroft[5], Christopher Schadt[6], Basem Al-Shayeb[1], Xiaoguang Dai[7], Christopher Mozsary[7], Scott Hickey[2], Christine He[2], John Beaulaurier[2], Sissel Juul[7]

[1]IGI | UC Berkeley, Berkeley, USA. [2]Oxford Nanopore Technologies Incorporated, San Francisco, USA. [3]Department of Earth and Planetary Science | UC Berkeley, Berkeley, USA. [4]Institute Pasteur, Paris, France. [5]Queensland University of Technology, Brisbane, Australia. [6]Oak Ridge National Lab, Oak Ridge, USA. [7]Oxford Nanopore Technologies Incorporated, New York, USA

Abstract

Borgs are linear extrachromosomal elements (ECEs) of up to 1.1 Mbp in length that associate with anaerobic methane-consuming archaea of the genus "Candidatus Methanoperedens". Here, we used Nanopore sequencing to validate the published genomes and define additional genomes, three of which were fully curated into the long inverted terminal repeat regions. The 13 complete and four near-complete genomes have the same architecture and share 40 conserved genes that define a largely syntenous genome backbone. We use these conserved genes to identify three new Borg types from peatland soil and to render an overview of the phylogeny of 28 Borg types that revealed two main clades. We reconstructed the first complete 4.00 Mbp genome for a *Methanoperedens* that is inferred to be a Borg host and predicted its methylation motifs, which differ from pervasive TC and CC methylation motifs of the Borgs. Thus, methylation may enable host/Borg genome differentiation. Very high Borg to potential host abundance ratios and structural predictions suggest that Borgs may be capable of encapsulation. The findings clearly define Borgs as a distinct class of ECE with shared genomic signatures, establish their diversification from a common ancestor with genetic inheritance, and raise the possibility of periodic residence outside of host cells.

Categories

2. Methanotrophs

Session 5

Methane Bioconversion to Volatile Fatty Acids by Aerobic Methanotrophs via a Novel Fermentation Pathway

Yicheng Ma, Tao Liu, Zhiguo Yuan, Jianhua Guo

The University of Queensland, Brisbane, Australia

Abstract

Microbial methane fermentation by methanotrophs has been proposed as a promising approach to convert natural gas to liquid chemicals (e.g. volatile fatty acids, VFAs) for transportation. Although methane fermentation to VFAs was previously observed under oxygen-limiting conditions, underlying mechanisms are still unclear. Here, we report a novel methane fermentation pathway, in which methane is first stored as intracellular storage compounds (ISCs) under nitrogen-limiting conditions, then ISCs are further fermented to VFAs under O2-limiting conditions. This pathway was adopted by multiple Type I and II methanotrophs, which was confirmed by RT-qPCR, 13CH4-labeled isotope experiments, metabolic analyses and proteomic sequencing. Our findings advance our understanding of methane fermentation mechanisms, and open new opportunities for achieving efficient liquid chemical production using natural gas as a feedstock.

Categories

2. Methanotrophs

Session 5

Developing *Cupriavidus necator* as a host for conversion of CO_2 to fatty acids

Jessica Roberts, Christopher Calvey, Emily Fulk, Lucas Friedberg, Reuben Swart, Aleena White, Violeta Sànchez i Nogué, Christopher Johnson

National Renewable Energy Laboratory, Golden, CO, USA

Abstract

Converting CO_2 to value-added products represents a key opportunity to incentivize CO_2 capture and mitigate global warming. The soil bacterium *Cupriavidus necator* can grow on CO_2 as its sole carbon source when provided with H_2 as an energy source and O_2 for aerobic respiration, both of which canbe generated by electrolysis of water using renewable electricity. Alternatively, CO_2 can be electrocatalytically reduced to generate formic acid, which *C. necator* can use as its sole source of carbon and energy. *C. necator* is also amenable to genetic manipulation and can be engineered to convert CO_2, directly or via formic acid, to numerous products across a range of applications. Here we will describe our progress toward developing *C. necator* as a microbial host to enable hybrid electrocatalytic/biocatalytic systems for production of fuels and chemicals from renewable electricity and CO_2. This presentation will focus, in particular, on our work engineering *C. necator* for production of fatty acids.

Categories

3. Aerobic hydrogenotrophs

Session 6

CFD-CRD predicts metabolic shifts in *C. autoethanogenum* based on the dissolved gas concentration gradients in industrial-scale syngas fermentation

Lars Puiman[1], Eduardo Almeida[1], Cristian Picioreanu[2], Henk Noorman[3,1], Cees Haringa[1]

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Abstract

Syngas fermentation is an established process for converting CO and H_2-rich waste gas streams into value-added chemicals. At scale, the company LanzaTech employs an external-loop gas lift reactor to produce ethanol. The typical gas and liquid flow pattern results in frequent fluctuations in dissolved gas concentrations (around 1 order of magnitude), from the microbial perspective.

To study how the dissolved gas concentration and its gradient would affect individual cells and the global reactor performance, we coupled a lumped (linlog-based) metabolic kinetic model of *Clostridium autoethanogenum* with an Euler-Lagrangian computational fluid dynamics (CFD) model. The dynamic kinetic model comprises 12 compounds and 11 intracellular reactions, while the CFD model solves turbulent fluid flow and mass transfer in the industrial-scale reactor. This yielded results with a high spatio-temporal resolution from both the reactor and the microbial perspective.

Based on different dissolved gas concentrations and consequently varying reduced ferredoxin concentrations, we identified three metabolic regimes: (1) acetogenesis at high concentrations, (2) solventogenesis at intermediate levels and (3), at low concentrations, an overflow regime wherein extracellular acetate is converted into ethanol. The gradients in dissolved gas concentration are observed as feast-famine cycles that lead to reduction-oxidation states of ferredoxin. High variability in ferredoxin concentration was found to increase the ethanol productivity and the gas-to-ethanol yield, at the expense of acetate production.

This two-way coupled CFD-metabolic model offers fundamental understanding of the microbial behaviour in (large-scale) syngas fermentation processes and the governing factors that lead to increased productivity and gas-to-product yields.

Categories

4. Industrial gas fermentation

Session 6

Scaling up and scaling down gas fermentation: A quantitative view on the large scale performance to be mirrored in the lab

Carolin Bokelmann, Pavan Kumar Venkiteswaran Sathyavageeswaran, Ralf Takors

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Abstract

The products of gas fermentation such as low chain alcohols and organic acids typically belong to the group of base and fine chemicals. Market driven cost margins force related bioprocesses to be realized in large reactor volumes (>> 100 m^3) for benefitting from the economy-of-scale principle. Already the design of such large gas fermentations represents a formidable task as fundamentals of mass transfer, bubble dynamics, and hydrodynamics are often missing. Furthermore, the scale-down of real large scale conditions into lab operations is equally challenging as it heavily relies on the detailed status quo inside large-scale bioreactors.

The current contribution exactly tackles this problem. By thoroughly applying large-scale simulations using Lattice-Boltzmann frameworks, different reactor types and operation modes are quantitatively studied and evaluated. Thereof, design principles for scale-down are derived leading to the optimum design of a so-called SMCB (single multi-comparment bioreactor), a scale-down device that has been recently presented by Gaugler et al. (Engineering in Life Science, 2022). Experimental and simulation results demonstrate the similarity of key design parameters such as mixing time in the SMCB setting. Furthermore, experimental tests with Clostridium autoethanogenum provide first evidence for the scale-up impacts on the strain performance.

Research is funded by the German BMBF ‘Bioeconomy International’ initiative in the project VW (Valorize Waste By Knowledge-Driven Scale-up of Gas Fermentation) which is a collaboration with the US partner LanzaTech.

Categories

4. Industrial gas fermentation

Session 6

Artificial enzyme cascades for C1-utilization

Volker Sieber

Technical University of Munich, Straubing, Germany

Abstract

Carbon Dioxide and Hydrogen are the sustainable feedstock of the future and many means of their utilization by chemical and biotechnological processes are under development. We apply a Power-To-X-To-Product approach. First, Methanol is produced from carbon dioxide and hydrogen by established heterogeneous catalysis. Using engineered artificial enzymes and synthetic enzyme cascades we convert green methanol into small sugars and amino acids. For example, we recently created a synthetic methanol alanine pathway (MAP) as a cell-free enzymatic cascade. The pathway consists of nine enzymes with an intrinsic cofactor recycling system and produces L-alanine from methanol with a maximum of 90% theoretical yield. In an alternative approach, divergent directed evolution of a formolase enzyme was used to produce the C4 sugar erythrulose from formaldehyde.

Categories

9. Others

Session 6

Understanding C1 assimilating microorganisms through Metabolomics

Catarina Rocha, Mariana Saavedra, Stefano Donati, Lars Nielsen

DTU Biosustain, Copenhagen, Denmark

Abstract

Biomanufacturing has been offered as a sustainable alternative for the production of food, feed, energy and many high-value compounds. The use of C1 feedstocks, such as atmospheric CO2 and some industrial waste gases, represents a promising way to move biomanufacturing away from sugar-based feedstocks while preventing the release of these gases to the atmosphere.

In recent years there has been an increasing interest in studying C1 assimilating microorganisms to discover the pathways that allow the use of C1 feedstocks as the sole carbon source. However, we need to better understand their metabolism in order to drive the discovery of the biofactories of the future.

Systems biology approaches, in particular quantitative metabolomics, enable the precise measurement of intracellular metabolite concentration. HILIC and Ion-pairing UPLC columns allow fast and reliable separation of peaks, contributing to the development of robust LC-MS methods that measure hundreds of metabolites in a short time.

In this work we present 2 targeted metabolomics methods using a U-13C labeled IS for the robust absolute quantification of intracellular metabolites: an ion-pairing method targeting metabolites involved in the central carbon metabolism and a HILIC method targeting metabolites involved in C1 metabolism.

Categories

9. Others

Session 6

Microbial gas fermentation as platform to produce isobutanol for sustainable aviation fuels

Karen Rodriguez Martinez[1], Shivani Garg[2], Anuragini Rastogi[2], Audrey Harris[2], Gary Schenk[3], Michael Koepke[2], Esteban Marcellin[1]

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Abstract

Concerns over high greenhouse gases emissions from transportation have made us consider lower-carbon fuels. Significantly, the aviation industry contributes to 12% of transportation emissions. Decarbonizing aviation, which relies heavily on high-density, fossil-derived fuels, has been challenging. Due to the complexity of electric aircrafts, the preferred path to decarbonize aviation is the production of sustainable aviation fuels (SAFs). Our research offers a promising solution: SAFs from recycled carbon. SAFs, derived from processes such as alcohol-to-jet that use ethanol and isobutanol, offer a viable approach to curb aviation emissions. However, using traditional agricultural resources to produce these fuels can create competition for food supplies.

Acetogens are anaerobic microorganisms capable of converting these gases into valuable compounds, such as acetate, ethanol, and 2,3-butanediol. Our focus is on producing isobutanol, a high-energy compound more advantageous than ethanol due to its chemical structure. We're using Clostridium autoethanogenum, an acetogen known to produce ethanol at a commercial scale and engineering it to produce isobutanol. The route to creating isobutanol involves the modification of valine biosynthesis from pyruvate.

We've used various strategies for this process, including different enzyme variants and transformation methods characterized using in vitro methods. We've also analyzed these strains to understand the factors influencing isobutanol production at the molecular level. Through this study, we aim to provide the necessary tools to increase isobutanol production, optimize the bioprocess and further engineer these metabolic processes. In summary, our research offers a feasible and sustainable method for aviation decarbonization, potentially shaping the future of aviation fuels.

Categories

1. Anaerobes (e.g. acetogens)

Session 6

Re-imagining manufacturing: Building a gas fermentation industry

Sean Simpson

LanzaTech Inc, Skokie, USA

Abstract

The climate crisis is the most urgent challenge to mankind and can only be resolved via rapid action to drastically reduce waste carbon emissions. Gas fermentation using carbon-fixing microorganisms is a fully commercial carbon recycling process technology that transforms above-ground sustainable and waste carbon resources into fuels, chemicals and nutritional products at a scale that can be truly impactful in mitigating the climate crisis. This technology offers an industrial approach to both enable manufacturing at its current scale and achieve sustainability targets.

LanzaTech is a pioneer and world leader in gas fermentation, having successfully scaled up the process from the laboratory bench to full commercial scale, with 3 commercial plants in operation and additional facilities in construction.

Compared to other gas-to-liquid processes, gas fermentation offers unique feedstock and product flexibility. The process can handle a diverse range of high volume, low-cost feedstocks. These include industrial emissions (e.g., steel mills, processing plants or refineries) or syngas generated from any resource (e.g., unsorted, and non-recyclable municipal solid waste, agricultural waste, or organic industrial waste), as well as CO2 with green hydrogen. In the first instance the LanzaTech process has been focused on the direct production of ethanol from these sustainable feedstocks. This ethanol can either be used directly as a blend stock in gasoline or dehydrated to ethylene for the manufacture of everything from sustainable aviation fuel to polyester resins, and surfactants.

Additionally, LanzaTech has developed a comprehensive synthetic biology capability for gas fermenting bacteria. This capability has enabled the company to demonstrate and, in some cases, scale the direct production of over 100 alternative chemical outputs from its gas fermentation process.

This paper will provide an insight into the LanzaTech journey from scrappy start-up to global technology leader through the commercialization of its gas fermentation process as a platform, and give a perspective on the future for the industry at large.

Categories

4. Industrial gas fermentation

Session 7

Cases studies and variable mass transfer modeling for Gas Fermentations in Trickle Bed Reactors

Hariklia Gavala, Sambit Dutta, Ioannis Skiadas

DTU Chemical and Biochemical Engineering, Kgs. Lyngby, Denmark

Abstract

Over the last 8 years, at DTU Chemical Engineering we have developed a Trickle Bed Reactor, TBR, a thermodynamics-assisted methodology for directing microbial enrichments specialized for gas fermentations as well as thermodynamics-consistent modeling of the biochemical reactions. TBR offers the dual benefit of high surface area, thus facilitating mass transfer, as well as high density of microbes that results in enhanced production rates. Dynamic modeling of both biochemical reactions involved, and mass transfer is a powerful tool that can be used for optimal up scaling, and reliable predictions that can be tailored to specific cases in terms of scale and products.

The presentation will start with an overview of our contributions in the field of gas fermentation with selected representative case studies and will subsequently focus on our newest work on modeling of variable volumetric mass transfer coefficient that allows optimal up-scaling of a rather complex reactor system. Predicting and controlling the volumetric mass transfer coefficient can be proved valuable, not only for maximizing the mass transfer rate, but equally importantly, to preventing inhibition and toxicity phenomena. Temperature, pressure, reactor geometry and gas and liquid flow rate can be adjusted to secure maximum efficiency of the gas fermentation process. As this approach and modeling tool can be adjusted to different reactors and microbial systems, it can be very useful for designing new and re-visiting established processes in the field.

Categories

9. Others

Session 7

Enhancing Value-added Production from Flue Gas-Derived CO_2 through Integrated Electrochemical and Gas Fermentation Approaches

Michael Resch

National Renewable Energy Laboratory, Golden, USA

Abstract

The utilization of flue gas-derived CO2 to produce valuable products offers a promising incentive for conventional carbon capture technologies, promoting a more economically favorable and sustainable process. As part of the CO2 Reduction and Upgrading for e-Fuels (CO2RUe) Consortium, this project focuses on integrating electrochemistry with gas fermentation and employs unique modeling techniques. By producing valuable products from flue gas-derived CO2, the net cost of conventional carbon capture technologies can be reduced, resulting in a more economically favorable and sustainable process. Moreover, it has the potential to enhance the carbon conversion efficiencies of biorefineries.

Collaborating with industrial partners, namely LanzaTech and Dioxide Materials, we are actively addressing the challenges associated with scaling up the conversion of biorefinery flue gas mixtures. Our vision involves co-locating such systems at industrial sources to enable direct conversion of concentrated or dilute sources of CO2.

During this presentation, we will provide an overview of our research efforts. Firstly, we will discuss the influence of flue gas components, such as H2S, on the CO2 electrolyzer's capacity to reduce CO2 into CO. Additionally, we will explore our molecular biology endeavors aimed at improving the carbon conversion efficiency of the gas fermenting microbe C. autoethanogenum, specifically in converting CO/H2/CO2 mixtures into fuels and chemicals. Furthermore, we will present the results of our recent technoeconomic analysis, which evaluates the renewable electricity requirements and associated costs for utilizing biorefinery CO2 waste streams at scales of up to 14 T/hour.

Categories

4. Industrial gas fermentation

Session 7

Biosynthesis of biodegradable polyhydroxyalkanoates (PHAs) for packaging materials from sustainable carbon sources – solving two problems with one solution

Leonie van 't Hag, Shahruk Nur-A-Tomal, Neil Cameron

Monash University, Clayton, Australia

Abstract

Carbon capture and utilisation has been recognised internationally as an essential step to accelerate the decarbonisation of various industries worldwide [1] . The biomanufacturing industry in particular is one that would largely benefit from more sustainable and scalable means of carbon (CO2) feedstock generation and utilisation for processes such as fermentation.

Additionally, food and agricultural waste (including municipal solid waste) are one of the most significant waste streams generated by human activity [2]. Plastic packaging waste is a continuing issue for waste management programs and the adverse effects of plastics are well known today. Generally, plastics are not biodegradable and accumulate in the environment for a long time, depending on their chemical nature, the characteristics of the environment where it is disposed off and can turn into microplastics. Therefore, we have developed biodegradable polyhydroxyalkanoates (PHAs) and their composites to replace synthetic soft plastics in packaging applications from a range of carbon feedstocks.

Pseudomonas putida can produce medium-chain-length (mcl) PHAs [3] which have comparable properties to conventional packaging plastics. In this study, the effect of feeding intermediate CO2 capture products, as well as glucose and fatty acids of a range of chain lengths (C8 – C14) from food waste on the production of mcl-PHAs by *Pseudomonas putida* has been studied. The PHA properties have systematically been characterised and their use in soft plastic films has successfully been achieved.

The carbon feedstocks could be used to tune the chemical structure of PHAs with a range thermal and mechanical properties suitable for different applications. This can significantly improve the circular economy of food & packaging.

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[2] H. Raclavskáet al, Biodegradable Waste Management in the Circular Economy 2022, 69 – 153.

[3] A. Prieto et al. 2014, A holistic view of polyhydroxyalkanoate metabolism in Pseudomonas putida, Environmental Microbiology, 18, 341-357.

ACKNOWLEDGEMENTS: This work was supported by a grant from the Victorian Government through the Victorian Higher Education State Investment Fund (VHESIF) – Circular Economy Accelerator Organics project (CEA-O).

Gas fermentation Conference 2024

Advancing the field for creating a new Industry

Categories

4. Industrial gas fermentation

Session 7

Orthogonal biomanufacturing from C1 feedstocks

Ramon Gonzalez

MojiaBio, Tampa, USA

Abstract

While one-carbon (C1) compounds are emerging as cost-effective and potentially carbon-negative feedstocks for biomanufacturing, the development of microbial biocatalysts for their conversion to value-added products remains a challenge. This is in part due to the approaches used for strain engineering, which rely on the canonical architecture of metabolism and entail concurrent engineering of pathways for substrate utilization, central metabolism, and product synthesis. The resulting crosstalk between product-forming and growth-sustaining functions that compete for the same carbon and energy carriers lead to inefficient microbes and bioprocesses that suffer from unreliable scale-up and deployment, expensive downstream processing, and high capital expenses.

We have addressed these shortcomings by pioneering a new approach herein referred to as *Ortho*gonal *Bio*manufacturing (OrthBio™). Our OrthBio™ platform relies on the engineering of an iterative C1 elongation pathway (C1+Bio™) based on formyl-CoA elongation (FORCE) reactions that are orthogonal to the host metabolism thus allowing product synthesis independent from cell growth. The FORCE reactions, catalyzed by 2-hydroxyacyl-CoA synthases (HACS), are acyloin condensations between carbonyl compounds, such as aldehydes and ketones, and the C1 moiety formyl-CoA. In this talk I will discuss the conceptualization, design and implementation of C1+Bio™ and its deployment for the synthesis of multicarbon products at industrially relevant titers, rates, and yields and with unprecedented scalability across production platforms and scales.

1- Lee, S.H., Chou, A., Nattermann, M., Zhu, F., Clomburg, J.M., Paczia, N., Erb, T.J., and Gonzalez, R. (2023). Identification of 2-hydroxyacyl-CoA synthases with high acyloin condensation activity for orthogonal one-carbon bioconversion. ACS Catal. 13: 12007-12020

2- Chou, A., Lee, S.H., Zhu, F., Clomburg, J.M., and Gonzalez, R. (2021). An orthogonal metabolic framework for one-carbon utilization. Nature Metabolism, 3:1385-1399.

3- Chou, A., Clomburg, J.M., Qian, S., and Gonzalez, R. (2019). 2-Hydroxyacyl-CoA lyase catalyzes acyloin condensation for one-carbon bioconversion. Nature Chemical Biology, 15:900-906

Categories

9. Others

Session 7

Living in Space and Lessons for Earth: A systems perspective.

Jitendra Joshi

Woodside Energy, Perth, Australia

Abstract

Earth is a biological life raft that sustains human life in the inhospitable environment of the cosmos. This planetary closed-loop system provides oxygen, water and essential nutrients while recycling waste, purifying contaminants and renewing resources. NASA is learning just how this life raft functions in order to create a portable replica of the biological system we call home.

Mimicking Earth's life support functions for long-duration space travel has it's unique challenges as there are no buffers of huge land and water bodies. In trying to close the materials cycling loops for space missions there are several lessons learned that can be applied back on Earth. These lessons include systems engineering principles for developing integrated systems that are efficient and resilient.

Categories

4. Industrial gas fermentation

Session 8

Exploring and Engineering Microaerobic Co-Culture for Carbon-Negative Bioproduction via Gas Fermentation

Guanyu Zhou, Dhruv Jatkar, Anthony Stohr, Benjamin Woolston

Northeastern University, Boston, USA

Abstract

Gas fermentation using acetogenic microbes has emerged as an attractive platform for the sustainable production of biofuels and chemicals. Despite the promise of gas fermentation, a major challenge is the anaerobic lifestyle of the microbial biocatalysts, which limits them to producing only low-margin compounds at industrially relevant levels. We are developing a synthetic co-culture approach to solve this problem, in which CO_2 fixation and product formation are split between an anaerobic and aerobic microbe in the same reactor. The acetogen converts the CO_2 to acetate at high yield, and the partner aerobic organism upgrades the acetate to a higher-value product using the energy available from respiration, while rapidly consuming the oxygen to establish the low-oxygen conditions necessary to enable acetogen growth. In essence, a symbiotic relationship is established in which the aerobe protects the acetogen from oxygen in exchange for fixed carbon. We first examined the potential of the co-culture computationally, showing through community flux balance analysis (FBA) that, across a range of products, the co-culture could outperform an acetogen mono-culture. We then demonstrated stable co-culture of *Clostridium ljungdahlii* and *Escherichia coli* in an air-sparged bioreactor on orthogonal sugar substrates. Then, using a parallel mini-bioreactor system we developed for experiments under different continuous gas conditions, we identified conditions that enabled autotrophic growth co-culture. Finally, again though FBA we identified additional cross-feeding interactions predicted to enhance co-culture performance, and examined these experimentally. This work puts forward a new approach to carbon-negative bioproduction at high yields and productivities in a single bioreactor.

Categories

1. Anaerobes (e.g. acetogens)

Session 8

Gas fermentation by novel biocatalysts within pressurized systems as new avenues for the sustainable production of biochemicals

Joana I. Alves[1,2], João PC Moreira[1,2], Ana L Arantes[1,2], Sónia G Barbosa[1,2], Lucília Domingues[1,2], Diana Z Sousa[3], M Madalena Alves[1,2]

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Abstract

The overuse of fossil based chemicals and fuels continues to be the main cause of greenhouse gas (GHG) emissions which lead up to major environmental issues. Therefore, the development of a circular economy and the use of renewable resources is still on demand, which is confirmed by the recent EU target for Renewable Energy Sources consumption by 2030 (raised to 32%). Ideally, in the frame of a circular economy, GHG should become feedstocks instead of residues. Thus, fermentation of (syn)gas (mainly CO, H_2 and CO_2) is a novel smart, green and socially engaged approach for an alternative valorization of gas waste streams, being fully in line with SDGs 12 and 13 by contributing to the production of biocommodities (e.g. butanol, propionate or acetone) and with simultaneously climate regulation via use and valorization of GHG, significantly reducing their release to atmosphere. Syngas fermentation is based on the ability of microorganisms to convert its constituents into value-added products. In recent years, successful attempts have been made to exploit the potential of autotrophic acetogens through genetic engineering strategies, manipulation of culture conditions, synthetic co-cultivation and bioreactor design. Gas fermentation still involves practical challenges due to limitations of the process. The major bottleneck of syngas fermentation that blocks the commercialization of this technology is gas-to-liquid mass transfer limitations, since it reduces the microorganisms' access to the substrate and consequently reduces the productivity rates. It is of utmost importance the development of alternatives that promote the enhancement of mass transfer, the improvement on the productivity rates from gas fermentation and the deep study of the biocatalysts involved in syngas bioconversion pathways.

The main results from the scientific research project INNOVsyn – Innovative strategies for syngas fermentation – showed that the use of pressurized bioreactors is beneficial for the growth of mixed cultures or defined co-cultures, increasing productivities and CO uptake rates and also triggers the activation of other metabolic pathways towards different products from syngas into methane, acetate, butyrate and propionate. The team also reached interesting results regarding the use of a synthetic co-culture composed by an acetogen and a propionigenic bacterium for syngas bioconversion to propionate. Recently, a novel autotrophic gas-fermenting acetogenic bacterium strain, *Acetobacterium wieringae* strain JM, was isolated. This strain is very versatile and it grows at high rates in different gas compositions without any additional carbon sources as supplementation, providing unprecedent expectations for novel highly efficient CO fermentation processes. A robust, stable and efficient transformation protocol for *A. wieringae* strain JM has been already developed to genetically modify this strain towards the production of non-native compounds, as acetone or isopropanol. The research that have been done combine novel biocatalysts with strategies for process optimization by using pressurized systems to maximize productivity and overcome gas-liquid

mass transfer limitations. Our research is in the path towards the industrialization of syngas fermentation.

Categories

1. Anaerobes (e.g. acetogens)

Session 8

Gas fermentation with acetogenic bacteria:Biochemistry, energetics and metabolic engineering

Volker Müller

Goethe University, Frankfurt, Germany

Abstract

Acetogenic bacteria are characterized by a special pathway for CO2 fixation, the Wood-Ljungdahl pathway (WLP). This pathway enables autotrophic growth on H2 + CO2 by acetate formation and acetogenesis is coupled to the net synthesis of ATP. Acetogens use substrate-level phosphorylation along with either one of the respiratory enzymes, Rnf or Ech to synthesize ATP. In addition, soluble, energy-saving, electron-bifurcating enzymes are employed to overcome energetic barriers. The structure and function of these complexes will be described.

Overcoming energetic barriers is the main obstacle in the production of compounds other than acetate from H2 + CO2. I will describe how metabolic engineering allows for the production of formate or lactate and how soluble “CO2” analogues such as formate and methanol are used and improve production of added-value compounds. Mixotrophic growth on gas plus organic substrates is another way to redirect electron and carbon flow.

Categories

1. Anaerobes (e.g. acetogens)

Session 8

Integration of Direct Air Carbon Capture (DAC) Technology with Gas Fermentation - Accelerating the Biomanufacturing Industry Decarbonisation Journey

Evangeline Leong[1], Paul Webley[1], Esteban Marcellin[2], Leonie van't Hag[1]

[1]Monash University, Clayton, Australia. [2]The University of Queensland, Brisbane, Australia

Abstract

Carbon capture and utilisation (CCU) has been recognised internationally as an essential step to accelerate the decarbonisation of various industries worldwide. The biomanufacturing industry in particular is one that would largely benefit from more sustainable means of CO2 feedstock generation and utilisation at a commercial scale. Gas fermentation has become a promising process for the production of various sustainable bioproducts such as alternative proteins, bioplastics and high-value intermediate chemicals.

Due to an unlimited feedstock of atmospheric CO2, this solution can be scaled up to deliver large-scale quantities of high-value bioproducts while tackling our climate issue. We have been building on our newly designed DAC2BIO unit for such an application which focuses on medium-concentration CO2 and H2O co-production. We first identified targeted bioproducts of interest and reversed engineered metabolic pathways that supported such downstream production. Next, we performed a systems-level design study to determine the requirements of the entire system and determine system synergies - from the DAC to biological CO2 utilisation and then conversion to achieve close to net zero processes with the support of ultra-low-cost solar photovoltaics.

This systems-level design will pave the way for affordable sustainable CO2 and build the ability to accelerate its large-scale deployment in gas fermentation applications within Australia and globally.

Categories

9. Others

Session 8

$CO_{(2)}$-metabolism: quantifying mixed carbon oxide and hydrogen gas fermentation by *Clostridium autoethanogenum*.

James K. Heffernan[1,2], R. Axayactl Gonzalez-Garcia[1,2], Timothy McCubbin[1,2], Michael Köpke[3], Kaspar Valgepea[4], Lars K. Nielsen[1,2], Esteban Marcellin[1,2]

[1]Australian Institute for Bioengineering and Nanotechnology, The Univesity of Queensland, St. Lucia, Australia. [2]ARC Centre of Excellence in Synthetic Biology, St. Lucia, Australia. [3]LanzaTech Inc., Skokie, USA. [4]ERA Chair in Gas Fermentation Technologies, Institute of Technology, University of Tartu, Tartu, Estonia

Abstract

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, CO_2 and H_2 shows strong possibility for enabling broader application of C1 recycling. Quantifying CO fermentation in the absence of H_2 results in an entirely different metabolism to CO_2/H_2 fermentation, where differences between carbon source (CO_2 or CO) may be confounded with available energy source (H_2 or CO).

To accommodate these potential variables into a comparison of CO_2 and CO metabolism, here we quantify chemostat steady-states (CSSs) under constant H2 supply with a gradual, stepwise switch from CO_2-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 CO_2 metabolism. We demonstrate that metabolism of CO_2/CO by C. autoethanogenum is highly flexible in the presence of H_2, maintaining co-utilization of CO_2 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.

Categories

1. Anaerobes (e.g. acetogens)

Session 9

Biogas conversion using methanotroph-photoautotroph cocultures: recent developments in coculture characterization, modeling and bioreactor design

Jin Wang, Qinghua He

Auburn University, Auburn, USA

Abstract

Agricultural, municipal, and industrial waste streams containing stranded organic carbon represent a significant and underutilized feedstock to produce fuels and chemicals. The adoption of commercially proven anaerobic digestion (AD) technology for biogas production has been hindered by the difficulty in direct biogas utilization (e.g., biomethane) due to contaminants such as H_2S and NH_3. On the other hand, biological CH_4 conversion to value-added products is hindered by CH_4's low solubility in aqueous solutions, leading to large reactor volume and high energy and water consumption.

To overcome these barriers, we propose a methanotroph-photoautotroph coculture-based platform for integrated biogas valorization and nutrient recovery. Our solution is inspired by nature's way of recovering energy and carbon from both CH_4 and CO_2 through the metabolic coupling of methane oxidation to oxygenic photosynthesis. This coupling enhances coculture growth through the exchange of *in situ-produced* O_2 and CO_2 and other emergent synergistic interactions.

Developing coculture-based biotechnology faces many fundamental scientific challenges, including tracking the growth of individual species in the coculture over time, quantifying and understanding inter-species metabolic interactions, and developing kinetic models for the coculture. There are also many engineering challenges for biological biogas conversion, including limited light penetration and mass transfer of gas substrate. In this talk, we discuss these fundamental and engineering challenges, propose experimental and computational solutions to address them and demonstrate the effectiveness of our solutions in several case studies.

Categories

8. Communities

Session 9

Designing Productive Microbial Consortia Living on Water, Light, Electricity, N_2, and CO_2

Jens Krömer

Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany

Abstract

Enriched microbial consortia are able to convert CO_2 to carboxylates using electrodes as a source of redox power. The disadvantage of such consortia is the difficult control of community composition and as a consequence an evolving mix of hard-to-separate products.

Using a bottom-up approach, we look into designing synthetic consortia based on farmer cells that can thrive on light, electricity, H_2O, CO_2, and N_2 and laborers that produce specific products and are amenable to metabolic engineering. The advantage of consortia is their robustness and the possibility to share different tasks among specialist cells. Enabling an informed design of the consortium requires systems biotechnological tools to characterize and understand the underlying processes in the consortium. Tools we are developing include proteomics, metabolomics, fluxomics, and genome-scale metabolic reconstructions.

But while the tools are a necessity, a bigger challenge for chemical production based on CO_2 in these consortia is overcoming redox constraints. We want to solve this by employing our extensive knowledge of the use of solid-state electrodes and mediator molecules as an inexhaustible source or sink of electrons. The advantage over simply feeding hydrogen is that electrodes provide an electronically controllable driving force for draining or feeding electrons at desired energy levels in a tunable fashion in situ.

We believe that this can be used to extend the range of possible products from C_1 and is not limited to CO_2 as the only C_1 feedstock.

Categories

8. Communities

Session 9

Merging carboxylate and syngas platforms: The power of mixotrophic communities

Sabine Kleinsteuber, Flávio Baleeiro, Heike Sträuber

Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany

Abstract

Anaerobic fermentation of agro-industrial waste and byproducts is a promising biorefinery technology to produce green chemicals and replace current production schemes that depend on fossil resources or environmentally destructive agricultural practices. Medium-chain carboxylates are such biobased chemicals with a wide range of applications, e.g., for the production of lubricants, detergents, food additives or cosmetics. They can be produced by open mixed cultures in a process known as microbial chain elongation. However, the yield of this heterotrophic process is limited by the availability of electron donors that are needed to convert short-chain carboxylates – the common intermediates of anaerobic digestion – to longer-chain products such as caproate and caprylate. Hence, efficient chain elongation is restricted to substrates that are rich in ethanol or lactate, e.g., brewery waste or ensiled crops. The substrate range of chain elongation can be extended by feeding gas mixtures of hydrogen and CO_2 or syngas as co-electron donor and autotrophic carbon source, but this approach comes with the challenges of limited gas-liquid mass transfer and methanogenesis as a competing hydrogenotrophic process. To overcome these obstacles, we developed a reactor system with gas recirculation for continuous mixotrophic carboxylate production and applied ethylene as an affordable and recyclable methanogenesis inhibitor. Electron balances and microbial community analyses revealed how syngas-aided chain elongation can turn carboxylate production by anaerobic fermentation from a CO_2-emitting into a net carbon-fixing process.

Categories

8. Communities

Session 9

An atlas of C1 pathways for X.

Damien Cleary

The University of Queensland, Brisbane, Australia

Abstract

More sustainable futures require that existing linear industrial processes make better use of waste. Currently, several microorganisms convert waste containing one carbon (C1) compounds to industrial products. However, the full rational use of these microbes requires a more detailed characterisation of the energetics and biochemistry of these pathways. To accelerate the commercialisation of industrial strains consuming C1 compounds (carbon dioxide, carbon monoxide, methane etc.), we have developed the C1 Atlas, a set of general C1 assimilation models, capable of producing a range of metabolic products from one carbon compounds.

Categories

9. Others

Session 9

Model-driven design of bacterial communities with solventogenic strains for expanding the product range from gas fermentation

Niels Nouse[1,2], Sara Benito-Vaquerizo[3], Hetty van der Wal[1], Truus De Vrije[1], Vitor Martins dos Santos[3], Peter J. Schaap[3], Jeroen Hugenholtz[2], Stanley Brul[2], Diana Z. Sousa[4], Maria Suarez-Diez[3], Ana M. Lopez Contreras[1]

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Abstract

Microbial fermentation of syngas and of CO_2/H_2 mixes appears as an attractive technology for production of commodity chemicals. Acetogens ferment syngas to produce acetate and ethanol, while solventogenic strains produce organic acids (acetate, butyrate) and solvents (acetone, butanol, ethanol) from sugars. Currently, new strategies are explored to increase the product range from gas fermentations and microbial communities are emerging as a suitable approach. Establishing these communities in the laboratory is a laborious process and systematic approaches are needed to accelerate the pace of innovation. Genome-scale metabolic models (GEMs) and constraint-based approaches such as Flux Balance Analysis (FBA) have shown their potential in accelerating the development of microbial cell factories. In this research, we have followed a modular approach to design fit for purpose communities, by combining modules representing organisms such as the previously developed GEMs of *Clostridium autoethanogenum*, *C. kluyveri*, *C. acetobutylicum*, *C. beijerinckii*, or the newly developed model of *Anaerotignum neopropionicum* [1]. The impact of process conditions on the feasibility and product range of the community informed experimental activities. Here, we describe a model-based approach for the development of synthetic microbial consortia to upcycle syngas and expand the product range to butyric acid as main product [2]. In addition, the effect of the gas composition on the product range was studied experimentally.

References

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Funding

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Categories

5. Synthetic Biology tools for gas fermentation

Session 9

Engineering synthetic co-cultures for sustainable oleochemical production from C1 feedstocks

Christopher Lawson

University of Toronto, Toronto, Canada

Abstract

Conversion of C1 feedstocks, including CO, CO_2, and methanol, into medium-chain fatty acids and alcohols represents a sustainable platform for chemical manufacturing. A promising strategy for conversion of C1 feedstocks to oleochemicals is to harness synthetic co-cultures of acetogenic and chain elongating bacteria that integrate the Wood-Ljungdahl pathway with reverse ß-oxidation. This allows for optimization of C1 conversion through division of labour and the use of metabolic engineering tools to improve productivity and expand the product range. However, rationale design of co-cultures from a diverse pool of acetogens and chain elongators is challenging and the ability to genetically modify many of these organisms is limited by barriers to DNA uptake and a lack of genetic tools. In this study, we screen different co-cultures of acetogens and chain elongators on various C1 substrates (CO, CO_2, and methanol) to identify consortia and conditions that maximize production of medium-chain fatty acids and alcohols. Subsequently, we attempt to improve plasmid uptake rates in top performing chain elongator strains by evading their native restriction-modification systems using a plasmid methylation pipeline prior to gene transfer. Our results offer preliminary insights on the physiology and performance of different acetogen/chain elongator co-cultures and establish critically needed molecular tools to enable their genetic manipulation. These efforts represent important steps in the development of synthetic co-cultures for sustainable biomanufacturing based on largely untapped groups of anaerobic bacteria.

Categories

8. Communities

Session 10

Engineering bacterial microcompartments for sustainable production of biochemicals

Danielle Tullman-Ercek

Northwestern University, Evanston, USA

Abstract

One promising route to sustainable bioproduction of fuels and chemicals is the engineering of organisms such as acetogens to efficiently convert abundant and low-cost carbon monoxide (CO) or carbon dioxide (CO2) and hydrogen (H2) containing gases to desirable products at high efficiency and low cost. This approach not only provides an avenue for repurposing greenhouse gases, but also minimizes the necessity for harsh chemicals and hazardous byproducts common in petroleum-based processes. However, many biochemicals are not yet produced biologically due to roadblocks in the cellular biosynthesis process. These roadblocks can include toxicity of intermediates, redox imbalances, and/or loss of product to off pathway reactions. Nature uses spatial organization strategies, such as sequestration in organelles, to alleviate these issues. Even in bacteria, there are organelle-like structures encapsulated by proteins known as bacterial microcompartments (MCPs) that are hypothesized to serve in these functions. However, much is still unknown about the systems, hindering their application in metabolic engineering efforts. With this presentation, I will present how we used a combination of cell-free metabolic engineering, in vivo studies, and modeling to explore the native regulation, assembly and function of microcompartments from a model organism, Salmonella enterica. In so doing, we identified the types of pathways that may benefit from encapsulation, and established some design rules for repurposing these organelles for engineered metabolic pathways. Moreover, I will discuss how this may be applied in the context of an acetogen.

Categories

5. Synthetic Biology tools for gas fermentation

Session 10

Understanding the metabolism of C1 assimilating microbes to engineer them for biomanufacturing

Stefano Donati

DTU Biosustain, Kgs. Lyngby, Denmark

Abstract

C1-based biomanufacturing is a promising technology that might help in establishing a circular bio economy, replacing multi-carbon feedstock directly with CO_2 or its reduced equivalents. In recent years, genetic engineering of environmental bacteria capable of growth on C1 enabled the first demonstrations of such processes at an industrial scale. Moreover, in the next years, synthetic C1 assimilating strains might provide a powerful more tractable option to establish bioprocesses. All these engineering attempts required a deep understanding of metabolism and of how it behaves in relevant industrial conditions.

In our group recently established at DTU Biosustain, we develop and employ technologies to study microbial C1 metabolism, with the goal of further understanding and engineering microbial metabolism for biomanufacturing applications. Collaborating with the local Biofoundry, we take advantage of methods such as generation of large libraries and their screening, multi-omics data analysis, genetic engineering and adaptive laboratory evolution. We will be presenting the latest methodologies and achievements in those areas.

Categories

9. Others

Session 10

Natural and Engineered Organelles for Catalysis in Confinement: Carboxysomes and other Bacterial Microcompartments

Cheryl Kerfeld

MSU and LBL, Berkeley, USA

Abstract

Bacterial microcompartments (BMCs) represent biological modularity as multienzyme-containing proteinaceous organelles. Bioinformatic analyses have revealed the widespread occurrence of BMCs across the Bacterial Kingdom. The generalized structure of BMCs establishes catalyst proximity and spatial control of local reactant and substrate concentrations, sequesters volatile or reactive intermediates, and controls metabolite and gas exchange with the surrounding environment. Accordingly, BMCs can be viewed as a biological paradigm for spatially confined chemistry. In addition to fundamental studies of the structure and function of BMCs, recent advances in programming and assembling BMCs in vivo and in vitro poise this biological architecture to become a platform for the study spatially confined chemistry. BMC architectures provide a template for combining synthetic chemistry with synthetic biology to resolve mechanisms for spatial control of reaction networks with unprecedented precision. Relative to lipid-bound compartments, the protein-based boundary of the BMC can be precisely structurally defined and the multiple shell constituents can be individually tuned for electron, substrate, product, and potentially gas transport properties. Knowledge of how BMCs self-assemble, circumscribe a private co-factor pool, and how they variously confine radicals, volatiles, and toxic intermediates poises this biological architecture to become a platform for exploring the mechanistic properties of catalysis in spatially organized, multi-scale, hierarchical host confinement.

Categories

5. Synthetic Biology tools for gas fermentation

Session 10

Exploiting a C1/C5 Bi-Substrate Scenario: Superior Growth Performance and Metabolic Insights in Methylotrophic Yeast *Pichia pastoris*

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Abstract

Methanol has emerged as a promising alternative feedstock in microbial cell factories for the production of chemicals and value-added products. However, its biological conversion is companied by substantial challenges, including methanol toxicity, low carbon yields, and inefficient utilization.

To overcome these hurdles, significant efforts are required to rewire the metabolic network of cell chassis for C1 assimilation. Alternatively, a more straightforward and practical solution involves using a co-substrate with methanol, or a C1 plus Cn scenario, where Cn could be C3 (such as glycerol), C5 (xylose, arabinose), or C6 (glucose, fructose, sorbitol).

In the case of native methylotrophs, like *Pichia pastoris*, co-feeding with methanol and other substrates has been widely used to boost recombinant protein production. However, batch fermentation of these substrates is limited by metabolic repression. Additionally, the mechanisms underlying the benefits of co-substrate feeding are not well-understood due to the complexity of studying C1/Cn carbon metabolism dynamics.

This study focuses on the C1/C5 (methanol/xylose) scenario in *P. pastoris*, which is widely utilized in protein expression and chemical synthesis. Xylose, a readily available sugar, is co-utilized with methanol in *P. pastoris*, rendering it an ideal substrate combination for industrial applications and mechanistic studies. The results indicate that an engineered *P. pastoris* strain demonstrates superior growth performance on methanol/xylose compared to single substrates. Additionally, insights into flux distribution patterns and metabolic regulation changes associated with this bi-substrate strategy were derived from 13C metabolic flux analysis and proteomics studies.

Categories

9. Others

Session 10

Strain engineering for conversion of non-canonical carbon sources to valuable products

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Abstract

Microbial engineering broadly seeks to address the bioproduction of a wide array of bulk, commodity, and fine chemicals. Substantial improvements in synthetic biology and metabolic engineering techniques have made many such bioproduction platforms within reach of industry scale-up. The majority of metabolic engineering paradigms focus on glucose and other sugars as carbon sources, due to their importance as sustainable starting materials. However, a massive potential also exists for engineering conversion of non-canonical carbon streams such as aromatics from lignin, degradation products from plastic and other waste, and C1 carbon sources. Since these carbon sources are less studied, targeted methods that rely on traditional knowledge of metabolic enzymes can be lacking. Here the role of functional genomics, laboratory evolution, genome-scale models, and systems biology-guided strain engineering proves to be essential. One example is that of an ensemble method to enable aromatics conversion to final products at close to 80% maximum theoretical yield. This and other examples will be reviewed in the context of their applicability for C1 carbon conversion.

Categories

9. Others

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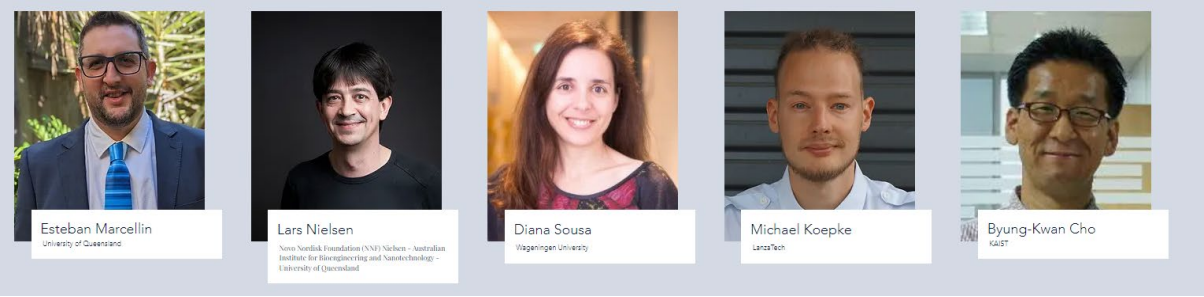

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