Inaugural UQ Biosustainability Hub Conference

PROGRAM BOOK

7 - 9 May 2025 | Moreton Island, Queensland, Australia

UQ Biosustainability Hub
www.biosustainabilityhub.org

Welcome to Inaugural UQ Biosustainability Hub Conference

Our vision

By the end of the decade, synthetic biology could be used extensively in manufacturing industries that account for more than a third of global output. The UQ Biosustainability Hub aims to capture a piece of that market share and drive a new industry in Australia.

This hub is a piece of cutting-edge research infrastructure, and it houses research expertise, combined with industry relationships to help transition the world to a sustainable, global bioeconomy.

Our mission

Develop economically viable solutions for every company that wants to transition to net zero

Conference Themes

- Multi-omics Analysis
- Computational Biology
- Translational / bioeconomy research
- Gas & Precision Fermentation
- Bioengineering - *Biomining, biomaterials, waste valorisation*
- Bioprocess Optimisation - *Scale-up, bioreactor optimisation, cell-free enzyme cascades*

Bonus Challenge: Logo Design

- The UQ Bio Hub needs a representative logo
- Reward given to the best logo at the end of conference (no guarantee that the logo will be used officially)

Acknowledgement of Quandamooka Country

The region of Moreton Bay and its surrounding islands is traditionally known as **Quandamooka**, home to the Ngugi people of Moreton Island and the Gorenpul and Nunukul clans of North Stradbroke Island. Moreton Island is called Mulgumpin (also spelled Moolgumpin or Moorgumpin), meaning "place of sandhills" in the Aboriginal language.

For over 50,000 years, Aboriginal Australians have relied on the land and sea for food, medicine, shelter, tools, and clothing. The Ngugi people have lived on Moreton Island for more than 2,000 years, drawing heavily on marine resources such as fish, shellfish, dugong, and turtle, which formed the cornerstone of their diet. Their marine-based lifestyle was complemented by plant foods and honey, with around 64 edible plant species identified on the island. The Ngugi people's connection to the land and sea is deeply spiritual, with many animals playing important roles in their cultural traditions and customs.

UQ Biosustainability Hub acknowledges Aboriginal and Torres Strait Islander peoples as the First Australians. We recognise their cultures, histories and diversity and their deep connection to the lands, waters and seas of Queensland and the Torres Strait.

We acknowledge the Ngugi people as the Traditional Custodians of Quandamooka Country, Mulgumpin (Moreton Island), the lands on which our conference is located and where we meet, work and learn.

We pay our respects to the Elders, those who have passed into the dreaming; those here today; those of tomorrow. May we continue to peacefully walk together in gratitude, respect and kindness in caring for this Country and one another.

Message from the Director

Welcome to the Biosustainability Hub Conference

The Biosustainability Hub believes that collaboration and diversity are key to driving innovation. By bringing together individuals with unique perspectives and expertise, we aim to tackle the hardest challenges of our time: decarbonising industry and achieving net-zero emissions by 2050.

Biomanufacturing offers unique opportunities to transform how we produce fuels, chemicals, materials, and food ingredients. Australia, has abundant renewable resources and strong research capabilities, hence is uniquely placed to deliver on the promises of synthetic biology and lead the development of a sustainable bioeconomy.

The transition to a sustainable bioeconomy demands innovative solutions in systems biology, engineering, and industrial biomanufacturing. The Hub integrates systems and synthetic biology, automation, and deep learning to accelerate the development of high-performance microorganisms for emissions reduction.

As we prepare to move into a unique and exciting new floor in 2025, it is essential that we foster collaboration and ensure that all groups understand each other's work. With numerous resources comes great responsibility—to deliver outcomes that meet the ambitious goals of the Hub and contribute meaningfully to Australia's bioeconomy.

The projects we develop can only succeed if they are both economically viable and accepted by customers. Ensuring cost-effectiveness, scalability, and market relevance is central to everything we do. Our research must be guided by scientific excellence and by practical application and industry needs.

Using biology, we produce sustainable fuels, chemicals, plastics, ingredients, novel foods, materials, new secondary metabolites, cell free proteins and even mine rare earth minerals. By harnessing non-fossil feedstocks - including waste gases, mine waste, and industrial byproducts - we help build a scalable, carbon-efficient bioeconomy for Australia.

Through metabolic engineering, high-throughput screening, and mathematical model optimisation, we develop microbial cell factories that minimise environmental impact. Yet, technoeconomic assessments often identify feedstock cost as a key barrier to commercial viability. Our approach addresses this by using waste gases as feedstocks, transforming greenhouse gases into valuable products.

The Hub hosts world-class resources, including Q-MAP's systems biology platform, which identifies metabolic bottlenecks and enhances strain performance through targeted engineering. Coupled with bioprocess optimisation at IDEA Bio, we scale production from milligrams to kilograms, ensuring industrial robustness and efficiency.

Our strategic partnerships with industry de-risk emerging technologies and accelerate their adoption. Positioned at the intersection of academia and industry, the Biosustainability Hub strengthens Queensland's leadership in industrial biotechnology, driving decarbonisation, enhancing resource circularity, and fostering a resilient bioeconomy for Australia.

The next three days offer a unique opportunity to explore the research conducted at the Hub, reimagine what is possible, and cast new ideas so that together we can deliver on these promises. Unfortunately, three days are too short, as we also want to socialise and build trust. I encourage everyone to read the abstract book below, as not everyone will have the opportunity to present a talk.

Thank you for joining us. We look forward to your engagement.

Sincerely,

Esteban Marcellin

Conference Map at Tangalooma Resort

Conference Agenda

WEDNESDAY 7TH APRIL 2025

06:30 – 09:30	• Brisbane to Tangalooma • BioHub Bingo
09:30 – 10:00	Morning Tea
10:00 – 10:45	• Welcome to Country • Welcome Talk by Professor Esteban Marcellin, Director of the UQ Biosustainability Hub
10:45 – 11:45 **CHAIRS** **MARIKO**	Workshop I. Biohub Group Leaders • Adrian Oehmen • Birgitta Ebert • Bronwyn Laycock • Damian Hine • Denys Villa Gomez • Esteban Marcellin (also speaking for Gary Schenk) • Huadong Peng • Yosephine Gumulya • Zeinab Khalil • Tim McCubbin (on behalf of Lars Nielsen)
11:45 – 12:45	Lunch
12:45 – 14:15 **CHAIRS** **JITENDRA & ADRIAN**	Seminar I – Gas Fermentation • Karen Rodriguez Pathway engineering and systems biology for optimised isobutanol production in the gas-fermenting acetogen *Clostridium autoethanogenum* • James Heffernan $CO_{(2)}$-Metabolism: Quantifying Mixed Carbon Oxide and Hydrogen Gas Fermentation by *Clostridium autoethanogenum* • Lars Puiman Bioreactor design and modelling for gas fermentation processes • Hemanshi Galaiya Filing the gap towards sustainable bioplastic production - unlocking the correlation between

	polyhydroxybutyrate production, redox balance and hydrogenase expression in *C. necator* H16 • Antonia Ebert Assessing multi one-carbon conversion in the aerobic carboxydotroph *Hydrogenophaga pseudoflava* • Nhat Huynh MMOneo: engineering an industrial host organism to convert methane waste via expression of methane monooxygenase
14:15 – 15:00	Check-in
15:00 – 16:00 **CHAIRS** **CAROLINE**	Workshop II. BioHub Industry Panel What do Industry-Academic Partnerships look like? • Axa Gonzalez • Bronwyn Laycock • Denys Villa Gomez • Adrian Oehmen • Jitendra Joshi
16:00 – 16:30	Afternoon tea
16:30 – 17:30 **CHAIRS** **HUADONG & BRONWYN**	Seminar II – Bioconversion & Bioeconomy Research • Jennifer Adami Bioengineered lignin conversion into high-performance fibre monomers • Melody Yap Metabolic and Evolutionary Engineering of *Yarrowia lipolytica* for Sustainable Lipid Production from Acetate • Rimjhim Agarwal Navigating new product development and competition in the Food and Beverage market: focussing on higher value markets by leveraging market dashboards. • Shabbir Ahmad Unlocking the potential of sustainable aviation fuel in Australia: Market Prospects, Feedstock Viability, and Policy Pathways
17:30 – 19:30	Free time

19:30 – 21:30	Dinner I (Moreton Grill Buffet by the beach)
THURSDAY 8TH APRIL 2025	
07:00 – 08:30	Breakfast
08:30 – 09:30 **CHAIR** **DENYS & JOSEPH**	Workshop III. Biohub Facilities How can IDEA Bio, Q-MAP support your project? Introduction by Axa and Esteban A Panel Discussion with: • Yu Sun • Helen Wong • Dara Daygon • Anh Phan • Subaru Muroi Esteban to introduce Heather, Hub Floor Manager
09:30 – 10:30 **CHAIRS** **YOSEPHINE & DAMIAN**	Seminar III – Innovative Ingredients • Nathan Qifeng Zhong Rare Sugar Production Using Cell Factories • Baode Sun Engineering *Yarrowia lipolytica* to produce psilocybin, a high-value therapeutic candidate • Isabella Casini Optimizing Microbial Metabolism for a Sustainable Future: The Role of Metabolic Modelling • Froylan Garnica Garcia Profitable Upcycling of Spent Genetically Modified Microbial Biomass from Precision Fermentation
10:30 – 11:00	Morning Tea
11:00 – 12:30 **CHAIRS** **INES & BIRGITTA**	Seminar IV - Microbial communities Bioengineering - biomining, biomaterials, waste valorisation • Ilan Blanville The potential of soil microbial communities to reduce nitrous oxide emissions in agricultural soils. • Ian Petersen

	Optimisation of phosphate-solubilising microbes for agricultural applications • Tamara Važić Cyanobacterial potential for adaptation, colonisation and carbonisation of bischofite-enriched sandy substrates in semi-arid and arid environments • Fernanda Soto Improving bioleaching efficiency of critical elements from bauxite residue. • Luke Webster Identification of critical metal binding proteins from mine waste • Rosemary Gillane Seeing the value in waste: Isolation of microbes from bauxite residue to recover critical minerals
12:30 – 16:30	Lunch to go + Free time
16:30 – 17:30	Scientific Speed Dating Hosted by Nathan, Hemanshi
17:30 – 19:30	Free time
19:30 – 21:30	Dinner I (Moreton Grill Buffet by the beach)

FRIDAY 9TH APRIL 2025

07:00 – 08:30	Breakfast
08:30 – 09:30 **CHAIRS** **AXA** **&** **ZEINAB**	Seminar V - Multi-omics Analysis & Computational Biology • Zhuoqi Xiao A comparative study of site-specific and random genomic integration in CHO cells for bispecific antibody production • Craig Barry Identifying critical mechanisms for optimizing yields of red blood cell cultures using temporal single cell proteomics • Rafael Eduardo Hernández-Guisao Metabolic Pathway Analysis of a condensed metabolic model of *Chlamydomonas reinhardtii* tailoring lipid profile and accumulation • Tim McCubbin A Metabolic-Model Driven Exploration of the Dynamic Shifts in Cyanobacterial Metabolism across a Batch Fermentation Process
09:30 – 10:00	Morning Tea
10:00 – 11:00	Check out
11:00 – 11:45	The Future of the Biohub & Closing talk
11:45 – 13:00	Lunch
14:30	Ferry to Brisbane (Trip 1)
16:00	Ferry to Brisbane (Trip 2)

Workshop I

BioHub Group Leaders

Panel Discussion

Chairperson

Mariko Terasaki Hart

Panel Members

Adrian Oehmen

Birgitta Ebert

Bronwyn Laycock

Damian Hine

Denys Villa Gomez

Esteban Marcellin

Huadong Peng

Yosephine Gumulya

Zeinab Khalil

Gary Schenk and Lars Nielsen were unable to join us at the conference.

We appreciate their important contributions to the UQ Biosustainability Hub.

Adrian Oehmen - The entrapment of sulfate-reducing bacteria improves the performance and stability of mining-influenced water treatment

Xinting Yin[1], Nicholas Gurieff[2],

[1]School of Chemical Engineering, The University of Queensland, St Lucia, Australia. [2]Rio Tinto, Brisbane, Australia

This study developed a method for sulfate removal from acid mine drainage (AMD) utilizing entrapped sulfate-reducing bacteria (SRB) in a polymeric matrix. In long-term observations of two parallel sequencing batch reactors (SBR) – one with non-entrapped SRB and the other with entrapped SRB, it was found that immobilized SRBs exhibited superior sulfate removal efficiency and was more resilient to operational disturbances, such as temperature changes. SRB entrapment has the potential to offer an innovative solution for AMD, contributing towards cost-effective and sustainable mining water treatment.

Mining activities often lead to the generation of acid mine drainage (AMD), which is characterized by high acidity and the presence of heavy metals. SRB have been identified as valuable anaerobic microorganisms in the treatment of AMD due to their ability to reduce sulfate and precipitate heavy metals. However, SRBs are typically unstable in traditional applications of AMD treatment and metal recovery. Hence, mining water treatment is generally limited to the use of constructed wetlands to ensure desired treatment results, which occupy large land areas. Also, SRBs are susceptible to toxic shocks due to disturbances. The application of hydrogel entrapped sulfate-reducing bacteria (SRB) is proposed here as a promising strategy to enhance bioreactor performance and protect cells against disturbances. In this study, two anaerobic lab-scale bioreactors were established to assess and compare the sulfate removal performance of entrapped and non-entrapped SRB for AMD treatment.

First, non-entrapped SRBs were cultivated in a 1L anaerobic SBR containing brewery waste sludge. Between days 0-20, 21-40, 41-69 and 70-217, the sulfate removal rate averaged, respectively, 0.14, 0.23, 0.33, and 0.55 g sulfate g cell^{-1} day^{-1}. During the various phases, the influent concentrations of COD and sulfate were altered according to sulfate removal rate and COD/SO_4 removal ratio during this acclimatization phase. During Phase IV, the enriched SRBs were entrapped in a polymeric hydrogel matrix. The sulfate removal rate of entrapped SRB showed rapid improvement across four operational phases, reaching 1.4 g sulfate g cell^{-1} day^{-1} in the final phase, surpassing the removal rate of non-entrapped SRB by nearly three-fold. It is worth noting that the average COD/SO_4 removal ratio of entrapped SRB decreased to about 0.8 g sulfate g COD^{-1}, which is lower than the average COD/SO_4 removal ratio of non-entrapped SRB (1.4 g sulfate g COD^{-1}). This suggests that entrapped SRB can achieve the same amount of sulfate removal while consuming less organic carbon source, saving operational costs. Additionally, during the 210 day operational period of the entrapped SRB bioreactor, it was established that the sulfate removal rate remained more stable in comparison to non-entrapped SRB, despite a change in temperature from 24°C to 15°C.

By applying immobilized SRB to mining-influenced water treatment, there can be significant savings in the mining-influenced water treatment cost. SRB entrapment safeguards the functional stability of SRBs in AMD treatment, ensuring protection from toxic shocks and facilitating simple maintenance and cleaning. Future work will explore its application for metal recovery.

Birgitta E. Ebert - Enhancing carbon yield and redox efficiency of *Pseudomonas putida* via synthetic chemolithoheterotrophy

Daniel Bergen[1], Simon Grieshaber[2], Oscar Puiggene[3], Esteban Marcellin[1], Robert E. Speight[4], Bastian Blombach[5], Pablo I. Nikel[3]

[1]The University of Queensland, St Lucia, Australia. [2]Tu Munich, Straubing, Germany. [3]DTU Denmark, Lyngby, Denmark. [4]CSIRO, Brisbane, Australia. [5]TU Munich, Straubing, Germany

Focus Area: Bioengineering, Precision Fermentation

Area of Expertise: Molecular Cloning, Modelling, Strain Engineering

Favorite Organisms: *Escherichia coli, Pichia pastoris, Pseudomonas putida, Saccharomyces cerevisiae*

In bioprocesses, redox metabolism is a key determinant of biosynthetic efficiency, yet in chemoheterotrophs, the regeneration of NAD(P)H is inherently linked to carbon oxidation, leading to substantial carbon loss as CO_2. Synthetic chemolithoheterotrophy offers a strategy to decouple redox cofactor regeneration from carbon metabolism by harnessing inorganic electron donors, thereby minimizing CO_2 emissions and maximized carbon efficiency. Metabolic models predict that this approach can significantly enhance carbon yields and enable mixotrophic growth.

Our experimental validation of this concept has demonstrated that recombinantly expressed O_2-tolerant NAD^+-reducing hydrogenases enable *Pseudomonas putida* to utilize hydrogen as an alternative electron donor, resulting in a 30% increase in biomass yield. The use of an NAD^+-dependent phosphite dehydrogenase with phosphite as an alternative electron donor has led to similar improvements in carbon efficiency. These findings highlight the potential of synthetic chemolithoheterotrophy in *P. putida* for sustainable biomanufacturing applications, especially of highly reduced molecules such as biofuels, lubricants, and other industrial chemicals.

By rewiring redox and carbon metabolism, our work advances *Pseudomonas* as a versatile microbial platform for H_2-driven bioproduction, paving the way for more sustainable and economically viable industrial biotechnology.

Damian Hine - How deploying the economics of innovation to increase the likelihood of good ideas reaching their intended markets and audiences

The University of Queensland, Brisbane, Australia

The commercialisation process has often been portrayed as being atheoretical. However, commercialisation is only a subset of innovation, which has a long and rich history in economics. The theory underpinning innovation research is founded on evolutionary economics – the study of change at the firm, product and technology levels. The focus of this work is on disruption – from the creation of disruptions to responding to disruptions. The use of relevant theory aligned research design and methodologies, while adhering to the scientific methods that have actually generated scientific breakthroughs is at the core of designing successful innovation strategies. This also keeps the scientist/innovator at the core of the innovation process. The economics, with a focus on assessing the feasibility, viability, and scalability of science and technology is used to facilitate the translation of research into impactful and commercially viable outcomes – or to realise where a research output may not survive to have the impact desired by its creator.

I use examples from a range of industries to highlight where and how the analytical frameworks and specific analyses are being deployed – such as in sustainable fuels, digital health technologies, microbial protein production, and plant-based biomanufacturing.

First to biofuels, an underdeveloped industry in Australia, but one with great potential to reducer our reliance on fossil fuels, diversify and strengthen our advanced manufacturing base, increase our fuel sovereignty and develop agricultural production that could support regional and local socio-economic development and community growth and resilience. In upstream development we are assessing alternative agricultural feedstocks (such as sugarcane bagasse, sorghum, pongamia and canola) in order to design a biofuels industry for Australia based on the principles of sustainability (environmental, social, economic, political), viability and scalability. Economic and financial studies of the alternative feedstocks (Sugar research Australia funded) are presented including efforts currently being undertaken to design an institutional investment vehicle to drive investment in a coordinated industry-wide fashion. This work is augmented by the design of a governance structure for the fledgling biofuels industry in Australia, so self-interest can be harnessed for common good.

Another more specific biofuels initiative (ARC Discovery and private sector funded) focuses on the downstream development and potential commercialisation of an enzymatic cascade to progress pyruvate to isobutanol to overcome current yield and titre limitations that have hamstrung this fuel globally. This work is being undertaken with SCMB (Schenk, Boden and Guddat), and Gevo, a major US biofuel refiner. For this we are conducting techno-economic analyses (TEA), market analyses, investment landscape analyses, regulatory landscape analyses

and supply chain and logistics (including risk) analyses to ascertain the economic viability and map the most effective commercial pathways for isobutanol.

Within FaBA, a major focus is on the development of data analytics that support evidence-based decision making for small firms and researchers during their new product development process. This includes rapid market analyses, through to dashboards designed to allow enterprises to look forward across the pre-competitive (prior to market launch) to competitive (on-market) product phases using historical longitudinal data sourced from over 45 different data sources (including ABS, Mintel, Statista, Factiva, Bureau Van Dyk, USDA Food Central, FAO Statistics, Australia Business Register database and so on). This is being established to aid the competitiveness of small F&B businesses who would otherwise rarely have access to such comprehensive analytics for little to no cost.

We apply established frameworks such as Technology Readiness Levels (TRLs), along with more novel frameworks such as the Commercialisation Tourbillon (a 12-step analytical framework that integrates established tools from diverse disciplines to rigorously assess the overall feasibility of translating and commercialising technologies) to map the developmental trajectories of promising innovations. Through this approach we map multiple potential product pathways with varying degrees of rarity and excludability, to identify the most promising and commercially viable paths to market. We also observe how regulatory bodies strategically design adaptive regulation from the outset, aiming to effectively accommodate emergent and potentially disruptive technologies within the regulatory landscape.

In conclusion, while the research initiatives are diverse, the research designs and motivations are consistent – to increase the proportion of good ideas that succeed on in hotly contested market environments. The application of predictive analyses to support strategic foresight coupled with methodological innovations, to develop effective commercialisation strategies that can successfully translate fundamental research outcomes into tangible and impactful solutions.

Denys Villa Gomez

The University of Queensland, Brisbane, Australia

The global imperative for achieving net-zero emissions demands transformative approaches that integrate circular economy principles with advanced biotechnological solutions. As Group Leader within the Biosustainability Hub, my research bridges the gap between industrial waste challenges and sustainable resource recovery, leveraging bioprocess engineering, synthetic biology, and gas fermentation to drive decarbonization and circularity. Our team is at the forefront of innovative biotechnology research, focusing on developing processes to recover valuable resources from waste, reduce carbon emissions through biological approaches, create sustainable solutions for industrial waste management, and bio-sequester greenhouse gases using waste materials.

To achieve these ambitious goals, we employ advanced techniques such as omics approaches, micro-spectral tools, synthetic biology, extremophile microbe cultivation, and bioprocess engineering. Our key focus areas include:

1. Developing bioprecipitation and bioleaching strategies to extract zinc, nickel, cobalt, and rare earth elements (REEs) from mining waste, such as red mud.
2. Driving microbial communities to sequester CO_2 via mineral carbonation and convert methane into fertilizers through gas fermentation.
3. Partnering with industry to deploy biohydrometallurgy for detoxifying acid mine drainage (AMD) and scaling bioextraction processes.

The practical application of our expertise is demonstrated through strategic partnerships with global industry leaders such as Rio Tinto™, SQM™, Woodside™, MOSAIC™, and Windfall Bio™. These collaborations have enabled the translation of our research into scalable solutions. For instance, by using metal-binding proteins and fungal strains, we are unlocking gallium and REEs from bauxite residue our research this in partnership with Rio Tinto™ . Similarly, our research collaboration with Windfall Bio™ has led to significant advancements in adapting and characterizing methanotrophic microbial consortia and establishing optimal scale-up procedures for production.

By merging synthetic biology with industrial needs, our research group is redefining waste valorisation and offering scalable pathways to decarbonise heavy industries. Through innovation in bioprocess engineering and strategic industry alliances, we are demonstrating that biotechnology can reconcile economic growth with planetary boundaries. As we continue to push the boundaries of what's possible, we are forging a sustainable future for global manufacturing and proving that cutting-edge biotechnology holds the key to addressing some of the most pressing environmental challenges of our time.

Esteban Marcelin - The Biosustainability Hub at UQ: Engineering Systems for a Sustainable Future

AIBN, The University of Queensland, St Lucia, Australia

The transition to a bio-based economy requires innovative approaches to engineering, systems biology, and industrial biomanufacturing. The Biosustainability Hub at UQ integrates synthetic biology, automation, and learning to accelerate the development of high-performance microbial strains for sustainable chemical, SAFs, foods (FaBA) and material production. By harnessing renewable feedstocks such as waste gases, biomass-derived sugars, and industrial byproducts, our research enables the replacement of fossil-based processes with scalable, carbon-efficient biomanufacturing. Through advanced metabolic engineering, high-throughput screening, and machine learning-guided optimization, we develop microbial cell factories capable of producing sustainable fuels, chemicals, and materials with minimal environmental impact. Our strategic industry partnerships facilitate the translation of these innovations from the lab to commercial-scale production, bridging fundamental science with translational applications.

Technoeconomic assessment of biological solutions often identifies feedstock cost as a key limitation to commercial viability. This challenge can be overcome by utilizing waste gases as feedstocks for biomanufacturing, transforming greenhouse gases into valuable bioproducts. At the Biosustainability Hub, we employ systems biology, using Q-MAP, to identify metabolic bottlenecks and enhance strain performance through targeted engineering. By integrating advanced bioprocess optimization strategies, using IDEA Bio, we take microbial strains from milligram-scale lab experiments to kilogram-scale production, ensuring their robustness and efficiency at industrial scale. This approach enables the development of cost-effective, scalable, and sustainable biomanufacturing processes that reduce reliance on fossil resources while valorizing carbon waste streams.

By collaborating with industry, we de-risk emerging technologies and accelerate their market adoption. Positioned at the intersection of academia and industry, the Biosustainability Hub strengthens Queensland's role as a hub for industrial biotechnology, driving decarbonization, enhancing resource circularity, and fostering a resilient bioeconomy.

Huadong Peng - Yeast Engineering Biology and Synthetic Biology for Sustainability (YESBio)

Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St Lucia, Australia

Focus Area: Precision Fermentation

Area of Expertise: Molecular Cloning, Strain Engineering

Favorite Organisms:*Microbial Communities/ mixed cultures, Pichia pastoris, Saccharomyces cerevisiae, Yarrowia lipolytica*

Engineering biology and synthetic biology have transformed the biotechnology industry, creating unprecedented opportunities to enhance the efficiency and scalability of biomanufacturing. This presentation will explore the development of novel synthetic biology tools and metabolic engineering strategies to optimise yeast cell factories and synthetic yeast communities for the biosynthesis of high-value compounds in sectors such as agri-food, biofuels, and biomedicine. First, I will introduce the development of a molecular toolkit for engineering synthetic yeast communities. A high-throughput automated screening platform was used to evaluate synthetic yeast auxotrophic co-cultures using a yeast single knockout library. Additionally, innovative molecular tools were designed to construct and validate two-member and three-member yeast co-cultures, demonstrating their ability to enhance the bioproduction of resveratrol, a key antioxidant. Next, I will present examples of modular metabolic engineering applied to the *de novo* biosynthesis of high-value compounds, including functional lipids such as cyclopropane fatty acids and the natural flavour compound raspberry ketone. Finally, I will introduce the current research focus of my team at the Australian Institute for Bioengineering and Nanotechnology (AIBN) and the UQ Biosustainability Hub: the Yeast Engineering and Synthetic Biology (YESBio) Platform. Through this platform, I aim to develop advanced yeast cell factories and synthetic microbial communities, providing sustainable biotechnological solutions to address challenges and opportunities in the agri-food and medical sectors.

How does my work support or relate to the vision and mission of the Biosustainability Hub?

My research aligns with the UQ Biosustainability Hub's vision by developing sustainable yeast cell factories and synthetic microbial communities for precision fermentation and biomanufacturing, reducing reliance on petroleum-based chemicals and resource-intensive food production. These innovations contribute to net-zero emissions and climate change mitigation by enabling low-emission biomanufacturing, carbon-efficient food systems, and circular bioeconomy solutions.

Yosephine Gumulya - Methane2Protein: Exploring the use of simulated microgravity bioreactors for methane conversion.

Wenbin Zhang, Lars Puiman, James Heffernan, Esteban Marcellin

University of Queensland, Brisbane, Australia

Achieving net zero emissions by 2050 requires effective methane removal given its significant impact on global warming. One promising strategy is transforming methane into single cell protein (SCP). However, this approach faces major challenges, notably the poor solubility of methane, which limits effective mass transfer. Previous studies on methane oxidising bacteria (MOB) for SCP production typically used stirred tank bioreactors that rely on high agitation to ensure adequate gas-liquid transfer, or bubble column reactors that depend on fluid dynamics for mixing. Here, we investigate the use of high aspect ratio vessels, bioreactors commonly used for simulating microgravity conditions, to enhance gas-liquid mass transfer, biofilm formation and protein production. We hypothesise that the absence of convection in simulated microgravity conditions creates to a diffusion dominated environment for mass transfer. Biofilms are essential for facilitating the growth of MOB in methane waste by providing a protective matrix that shields bacteria from harmful inhibitors. In typical stirred tank bioreactors, mechanical agitation disrupts biofilm integrity. The low shear stress environment of simulated microgravity bioreactors may allow for more stable biofilm development, leading to more robust and active MOB. This optimisation could significantly increase the biomass and protein yield from MOB, transforming methane waste into a sustainable protein source.

Zeinab Khalil

The University of Queenslnad, Brisbane, Australia

Dr. Zeinab Khalil is an ARC Future Fellow and the Director of the Soils for Science Program at the Institute for Molecular Bioscience, The University of Queensland. As a highly accomplished researcher and qualified pharmacist, she specializes in natural product discovery, medicinal chemistry, and microbiology. Dr. Khalil completed her Bachelor's degree in Pharmacy, followed by a Master's degree and a PhD focused on natural product discovery and microbial ecology. Leading the Soils for Science initiative, Dr. Khalil has established a pioneering platform dedicated to uncovering novel antibiotics and biopesticides from soil microbiomes. Her innovative research integrates genomics, metabolomics, and advanced natural product chemistry, contributing significantly to combating antimicrobial resistance, enhancing agricultural productivity, and promoting environmental sustainability. Through this initiative, her team has generated over 3,000 microbial strains, with more than 30% demonstrating promising antibiotic and biopesticide properties. Dr. Khalil has successfully secured over $4 million in research funding and has published more than 104 scientific papers, with over 2400 citations (h-index 32). Her extensive national and international collaborations include partnerships with QAFFI, the Bush and Botanical Indigenous Enterprise Cooperation, and various global research institutions (e.g. Novo Nordisk Foundation) and industry partners (e.g. Agilent Technologies). These collaborations aim to accelerate the discovery of novel antibiotics and sustainable solutions for agriculture. Her work continues to bridge academic research and industry collaborations, ensuring impactful solutions to some of the world's most pressing health and environmental challenges.

Seminar I

Gas Fermentation

Chairperson

Jitendra & Adrian

Presenters

Karen Rodriguez

James Heffernan

Lars Puiman

Antonia Ebert

Hemanshi Galaiya

Nhat Huynh

Pathway engineering and systems biology for optimised isobutanol production in the gas-fermenting acetogen *Clostridium autoethanogenum*

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

[1]The University of Queensland, Brisbane, Australia. [2]LanzaTech, Skokie, USA

Focus Area: Bioprocess Optimisation, Gas Fermentation

Area of Expertise: Bioprocess engineering/optimisation, Omics - Metabolomics, Proteomics, etc., Systems Biology

Favorite Organisms: *Clostridium autoethanogenum, Microbial Communities/ mixed cultures"*

Concerns over rising greenhouse gas emissions from the transportation sector have driven the search for low-carbon fuel alternatives. The aviation industry alone accounts for 13% of transportation-related emissions, yet decarbonising this sector remains challenging due to its reliance on high-energy-density fossil fuels. Given the limitations of electric aircrafts, the preferred pathway to reducing aviation emissions is the development of sustainable aviation fuels (SAFs). However, most biofuels currently produced are derived from edible biomass (first-generation biofuels), creating competition with food supply and arable land use.

Waste gases from agricultural, industrial, and municipal sources present a promising alternative feedstock for SAFs via microbial gas fermentation. Acetogens, a group of anaerobic microorganisms, are particularly well-suited for gas fermentation due to their ability to convert CO, CO_2, and H_2 into valuable compounds, including acids and alcohols. Among them, *Clostridium* species have been extensively engineered to expand their product spectrum, providing viable low-carbon fuel alternatives. *Clostridium autoethanogenum* is a strong candidate for metabolic engineering due to its well-established genetic toolbox, proven scalability, and efficiency in gas-to-liquid conversion.

Our research focuses on harnessing acetogens as CO_2-fixing cell factories to produce advanced biofuels, specifically targeting non-native isobutanol production via gas fermentation. Isobutanol, a C4 alcohol with superior fuel properties, is a promising intermediate for alcohol-to-jet SAF conversion processes, offering a pathway to reducing the aviation sector's carbon footprint. We employed *C. autoethanogenum* as a platform for heterologous isobutanol production, leveraging its robustness and adaptability for continuous bioreactor performance.

Engineered strains were developed using a tailored enzymatic pathway derived from valine biosynthesis, incorporating diverse enzyme variants catalysing isobutanol synthesis from pyruvate. Initial fermentation studies assessed the impact of enzyme combinatorial assembly and pathway intermediate availability on isobutanol production. A systematic analysis of high-performing strains identified metabolic bottlenecks constraining production, revealing that thermodynamic constraints play a significant role in isobutanol biosynthesis.

This work advances our understanding of acetogen carbon fixation and metabolism, highlighting the interplay between metabolic robustness, energy conservation, and redox balance in heterologous isobutanol production. Key metabolic and culture-based strategies are identified to enhance isobutanol yields, contributing to the development of scalable and sustainable biofuel technologies for aviation.

CO(2)-Metabolism: Quantifying Mixed Carbon Oxide and Hydrogen Gas Fermentation by *Clostridium autoethanogenum*

James Heffernan, Lars K. Nielsen, Esteban Marcellin

AIBN, UQ, Brisbane, Australia

Focus Area: Bioengineering, Bioprocess Optimisation, Computational Biology, Gas Fermentation, Multi-omics Analysis, Translational / bioeconomy research

Area of Expertise: Adaptive Laboratory Evolution, Systems Biology Bioprocess engineering/optimisation, Omics - Metabolomics, Proteomics, etc.

Favorite Organisms: *Clostridium autoethanogenum, Cupriavidus necator, Escherichia coli, Hydrogenophaga pseudoflava, Methanotroph, Pichia pastoris*

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 H2 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 H_2 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 CO2/CO by *C. autoethanogenum* is highly flexible in the presence of H_2, maintaining co-utilization of CO2 and CO over a wide range of gas compositions. Further, a multi-omics analysis elucidates novel mechanisms of C1 metabolism and redox homeostasis. Surprisingly, we also find conditions with high 2,3-butanediol flux, a feature thought to be driven by CO-based metabolism. Use of these findings could be influential to broad C1 gas-to-liquid processes, providing a novel avenue for the circular carbon economy and sustainable production platforms.

Bioreactor design and modelling for gas fermentation processes

Lars Puiman, James Heffernan, Timothy McCubbin, Esteban Marcellin

The University of Queensland, Brisbane, Australia

Focus Area: Bioprocess Optimisation, Gas Fermentation

Area of Expertise: Automation, Bioinformatics, Data Scientist, Bioprocess engineering/optimisation, Fermentation Services, Modelling

Favorite Organisms: *Clostridium autoethanogenum, Cupriavidus necator, Cyanobacteria, Methanotroph, Microbial Communities/ mixed cultures*

The increasing levels of greenhouse gases like CO_2 and CH_4 in earth's atmosphere and associated climate change emphasize the need for innovative carbon converting technologies. In the past few years, gas fermentation arose as a prominent technology to capture and convert C1-rich waste streams into useful products [1]. The successful application of gas fermentation processes is highlighted by its commercial implementation by numerous companies like LanzaTech, SolarFoods and Calysseo. Although these companies operate on highly concentrated C1 waste streams, the current challenge is to convert less concentrated and more abundant waste streams. This includes sources like biogas, CO_2 from waste incineration and diluted methane emissions stemming from agriculture and nature.

Converting these sources is challenging, not only due to the inherently present mass transfer limitations and the low volumetric rates associated with it, but also due to operational safety aspects related to flammability and explosivity [2]. The significant reactor volume that is required for successful industrial operation of gas fermentation processes, leads to concentration gradients, which can have an unpredictable effect on the outcome of the fermentation process [3].

Computational modelling is a relatively cheap and low-risk tool that can be used to estimate the severity of these challenges and identify ways to overcome them. For example, it helps estimating which operating conditions can be used to achieve high productivities and gas conversion rates and whether the operation might be safe or not. High-resolution tools such as Computational Fluid Dynamics can be used to design industrial-scale reactors by optimizing geometry and operating conditions but may also be used the estimate the effect of the local conditions and the concentration gradient on the microbial population.

This work showcases how computational and CFD modelling can be applied to a variety of (gas) fermentation processes. We will demonstrate how modelling may be used to overcome the technical challenges in gas fermentation, paving the way towards net zero carbon emissions.

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2. K. Quataert, E. Verhoeven, K. De Winter, H. Waegeman, K. Quataert, E. Verhoeven, · K De Winter, · H Waegeman, Piloting, Scale-Up, and Demonstration, (2023) 387–405. https://doi.org/10.1007/978-3-031-27811-2_34.
3. G. Nadal-Rey, D.D. McClure, J.M. Kavanagh, S. Cornelissen, D.F. Fletcher, K. V. Gernaey, Understanding gradients in industrial bioreactors, Biotechnol. Adv. 46 (2021). https://doi.org/10.1016/j.biotechadv.2020.107660.

Filing the gap towards sustainable bioplastic production - unlocking the correlation between polyhydroxybutyrate production, redox balance and hydrogenase expression in *C. necator* H16

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[1]University of Queensland, Brisbane, Australia. [2]Technical University of Denmark, Lyngby, Denmark

Focus Area: Bioengineering, Gas Fermentation

Area of Expertise: Adaptive Laboratory Evolution, Fermentation Services, Molecular Cloning

Favorite Organisms: *Cupriavidus necator*

Gas fermentation offers a promising pathway for sustainable bioproduction by utilising microorganisms to convert industrial waste gases into valuable materials. *Cupriavidus necator* H16, a versatile hydrogen-oxidising bacterium, has emerged as an ideal candidate for biotechnological applications, particularly in the production of polyhydroxybutyrate (PHB) bioplastics from CO_2 and H_2. However, optimising hydrogenase activity — a key enzyme that facilitates hydrogen metabolism — remains a challenge, as its regulation directly impacts carbon flux and overall PHB yields.

This study investigates the dynamic relationship between hydrogenase activity, intercellular redox balance and PHB biosynthesis during gas fermentation, with a specific focus on maintaining safety margins in H_2:O_2 ratios. While *C. necator* efficiently utilises H_2 as an electron donor, our findings indicate an unexpected repression of hydrogenase xpression, even when CO_2 and H_2 is non-limiting. This phenomenon suggests a metabolic trade-off between energy generation and carbon sequestration, where PHB synthesis is prioritised over hydrogen oxidation despite similar growth rates to H_2 limited cultures.

To investigate this relationship, we employed turbidostat gas fermentation experiments, with various O_2 and H_2 levels and analysed the corresponding proteomes. Preliminary results reveal a correlation between increased PHB production and downregulation of hydrogenase expression, raising important questions about intracellular redox balance and regulatory mechanisms governing microbial metabolism under gas-fed conditions. Understanding these metabolic constraints is critical for optimising large-scale bacterial gas fermentation systems, aligning directly with the Biosustainability Hub's mission to drive economically viable biotechnological solutions that support industry in transitioning to net zero. Through metabolic engineering, we aim to fine-tune the balance between hydrogenase activity and PHB yields, to ultimately reduce our reliance on petrochemical-derived plastics while transforming industrial waste gases into high-value products. Overall, advancing our understanding of hydrogenase regulation will facilitate the development of scalable, safe, and efficient microbial gas fermentation technologies, reinforcing the Hub's position as a leader in biosustainable innovation.

Assessing multi one-carbon conversion in the aerobic carboxydotroph *Hydrogenophaga pseudoflava*

Antonia Ebert[1], James Heffernan[1], Yulu Wang[2], Craig Morton[3], Robert Speight[2], Bastian Blombach[4], Esteban Marcellin[1]

[1]The University of Queensland, Brisbane, Australia. [2]CSIRO, Brisbane, Australia. [3]CSIRO, Clayton, Australia. [4]Technical Univerity of Munich, Straubing, Germany

Focus Area: Gas Fermentation, Multi-omics Analysis

Area of Expertise: Adaptive Laboratory Evolution, Omics - Metabolomics, Proteomics, etc, Systems Biology

Favorite Organisms: *Hydrogenophaga pseudoflava*

Microbial waste gas conversion into valuable products is hoped to be a major game-changer in mitigating the release of greenhouse gases into the atmosphere. Aerobic gas fermenting microbes can produce energy intensive compounds, e.g., polyhydroxyalkanoates, autotrophically from CO_2, CO, H_2, and CH_4, major components of e.g., syn- and biogas. Based on the utilized substrate, these bacteria are grouped as carboxydotrophs (CO, CO_2, H_2), hydrogenotrophs (CO_2, H_2) or methanotrophs (CH_4). Here, the carboxydotrophic *Hydrogenophaga pseudoflava* strain DSM 1084 is characterized as an autotrophic host for the conversion of CO, CO_2, and H_2. Our focus lies in understanding the native aerobic carboxydo-hydrogenotrophic metabolism through phenotypical characterization in bioreactors. Particularly the growth regulation and activity of the main autotrophic enzymes – hydrogenase and carbon monoxide dehydrogenase – during the feed of different gas mixes. Maximal growth rates of 0.14 ± 0.008 h^{-1} were observed during growth with 65 % H_2, 10 % CO_2, 4 % O_2 in argon and nitrogen atmosphere. We aim to establish novel waste-gas-based bioproduction processes in which feedstocks can be highly flexible. Therefore, we will expand the strains substrate spectrum to formate, methanol and methane through heterologous expression and re-activation of natively harboured enzymes. Our results demonstrated functionality of the methanol and formaldehyde dehydrogenases, validating the strain as a multi one-carbon assimilation host. As *H. pseudoflava* does not comprise a functional soluble formate dehydrogenase, we heterologously express the soluble *fds* cluster of *Cupriavidus necator* H16. Combination of both the phenotypical analysis and synthetic biology will aid in the development of *H. pseudoflava* as a host for capturing mix gas streams from industry and waste processing.

This research aligns well with the gas fermentation theme of the Biosustainability Hub, aiming to minimize the release of greenhouse gases into the atmosphere to mitigate climate change and contribute to a circular economy. Further, possible products of *H. psuedoflava* include biomass, for food and feed production, as well as biopolymers which have a prolonged capture over fuels.

MMOneo: engineering an industrial host organism to convert methane waste via expression of methane mono-oxygenase.

Nhat Huynh, James Heffernan, Yosephine Gumulya, Esteban Marcellin

The University of Queensland, Brisbane, Australia

Focus Area: Bioprocess Optimisation, Gas Fermentation, Precision Fermentation

Area of Expertise: Bioprocess Engineering/optimisation, Molecular Cloning, Recombinant Protein Production

Favorite Organisms: *Escherichia coli, Methanotroph, Pichia pastoris*

Reducing methane emissions is crucial for achieving immediate climate benefits, given its potency as a greenhouse gas. While significant efforts are underway to transition from fossil fuels to clean energy, methane mitigation remains a challenge. One promising approach is converting methane waste into feedstock for biomanufacturing, enabling economically viable biological solutions, in which the use of C1 compounds like methanol in yeast-based bioproduction has already been demonstrated for the synthesis of high-value products. In nature, methanotrophs can convert methane into methanol and various value-added chemicals through methane monooxygenase (MMO), an enzyme requiring either copper (membrane bound, particular MMO) or iron (cytoplasmic soluble MMO) as a cofactor. However, methanotrophs have limitations for industrial bioprocessing, namely strain engineering and methanol accumulation.

To overcome these challenges, integrating the MMO enzyme from methanotroph to an industrial host with native methanol metabolism and the availability of molecular toolkit presents a promising alternative. Our research aims to advance the “methane-to-X” biomanufacturing platform using *Pichia pastoris* by integrating recombinant soluble MMO (sMMO) protein expression. sMMO is a multi-subunit enzyme composed of three functionally distinct components: hydroxylase, reductase, and a regulatory protein. However, its catalytic oxidation has remained challenging due to the enzyme’s structural and functional complexity. Building on the findings of Yu et al. (2024), where a mini-soluble MMO was successfully heterologously expressed in *E. coli* with ferritin as protein scaffold, our next steps focus on expressing this recombinant protein in *Pichia pastoris*. Targeting specific subcellular organelles, such as peroxisomes, may enhance enzyme activity and stability. Additionally, it is expected that deep cellular characterization of *Pichia* strains will be conducted using multi-parallel bioreactors. Multi-omics data will inform systems biology analyses and bioprocess optimization, with candidate strain performance validated through metabolic flux modelling.

This project is expected to provide key insights into MMO function while establishing a *Pichia*-based “methane-to-X” platform. This platform could benefit Australia’s primary industries by supporting the production of innovative food ingredients and single-cell protein, aligning with the gas fermentation theme of the Biosustainability Hub.

Yu, Y., Shi, Y., Kwon, Y.W. *et al.* A rationally designed miniature of soluble methane monooxygenase enables rapid and high-yield methanol production in *Escherichia coli*. *Nat Co mmun* **15**, 4399 (2024)

Workshop II

BioHub Industry

Panel Discussion

Chairperson

Caroline Scott - DVC RI, Strategic Partnerships

Panel Members

Denys Villa Gomez - School of Civil Engineering

Adrian Oehmen - School of Chemical Engineering

Jitendra Joshi - Woodside Energy

Axa Gonzalez - IDEA Bio

Bronwyn Laycock - School of Chemical Engineering

Caroline Stott

DVC RI - Energy Transitions, Strategic Partnerships - Government and Industry, The University of Queensland

With a focus on energy transitions, Caroline Stott connects researchers with industry and government partners to explore collaborative research activities, establish commercial partnerships and develop scalable business models.

Caroline has held management and commercialisation roles at Queensland University of Technology, Griffith University, The University of Queensland, the International Energy Centre and the Brandenburg University of Technology (in Germany).

She has a strong understanding of strategic research initiatives and large-scale engagements within university environments. She is adept at aligning internal capability with external opportunities to grow research capacity, further the impact and translation of research, and support long-term, mutually beneficial partnerships. Caroline holds undergraduate degrees in Science (Biomedical Science) and Arts (Economics and German), and a Master's in Project Management. She was formerly Chair of the Future Energy Lead ers group within the Energy Policy Institute of Australia.

Profile: research-support.uq.edu.au/profile/12908/caroline-stott

Denys Villa Gomez

Group Leader at School of Civil Engineering

The University of Queensland

Denys Villa Gomez holds a joint appointment at the University of Queensland as Senior Lecturer at the School of Civil Engineering and as a Research Fellow at the Australian Institute for Bioengineering and Nanotechnology. She obtained her PhD at the world-leading institute's IHE-Delft/Wageningen University, The Netherlands in 2013. She applies advanced methodologies such as omics approaches and micro spectral tools to develop biotechnology processes that reduce carbon emissions and recover resources from mine waste and wastewater. She is the leader of the key area "Synbio Mining" within the recently created UQ Biosustainability Hub and chief investigator at the ARC Training Centre in Critical Resources for the Future. She has published over 40 peer-reviewed journal and conference papers cited more than 500 times, and has served as editor and reviewer for leading journals and advisory roles in industry and scientific committees (e.g. International Mine Water Association).

Profile: about.uq.edu.au/experts/13368

Adrian Oehmen

Group Leader at School of Chemical Engineering

The University of Queensland

Adrian Oehmen is an Associate Professor in the School of Chemical Engineering at the University of Queensland. He leads research in the area of bioprocess engineering, particularly focussing on wastewater treatment and resource recovery. His research interests include enhanced biological phosphorus removal (EBPR or BioP), metabolic modelling, biopolymer (polyhydroxyalkanoate – PHA) production, micropollutant removal and greenhouse gas (N2O) assessment and mitigation. He also focusses on other aspects of bioprocess engineering, including microbial encapsulation, bioprocess modelling and food and beverage applications. He has published more than 100 papers in international scientific journals, led or collaborated on more than 30 research projects (many with industry). He is active within the International Water Association (IWA), serving on specialist group and conference committees and is an Associate Editor of Water Research.

Profile: about.uq.edu.au/experts/ 21291

Jitendra Joshi

Head of Technology for New Energy Solutions

Woodside Energy

Dr. Jitendra Joshi serves as the Head of Technology for New Energy Solutions at Woodside Energy in Perth, Australia. Jitendra's responsibilities include formulation of strategy for converting Greenhouse gases to value-added products and integrating renewables in Carbon transformation and Hydrogen generation. Jitendra's team at Woodside Energy implementing combination of biological and thermo-chemical pathways to realize the full potential of Greenhouse utilization and Hydrogen production.

Previously, Dr. Joshi was the Lead for Technology Integration within the Human Exploration and Operations Mission Directorate (HEOMD) at NASA. He has over two decades of Science and Technology project management experience with leadership roles in several research and technology development projects.

Profile: plants4space.com/team/jitendra-joshi

Axayacatl Gonzalez

IDEA Bio Facility Manager, The University of Queensland

Dr Axa specialises bioprocesses engineering and systems and synthetic biology. The key component of his research is the integration of microbiology, molecular biology, metabolic engineering and bioprocessing into a systems and synthetic biology approach that can lead to a better understanding of bioprocesses. Either producing biofuels, sustainable chemicals or antibiotics, he has participated in the development of significant contributions.

He has made important contributions in his discipline, providing a clearer understanding of cell metabolism, and developed strategies to improve the performance of cell factories using bioprocess strategies and metabolic engineering approaches.

As an early-career researcher, he has worked at the ARC-Training Centre for Biopharmaceutical Innovation (ARCT-CBI, 2018), the Queensland Strain Factory (QSF, 2019), ARC Centre of Excellence in Synthetic Biology (COESB, 2021) and is currently a Senior Fermentation Engineer for the Integrated Design Environment for Advanced biomanufacturing (IDEA bio) at AIBN-UQ.

In 2022, he was awarded an Early and Mid-Career Researcher Seen funding award by the ARC Centre of Excellence in Synthetic Biology to develop novel biofertiliser using synthetic microbial communitites. In collaboration with colleagues from the AIBN and SAFS. He also received a seed grant from the QAAFI and DAF Zero Net Emissions Grand Challenge Fund to develop a robust pipeline to generate synthetic microbial communities that has the potential to reduce NOx emission in farm lands.

His research has also allowed him to develop collaborations within the ARC Centre of Excellence in Synthetic Biology, including projects with Macquarie University, Queensland University of Technology; UQ, the School of Agriculture and Food Sciences and internationally with Instituto Politecnico Nacional (Mexico) and Technical University of Denmark (DTU). All these collaborations share a core goal: to build and characterise new microbial cell factories to produce a high-value products: biofuels, biofertilisers, and biopharmaceuticals.

Profile: aibn.uq.edu.au/profile/4058/ricardo-axayacatl-gonzalez-garcia

Bronwyn Laycock

Group Leader at School of Chemical Engineering

The University of Queensland

Professor Bronwyn Laycock has a diverse background in translational research, working not only in academia but also in industry and as a consulting chemist as well as at CSIRO. Her research activities have ranged from bio/degradable polymers, composites, organic and organometallic synthesis, waste conversion technologies, and pulp and paper chemistry, to general polymer chemistry. She is currently working across a range of projects with a focus on materials for circular economy applications and management of the transition to the new plastics economy. The application areas in her research program include biopolymers (particularly polyhydroxyalkanoates), polymer lifetime estimation and end-of-life management/conversion technologies, biocomposites, controlled release matrixes for pesticide and fertiliser applications, polyurethane chemistry, and biodegradable packaging.

She has a strong history of successful commercialisation and impact, being a co-inventor on CSIRO's extended wear contact lens program (recognised as its fourth most significant invention) - for which she was awarded a joint CSIRO Medal for Research Achievement 2009. As a Project Leader and Deputy Program Leader within the CRC for Polymers, she also managed a project that delivered an oxodegradable thin film polyethylene that was commercially licenced by Integrated Packaging. This work earned the team a Joint Chairman's Award for research/commercialization (CRC for Polymers) and an Excellence in Innovation Award (CRC Association).

Profile: about.uq.edu.au/experts/1873

Seminar II

Bioconversion

& Bioeconomy Research

Chairperson

Huadong & Bronwyn

Presenters

Jennifer Adami

Melody Yap

Rimjhim Agarwal

Shabbir Ahmad

Bioengineered lignin conversion into high-performance fibre monomers

Jennifer Adami, Birgitta Ebert

AIBN, Brisbane, Australia

Focus Area: Bioengineering, Bioprocess Optimisation, Synthetic Biology

Area of Expertise: Molecular Cloning, Strain Engineering

Favorite Organisms: *Escherichia coli, Pichia pastoris Pseudomonas putida*

Lignin, a major byproduct of the paper and agricultural industries, represents Earth's largest renewable source of aromatic compounds. However, it remains largely untapped due to its structural complexity. This project aims to unlock lignin's potential by engineering *Pseudomonas putida KT2440* to convert lignin-derived monomers into high-value aromatic compounds, particularly 4-hydroxybenzoate (4HB)—a key precursor for Vectran™, a high-performance polymer renowned for its remarkable strength and exceptional resistance. Our approach combines metabolic engineering, cytochrome P450 discovery, and ancestral sequence reconstruction (ASR). To expand the metabolic range of *P. putida*, heterogeneous pathways for non-native lignin-derived compounds will be introduced through genetic engineering. While the project aims to optimize lignin depolymerization for coniferal-type lignin monomers that can be readily converted into 4HB, the feedstock will also contain other lignin monomers and derivatives such as guaiacol-type and isoeugenol-type compounds. As *P. putida* requires metabolic engineering to utilize guaiacol and its derivatives, relevant enzyme candidates will be identified from literature and databases to later express them in the host strain. Initial metabolic pathway analyses revealed an interconnectedness between protocatechuic acid (PCA) and lignin depolymerization products in contrast to 4HB. To maximize carbon conservation and improve yields, we will enhance pathways leading to PCA and develop strategies for its efficient conversion into 4HB. Additionally, we will screen bacterial and eukaryotic P450 enzymes, as they can catalyze key oxidative reactions. A database search already identified promising candidates and further bacterial and eukaryotic P450 enzymes will be screened by using ancestral sequence reconstruction (ASR). By advancing microbial lignin valorization, this work contributes to a circular bioeconomy, offering a sustainable alternative to petrochemical-based materials.

Metabolic and Evolutionary Engineering of *Yarrowia lipolytica* for Sustainable Lipid Production from Acetate

Melody Yap[1,2,3], Huadong Peng[4,2,5]

[1]Australian Institute for Bioengineering and Nanotechnology, Saint Lucia, Australia. [2]ARC Centre of Excellence in Synthetic Biology, Saint lucia, Australia.[3]School of Agriculture and Food Sustainability, Saint lucia, Australia.[4]Australian Institute for Bioengineering and Nanotechnology (AIBN), Saint lucia, Australia. [5]Food and Beverage Accelerator (FaBA), Saint Lucia, Australia

Focus Area: Bioprocess Optimisation, Precision Fermentation

Area of Expertise: Adaptive Laboratory Evolution, Molecular Cloning, Strain Engineering

Favorite Organisms: *Yarrowia lipolytica*

Microbial lipid production offers a promising alternative for sustainable biofuels and omega-3 fatty acids. Yarrowia lipolytica can utilize acetate, a carbon source derived from industrial waste streams, offering a more direct lipid biosynthesis pathway than glucose. However, high acetate concentrations can inhibit growth, limiting its industrial potential. Improving acetate tolerance is therefore crucial for enabling efficient lipid production and upcycling of low-cost carbon sources. To investigate growth trends, Y. lipolytica was cultured under various pH and acetate conditions. Adaptive evolution enhanced its tolerance to acetate, supporting its applicability in industrial settings. Genetic modifications further boosted lipid accumulation by targeting key metabolic pathways involved in biosynthesis. Bioprocess optimization using the best-performing engineered strain explored co-substrate strategies, including partial or full replacement of acetate with glycerol or glucose, to maximize lipid yields. Altogether, this work highlights the synergistic role of evolutionary and metabolic engineering in optimizing Y. lipolytica for high-efficiency lipid production from unconventional carbon sources.

By leveraging microbial cell factories and medium optimisation strategies, this project facilitates the efficient and eco-friendly conversion of waste-derived substrates into sustainable bioproducts, minimising environmental impact and advancing circular bioeconomy initiatives. This enabling economically viable biotechnological solutions for a net-zero future.

Navigating New Product Development and Competition in the Food and Beverage market: Focussing on higher value markets by leveraging market dashboards.

Rimjhim Agarwal[1], Damian Hine[1], Edgar Brea[2], Galey Tenzin[3]

[1]QAAFI, Brisbane, Australia. [2]UQ Business School, Brisbane, Australia. [3]School of Agriculture and Food Sustainability, Brisbane, Australia

Focus Area: Translational / bioeconomy research

Area of Expertise: Translation and commercialisation

This paper highlights how three scalable analytical offerings - Rapid Analyses, Mid-level Analyses and Bespoke Analysis - help understand and identify market opportunities and business competitiveness via product, business and industry level analyses in the pre-competitive (TRL1-9) and competitive (on market) landscape. These analyses employ over 40 data sources to understand markets (e.g. market research reports, data repositories, and Australian and international longitudinal datasets), market opportunity and customer needs (e.g. product and market trends, product reviews, online forums), competition (e.g. product launches, business births deaths and marriages), competitive performance (e.g. market concentration, industry value add), R&D analyses (R&D intensity, R&D spend) and technology trends, IP creation and protection (e.g. publications, patents, trademarks) as well as customer pain points (e.g. online fora, product revies). Through an integrated use of analyses and dashboards, we are working to build evidence-based support for NPD and commercialisation strategies.

The **interactive dashboards** are designed to help businesses (and researchers) developing new F&B products to gauge their likely competitiveness in existing markets, as well as potential markets and market niches at different stages of their R&D and NPD (pre-competitive) process. By navigating through the relevant dashboard pages and sections, those with products at various TRLs can understand better the product, market and technology trends, and the competitive landscape that they will face if they progress to market. They can guide the development of new market entry strategies, novel and improved products, or alternative markets for existing products using a holistic approach to products, co-products and by-products; and make sustainable use of the agricultural commodities and ingredients. To keep pace with emerging trends and evolving market dynamics, the dashboard functionalities are designed to be dynamic, and so far semi-automated to continuously update.

The **Rapid Analyses** offer quick, high-level key insights into local, national, regional and international markets. An example is the rapid noodle market appraisal. It offers the global, regional and country-specific market sizes and growth, further disaggregated by product type (e.g., instant, plain, chilled), major companies and brands, and market

concentration (Herfindahl-Hirschman Index) reveal the industry's competitive landscape, patent, new product launches, and product claims offer insights into the technology trends.

Mid-level analyses involve a more extensive pre-competitive analysis by mapping the TRLs (level 1-9), for relevant products/technologies and associated R&D activities; and the competitive analysis of identifying market potential, competition including competitors' distribution channels, R&D activity and customer needs and preferences.

Bespoke analyses are more resource intensive, help identify the most attractive markets from a range of adjacent market niches with critical insights from each market to inform product development strategies. By addressing specific challenges and leveraging unique opportunities, it provides tailored insights and strategies to enhance new product development, linking scientific research and innovations with market needs.

Leveraging these multi-level analyses and dashboards, helps businesses and researchers transform innovative scientific research into market-ready products that drive economic growth and sustainability

Unlocking the potential of sustainable aviation fuel in Australia: market prospects, feedstock viability, and policy pathways

Shabbir Ahmad, Damian Hine, Galey Tenzin, Jon Aster

The University of Queensland, Brisbane, Australia

Focus Area: Translational / bioeconomy research, Economic feasibility analysis

Area of Expertise: Modelling, Economic and financial modelling

The aviation sector contributes 2% of global carbon dioxide emissions, with transport accounting for 23%. Recent geopolitical and supply chain disruptions highlight Australia's need to improve national fuel sovereignty. In 2024, Australia consumed 63 billion litres of fuel, 57 billion of which were imported, mostly fossil-based diesel and aviation fuel. Alternative energy solutions, both transitional and long-term, are reshaping the sector, with rising SAF consumption driven by policies such as Japan's 10% SAF mandate by 2030 and 100% by 2050. However, Australia still lacks a national SAF policy, unlike the EU, the USA, and Brazil. This study explores non-edible feedstocks like Pongamia and crop residues from sugarcane and sorghum for SAF production by examining biofuel market dynamics, agricultural feedstock productivity, technological challenges, regulations, financial viability, and policies for scaling SAF feedstock production.

Analysis shows that ethanol productivity from sugarcane and sorghum stands at 82.3 and 160 litres per tonne, respectively, but a 30% efficiency improvement is required for profitability, highlighting the need for technological advancements. Pongamia, a focus for Japan and major Australian firms, is well-suited to Queensland's agro-climatic conditions and existing infrastructure. Despite its high oil yield and climate resilience, yield variability affects financial viability. Targeted breeding, genetic development, agronomic optimization, and better oil extraction methods are needed, along with improved yield productivity and ethanol conversion for all feedstocks.

Economic viability analysis shows sugarcane's SAF cost structure has high initial costs, with feedstock at AUD 31.46–43.72 per tonne, ethanol production at AUD 0.34–0.61 per litre, and SAF conversion costs projected to decline from AUD 5.15 per litre in 2025 to AUD 2.92 per litre in 2050. A 1.0% productivity boost in each five-year could further reduce the SAF costs to AUD 2.51 per litre. Sorghum has a relatively lower cost structure, with production costs dropping from AUD 3.47 per litre in 2025 to AUD 1.46 per litre in 2050 under optimal ethanol conversion. By-products like Pongamia seed meal enhance profitability, while carbon credits and policy incentives help mitigate costs, but feedstock efficiency is key to competitive SAF pricing.

NPV analysis reveals that sugarcane-based SAF results in negative NPV unless ethanol conversion efficiency improves by at least 30%. With a 30% improvement at a 10% discount rate, it breaks even, while a lower discount rate generates positive NPV and cash flows, making it viable. Sorghum follows a similar trend, with baseline production leading to negative NPV and 30% ethanol yield growth enabling profitability. Pongamia's NPV analysis highlights the importance of by-product revenue, with mid-yield scenarios (32 t/ha) achieving moderate profitability, while high-yield projections (38.4 t/ha) with R&D investment result in strongly positive NPV.

With growing demand for low-carbon aviation fuels, investing in SAF infrastructure and technology is crucial for Australia's transition. Future research can focus on improving feedstock productivity, optimize supply chains, assess regulations, and integrate carbon markets for a competitive SAF industry. A simulation-based scenario analysis can guide policy, but agricultural feedstocks are likely to be a transitional solution until low-carbon fuel technologies mature.

Workshop III

BioHub Facilities

Panel Discussion

How can IDEA Bio and Q-MAP support your project?

Introduction by Esteban and Axa

Chairperson

Denys & Joseph

Panel Members

Yu Sun - IDEA Bio

Subaru Muroi - IDEA Bio

Helen Wong - IDEA Bio & QMAP

Dara Daygon - QMAP

Heather Pegg - Floor manager

Yu Sun - Optimizing Fermentation Processes for Sustainable Biomanufacturing

Focus Area: Bioprocess Optimisation, Precision Fermentation

Area of Expertise: Bioprocess Engineering/optimisation, Fermentation Service

Favorite Organisms: *Escherichia coli, Pichia pastoris, Saccharomyces cerevisiae*

The shift towards net-zero emissions requires innovative and cost-effective bioprocessing solutions to support sustainable industrial practices. Fermentation is a promising approach for the sustainable production of biomaterials, bio-based chemicals, and therapeutic compounds due to its ability to leverage microbial metabolism for efficient bioproduction.

As a fermentation scientist at IDEA Bio, I focus on developing optimized fermentation processes to enhance product yields while maintaining reproducibility and economic feasibility. In collaboration with researchers and industrial partners, I work on refining fermentation strategies for various microbial strains, including Pichia pastoris and Saccharomyces cerevisiae. These microorganisms serve as hosts for the production of recombinant proteins and small active molecules. My current project involves optimizing the fermentation of Saccharomyces cerevisiae to improve the yield of psilocybin, a bioactive compound with pharmaceutical potential. Our bioreactor systems range from 15mL to 15L, enabling us to scale processes from early-stage development to pilot studies. By adjusting process parameters, optimizing nutrients, and implementing bioreactor control strategies, my work aims to improve fermentation efficiency and consistency. I also provide hands-on training to young researchers, guiding them in using bioreactors to conduct their experiments effectively.

The insights gained contribute to a better understanding of microbial fermentation dynamics and will support future industrial applications. By addressing key challenges such as media formulation and process monitoring, fermentation-based production could play a pivotal role in advancing sustainable biomanufacturing and supporting the broader transition toward net-zero emissions.

Helen Wong - Integration of Automation Using Lab Robotics

Focus Area: Multi-omics Analysis

Area of Expertise: Automation

As the Automation Specialist for the Biosustainability Hub, I work across 2 groups within the hub, namely QMAP and IDEA Bio. My role primarily involves integrating automation to a majority of repetitive, time-consuming and laborious laboratory tasks to decrease the prevalence of human error, time spent, and plastic tip and/or needle waste. These tasks are often conducted by researchers (as well as anyone working in the laboratory) and can be inefficient when dealing with an extremely large quantity of samples or when multiple transfer steps are required in manual sample handling; as they may involve manual pipetting transfers, incubation, plate shaking, centrifugation and colony plate picking. By integrating automation, I'm able to provide an efficient solution to these varying sample handling steps.

How do I integrate automation? I do this by utilising liquid handling robots and colony picking robots which contain built-in robotic arms, a deck space for different types of labware and tip washing stations. These can be programmed to do different types of liquid and colony transfers such as 96-well and 384-well plate transfers. In the instance where customised labware is required, I design 3D-printable labware components to suit the desired workflow.

I routinely run sample preparation automation protocols, method development and optimisation of a range of methods used in synthetic biology such as proteomics, metabolomics sample processing to provide a high-throughput solution to the manual strain involved in handling large sample quantities. I additionally work on customising methods for researchers and students as well as implementing and developing a standard range of automation methods that researchers can use and modify in their own time to suit their specific projects. These involve commonly used methods such as PCR dispenses, colourimetric assays, and liquid or microbial colony transfers. Prior to anyone wanting to use existing or customised protocols, I'll organise trainings to enable students and researchers to run these robotics independently.

More of my work involves managing the servicing, calibration and preventative maintenance of these robotics as well as liaise with their respective suppliers to ensure that the proper consumables, solvents and hardware components can be used in each robot. My collaborative works both externally and internally within the hub include, standardising protocol transfers between the same type of robot used between different labs, and in streamlining manual data entry from client submissions to automation robotic file paths to proteomics analytical run sequences.

Lab automation offers a solution to researchers in the Biosustainability Hub wanting to process large sample quantities in less time. By using less plastic, reducing time, and decreasing errors, lab automation robotics perpetuates a sustainable approach to our shared vision of transitioning into net zero.

Dara Daygon - Applications of LC-MS based metabolomics

Focus Area: Multi-omics Analysis

Area of Expertise: Omics - Metabolomics, Proteomics, etc.

My research work focuses on metabolomics-driven biochemical analysis. As a member of QMAP's Metabolomics team, I utilise mass spectrometry techniques to analyse expression patterns of important metabolic pathways. This includes the central carbon metabolism - a critical hub in cellular energy production and biosynthesis, amino acid synthesis, and labeled isotope tracing. Our team continuously develops analytical strategies to enhance our capabilities and provide tailored support for both internal and external projects. For example, we have developed methods to extract and quantify psychoactive compounds in *Psilocybe* mushrooms including all the tryptamine derivatives and substrates involved in its biosynthesis. In collaboration with other research groups, my work extends to clinical and model organism studies aimed at understanding disease mechanisms and identifying therapeutic targets. By profiling metabolites, we investigate how metabolic disruptions contribute to lifestyle-related diseases, depression, and neurodegenerative disorders. These interdisciplinary efforts facilitate a systems-level understanding of disease processes, accelerating the development of diagnostic tools, treatments, and biomarker validation. I also co-supervise PhD students working on complex plant traits to develop climate-resilient crops and promote sustainable agricultural practices. My research supports the mission and vision of the Biosustainability Hub by contributing to the characterisation of microbial strains for biotechnological applications, identifying metabolic bottlenecks in precision fermentation, and optimising analytical techniques for the quantification of target products.

Anh Phan - Proteomics Specialist

Focus Area: Multi-Omics Analysis

Area of Expertise: Omics - Metabolomics, Proteomics, etc.

I currently hold the position of Senior Research Technician/Proteomics Specialist at the Queensland Metabolomics and Proteomics Facility (Q-MAP), under the Bio-Sustainability Hub at AIBN, UQ. Together with the Q-MAP team, we provide analytical services, scientific support, and training to higher degree research students, researchers, and industry partners. To support research activities at the Bio-Sustainability Hub and external research communities, I contribute to proteomics analytical workflow, participate in method development and improvement, and work to maximize the capabilities of Q-MAP facility. My goal is to ensure the delivery of the highest quality results to all stakeholders. Specializing in Proteomics analysis with experience in operating Thermo Orbitrap Mass spectrometry Technology Platform coupled with nanoflow HPLC and FAIMS Duo Pro Interface, I conduct experiments and analysis related to protein identification and quantification

across a range of biological samples (e.g., cells, tissues, blood plasma, etc.), primarily using Data-Independent Acquisition (DIA) and Data-Dependent Acquisition (DDA) methods.

The proteomics analyses conducted at the Q-MAP facility enable researchers at the Bio-Sustainability Hub to gain insights into cellular protein expression, post-translational modification, and the functions of proteins involved in metabolic and cellular processes. This is a key component of advanced research and innovation translation at the Bio-Sustainability Hub.

Subaru Muroi - Optimizing Bioprocesses with AI: Bayesian Optimization using Gaussian Process Regressors

Focus Area: Computational Biology, Multi-omics Analysis

Area of Expertise: Bioinformatics, Data Scientist, Machine Learning, Modelling, Omics - Metabolomics, Proteomics, etc., Systems Biology

The rapid advancement of large language models (LLMs) and AI across industries has generated unprecedented interest in machine learning (ML). While AI has already transformed marketing, finance, and automation, its applications in the sciences—particularly in bioprocess optimization—are now being explored more rigorously. At IDEABio, we leverage AI-driven approaches to solve complex optimization challenges in biological experiments, focusing on Bayesian optimization with Gaussian Process Regressors (GPRs). GPRs are powerful probabilistic ML models capable of defining any continuous function. However, they struggle with real-world applications that involve categorical variables, which require specialized mathematical methods to integrate with continuous data. Our work aims to resolve this challenge by developing an approach that effectively combines categorical and continuous variables to optimize biological processes such as media formulation. In biological research and bioprocessing, media—the nutrient source for cell growth and production—is a key factor influencing outcomes such as protein yield or biomass accumulation. Traditional experimental designs, such as full-factorial or Design of Experiments (DoE) approaches, quickly become infeasible due to the vast number of variable combinations. Bayesian optimization provides an efficient alternative by strategically balancing exploration (testing uncertain areas of the function) and exploitation (focusing on promising variable combinations). By linking a GPR model to a target variable (e.g., protein yield), we can systematically optimize experimental conditions while minimizing the number of trials needed. The model iteratively suggests new experimental conditions, guiding scientists toward an optimal solution with significantly reduced experimental effort. This is particularly valuable in bioprocess engineering, where optimizing media formulations involves both continuous variables (e.g., nutrient concentrations, temperature) and categorical variables (e.g., media types, proprietary additives).

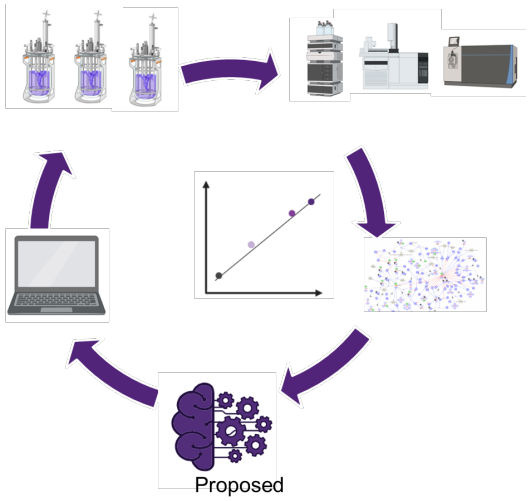
Our approach allows for continuous refinement of the model, enabling knowledge transfer across different optimization problems. For example, a media optimization model can later be expanded to include bioreactor process parameters, ensuring that the entire system—rather

than isolated components—is optimized. This flexibility is crucial for industries utilizing precision fermentation, where iterative improvement based on experimental results is key to efficiency and scalability. At IDEABio, we have the facilities to validate these AI-driven optimizations through direct experimental comparison. By integrating ML into biological experimentation, we not only accelerate the discovery of optimal conditions but also enable continuous model improvement, making AI a powerful tool for advancing bioprocessing technologies. This work highlights the transformative potential of AI in scientific research, bridging theoretical machine learning with real-world biological applications. The ability to predict optimal conditions, minimize experimental costs, and adapt dynamically to new challenges represents a paradigm shift in bioprocess engineering, paving the way for more efficient and intelligent experimental design.

Heather Pegg - AIBN Biosustainability Hub Floor Manager

As the Biosustainability Hub Floor Manager, I'm excited to play a key role in creating an environment where researchers can thrive. I ensure our research facilities and equipment run smoothly and efficiently while maintaining safety and compliance standards. By working closely with other Floor Managers, I coordinate activities and offer support to our talented researchers. I manage equipment maintenance, allocate space, and oversee safety measures, all while providing guidance and support for training, lab access, and regulatory documentation. I also assist with health and safety tasks, to ensure we follow the best practices. Through my work, I help researchers focus on their groundbreaking research and discoveries, while I handle the infrastructural and operational side of things. Ultimately, my mission aligns with our broader goal to develop economically viable solutions - creating a sustainable future through innovation and research.

System Biology in a bio-foundry world



Research Facilities @ AIBN, The University of Queensland

Integrated Design Environment for Advanced biomanufacturing (IDEA Bio)
Queensland Metabolomics and Proteomics (QMAP)

Prof Esteban Marcellin	Director, IDEA Bio and QMAP
Prof Lars Nielsen	Director, IDEA Bio
Dr Axa Gonzalez	IDEA Bio Manager & Test Lead
Mr Laurin Walther	Fermentation Specialist
Ms Yu Sun	Fermentation Specialist
Mr Nhat Huynh	Fermentation Specialist
Ms Helen Wong	Automation Specialist
Dr Tim McCubbin	Design and Learn Lead
Dr Subaru Muroi	Machine Learning & AI Specialist
Dr Justin Chitpin	System Biologist
Mr Haxby Hefford	Software Engineer
Dr Dara Daygon	GCMS Specialist
Ms Terra Stark	GCMS Specialist
Dr Gabi Netzel	HPLC Specialist
Dr Anh Phan	Proteomics Specialist
Dr Gert Talbo	Proteomics Specialist
Dr Shana John	Proteomics Specialist
Ms Sally Yukiko	Administration Officer

Laurin Walther - Optimizing Fermentation Processes for Sustainable Biomanufacturing

Focus Area: Bioprocess Optimisation, Precision Fermentation

Area of Expertise: Bioprocess Engineering/optimisation, Molecular cloning

Favorite Organisms: *Pichia pastoris, Saccharomyces cerevisiae, Yarrowia lipolytica*

As a Fermentation Scientist at IDEA Bio, I am engaged in both academic research and industry-driven projects, specialising in microbial fermentation and bioprocess development. My work spans a variety of microorganisms, optimising fermentation strategies to enhance productivity and scalability. A key-project I'm involved in is a framework project aimed at producing high titers of psilocybin in *Saccharomyces cerevisiae*, where I contribute to strain engineering, media optimisation, and process development to improve yields and efficiency.

Beyond research, I am actively involved in training and mentoring students using fermenters provided by IDEAbio. I provide hands-on instruction in operating a wide range of bioreactors, from 15 mL to 15 L, equipping them with essential skills in bioprocess engineering and microbial cultivation.

A crucial aspect of my role is closing the gap between research and commercialisation. By developing economically feasible bioprocesses, I help translate laboratory breakthroughs into scalable, cost-effective industrial applications. This involves optimising microbial performance under controlled conditions, refining process parameters, and ensuring that fermentation-based production systems can be implemented at an industrial scale. My goal is to contribute to the Biosustainability Hub by advancing bioprocesses that reduce waste streams, improve resource efficiency, and ultimately lower the carbon footprint of most compounds used today. Through continuous innovation, I strive to make biotechnology a key driver in sustainable biomanufacturing and environmental impact reduction.

Haxby Hefford - Creating, managing, and analyzing data for IDEA Bio

Focus Area: Computational Biology

Area of Expertise: Automation, Machine Learning, Modelling

As a software engineer at IDEA-Bio, my primary focus is on optimizing the collection, storage, and analysis of bioreactor and 'omics data, with an emphasis on extracting and managing data from the Laboratory Information Management System (LIMS). This involves developing and enhancing digital infrastructure to ensure that data is efficiently processed, accurately extracted, and made accessible for analysis. A major part of this work is streamlining the extraction of data from LIMS and integrating soft sensors into the data workflow for real-time process optimization.

LIMS is the backbone of laboratory data management, storing essential information such as sample details, experimental conditions, measurements, and results. In the biotech industry, especially in bioreactor operations, data is generated from multiple sources, including sensors, chromatographs, and mass spectrometers. Extracting this data efficiently is only part of the challenge—the real value comes from transforming it into a structured format that enables integrated analysis and meaningful insights. Without well-optimized pipelines, researchers would have to spend a significant amount of time manually retrieving and processing data, which slows down experimentation and decision-making. A core aspect of my role is developing custom data extraction pipelines that interface with LIMS. These pipelines automate the process of pulling data, standardizing it into a format that aligns with analytical workflows, and integrating it into downstream applications. Whether the data is used for statistical analysis, machine learning models, or real-time monitoring, ensuring consistency and accuracy is critical. By automating these processes, I help researchers focus on interpreting results rather than dealing with tedious data wrangling. Beyond just data extraction, I also work on implementing soft sensors—computational models that estimate difficult-to-measure process variables based on available real-time data. These soft sensors enhance decision-making by providing continuous insights into bioreactor performance, allowing researchers to detect trends, optimize conditions, and respond to potential issues before they escalate. Integrating predictive analytics into the data pipeline makes it possible to optimize bioprocesses dynamically, improving efficiency and reliability. I also collaborate closely with computational researchers, discussing ideas and exploring new ways to enhance data analysis. With experience in modeling and machine learning, I'm always interested in finding ways to integrate these techniques into our workflows. Whether its applying machine learning to predict process outcomes or developing statistical models to better understand bioreactor dynamics, I enjoy working at the intersection of software engineering and scientific discovery.

Ultimately, my role at IDEA-Bio is about enabling better, faster, and more informed decision-making through optimized data infrastructure. By building efficient data pipelines, improving accessibility, and leveraging predictive analytics, I help drive innovation in biotechnology. The integration of real-time data, automated processing, and advanced analytics contributes to more effective research and development, ultimately leading to more scalable and reproducible bioprocesses.

Justin Chitpin - Computational pipeline for rational strain design by combining multi-omic characterisation and metabolic modelling

Subaru Muroi, Haxby Hefford, Timothy McCubbin, Esteban Marcellin, Lars Nielsen

Focus Area: Multi-Omics Analysis, Computational Biology

Area of Expertise: Bioinformatics, Data Scientist, Machine Learning, Modelling, Omics - Metabolomics, Proteomics, etc. Systems Biology

Biofoundries, such as IDEA Bio, seek to enable strain engineering to produce strains with superior phenotypes of interest, often with the goal of producing a metabolite or protein at high concentrations. This is performed in a Design-Build-Test-Learn (DBTL) cycle. However, the "Learn" component of this cycle is often done poorly, with most foundries relying on build capability, high-throughput screening, and design of experiment type approaches to yield high-performing strains. This remains costly and time intensive, and scales poorly to longer pathways owing to the combinatorial explosion in the design space.

At IDEA Bio, we prioritise the rational design of new strains by analysing detailed -omics data sets, collected from instrumented bioreactors, and combining this with metabolic modelling and machine learning approaches to identify metabolic bottlenecks or features of high or low producers. Many of these same approaches can be applied to provide insight into -omics data sets, even when the overall goal is not metabolic engineering related.

To achieve these goals, we are developing a computational pipeline that leverages -omics data with metabolic models to provide a deeper understanding of cellular metabolism. This pipeline is also available to members of the Biosustainability Hub to analyse bioreactor runs or -omics data sets. We address these goals in three distinct but related ways that, when combined, can generate precise metabolic hypotheses to improve strain design or understand how cell metabolism differs between conditions. The first way we do this is by using statistical and machine learning techniques to interpret -omics data and map our findings onto metabolic models. These visualisations enable us to relate changes in metabolite or protein levels to individual metabolic pathways that could be targeted to improve production. The second way we make sense of high-throughput data is to directly integrate these measurements into metabolic models. This approach allows us to construct dynamical systems that explain the observed biological measurements under steady state (e.g. chemostats) or non-steady state conditions (e.g. batch fed reactors). These systems can be analysed by mathematical methods to quantify the influence of enzyme levels over metabolic pathways to infer metabolic bottlenecks. Finally, these metabolic models can be perturbed in silico and fed into machine learning models to predict how biological modifications to the strain or bioreactor environment (e.g. media) influence the production of desirable metabolites or proteins.

My expertise that I bring as a service available through IDEA Bio is in metabolic modelling and -omics data analysis. In the context of the Biosustainability Hub, this systems biology framework

to learning and testing is essential to characterise the metabolism of a strain and identify metabolic targets to engineer higher-performing strains. Finally, members of our group are also developing cutting-edge methods within their respective expertise to offer exciting new data analysis and rational design tools. For example, I am working on a method to improve pathway enrichment analysis by considering the relations between separate pathways through the concept of atomic elementary flux modes.

Gabi Netzel - Metabolomics Using UHPLC

Focus Area: Multi-Omics Analysis

Area of Expertise: Omics - Metabolomics, Proteomics, etc.

As a member of the Metabolomics Team, I have many years of experience in chromatographic analysis using various detectors. Currently, I am responsible for the Ultra High Pressure Liquid Chromatography (UHPLC) systems. I manage a Vanquish Core system with a Fluorescence Detector (FLD), which is dedicated to amino acid analysis but can also be used for other fluorescence-active compounds. Additionally, I oversee two Vanquish Duo systems equipped with Diode Array (DAD), Charged Aerosol (CAD), and Refractive Index (RI) detectors. These systems are used for analyzing organic acids, aromatic compounds, triterpenoids, tetraterpenoids, and more.

Our most recent addition is a highly sensitive IC-6000 Duo PAD instrument, dedicated to analyzing sugars, particularly mono-, di-, and oligosaccharides. If you are interested in quantifying a specific compound, I will do my best to include it in an existing method or develop a new one. While it may take some time to perfect a method, I usually succeed. If I cannot assist you, my colleagues Dara (LCMS specialist) and Terra (GCMS specialist) might be able to help. Although my primary focus is UHPLC and the quantification of known compounds, our Metabolomics team recently gained access to the HF Orbitrap system. This instrument can be used for open profiling by UHPLC with peak annotation using Compound Discoverer software, or it can potentially identify unknown peaks in samples. My vision is to develop sustainable and innovative methods that support researchers and industry in creating carbon-neutral, economically viable products and materials.

Robin Palfreyman – Bioinformatician

Focus Area: Multi-Omics Analysis, Computational Biology

Area of Expertise: Bioinformatics, Data Scientist, Omics - Metabolomics, Proteomics, etc.

As a bioinformatician with a background in biochemistry and software development, I provide comprehensive bioinformatics support to Q-MAP, IDEA Bio, the Biosustainability Hub, the university community, and external collaborators. My responsibilities include the analysis, manipulation, interpretation, and integration of complex omics datasets, specifically genomic, transcriptomic, proteomic and metabolomic data. Through my work with IDEA Bio and Q-MAP, the Queensland node of Metabolomics Australia, I enable researchers to extract meaningful biological insights from their data, thereby advancing their ability to engineer targeted processes. Within Q-MAP and IDEA Bio, I play a critical role in the organisation and integration of metabolomic, proteomic and fermentation data generated by Q-MAP and the IDEA Bio bioreactors, ensuring seamless accessibility for modelers and data analysts. Furthermore, I am responsible for the development, maintenance and administration of the Q-MAP sample management system, which effectively tracks samples, analyses and financial transactions, while supporting the node's ISO 9001 certification requirements.

Terra Stark - GC-MS specialist

Focus Area: Multi-Omics Analysis

Area of Expertise: Omics - Metabolomics, Proteomics, etc.

I'm based at the Q-MAP facility, part of the Metabolomics team. Here, I specialise on the metabolomics side of research that involves gas chromatography–mass spectrometry (GC-MS) analyses. I work closely with students and researchers—both within and outside the university—to analyse a wide variety of samples, including bacteria, plants, and animal specimens. Most of the time, I use targeted and comprehensive profiling to study polar metabolites and volatiles, along with analysing greenhouse gases, fatty acids, and bio-oils. I also develop custom methods for clients who want to target specific compounds. The process usually starts with a consultation to understand the client's research goals. Then, I extract metabolites from their samples (either independently or with their involvement), run the samples through the GC-MS using the right analytical method, and finally process the data to generate a statistical report or quantitative results for targeted metabolites. GC-MS is a powerful tool for metabolomics, and my work contributes to the Biosustainability Hub's mission by helping researchers gain deeper insights into key molecular activities. This can range from optimizing strains and models to uncovering new metabolic mechanisms. I'm fortunate to be part of a highly collaborative team, where I can always rely on my fellow Q-MAP colleagues' support and expertise to ensure we achieve the best possible results.

Shana John - Advancing Analytical Chemistry for a Sustainable Bioeconomy

Focus Area: Multi-Omics Analysis

Area of Expertise: Omics - Metabolomics, Proteomics, etc.

Dr. Shana John is an accomplished analytical chemist with extensive experience in metabolomics, lipidomics, proteomics, chemical analysis, and bioprocess development. With a strong background in research and laboratory management, Dr. John has contributed to advancements in precision analytical techniques.

Dr. John holds a PhD in Analytical Chemistry and has spent over a decade working at the intersection of chemistry, biochemistry, and molecular analysis. Her expertise includes cutting-edge chromatographic and mass spectrometric techniques (HPLC, LC-HRMS, GC-MS and ICP-MS), enabling high-precision chemical characterization of complex biological and environmental systems. With a passion for sustainability and innovation, Dr. John has played a critical role in developing analytical methodologies that support bio-based processes, environmental monitoring, and pharmaceutical manufacturing. She has developed and validated analytical methodologies for trace-level detection of contaminants, biomolecules, and pharmaceutical compounds. Her research has been published in reputed refereed journals, and she has actively contributed to international conferences, reinforcing her growing reputation in the field. Beyond research, Dr. John has a strong track record of mentoring Honours and Research Higher Degree students, fostering the next generation of scientists. Her leadership in laboratory operations, adherence to GLP, GMP and quality assurance protocols, and experience securing external research funding demonstrate a commitment to bridging the gap between academic discovery and commercial application.

Aligned with the vision of transitioning the world to a sustainable bioeconomy, Dr. John is dedicated to developing economically viable, science-driven solutions for industries striving toward net zero emissions. Her work in biochemical profiling and chemical analysis supports the creation of innovative, resource-efficient bioprocesses that drive the bioeconomy forward.

Sally Rosa Yukiko - Enabling Innovation: Driving Research Operations for a Sustainable Bioeconomy

Behind every success of scientific initiatives and industry changing innovation, there is a strong foundation of research enabling support driving progress. My role on Research Operation and Enabling Support is to provide critical infrastructure that allows our researchers to focus on developing solutions for a sustainable future. I oversee a broad range of responsibilities, from managing research grants, financial reporting, operational logistic to stakeholder engagement, as well as maintaining Quality Management Standard for our facility. I ensure that the operational backbone of scientific research is strong, efficient, and strategically aligned with global sustainability goals.

My contribution to the transition of global bioeconomy lies in facilitating wide range of research administration process in alignment with university guidelines, ensuring seamless project execution. I organize conferences, industry engagement events, and community outreach initiatives to enhance the awareness of our research impact and cultivate meaningful collaboration. In addition to that by arranging networking events and liaising with professional teams across research and facility operations, I create opportunities for collaboration, fostering stronger connections between researchers, industry leaders, and the broader community. I am here to make sure that the Biosustainability Hub's research environment is not just functional but inspiring – so innovation can happen without a hitch! Feel free to reach out if you have any questions or need assistance with research administration and operation matter. I'm always happy to help and provide guidance!

Seminar III

Industry Projects – Innovative Ingredients

Chairperson

Yosephine & Damian

Presenters

Nathan Qifeng Zhong

Baode Sun

Isabella Casini

Froylan Garnica Garcia

Rare Sugar Production Using Cell Factories

Nathan Qifeng Zhong[1,2], Esteban Marcellin[1,2,3], Axa Gonzalez[2,4,3]

[1]Food and Beverage Accelerator (FaBA), Brisbane, Australia. [2]Australian Institute for Bioengineering and Nanotechnology (AIBN), Brisbane, Australia. [3]ARC Centre of Excellence in Synthetic Biology, Brisbane, Australia. [4]Integrated Design Environment for Advanced Biomanufacturing (IDEA Bio), Brisbane, Australia

Focus Area: Precision Fermentation

Area of Expertise: Molecular Cloning, Systems Biology, Strain Engineering, Omics - Metabolomics, Proteomics, etc.

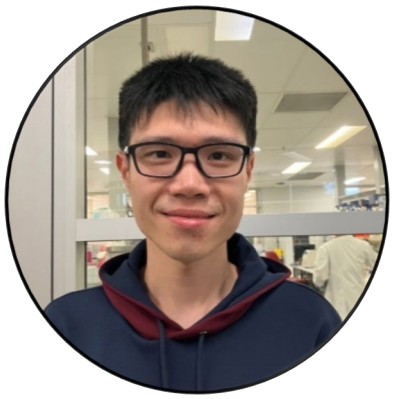
Favorite Organisms: *Escherichia coli*

Australia is one of the world's largest sugar exporters. To maintain its position as a leader in the industry, innovation in high-value sugar production is important. This project aims to develop a sustainable and economically viable approach to produce high-value rare sugars through precision fermentation. To create rare sugar-producing cell factories, we used generally recognised as safe (GRAS) microorganisms as host strains, which have been engineered to convert common sugar into high-value sugar. We have designed and implemented genetic engineering tools to redirect the carbon metabolic flux towards enhancing rare sugar production while balancing energy generation pathways. By refining these engineered strains, we seek to develop an efficient biological workhorse for rare sugar production, adding value to Australia's sugar industry and strengthening Australia's position in the global market.

Engineering *Yarrowia lipolytica* to produce psilocybin, a high-value therapeutic candidate

Baode Sun[1,2], Axayacatl González[1,2,3], Tim McCubbin[1,2,3], Esteban Marcellin[1,2,3,4], Huadong Peng[1,2,4]

[1]Australian Institute for Bioengineering and Nanotechnology, Brisbane, Australia. [2]ARC Centre of Excellence in Synthetic Biology, Brisbane, Australia. [3]Integrated Design Environment for Advanced biomanufacturing (IDEA bio), Brisbane, Australia. [4]Food and Beverage Accelerator (FaBA), Brisbane, Australia



Focus Area: Bioengineering

Area of Expertise: Bioprocess engineering/optimisation, Molecular Cloning, Strain Engineering

Favorite Organisms: *Escherichia coli, Saccharomyces cerevisiae, Yarrowia lipolytica*

Psilocybin is a psychoactive alkaloid derived from a fungal genus *Psilocybe*, commonly referred to as “magic mushrooms”. While it is a notable member of the psychedelic family given its recreational use, recently psilocybin has shown to be a potential therapeutic agent for alleviating different mental health conditions, such as depression, substance abuse, post-traumatic stress disorder (PTSD), chronic pain and end-of-life anxiety. The Food and Drug Administration (FDA) has granted “Breakthrough Therapy Designation” to a psilocybin analogue for major depression treatment. In Australia, the Therapeutic Goods Administration (TGA) has permitted patients with serious mental health conditions to obtain medicines containing psilocybin from authorised psychiatrists starting in 2023. Given the increasing interest in psilocybin, establishing a sustainable approach to its production is critical to meeting the demand for both research applications and future markets. In this recent work, we demonstrated the *de novo* biosynthesis of psilocybin and its dephosphorylated metabolite psilocin in the yeast *Yarrowia lipolytica* by expressing a heterologous biosynthesis pathway originating from *Psilocybe cubensis*. This synthetic pathway contains five genes originating from *P. cubensis* and a tryptophan decarboxylase coding gene from *Catharanthus roseus*. Then shake flask cultivation showed that the engineered *Y. lipolytica* strain successfully produced psilocybin and psilocin with titres higher than engineered yeast *Saccharomyces cerevisiae* with the same biosynthetic pathway. Our preliminary results indicate *Y. lipolytica* is a promising host for psilocybin production and its derived compounds.

Microbial production of psilocybin and its derivatives offers an efficient and environmentally friendly alternative for high-value therapeutics, providing a significant advantage over conventional chemical synthesis methods. This approach supports climate change mitigation efforts and contributes to zero-emission biomanufacturing.

Optimizing Microbial Metabolism for a Sustainable Future: The Role of Metabolic Modelling

Isabella Casini

Australia Institute for Bioengineering and Nanotechnology, St. Lucia, Australia

Focus Area: Computational Biology

Area of Expertise: Bioinformatics, Data Scientist, Bioprocess engineering/optimisation, Fermentation Services, Modelling, Omics - Metabolomics, Proteomics, etc., Systems Biology

Favorite Organisms: *Clostridium autoethanogenum, Pichia pastoris*

My work focuses on metabolic modelling, a computational (*in silico*) approach to analyse and optimize microbial metabolism for various applications, including biotechnology and sustainable production. Genome-scale metabolic models (GEMs) represent mathematically the biochemical reactions that occur in cells and the genes responsible for the enzymes catalysing those reactions. I construct, refine, and employ GEMs to 1) estimate fluxes through metabolic pathways, 2) identify potential bottlenecks, 3) predict maximum theoretical yields, and 4) determine essential genes. I use previously generated fermentation data, such as uptake and production rates, and omics data, such as transcriptomics and proteomics, to constrain the model. These *in silico* models aid directly and indirectly in the design and analysis of engineered strains, thus contributing towards more efficient precision fermentation. Precision fermentation can provide more sustainable biotechnological solutions to support more traditional processes. Further, i*n silico* work can increase the efficiency of wet laboratory work, reducing resources (*e.g.*, time and energy) required. Through metabolic modelling, I contribute to the design of microbial systems that can produce valuable compounds — such as biofuels and dairy proteins—while minimizing environmental impact and resource consumption.

Profitable Upcycling of Spent Genetically Modified Microbial Biomass From Precision Fermentation.

Froylan Garnica Garcia

AIBN, St. Lucia, Australia

Focus Area: Precision Fermentation, Bioengineering, Translational/ Bioeconomy Research

Area of Expertise: Bioinformatics, Data Scientist, Bioprocess engineering/optimisation, Fermentation Services, Recombinant Protein Production, Translation and Commercialisation

Favorite Organisms: *Bacillus subtilis, Trichoderma harzianum, Bacillus amyloliquefaciens*

By the first half of the 21st century, the global population is expected to reach nearly 10 billion people. With this rapid growth, the demand for essential goods and services, particularly food and medicines, will also rise significantly. Precision fermentation is an emerging technology that is poised to play a crucial role in meeting this demand. This technology enhances traditional fermentation by using genetically engineered microorganisms to produce valuable molecules of interest, such as enzymes, vitamins, and proteins. Compared to conventional production methods, precision fermentation offers significant environmental and economic benefits across the pharmaceutical, food, manufacturing, and agricultural industries. These advantages include reducing reliance on animal derived products, conserving water and energy, and significantly increasing production yields.

Despite its many benefits, precision fermentation faces notable challenges. One of the most pressing issues is the large volume of spent microbial biomass (SMB) left over at the end of the fermentation process. Furthermore, Australian regulations on genetically modified organisms (GMOs) require that all genetically modified (GM) microorganisms used in precision fermentation be completely deactivated before their disposal. This is because GM microorganisms pose potential environmental risks, including horizontal gene transfer and disruptions to microbial ecosystems, among others. Nevertheless, this mandatory deactivation of GM microorganisms imposes an additional financial burden on Australian biotech companies that use precision fermentation for commercially valuable compounds. As precision fermentation gains traction, the need for sustainable and cost-effective SMB management solutions is becoming increasingly urgent.

SMB is rich in macronutrients such as carbon and nitrogen and contains essential micronutrients and trace elements, making it a potentially valuable resource for various industries. Previous studies indicate that the composition of SMB varies slightly depending on the microorganism species used in fermentation. Taking that nutrient-rich nature into account, repurposing SMB instead of treating it as waste could provide new opportunities for sustainability and economic gain. The challenge lies in identifying

feasible and profitable applications for SMB while complying with Australia's stringent GMO regulations.

This is why, my PhD research aims to advance the precision fermentation industry toward a more circular and sustainable bioeconomy by developing viable SMB upcycling strategies. My research will focus on identifying potential solutions for the SMB problem that align with Australian regulatory frameworks and industry needs. To achieve this, I will conduct a techno-economic analysis and a life-cycle assessment to evaluate the financial and sustainable feasibility of these potential solutions for SMB utilization. These assessments will help determine the most practical solution for the Australian biotech sector.

Once the most promising upcycling solution has been identified, I will carry out a series of experiments to thoroughly assess its effectiveness. The final step will involve engaging with the Australian biotech industry to present the findings and facilitate real-world implementation. By transforming SMB from a costly waste product into a valuable resource, my research will contribute to making precision fermentation more sustainable and economically attractive. This work has the potential to drive significant advancements in the Australian biotechnology sector, helping to create a more resilient and resource efficient bioeconomy.

Seminar IV

Bioengineering

biomining, biomaterials, waste valorisation

Microbial communities

Chairperson

Ines & Birgitta

Presenters

Ilan Blanville

Ian Petersen

Tamara Važić

Fernanda Soto

Luke Webster

Rosemary Gillane

The potential of soil microbial communities to reduce nitrous oxide emissions in agricultural soils.

Ilan Blanville[1], Tim McCubbin[1,2], Axa Gonzalez[1,3,2]

[1]Australian Institute for Bioengineering and Nanotechnology (AIBN), Brisbane, Australia. [2]ARC Centre of Excellence in Synthetic Biology, Brisbane, Australia. [3]Integrated Design Environment for Advanced Biomanufacturing (IDEA Bio), Brisbane, Australia

Focus Area: Bioengineering, Bioprocess Optimisation, Computational Biology, Multi-omics Analysis

Area of Expertise: Automation, Bioinformatics, Data Scientist, Bioprocess engineering/optimisation, Fermentation Services, Modelling

Favorite Organisms: *Microbial Communities/ mixed cultures*

The mitigation of nitrous oxide (N_2O) emissions from agricultural soils remains an elusive task. However, the use of soil microorganisms can serve as a novel biological strategy to suppress N_2O emissions in farmland. This project aims to screen, identify, characterise, and select native soil microbial communities from agricultural soils and harness their nitrogen transforming metabolic capabilities. Three soil samples were collected across the northern grain region of Australia. We implemented a selective pressure using (KNO_3^-) and (KNO_2^-) as sole N sources. Five individual isolates of interest were recovered and sequenced using NGS. The results allowed the identification of 4 bacterial genera demonstrating metabolic capabilities associated with nitrification, denitrification, and growth promotion in grain crops. The study presents an avenue for building engineered soil microbial populations that can reduce N_2O emissions in different agricultural systems, while also supporting crop production.

Optimisation of phosphate-solubilising microbes for agricultural applications

Ian Petersen

AIBN (UQ), Brisbane, Australia. AGFS (UQ), Brisbane, Australia

Focus Area: Bioprocess Optimisation, Multi-omics Analysis

Area of Expertise: Bioprocess engineering/optimisation, Fermentation Services, qPCR, Glasshouse Trials, Fungal Cultivation

Favorite Organisms: *Microbial Communities/ mixed cultures, Aspergillus niger*

As global rock phosphate reserves continue to be depleted, there are rising concerns for the future of agriculture, where the vast majority of phosphate fertiliser is derived from these deposits. Looking at ways to reduce our fertiliser usage and establish a more circular anthropogenic phosphorus cycle will help to secure adequate agricultural productivity for our growing population.

This project is looking to use phosphate solubilising microbes (PSM) which are natively found in certain soils to address this issue. PSM can break down existing soil phosphate reserves that are ordinarily unavailable for plant uptake, unlocking a new source of plant phosphorus that can help to reduce fertiliser requirements. In particular, soils that have previously been heavily fertilised contain substantial amounts of phosphate in these otherwise plant-unavailable forms.

Fungal strain A510 was isolated from soils in Brisbane, QLD and shown to have substantial phosphate solubilisation activity in liquid culture. However as often seen with bioinoculants, glasshouse trials with a variety of soils showed mixed results. Focussing on bioreactor cultures, various medium compositions were compared and it was seen that the ratio of carbon to nitrogen had a key impact on the profile of organic acids produced. Although organic acid production is one the of the main mechanisms behind phosphate solubilisation, the efficacy highly depends on the specific type of organic acid.

Proteomic analysis of the cultures revealed some of the metabolic changes taking place in the presence of phosphate limitation, and although some soil incubation assays have shown that the fungus can solubilise phosphate in soil, a final trial (completed, but with some remaining samples to process in the coming weeks) is looking to examine the impact of varying the carbon to nitrogen ratio of the soil on both the proliferation of the fungus and the resulting ability to solubilise phosphate.

It is hoped that some of these results will help to inform optimal application of this strain as a biofertiliser. For example, the current results suggest that a preincubation period in a nutrient rich medium such as compost may increase overall organic acid production and improve outcomes in soils, rather than a direct soil application. The results of this final trial may also highlight a particular carbon to nitrogen ratio for this preincubation medium or the soil itself that offers the best production of optimal organic acids that can improve phosphate solubilisation for plant uptake.

Cyanobacterial potential for adaptation, colonisation and carbonisation of bischofite-enriched sandy substrates in semi-arid and arid environments

Tamara Važić[1], Gordon Southam[2], Samandi Guathunga[2], Maria Grassino[3], Denys Villa Gomez[3,4]

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Focus Area: Bioengineering Favorite Organisms: *Cyanobacteria*

Cyanobacteria are photosynthetic prokaryotes found in aquatic and terrestrial environments. While their excessive proliferation in water bodies can disrupt ecosystems and pose health risks, cyanobacteria can be a valuable resource in harsh terrestrial environments where they are primary colonisers due to their high adaptability and low requirements. Considering the challenges of revegetation in semi-arid and arid regions, the potential of artificially induced biological soil crusts for substrate stabilisation, and the role of cyanobacteria in enhancing carbon sequestration through the biomineralisation of magnesium (Mg), the aim of this study is to examine the capacity of a cyanobacterial consortium to adapt, colonise, and promote carbonisation in magnesium-rich sandy substrates commonly found in tailings at mining sites in the Atacama Desert.

The evaluation of the cyanobacterial consortium's potential to adapt and colonise a sandy substrate containing varying amounts of bischofite comprised three experimental phases: (1) *Salt effect in liquid phase*—effects of targeted concentrations of bischofite solutions (0 g/L, 22 g/L, 31 g/L, 62 g/L, 125 g/L, 625 g/L); (2) *Desiccation phase*—the impact of desiccation and the consequent increase in salt concentrations; (3) *High light intensity phase*—effects of 5 hours/day of high light intensity under a 27-37°C temperature regime and low water content. Colonisation ability was evaluated through visual observation and measurement of cyanobacterial biomass (Chl *a* concentration). Adaptability to salt and light stress was assessed spectrophotometrically by detecting and quantifying protective metabolites, including UV-protective pigments (scytonemin, carotenoids), intracellular osmoprotectants (sucrose, trehalose), extracellular polysaccharides using hydrolysis, and heat-shock proteins. Changes in the cyanobacterial community were assessed through metagenomic analysis of the consortium at the end of the experiment. The carbonisation process of Mg from bischofite was evaluated by quantifying magnesium carbonate in the substrate and analysing samples using scanning electron microscopy (SEM).

The findings indicated that the cyanobacterial consortium exhibited high salt tolerance and adaptability at all applied concentrations of bischofite in the liquid phase, and furthermore, an increase in salt concentration during the desiccation phase. However, exposure to high light intensity, along with salt stress, led to a certain degree of mortality in the observed samples.

The results suggest that cyanobacteria have the potential to colonise and stabilise magnesium-rich sandy substrates and enhance carbon sequestration through biomineralisation.

Improving bioleaching efficiency of critical elements from bauxite residue.

Fernanda Soto[1,2], Luke Webster[1,2], Rosie Gillane[1,2], Esteban Marcellin[1], Denys Villa Gomez[2,1], Sue Harrison[3]

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Focus Area: Bioengineering Area of Expertise: Bioprocess engineering/optimisation

Favorite Organisms: *Aspergillus niger, Penicillium oxalicum*

Bauxite residue (BR) is a by-product of the alumina refining industry, with a current stockpile of over 4 billion tonnes of BR worldwide. This inventory is expected to grow as the demand for primary aluminium is forecast to increase by over 50% by 2050[1]. Critical minerals, such as vanadium (V) and rare earth elements (REEs), are contained within BR, and these are essential for developing clean energy technologies necessary for advancing toward net-zero emissions[2]. This investigation will develop a strategy for selectively extracting these critical metals from BR while retaining the aluminium and iron-bearing minerals in the solid phase, using bioleaching agents produced by filamentous fungal strains isolated from bauxite mine sites worldwide [3,4,5]. Initial experiments consisted of determining the growth and tolerance of *A. niger* and *P. oxalicum* to different bauxite residue samples. *A. niger* was shown to tolerate concentrations up to 10% w/v with slight inhibition. *P. oxalicum* was strongly inhibited at all the studied concentrations.

Both strains showed a high neutralisation capacity, being able to decrease the pH by 0.6 to 2.7 for *A. niger* and between 0.2 and 0.9 for *P. oxalicum*. It was observed that organic acids performed better in recovering critical metals (V and REEs) from BR compared to mineral acids when tested at the same normality and molarity. This highlights the role of metal - complex solubility in the separation from the solid phase. Organic acids also demonstrated superior selectivity for the previously mentioned critical metals compared to iron and showed better selectivity for Sc and V than for aluminium. These results will drive adaptive laboratory evolution and genetic engineering strategies to improve the lixiviant synthesis, bioleaching efficiency and organism tolerance to heavy metals and alkaline pH, providing a novel strategy for alumina refinery by-product valorisation.

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Identification of critical metal binding proteins from mine waste

Luke Webster, Rosemary Gillane, Fernanda Soto, Robin Palfreyman, Ajit Desigan, Esteban Marcellin, Denys Villa-Gomez

University of Queensland, Brisbane, Australia

Focus Area: Bioengineering, Multi-omics Analysis, Computational Biology

Area of Expertise: Bioinformatics, Data Scientist, Molecular Cloning, Omics - Metabolomics, Proteomics, etc., Protein Engineering, Recombinant Protein Production, Strain Engineering, Systems Biology

Favorite Organisms: *Cyanobacteria, Escherichia coli, Microbial Communities/ mixed cultures, Pseudomonas putida, Dissimilatory metal reduci ng bacteria*

Critical elements, such as rare earths, gallium, and germanium, are becoming increasingly important to the global economy due to the advancement of alternative energy and electronic technologies. Australia produces an estimated 30 million tonnes per year of alumina refinery waste (red mud), which contains an array of critical elements. Biomining could be the key to harnessing this untapped critical element supply, as current chemical separation methods are costly and environmentally damaging. However, a significant knowledge gap exists in understanding microbial interactions with non-biological metals, and industrial progress has been limited to a few model organisms. This work aims to identify interactions between potential biomining organisms and critical elements, with the goal of discovering highly selective metal binding proteins to be used in biomining technologies. Proteomic changes in response to the critical elements La, Dy, Ga and Ge were investigated in the candidate microorganisms *P. putida*, *S. oneidensis*, *G. metallireducens*, *A. niger*, and *P. oxalicum*. IMAC-based protein chromatography was used in conjunction with proteomics to identify metal-binding proteins for La. Meta-analysis of the proteomics results has determined key protein domains responsible for metal metabolism in microorganisms and may hold deeper insights related to metal binding. From this work, 10 candidate proteins have been selected to be manufactured for further analysis of metal-binding using a combination of chromatographic, spectroscopic, and assay-based methods. Changes in enzymatic pathways due to metal presence have also been modelled from this data, helping to elucidate the complex metal metabolism in these microorganisms. Further work will be focused on adapting this method for high-throughput applications, enabling a rapid identification pipeline starting from mine waste microbial characterisation, through to curated metal-binding proteins and metabolic potential within the mine site.

Many sustainable and renewable technologies are wholly reliant upon sourcing of critical metals, yet can these technologies really be considered sustainable if the underlying resource extraction is not? For the world to successfully transition to a green bioeconomy, sustainable solutions to mining are not only desirable, but necessary. This research aims to aid the mining sector transition to biosustainable, yet valorised methods, which may provide a cascade effect on the sustainability of green technologies requiring critical elements.

Seeing the value in waste: Isolation of microbes from bauxite residue to recover critical minerals

Rosemary Gillane[1], Luke Webster[1], Fernanda Soto[1], Nick Gurieff[2], Denys Villa Gomez[1]

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Focus Area: Bioengineering, Multi-omics Analysis

Area of Expertise: Bioprocess engineering/optimisation, Molecular Cloning, Omics - Metabolomics, Proteomics, etc., Strain Engineering, Systems Biology

Favorite Organisms: *Microbial Communities/ mixed cultures, Escherichia coli*

Critical minerals are essential for modern technology and the push towards green energy solutions, including electronics, renewable energy, and electric vehicles. Their scarcity can impact supply chains, innovation, national security and severely hampers our ability to reach net zero carbon emission goals. Bauxite residue, colloquially known as red mud, is a byproduct of alumina refining from bauxite ore. Australia alone, has an estimated stockpile of 200 million tonnes of bauxite residue, much of which has high concentrations of critical minerals including rare earth elements. Red mud is not only an untapped source of critical minerals but offers a unique opportunity to explore the microbial potential of these sites. We have been able to recover anaerobic and aerobic bacteria from red mud samples with two strains capable of surviving pHs up to 11. Bacterial isolates from high metal environments often result in adaptations of growth to specific metals (including rare earth elements). 19 samples have been whole genome sequenced, resulting in some strains that have not been characterised. We plan to further study isolates for tolerance to growth in red mud and capacity of proteins with metal binding to critical metals.

This research is crucial for advancing sustainable biological technologies capable of extracting critical metals from waste streams. The extreme conditions in which these isolates thrive also offer the potential to discover organisms that can utilise atmospheric carbon and produce valuable products, such as antimicrobial compounds, providing a competitive edge.

Seminar V

Multi-omics Analysis

& Computational Biology

Chairperson

Axa & Zeinab

Presenters

Zhuoqi Xiao

Craig Barry

Rafael Eduardo Hernández-Guisao

Tim McCubbin

A Comparative Study of Site-Specific and Random Genomic Integration in CHO Cells for Bispecific Antibody Production

Zhuoqi Xiao

Marcellin group, Brisbane, Australia

Focus Area: Bioengineering

Area of Expertise: Molecular Cloning, Recombinant Protein Production, Strain Engineering

Favorite Organisms: *Escherichia coli, Chinese Hamster Ovary*

Bispecific antibodies (BsAbs) represent a new class of biotherapeutics with applications beyond immunotherapy, including oncology, infectious diseases, and autoimmune disorders. Compared to traditional monoclonal antibodies, BsAbs offer greater specificity and efficacy. An example of a clinically approved IgG-like BsAb is Mosunetuzumab, which targets both CD20 and CD3 to redirect T cells for the treatment of B-cell malignancies. However, a significant challenge remains in achieving stable genome integration of the BsAb, which affects both expression and scalability. This project aims to compare two methods of generating stable producer cell lines for BsAbs in Chinese Hamster Ovary (CHO) cells: site-specific genomic integration and random integration approaches. Random integration, while commonly used, results in variable insertion sites that can impact gene expression, leading to inconsistent protein yields over time. In contrast, site-specific integration enables controlled transgene placement, reducing variability and improving long-term stability.

To achieve precise genomic insertion, we employed recombinase-mediated cassette exchange (RMCE), a targeted gene integration technique that allows for the controlled placement of the heavy and light chain genes of Mosunetuzumab into predefined loci. RMCE ensures that BsAb expression is not influenced by positional effects, facilitating more predictable and reproducible protein production. Additionally, RMCE minimises clonal variability, making it a more robust approach for generating stable producer cell lines. We anticipate that the production titres of BsAb in randomly integrated cell lines will decrease due to genomic instability, despite the higher initial titres. In contrast, site-specific integration is expected to maintain a stable genomic presence and consistent production levels. PCR, qPCR, and ddPCR techniques will be conducted to confirm gene integration position and copy number. The expression levels will be quantified using Biacore™ and Octet™ systems. Protein integrity will be assessed through SDS-PAGE, Western blot, and Dot blot. We hypothesise that stability studies conducted over 80 generations will demonstrate that site-specific integration will result in more consistent BsAb expression. In contrast, random integration will exhibit higher variability in yield and stability, despite initially showing higher expression levels.

This research focuses on optimising stable CHO cell lines to produce BsAbs, contributing to scalable bioprocessing solutions that improve access to vital therapeutics. In line with the Hub's mission, this work supports the development of innovative and efficient biomanufacturing strategies. By streamlining resource-intensive production cycles, we aim to transform biologics manufacturing into a more economically sustainable endeavour, ultimately paving the way for broader patient access to these life-saving treatments.

Identifying critical mechanisms for optimizing yields of red blood cell cultures using temporal single cell proteomics

Craig Barry[1], Sara Chiaretti[2], Rebecca Griffiths[2], Esteban Marcellin[1,3], Lars Nielsen[1]

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Focus Area: Computational Biology, Bioprocess Optimisation, Multi-omics Analysis

Area of Expertise: "Bioinformatics, Data Scientist, Bioprocess engineering/optimisation, Machine Learning, Modelling, Omics - Metabolomics, Proteomics, etc., Recombinant Protein Production

Favorite Organisms: *Chinese Hamster Ovary, Human, mouse, virus*

As the global population grows, the demand for healthy blood donors increases. This is especially true for donors of rare blood types that constitute only a small percentage of the population. The development of in vitro red blood cell (RBC) culture offers a potential solution to meet this critical need, particularly for rare blood types. This involves the expansion and differentiation of hematopoietic stem cells (HSCs) into mature RBCs in liquid cultures. This biological process of differentiation is called erythropoiesis, and results in highly heterogeneous cultures, comprising cells at various stages of differentiation. A key stage of producing mature RBCs is the process of enucleation, where the cell expels its nucleus and other organelles. Enucleation efficiency varies significantly depending on the source of HSCs, with efficiencies reported as low as 40% in cord-derived HSCs.

Developing a rational engineering strategy to improve RBC maturation efficiency is challenging due to the complex, poorly understood mechanism which drives enucleation. Working toward an optimisation strategy, requires a strong characterisation of the underlying biological process which governs the success or failure of enucleation. To investigate these mechanisms, our study uses a pioneering single-cell proteomics approach to dissect the heterogeneity of these cell cultures and identify specific cell populations during enucleation. This approach allows us to capture the dynamics of protein profiles which give rise to enucleated cells. These insights offer a promising pathway to optimize the yields of mature RBCs, paving the way for scalable in vitro production.

Metabolic Pathway Analysis of a condensed metabolic model of *Chlamydomonas reinhardtii* tailoring lipid profile and accumulation.

Rafael Eduardo Hernández-Guisao[1], Elvia Inés García-Peña[2], Edgar Salgado-Manjarrez[2]

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Focus Area: Computational Biology, Gas Fermentation, Multi-omics Analysis

Area of Expertise: Bioprocess engineering/optimisation, Modelling, Omics - Metabolomics, Proteomics, etc., Systems Biology

Favorite Organisms: *Cyanobacteria, Chlamydomonas reinhardtii*

Eukaryotic microalgae are autotrophic microorganisms that do not require complex culture medium. Instead, they only need light, inorganic nitrogen, and salts to fix carbon dioxide and convert it into high value products. This capacity make them excellent candidates for the transition to a sustainable economy as they are able to consume carbon dioxide and produce value-added metabolites. Among these, *Chlamydomonas reinhardtii*, a model microorganism, accumulate lipids that can be further converted into biodiesel. The quality of biodiesel, in terms of the cetane number, is inversely proportional to the number of unsaturation (double bonds) in the lipid's structure. Therefore, the more saturated the lipid, the more suitable the lipids is for biodiesel production. Several studies have been conducted regarding the mechanism of lipids accumulation and production but none of them has addressed tailoring lipids profile and accumulation from a metabolic pathways analysis point of view. With this approach the metabolism is analyzed through a set of vectors called Elementary Flux Modes (EFM), whose are minimum set of vectors that span the solution space of the pseudo steady-state condition of the metabolic network. In this work, we investigated the metabolism of *C. reinhardtii* focusing on lipids accumulation and profile through metabolic pathway analysis. To achieve this, we used a condensed metabolic model of *C. reinhardtii* consisting of 4 compartments, 349 reactions, and 293 metabolites. As an estimation of the solution space, 10^4 EFM were randomly sampled from the solution space optimizing the weighted sum of two reactions (not in loop) in each iteration subject to the steady state and irreversibility constraints. Using Pearson's coefficient we observed a strong positive correlation ($\rho>0.9$) between lipids accumulation and the reactions related to dihydroxyacetone phosphate and glycerol phosphate transport and interconversion in the chloroplast. The previous suggest that overexpressing the proteins involved in such reactions might be a good strategy for increasing the lipid production. By analyzing only those EFM with positive lipid production, we found 37 essential reactions. The removal of any of these reactions would result in zero lipid production. Carbon efficiency (CE) was calculated by dividing the number of C moles in each product by the number of total C moles available in the substrates. With this method, we found that the maximum carbon conversion towards lipids was 58.4%. The EFM with the highest CE converts acetate, light, and oxygen into lipids, CO2, and water. Interestingly in this EFM, we found that 30% of the oxygen used by the network was diverted towards lipids unsaturation. Therefore, limiting oxygen availability might be a good starting point for modifying lipid profile in this microalga. Additionally, by simulating a complete saturated lipid profile (no double bonds), we found that 100% CE towards lipids was achieved in one mixotrophic EFM, but this would require five times more energy input.

A Metabolic-Model Driven Exploration of the Dynamic Shifts in Cyanobacterial Metabolism across a Batch Fermentation Process

Tim McCubbin[1,2], Joseph Raphael[1,2], Lars Puiman[1,2], Hossein Firoozabadi[3], Esteban Marcellin[1,2], Lars Nielsen[1,2,3]

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Focus Area: Computational Biology, Multi-omics Analysis

Area of Expertise: Automation, Bioinformatics, Data Scientist, Modelling, Systems Biology, Omics - Metabolomics, Proteomics, etc.

Favorite Organisms: *Clostridium autoethanogenum, Cyanobacteria, Escherichia coli, Methanotroph, Pichia pastoris, Saccharomyces cerevisiae*

The photosynthetic, autotrophic metabolism of cyanobacteria makes them an attractive platform for the production of high value chemicals. Owing to their photosynthetic nature, cyanobacterial metabolism varies greatly across the cultivation owing to the phenomenon of self-shading, where higher density cultures reduce light penetration, leading to dark zones in the bioreactor where respiratory metabolism or fermentation can occur. Most studies to date characterise cyanobacteria in early exponential, non-light limited growth, which is not reflective of the higher density conditions required of an industrially relevant bioprocess. We argue that the lack of characterisation of metabolism under these conditions reduces the ability to rationally design metabolic engineering strategies.

In this work, we seek to create a mechanistic model of the cyanobacterial batch bioprocess. We first designed and manufactured flat-panel bioreactors, which were subsequently used to culture wild-type freshwater and marine *Synechococcus elongatus* strains. Metabolomics and proteomics data collected over the batch cultivation process demonstrates distinctly different metabolisms between the freshwater and marine strains. Using metabolic models, we are able to deconvolute the differentially expressed proteins between phases to predict distinct metabolic operation modes which occur in different regions of the bioreactor. To better explore these metabolic modes, we next seek to couple protein-constrained models with mixing models of the photobioreactors. The resulting mechanistic model of the photobioreactor will allow the prediction of metabolic fluxes in a spatially defined way, and allow for the prediction of more global optimisation strategies across the alternative metabolic modes; facilitating more optimal strain design strategies.

This analysis provides new insights into the likely roles of metabolic pathways and enzymes in Synechococcus metabolism. The mechanistic fermentation model is expected to provide new avenues for the rational design of strategies to overcome inherent limitations of the light-driven cultivation process, and consequently, help to improve the difficult economics of cyanobacterial fermentation processes.

Other abstracts – Precision Fermentation

Abstracts

Neha Lal

Kevin Hu

Laetitia Gonzales

Peizhen Gao

Hanzhi Sun

Optimising Recombinant Dairy Protein Production through Precision Fermentation

Neha Lal[1,2], Esteban Marcellin[1,2,3]

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Focus Area: Precision Fermentation, Bioprocess Optimisation

Area of Expertise: Bioprocess engineering/optimisation, Fermentation Services, Recombinant Protein Production

Favorite Organisms: *Pichia pastoris*

As part of the Food and Beverage Accelerator (FaBA) initiative, my research focuses on optimising the production of recombinant dairy proteins through precision fermentation in instrumented bioreactors. Working within a multidisciplinary team and collaborating with industry partners, I contribute to the development of scalable bioprocesses to enhance the efficiency and sustainability of protein production. Precision fermentation employs engineered microbial strains as microbial cell factories, enabling the targeted production of high-value proteins identical to their naturally occurring counterparts. My work primarily involves bioprocess optimisation in small-scale bioreactors (~500 mL), with recent efforts extending to larger-scale systems to assess scalability. By optimising the fermentation media, conditions, feed strategies, and bioreactor operation, my work supports the development of more effective and sustainable production processes that complement traditional agriculture and diversify protein production methods to meet growing global demand and specific consumer needs.

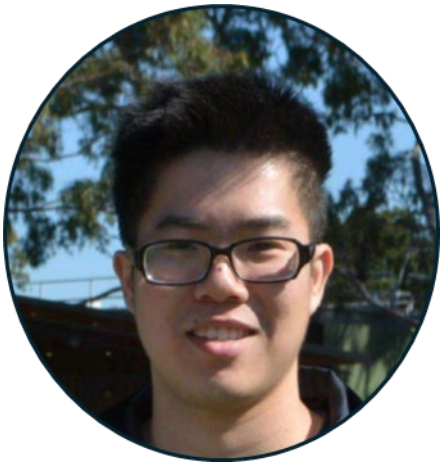
Beyond improving production efficiency, precision fermentation presents a viable climate-conscious alternative to conventional agriculture, as it can enable large-scale protein production with a significantly lower carbon footprint. Furthermore, by integrating industrial by-products such as sugarcane waste as feedstocks, precision fermentation has the potential to support a circular bioeconomy and reduce the need for typical raw materials. Precision fermentation also offers a promising solution to the ethical concerns associated with animal-based protein production, as by decreasing reliance on livestock, this method addresses key issues related to animal welfare, land use, and resource-intensive farming practices. These advantages have positioned precision fermentation as a key technology for sustainable biomanufacturing, and its adoption, alongside traditional approaches, is essential to reduce the environmental impact of protein production and ensure that food systems remain within planetary boundaries.

My research interests lie in fermentation science, bioprocess optimisation, and scale-up strategies for industrial biotechnology applications. Through this work, I am strengthening my expertise in precision fermentation and gaining hands-on experience in real-world bioprocess development. This role has allowed me to deepen my understanding of fermentation kinetics, oxygen transfer, and microbial metabolism while contributing to the advancement of sustainable protein production technologies that align with environmental and ethical priorities.

Developing Life Cycle Assessment and Techno-Economic Analysis Frameworks for precision fermentation of milk proteins and its integration into current dairy infrastructure.

Kevin Hu, Birgitta Ebert

AIBN, University of Queensland, Brisbane, Australia



Focus Area: Precision Fermentation, Bioengineering, Bioprocess Optimisation

Area of Expertise: Bioprocess engineering/optimisation, Fermentation Services, Recombinant Protein Production, Molecular Cloning, Strain Engineering

Favorite Organisms: *Escherichia coli, Microbial Communities/ mixed cultures, Pichia pastoris, Saccharomyces cerevisiae*

In collaboration with Fonterra, this FaBA research project evaluates the technical and economic feasibility of integrating precision fermentation into current dairy infrastructure. Specifically, the project involves the design and implementation of upstream fermentation and downstream product purification strategies, including the utilisation and recycling of side-streams generated both from microbial cultivation and dairy processing. Additionally, underutilised assets of dairy processing plants are also considered and capitalised in the bioprocess design. Techno-economic analyses will be performed to evaluate the feasibility and viability of the integrated precision fermentation system to derive whether it can provide a cost-effective pathway to realise precision fermentation of recombinant dairy products at scale.

Overall, the project contributes to the translation and transfer of knowledge, technology and practices in precision fermentation from laboratories within the Biosustainability Hub to our partner in the dairy industry, with the intent of reenforcing the reputation of the Hub, and the FaBA program, in precision fermentation for food applications.

Advancing Synthetic Biology for Sustainable Protein Production in the Food Industry: Optimizing Fermentation Processes and Scaling Up Recombinant Protein Production

Laetitia Gonzales[1,2], Esteban Marcellin[1,2,3]

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Focus Area: Precision Fermentation, Bioprocess Optimisation

Area of Expertise: Bioprocess engineering/optimisation, Fermentation Services, Recombinant Protein Production

Favorite Organisms: *Pichia pastoris*, *Escherichia coli*

I joined the Australian Institute for Bioengineering and Nanotechnology (AIBN) at the University of Queensland eight months ago, where I have been working on a project focused on utilizing synthetic biology-derived strains to produce recombinant proteins. These proteins have numerous applications, particularly in the food industry, as part of the Faba program.

The goal of the project is to optimize fermentation processes using genetically engineered strains to achieve high yields of recombinant proteins. These proteins are essential for applications in the food sector, including enhancing product functionality, improving nutritional value, and meeting specific consumer demands.

As part of a multidisciplinary team, I am involved in selecting strains with the ability to produce high-quality recombinant proteins in bioreactors, and to carefully optimize the fermentation process. Additionally, I focus on scaling up the process to larger bioreactors. My work contributes to advancing synthetic biology in the food industry, offering more sustainable, efficient, and versatile methods of protein production to meet the growing demand for alternative protein sources.

Originally, I am a Bioprocess Technician with nearly 10 years of experience in the Biopharmaceutical and Food & Beverage industries. I specialize in fermentation, process optimization, and scale-up. I have worked collaboratively on projects involving yeast and bacterial cultures in bioreactors ranging from 500ml to 100L. My focus has been on recombinant protein production, strain screening, and the optimization of fermentation processes to improve yields, efficiency, and overall productivity. I have actively contributed to the scale-up of bioreactor cultures and the development of new fermentation processes, ensuring successful implementation and improved production outcomes.

Metabolic engineering of Yarrowia lipolytica for sustainable bioproduction of raspberry ketone and other aromatic compounds

Peizhen Gao

Australian Institute for Bioengineering and Nanotechnology, Brisbane, Australia. ARC Centre of Excellence in Synthetic Biology, Brisbane, Australia. Food and Beverage Accelerator, Brisbane, Australia

Focus Area: Precision Fermentation

Area of Expertise: Strain Engineering

Favorite Organisms: *Yarrowia lipolytica*

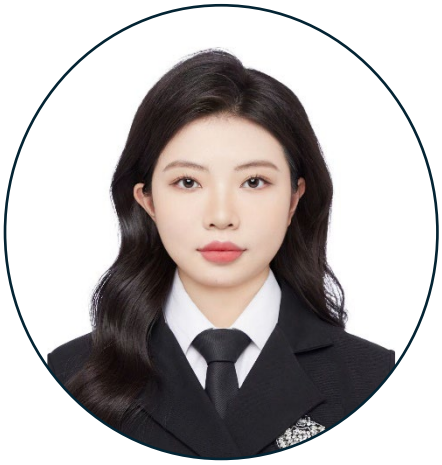
Precision fermentation has emerged as a transformative biotechnology platform for sustainable production of high-value compounds through genetically engineered microorganisms, with commercial success in many products. Raspberry ketone (4-(4-hydroxyphenyl) butan-2-one) represents an attractive target for precision fermentation approaches. As the primary aroma compound in raspberries, this versatile molecule serves as a popular flavouring agent in food products, a fragrance component in cosmetics, and has garnered attention for potential applications in weight management and metabolic health. The growing market has created significant demand for bio-based production methods that can deliver natural-equivalent raspberry ketone at commercial scale. While RK has been successfully produced in various hosts with notable progress, yield-limiting challenges remain to restrict production below industrial requirements. To address these challenges, our research harnesses recent advances in synthetic biology to optimise raspberry ketone biosynthesis in *Yarrowia lipolytica*, which is selected for its robust growth characteristics, high lipid metabolism, and natural tolerance to aromatic compounds. We have successfully demonstrated de novo biosynthesis of raspberry ketone in *Y. lipolytica* through strategic integration of the required biosynthetic genes into specific genomic loci. Comprehensive characterisation has been conducted to assess growth patterns under various conditions, establish the compound's toxicity profile within the host, map production kinetics throughout fermentation, and analyse metabolite distributions to identify pathway bottlenecks. Future efforts will focus on enhancing productivity through metabolic engineering strategies, including fine-tuning metabolic flux, protein engineering, multi-omics integration, and downstream process optimisation. Additionally, we aim to expand the product portfolio to other high-value aromatic compounds, further improving the economic viability of the bioprocess.

Our approach represents a significant step toward developing commercially viable biological processes for producing valuable aromatic compounds while reducing environmental impact, directly support the Hub's vision of achieving a sustainable, global bioeconomy and developing net-zero emissions solutions.

Engineering Yarrowia lipolytica to produce high-value flavour and pigments

Hanzhi Sun, Peizhen Gao, Esteban Marcellin, Huadong Peng

AIBN, Brisbane, Australia



Focus Area: Precision Fermentation

Area of Expertise: Strain Engineering

Favorite Organisms: *Yarrowia lipolytica*

As the demand for sustainable and bio-based production of valuable compounds grows, microbial biosynthesis has emerged as a promising alternative to traditional extraction and chemical synthesis methods. Raspberry ketone (RK) is widely used in food and cosmetic industries for its unique fruity flavour. However, traditional extraction methods from plants are inefficient and costly, while chemical synthesis raises environmental concerns. These challenges make microbial biosynthesis an attractive alternative. This study explores metabolic engineering strategies of yeast *Yarrowia lipolytica* for efficient raspberry ketone production. *Y. lipolytica* is a promising host due to its robust growth at high cell densities, broad substrate utilization capacity, and abundant acetyl-CoA and malonyl-CoA pools, key precursors for RK biosynthesis. Bioproduction experiments confirmed that the optimised strain successfully achieved de novo RK synthesis in minimal medium. This is the first reported case in *Y. lipolytica*, paving the way for further optimisation.

Building on this work, my research will now focus on the metabolic engineering of *Y. lipolytica* for the biosynthesis of two high-value pigments: pyomelanin and indigo. Pyomelanin is a brown-black pigment derived from homogentisic acid (HGA) polymerisation. It exhibits antioxidant and UV-protective properties, making it widely applicable in cosmetics, biomedicine, and material science. Indigo is an important natural dye widely used in textile dyeing, art, and cosmetics. Traditionally, indigo is obtained through plant extraction or chemical synthesis, but chemical synthesis contributes to environmental pollution issues. Microbial biosynthesis offers a sustainable and eco-friendly alternative, aligning with the growing demand for bio-based pigments.

The synthesis of raspberry ketone, Pyomelanin and Indigo in *Y. lipolytica* provides an environmentally friendly alternative to traditional extraction and chemical synthesis, driving sustainable bioproduction. This approach aligns closely with the vision of the BioSustainability Hub, advancing the development of eco-friendly and scalable biomanufacturing solutions.

Other abstracts - Building Microbial Communities

Abstracts

Elvia Inés Garcia-Peña

Axayacatl Gonzalez

Zhaoyue Gu

Microbial photoheterotrophic consortia for biohydrogen and polymer production under non-growing conditions: insights into metabolic processes and microbial interactions

Elvia Inés Garcia-Peña

CIUDAD DE MEXICO, CIUDAD DE MEXICO, Mexico

Focus Area: Bioengineering, Precision Fermentation

Area of Expertise: Adaptive Laboratory Evolution, Bioprocess engineering/optimisation, Systems Biology, Biofuel Production, Waste Management

Favorite Organisms: *Microbial Communities/ mixed cultures*

Natural photoheterotrophic mixed cultures (PHMCs) are technologically interesting models due to their ability to produce biohydrogen (bioH_2), polymers such as polyhydroxyalkanoates (PHA), and pigments. These natural PHMCs can be used in sequential processes involving dark fermentation (DF) and photofermentation (PF). This is because PHMCs predominantly comprise by *Rhodopseudomonas palustris*, with *Clostridium* sp present in lower proportions.

It has been demonstrated that *R. palustris* has a versatile metabolism that enables the utilization of low-cost substrates such as organic waste and wastewater, as well as, the organic acids (OAs) present in dark fermentative effluents (DFEs). The physiological and metabolic behavior of natural PHMCs, monocultures of *R. palustris* and Clostridium sp, and a designed microbial consortium (DMC) that mimics natural consortia were evaluated using a synthetic mixture of OAs to simulate DF effluents under nitrogen starvation conditions. Under non-growing conditions, the DMC achieved the highest biohydrogen (bioH_2) production (10 mmol) and bioH_2 yield (78.6 mmol H_2/g COD), along with PHA accumulation. This was followed by PHMC-C2 (7.5 mmol H_2; 59.6 mmol H_2/g COD) and the monocultures. The higher bioH_2 and PHA production observed in the PHMC and DMC suggests that these conditions promote the use of non-conventional energy pathways and interactions among microbial populations, enabling survival and sustained bio H_2 production.

This study provides the base for more in-depth research on the metabolic behavior of natural and designed photosynthetic cultures. Preliminary work is being done to employ proteomic tools for a deeper understanding of these metabolic processes.

Building communities: A synbio approach to assemble robust microbial communities using Pseudomonas spp to model an aerobic denitrification process

Ilan Blanville[1], Timothy McCubbin[1,2], Chelsea Janke[3], Esteban Marcellin[1,4,2], Axayacatl Gonzalez[4,1,2]

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Focus Area: Bioengineering, Bioprocess Optimisation, Gas Fermentation, Multi-omics Analysis, Precision Fermentation

Area of Expertise: Adaptive Laboratory Evolution, Administration, Bioprocess engineering/optimisation, Fermentation Services, Molecular Cloning, Omics - Metabolomics, Proteomics, etc., Protein Engineering, Recombinant Protein Production, Strain Engineering

Favorite Organisms: *Clostridium autoethanogenum, Cyanobacteria, Escherichia coli, Microbial Communities/ mixed cultures, Pichia pastoris, Pseudomonas putida, Saccharomyces cerevisiae, Yarrowia lipolytica*

Sustainable agriculture practices are possible using beneficial soil microorganisms. These microbes are essential for the subsistence of life and play an imperative role in maintaining soil productivity. Still, studying microbial communities in soils becomes challenging due to the complex interaction across the different populations. Our research relies on the available metagenomic data to understand the diversity of microbial communities in Australia's soils to build synthetic microbial communities that mimic soil communities using easy to engineer microbes and to build novel pathways for the synthesis of industrially relevant molecules through genome mining. To achieve these goals, we seek (1) to develop a simplified pipeline to identify the key microbial communities involved in the nitrification and denitrification processes in agricultural soils; (2) to engineer Pseudomonas spp as model organism to express a gene libraries and assemble synthetic communities; (3) to engineer soil communities that can reduce the impact of nitrogen use in soil.

This research aligns the UQ Biosustainability hub to develop sustainable practices that impact the agriculture section by reducing the use of chemical fertiliser, reducing the carbon and nitrogen emission associated with these processes.

Growth dynamics of synthetic microbial communities in Chi.Bio bioreactor

Zhaoyue Gu, Huadong Peng

AIBN, QLD, Australia

Focus Area: Bioprocess Optimisation

Favorite Organisms: *Saccharomyces cerevisiae*

Advances in synthetic microbial communities offer promising applications in the biomanufacturing processes. However, how to control and maintain the long-term stability of synthetic microbial communities remains unclear. This study investigated the long-term stability of synthetic yeast communities using the Chi.Bio open-source bioreactor platform. Our previously reported two pairs of cross-feeding synthetic yeast *Saccharomyces cerevisiae* communities were selected and tested in this study. First, wild type *S.cerevisiae* was used to test and optimise the Chi.Bio bioreactor parameters, including stirring speed, fluorescence intensity gain setting. We selected stirring speed of 0.8 and fluorescence intensity for different fluorescence proteins (FP) including green FP (sfGFP), blue FP (mTagBFP2) and red FP (mRuby2) based on the growth dynamics of monocultures. Then, we assessed the growth dynamics of the two pairs of synthetic yeast communities in synthetic minimal medium using Chi.Bio bioreactor. Finally, we adjusted the target optical density values after 24h or 48h using peristaltic pump, and these synthetic yeast communities showed promising to achieve target OD values. These growth data demonstrated the long-term stability of synthetic yeast communities. Especially three-strain communities showed better stability after OD adjustment twice, which indicates multiple member communities may show advantages in maintaining system stability than monocultures and two-member communities. Our study highlights the potential of Chi.Bio bioreactors in studying the long-term stability of synthetic microbial communities, providing a basis for optimising metabolic interactions in biotechnological applications.

Synthetic microbial communities outperform monocultures in biomanufacturing. Understanding their growth dynamics enhances control, enabling sustainable bioproduction and advancing net-zero goals.

Other abstracts - Organic Compounds and Biomaterials

Abstracts

Hannes Ehlert

Yutong Han

Boyu Zhu

.Seam

Joseph Raphael

Tinggeng Lai

Shom Cyril Philip

Jolynn Kiong

Ahmed Adel Hamed Ahmed

Improving the production of Mogroside-V in *Saccharomyces cerevisiae* by developing a sucrose responsive GAL promoter system

Hannes Ehlert[1,2], Birgitta Ebert[1,2], James DeVoss[1]

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

Focus Area: Precision Fermentation

Area of Expertise: Molecular Cloning, Protein Engineering, Recombinant Protein Production, Strain Engineering

Favorite Organisms: *Escherichia coli, Saccharomyces cerevisiae*

Mogroside-V is a glucosylated triterpenoid with an intense sweetening property 250 times that of household sugar with only a fraction of the caloric value. The agricultural production of the compound is limited due to the specific environmental conditions required by the native plant *Siraitia grosvenorii*. The extract is also often contaminated by off-tasting precursors and byproducts that make the biotechnological production of Mogroside-V attractive.

Recently, the *de novo* synthesis of Mogroside-V in the chassis organism *Saccharomyces cerevisiae* has been reported. The heterologous pathway was expressed using the GAL promoters, which are autoinducible in the absence of glucose after deleting the GAL80 gene. To still achieve the glucosylation of the triterpenoid backbone, ethanol and glucose need to be fed in a finely tuned ratio, which complicates the production.

We are developing a regulatory circuit for the autoinduction of the strong GAL promoters in the presence of the cheap carbon source sucrose. Sucrose can be split into fructose, which can be shuttled into the carbon metabolism of the cell, and glucose, which can be used to decorate the carbon backbone of the target compound. Using the auxin degradation toolkit and tightly regulated, sucrose-responsive promoters, we can selectively degrade GAL repressors in the presence of sucrose and the absence of glucose. This activates the GAL promoters, allowing for strong, autoinducible protein expression after a diauxic growth in which first glucose is consumed for cell growth until the culture switches into production mode utilizing sucrose. We believe this system will benefit the production of all glucosylated natural products and make sucrose more accessible as a carbon source for precision fermentation.

Optimizing cytochrome P450 monooxygenase activity in yeast to enhance triterpenoid production

Yutong Han

University of Queensland, Brisbane, Australia

Focus Area: Bioengineering

Area of Expertise: Molecular Cloning, Strain Engineering

Favorite Organisms: *Saccharomyces cerevisiae*

Triterpenoids are a diverse class of bioactive compounds with extensive applications in the food, pharmaceutical and cosmetics industry. Many triterpenoid saponins serve as natural emulsifiers, foaming agents, and stabilizers in beverages, confectionery, and dairy alternatives, contributing to texture, mouthfeel, and stability. Their bioactive properties also make them valuable in functional foods and nutraceuticals. However, plant-derived production faces challenges such as low yields and variability, driving interest in microbial synthesis. Microbial biosynthesis offers a scalable alternative, with *Saccharomyces cerevisiae* emerging as a promising host for triterpenoid production.

This study uses quillaic acid as a model triterpenoid to explore microbial production strategies. Biosynthesis of this highly oxygenated triterpenoid requires three cytochrome P450 monooxygenases catalysing six oxidation steps. This makes it a particularly challenging target since optimizing CYP450 expression in *S. cerevisiae* remains a challenge due to metabolic burdens associated with NADPH supply, cytochrome P450 reductase (CPR) selection, and expression balance.

In this study, we implemented multiple optimization strategies to improve the activity of CYP450 in yeast, including CYP450 expression tuning and selection of efficient CPRs. Additionally, by aligning CYP450 expression with the ethanol phase, we enhanced metabolic flux toward quillaic acid synthesis, leading to an 85-fold increase in titer. Proteomic analysis showed how this phase-specific regulation affects both native and introduced pathways in yeast. With these strategies, we established a robust platform for quillaic acid production, achieving a quillaic acid titre of 385 ± 14 mg/L in flask fermentation through targeted CYP450 expression enhancements. To further enhance production, we conducted ethanol-pulsed fed-batch fermentation in bioreactors, which increased the titer to 471 ± 20 mg/L.

This work establishes a foundation for efficient microbial triterpenoid biosynthesis, offering a sustainable alternative to plant-derived sources for industrial applications.

Biotransformation of CO2-derived acetate to fatty acid derivatives in Pseudomonas

Boyu Zhu, Esteban Marcellin, Birgitta Ebert

AIBN, The University of Queensland, Brisbane, Australia

Focus Area: Precision Fermentation

Favorite Organisms: *Pseudomonas putida*

Pseudomonas species are known for their metabolic versatility and robustness, making them attractive hosts for industrial biotechnology. My study focuses on the metabolic engineering of *Pseudomonas* strains to efficiently convert acetate into fatty acid derived molecules of industrial interest Potential target products are methyl ketones as aviation fuel and lubricant precursors and bifunctionalised fatty acids for polymer production.

My work will include introducing targeted genetic modifications of the central carbon metabolism and fatty acid biosynthesis to enhance flux toward target products. The introduction and overexpression of specific pathway enzymes, along with deletion of competing routes, shall further redirect metabolic flow toward fatty acid accumulation.

This project is embedded into the ARC Industry Transformation Research Hub RECARB and will be conducted in close collaboration with PhD student Nurul Izzati (AIBN), who will develop a gas fermentation process to convert CO_2 into acetate. The overall process aims to develop a microbial platform for carbon-negative production of value-added products and hence contributes to developing a sustainable bio-based economy.

Lipid pathway engineering in yeast *Yarrowia lipolytica*

. Seam[1,2], Baode Sun[1,2], Dr. Huadong Peng[1,2]

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Focus Area: Bioprocess Optimisation, Precision Fermentation

Area of Expertise: Adaptive Laboratory Evolution, Molecular Cloning, Strain Engineering

Favorite Organisms: *Yarrowia lipolytica*

Microbial lipid production is a cornerstone of sustainable biomanufacturing, which offers renewable alternatives to petroleum-based products and reduces fossil fuel dependence. Our research investigates lipid biosynthesis in Yarrowia lipolytica, an oleaginous yeast known for its metabolic versatility and ability to use various carbon sources. We focus on acetate as a substrate due to its abundance, low cost, and availability from waste streams, which makes it an economically and environmentally ideal feedstock. However, acetate is toxic at high concentrations and often decreases cell viability and productivity, which limits its industrial applicability. Our approach combines genetic engineering and adaptive laboratory evolution (ALE). Initially, we genetically modified *Y. lipolytica* to overexpress key genes involved in fatty acid biosynthesis to enhance the conversion of acetate into lipid precursors, thus pushing the carbon flux toward lipid accumulation. Then, ALE was performed to develop acetate-tolerant strains, allowing higher concentrations to be utilised without compromising cell viability. These evolved strains were selected under progressively increasing acetate concentration to ensure improved growth and metabolic efficiency. With this dual approach, we aim to overcome the limitations of acetate utilisation, thereby advancing the efficiency of lipid production in *Y. lipolytica* for industrial applications.

Our work supports the Biosustainability Hub's vision of sustainable industrial innovation through the use of yeast-based biomanufacturing solutions and precision fermentation that reduce dependence on fossil resources, promote a circular bioeconomy, and significantly lower production costs and resource demands.

Continuous production of Terpenoids in cyanobacteria

Joseph Raphael, Tim McCubbin, Esteban Marcellin

AIBN, Brisbane, Australia

Focus Area: Bioengineering, Bioprocess Optimisation, Computational Biology, Multiomics Analysis

Area of Expertise: Adaptive Laboratory Evolution, Bioprocess engineering/optimisation, Fermentation Services, Omics - Metabolomics, Proteomics, etc.

Favorite Organisms: *Cyanobacteria*

Cyanobacteria are the first oxygenic photosynthetic microorganisms on earth and contributed to the sharp rise in atmospheric oxygen between 2.45 and 2.32 billion years ago. They have been identified as a promising chassis organism for terpenoids, owing to the native MEP pathway as well P450 enzymes. Currently, the yield of terpenoids produced from cyanobacteria are far lower than heterotrophic organisms such as yeast and E.coli. Furthermore, the production of terpenoids in a chemostat has not been developed for cyanobacteria. Using our in-house 3D printed flat panel reactors, we grew recombinant valencene and squalene producing *Synechococcus* strains and obtained steady state growth at both carbon limited and light limited growth phases for the valencene strains. We observed cell toxicity for squalene producing strains and found that oxygen led to spontaneous oxidation of squalene to a toxic product, compromising cell health. Our results showcase the importance of bioprocessing optimisation for chemostat cultivation of cyanobacteria with a lens for terpenoid production. We anticipate that this study will be a starting point for continuous production of high-value products in cyanobacteria that both academics and industry can build on.

My work relates to the vision of the Biosustainability Hub as through bioprocess optimisation we have developed the first ever chemostat system for culturing cyanobacteria.

Multi-omics and Physiological Insights into Enhanced Squalene Production in Cyanobacteria

Tinggeng Lai

AIBN, Brisbane, Australia

Focus Area: Bioengineering, Bioprocess Optimisation, Computational Biology, Multiomics Analysis

Area of Expertise: Bioinformatics, Data Scientist, Fermentation Services, Modelling, Omics - Metabolomics, Proteomics, etc.

Favorite Organisms: *Cyanobacteria*

Cyanobacteria are photosynthetic bacteria that use sunlight and CO_2 for growth and metabolism. This ability positions them as promising carbon-negative cell factories for sustainable bioproduction of chemicals. Squalene is a valuable triterpenoid with diverse industrial applications, such as being an adjuvant in pharmaceuticals or as a precursor to the emollient squalane used in cosmetics. Through a combination of genetic and bioprocess engineering, our team has heterologously expressed squalene in the cyanobacteria *Synechococcus elongatus* PCC 7942 at the highest reported yield to date- 29.34 µg.mg^{-1} CDW. Our 1-liter photobioreactor fermentations were able to replicate industrial cultivation conditions and resulted in cell growth to OD_{750} ~11. While several studies have reported the heterologous production of terpenoids in cyanobacteria, few have utilised comprehensive systems biology approaches to characterise metabolic and physiological impacts of genetic engineering on the cell. Therefore, our team undertook a multiomics comparison between the wild-type and engineered strain, integrating transcriptomic, proteomic, and metabolomic data. Results show that squalene production negatively impacts the expression of photosynthetic proteins, as well as carbon and energy storage molecules. An accumulation of MEP intermediates HMBPP and DXP indicates that metabolic bottlenecks requiring alleviation exist within the system. Additionally, photophysiological assessments coupled with flow cytometry also reveal a decline in photosynthetic efficiency and cellular membrane integrity in squalene-producing strains compared to the wild type. We also observed upregulation of nutrient uptake regulons, suggesting nutrient deficiencies during bioproduction. This study shows that while our bioproduction strain may outperform its predecessors in yield, there are still many avenues for improving genetic design, strain health and fermentation conditions. Overall, we hope these results will support the development of cyanobacteria as a platform for the production of terpenoids in an industrially viable and sustainable manner.

Shom Cyril Philip

Institute for Molecular Bioscience, Brisbane, Australia

Shom Cyril Philip is an Honours student in his final semester at the University of Queensland, majoring in Microbiology, Immunity, and Infection. He is currently conducting his research in Dr Khalil's group at the Institute for Molecular Bioscience, where he focuses on the discovery and development of bioactive compounds with antimicrobial and immunomodulatory potential. His academic journey has been shaped by a passion for understanding host-pathogen interactions and developing innovative therapeutic strategies. During his Honours research, Shom has been investigating the potential of soil microbial isolates in insecticidal roles and against common resistant pathogens like *Mycobacterium tuberculosis* and MRSA. His work integrates LC-MS-guided metabolomics, bioassay-guided fractionation, cell culturing, and advanced data analysis techniques, providing him with hands-on experience in the natural product discovery pipeline. Beyond his research, Shom is deeply engaged in the scientific community, contributing to discussions on antimicrobial resistance, drug discovery, and sustainable bioprospecting. His work aligns with global efforts to identify new therapeutic agents from natural sources, addressing the urgent need for novel antibiotics and immunotherapies. With aspirations to further his studies in microbiology and pharmacology, Shom is committed to translating research into meaningful advancements in infectious disease treatment.

Jolynn Kiong

Institute for Molecular Bioscience, Brisbane, Australia

Jolynn Kiong is a Senior Research Assistant in Dr Khalil's research group at the Soils for Science lab, Institute for Molecular Bioscience, The University of Queensland. She holds a Bachelor's degree in Microbiology and graduated with a First-Class Honours in Medicinal Chemistry in 2021, focusing on the development of vaccines against Group A *Streptococcus*. With her enthusiasm for microbiology research, she has published four peer-reviewed journal articles and plays a key role in the establishment of both operational and laboratory workflows in microbial biodiscovery. Her contributions include developing protocols for biological assays including antibacterial and antifungal assays, enabling the systematic screening of microbial extracts for therapeutic potential. She also supports DNA sequencing and taxonomic identification of microbial isolates, helping to build a high-quality microbial library that underpins downstream discovery efforts. In addition, Jolynn is actively involved in research investigating biopesticides derived from soil microbes to control canegrub, a major pest affecting Australian sugarcane crops. Her work in this area contributes to sustainable agricultural solutions by reducing reliance on chemical pesticides. She also mentors students in their research and training, sharing her expertise in laboratory techniques and assay development. Additionally, her strong administrative skills support the streamlining of operational processes, enhancing research efficiency and overall impact. Through her work, Jolynn remains committed in fostering innovation in microbiology and biosustainability, ensuring that the research carried out translates into real-world solutions for a healthier future.

Integrated Genomics and Metabolomics Approaches for the Discovery of Novel Antibiotic Metabolites from Actinomycetes

Ahmed Adel Hamed Ahmed

The University of Queensland, Brisbane, Australia

Ahmed Adel Hamed Ahmed is a PhD student at the University of Queensland. He completed his Bachelor's degree in Pharmaceutical Sciences in 2016, graduating as one of the top-ranking students, which earned him a teaching assistant position at his university. Motivated by his passion for antibiotic drug discovery from natural sources, particularly microbial origins, he chose to pursue his graduate studies in the Natural Products Department. He successfully completed his Master's degree in August 2023, during which he demonstrated strong independence and research skills by focusing on the isolation and identification of bioactive metabolites from microbial sources. His work resulted in two published papers in *Molecules* and *Metabolites* journals, contributing valuable knowledge to a novel research area in Egypt. He is among the few researchers in the region with extensive expertise in LC-MS-based metabolomics, molecular networking, and processing of LC-MS datasets. Additionally, his research is pioneering the investigation of endophytes within Egyptian plants and the exploration of soil-derived actinomycetes from Egyptian environments. Currently, in the first year of his PhD, Ahmed is focusing on isolating antibiotic metabolites from actinomycetes through integrated genomics and metabolomics approaches. He is eager to expand his skills and contribute to the discovery of novel antibiotics to address the growing challenge of antimicrobial resistance.

Other abstracts - Waste to Products

Abstracts

Damien Cleary

Nurul Izzati

Geoffrey Otim

David Ubi

Xinyu Shi

Jonathan Humphries

Ruien Yu

Xinting Yin

A taxis of necessary biological names

Damien Cleary

The University of Queensland, Brisbane, Australia

Focus Area: Gas Fermentation, Computational Biology

Area of Expertise: Bioinformatics, Data Scientist, Machine Learning, Modelling, Systems Biology

Industrial molecules are central to the availability of food, fuel, and materials of modern society. Achieving net-zero emissions by 2050 will require many current emission-intensive production methods to be redesigned. Bioprocesses, which operate at ambient temperatures and pressures are low-emission alternatives. To identify economically viable bioprocesses from the space of possibilities, metabolic models which predict product titre and yield can be used to assess a pathways feasibility. Robust models require reaction networks that use a precise and unambiguous nomenclature for compounds and reactions. Structure-based labels preserve key substrate and production information and allow unique canonical labels to be generated. The IUPAC standard reaction identifier the RInChI, extends the International Chemical Identifier (InChI) molecular representation to reactions and encodes the structure of reactants, products, and auxiliary molecules (e.g. enzymes) as a six-layer hierarchical string. In this work, we demonstrate the utility of the RInChI in constructing, developing, and validating reaction networks. We compare the RInChI against traditional registry-based approaches, develop a reaction network of global one carbon metabolism across the tree of life and use this for the development of metabolic models of one carbon assimilation. The approach is then used as the basis for the de-novo synthesis of novel one-carbon assimilation pathways for the design of bioprocesses producing industrial molecules and their precursors from industrial one carbon waste.

Metabolic engineering of *Pseudomonas putida* for biolubricant production via CO_2 fixation by engineered *Thermoanaerobacter kivui*

Nurul Izzati, Esteban Marcellin, James Kemp Heffernan, Karen Rodriguez Martinez, Birgitta E. Ebert

Australian Institute for Bioengineering and Nanotechnology, Brisbane, Australia

Focus Area: Bioengineering, Gas Fermentation

Area of Expertise: Recombinant Protein Production, Systems Biology

Favorite Organisms: *Escherichia coli, Pichia pastoris, Saccharomyces cerevisiae*

In response to the urgent need to mitigate the global climate crisis, reducing greenhouse gas emissions, particularly carbon dioxide (CO_2), has become a critical priority. CO_2 can serve as a valuable carbon source for certain microorganisms, offering a potential solution for carbon utilization. Acetogenic bacteria, such as *Thermoarobacter kivui*, utilize the Wood-Ljungdahl pathway (WLP) for CO_2 fixation, presenting an opportunity to enhance carbon fixation efficiency and direct metabolic flux towards the production of acetate. Acetate can also serve as a carbon source for some microorganisms. Acetate is utilized by *Pseudomonas putida* as feedstock and can be converted into commercial bioproducts such as biolubricants by engineered microbes. The conversion involves several enzymes to produce succinate, a building block for many valuable polymers. Biolubricants, derived from these polymers, are more degradable and sustainable compared to petroleum-based lubricants. The global demand for lubricants is substantial, reaching 30-40 million tons per year, with 95% being petroleum-based. These petroleum-based lubricants are less degradable and toxic to the environment. Therefore, biolubricants are highly desired due to their sustainability, non-toxic waste, and high viscosity indexes.

This study aims to enhance the titer of acetate by utilizing *T. kivui* and to produce methyl ketone, a precursor for biolubricants, by engineering the metabolic pathway of *P. putida*. T. kivui is a thermophilic acetogen bacterium that thrives at 66°C, advantageous for industrial applications. Utilizing acetate from biochemical processes, including those involving *T. kivui*, is cost-efficient as it metabolizes atmospheric CO_2 and requires low energy for growth. Several studies have aimed to enhance acetate production using *T. kivui* by optimizing CO_2 concentration and controlling environmental factors such as pH and temperature. However, current research primarily focuses on growth and bioreactor optimization, leaving genetic engineering as a promising yet underexplored avenue. Therefore, this study aims to increase acetate production by leveraging genetic engineering tools. The second phase of this study will focus on producing methyl ketone. *Pseudomonas putida* is chosen for its high adaptability to chemical and physical stress, making it preferable for heterologous expression. Gene manipulation and metabolic engineering are commonly employed to fit the process for targeted product production. *P. putida* has been used to generate many polymers and aromatic compounds, such as degradable plastics, demonstrating its potential as a host for lubricant production. To enhance the success of lubricant production, the metabolic pathway of acetate metabolism will be studied to identify the appropriate genes for engineering.

Advancing a Circular Carbon Economy Through Synthetic Biology and Global Collaboration

Geoffrey Otim

Australian Institute for Bioengineering and Nanotechnology, Brisbane, Australia

SynBio Africa, Kampala, Uganda

Focus Area: Gas Fermentation

Favorite Organisms: *Escherichia coli, Saccharomyces cerevisiae*

Geoffrey Otim is a life scientist, synthetic biology leader, and PhD researcher at the University of Queensland, driven by a passion for sustainable biomanufacturing, biosecurity, and global health. His current research focuses on engineering microbial strains and optimizing gas fermentation processes for the bioconversion of syngas and industrial waste gases (CO_2, CO, H_2) into sustainable aviation fuel (SAF) and valuable biochemicals. This work directly supports the development of a circular carbon economy and aligns with the Biosustainability Hub's mission to enable economically viable transitions to net-zero for industries worldwide.

Geoffrey brings over a decade of diverse scientific and policy experience to the field. He previously served for eight years at the WHO Polio and Measles Regional Reference Laboratory in Uganda, contributing to disease surveillance, outbreak response, and vaccine-preventable disease research. His MSc work at Université Paris Cité focused on cryptic microbial secondary metabolite (natural product) discovery, and at the National University of Singapore's SynCTI, he worked on gut microbiome engineering for therapeutic application.

As a recognized pioneer of synthetic biology in Africa, Geoffrey founded East Africa's first iGEM team and established SynBio Africa, where he organized the continent's first international synthetic biology and biosecurity conference. He also led SynBio Africa Global Catastrophic Biological Risks Initiative and has represented the continent at many global science and policy platforms, including the OECD Summit, EBRC Global Forum, SynCell Global Summit, the UN Convention on Biodiversity, and the Global Health Security Conferences, among others. For his contributions, he was honored with the 2023 SynBioBeta Impact Award.

With interdisciplinary qualifications in life sciences, diplomacy, and health management, Geoffrey combines technical depth with strategic vision. His work embodies the Biosustainability Hub's vision of transitioning to a global bioeconomy, leveraging synthetic biology and innovation to address urgent climate and health challenges, especially within emerging economies.

David Ubi

University of Queensland, Brisbane, Australia

Mr. David Sam Ubi is a Ph.D. student at the Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland, Australia. He holds a Bachelor's degree (B.Sc) in Microbiology, and a Master's degree (M.Sc.) in Food and Industrial Microbiology, from the University of Calabar, Calabar, Nigeria, which he completed in 2016 and 2023 respectively. His academic achievements and research experiences during his undergraduate and postgraduate studies sparked his interest in pursuing a career in research and development.

David's research interests lie at the intersection of microbial production biotechnology: microbial bioactive metabolites (biosurfactants, biocatalysts, biotoxins, antibiotics, biofuels etc.,)- structure, function, engineering, production, and application in healthcare and the environment. His Ph.D. research focuses on gas fermentation, metabolic engineering, strain improvement, and (bio)process design for sustainable biofuel production, under the supervision of Prof., Esteban Marcellin and Dr., Lars Puiman.

David Sam has authored/co-authored several publications. He has received several awards and honours including Petroleum Technology Development Funds (PTDF) scholarship (for both B.Sc. and M.Sc. programs), The Chinese Government scholarship, and now the Australian Government Research Training Program scholarship. He is a member of the Nigerian Society for Microbiology (NSM), and American Society for Microbiology (ASM). As a Ph.D. student, he is committed to advancing knowledge and innovation in his field. He is passionate about interdisciplinary research and collaboration, and he believes that his research has the potential to make a positive impact on society.

The impact of anaerobic retention time on side-stream wastewater treatment processes

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Focus Area: Gas Fermentation, Computational Biology

Area of Expertise: Bioinformatics, Data Scientist, Machine Learning, Modelling, Systems Biology

Favorite Organisms: *Microbial Communities/ mixed cultures*

Nitrogen (N) and phosphorus (P) are essential nutrients in domestic wastewater that require removal to prevent eutrophication in receiving water bodies. The conventional anaerobic-anoxic-aerobic (A2O) process effectively removes N and P via nitrification by nitrifiers, denitrification by

denitrifiers, and P release/uptake by polyphosphate-accumulating organisms (PAOs). However, extreme weather events, such as heavy rainfall, dilute influent carbon substrates, impairing N and P removal and leading to poor effluent quality. To address this challenge, the side-stream enhanced biological phosphorus removal (S2EBPR) process has been developed. By relocating the anaerobic tank to a side stream that receives concentrated returned activated sludge (RAS) from the secondary settler with an extended hydraulic retention time (HRT) for fermentation, S2EBPR self-supplies carbon substrates to the mainstream, stabilizing biological nutrient removal (BNR) performance. Unlike the A2O process, which primarily supports conventional PAOs like *Accumulibacter*, S2EBPR favors fermentative PAOs, such as *Tetrasphaera*. These organisms not only remove P but also ferment complex molecules (e.g., glucose, amino acids) into volatile fatty acids (VFAs), enhancing substrate availability for conventional PAOs.

Although S2EBPR clearly offers potential bioprocess advantages, optimizing its operational parameters, particularly side-stream HRT, remains underexplored. Longer HRTs may improve fermentation and carbon substrates supply but increase construction and maintenance costs. Reported side-stream HRTs vary widely (1-60 hours), yet systematic studies comparing system performance and microbial dynamics (e.g., PAOs and glycogen-accumulating organisms, GAOs) are limited. In this study, an 8L S2EBPR reactor with a four-sub-cycle design was operated at side-stream HRTs of 24 hours (Stage 1), 16 hours (Stage 2), and 8 hours (Stage 3), using synthetic wastewater (COD:N:P = 500:40:10). Dissolved oxygen was maintained at 3-3.5 mg/L in the aerobic phase, and pH was controlled at 7.0-7.5 with automatic dosing of 0.5M NaOH and 0.1M HCl. Cycle studies were conducted in duplicate under each stable stage to evaluate P and N removal kinetics, while sludge samples from each stable stage were analysed using fluorescence-activated cell sorting (FACS), followed by quantitative fluorescence *in situ* hybridisation (FISH).

Results revealed consistent performance across all HRTs, with P and N removal efficiencies of ~97% and ~90%, respectively. Notably, nitrite accumulation was observed in the aerobic phases, peaking at 8 hours HRT (~70-80%, Stage 3), indicating potential synergy between shortcut N removal and S2EBPR, which would lower aeration costs and improve organic carbon efficiency. Sludge production was minimized at 16 hours side-stream HRT, reduced by 9.7% and 15.6% compared to 24 hours and 8 hours, respectively, which is the most critical operational cost in wastewater treatment plants (WWTPs). These findings provide critical insights for optimizing S2EBPR design, offering a balance of performance, cost-efficiency, and sludge management for industrial applications, particularly in the operation of WWTPs in regions with frequently changing weather conditions.

This research advances the Biosustainability Hub's vision of transitioning to a sustainable bioeconomy by enhancing the efficiency of biological nutrient removal in wastewater treatment, while supporting the mission of developing economically viable solutions through optimized operational parameters that reduce costs and sludge production for net-zero-aligned industrial applications.

The Effect of Temperature and pH on Sulphate Reducing Bacteria Performance using Microbial Entrapment Technology

Jonathan Humphries

The University of Queensland, Brisbane, Australia

Focus Area: Bioengineering

Area of Expertise: Bioprocess engineering/optimisation, Metallurgy

Favorite Organisms: *Microbial Communities/ mixed cultures*

Acid mine drainage (AMD) has damaged the environment and human life for as long as there has been mining. Various techniques have been developed to treat AMD with a focus on removing the toxic heavy metals present and reducing sulphates present in the waste by raising the pH. Traditional treatment techniques rely on alkali chemicals to neutralise the waste and precipitate metal hydroxides or either actively or passively remove metal waste. Biological treatment methods have been developed with sulphate reducing bacteria (SRB) reducing the sulphate in AMD to sulphide (S^{2-}) which then allows metal sulphide precipitation. SRBs are either immobilized or free floating with immobilised bacteria having higher productivity than free floating bacteria. Immobilisation of SRBs is a technique which has shown an increase in stability of the bacteria leading to greater amounts of heavy metal removal. The effect of parameters, such as pH and temperature, on the productivity of the immobilised beads is important to understand as this leads to increased understanding of the ideal operating conditions of the reactor and the relative bounds before destruction of the bacteria. In mobile bacterial sludge it has been found that temperature is proportional to bacterial activity. The ideal operating temperature for SRBs is between 25-35°C with temperatures outside this range lowering the activity of the bacteria. The ideal pH for SRB growth is above 6, ideally between 7-7.5, with pH values outside of this range lowering the activity of the bacteria. When SRBs are exposed to pH values around less than 6 activity decreases leading to less sulphate reduction. The study investigates whether immobilisation of these SRBs in immobilised beads would increase their resistance to pH and temperature changes leading to greater activity at a wider range of temperature and pH values allowing more opportunities for biotreatment of AMD.

This work contributes to the Biosustainability Hub's vision as it aims to allow treatment of mining waste biologically allowing a greener future through biological means. This adds to the vision of the Biosustainability Hub transitioning mining companies to a green bioeconomy.

The Impact of Organic Carbon Starvation on Conventional and Side-stream Wastewater Treatment Processes

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The University of Queensland, Brisbane, Australia

Focus Area: Bioengineering, Bioprocess Optimisation, Translational / bioeconomy research

Area of Expertise: EBPR wastewater treatment

Favorite Organisms: *Microbial Communities/ mixed cultures*

Biological nutrient removal (BNR) systems synergistically remove nitrogen (N) through nitrification by nitrifiers, denitrification by denitrifiers, and phosphorus (P) release/uptake by polyphosphate-accumulating organisms (PAOs). Organic carbon scarcity disrupts these processes by limiting electron donors for denitrification and impeding PAOs' metabolic demands. In real-world wastewater treatment plants (WWTPs), influent carbon variability — caused by seasonal dilution, industrial discharges, or sewerage dynamics — frequently triggers organic carbon starvation, destabilizing the enhanced biological phosphorus removal (EBPR) performance. Side-stream EBPR (S2EBPR) configurations address this challenge by diverting a sludge stream to an anaerobic fermenter, where endogenous VFAs are generated to supplement carbon-deficient mainstream inflow. Despite this innovation, the extent to which S2EBPR's carbon resilience depends on side-stream hydraulic retention time (HRT) remains unquantified. This study aims to investigate the impacts of organic carbon starvation on both conventional EBPR and S2EBPR systems with different side-stream HRTs. By analyzing microbial activity parameters, including phosphorus release/uptake rate and nitrification/denitrification dynamics, this research seeks to provide insights into system robustness under low-carbon disturbance. The findings will contribute to optimizing EBPR strategies for WWTPs facing carbon-limited influents, ensuring stable and efficient phosphorus removal.

In this study, three laboratory-scale sequencing batch reactors (SBRs) were operated with 8-L working volumes under the same condition. The first and second reactors were configured as S2EBPR systems operated at side-stream HRTs of 8 h and 16 h, respectively. The third reactor served as a conventional EBPR system, functioning as a baseline for comparative analysis of S2EBPR efficacy. All reactors followed an 8-hour cyclic operation, comprising four sequential sub-cycles (10%, 30%, 30%, and 30% of the total reaction time, summing to 6 h), followed by a 2-h settling, decanting and sludge exchange phase. Within each sub-cycle, an anoxic phase occupied 40% of the time, while the remaining 60% was allocated to the aerobic phase. The pH was maintained within 7.0–7.5 through automated dosing of 0.1 M HCl and 0.5 M NaOH, while the dissolved oxygen (DO) concentration in the aerobic phase was regulated at 3.0–3.5 mg/L via intermittent aeration. The reactors were fed with synthetic wastewater at a chemical oxygen demand (COD):N:P ratio of 500:40:10 to start up. To simulate carbon starvation, the COD:N:P ratio was reduced to 250:40:10 by reducing the concentration of carbon substrates by 50% while maintaining N and P levels. The reactors were operated at an SRT of 12 days, with performance evaluated across three phases:

1. Steady-state baseline (start-up and stabilise),
2. Carbon starvation (1 SRT),

3. Recovery (1 SRT).

To maintain data integrity and reliability, the phases 2 and 3 were duplicated. Mixed sludge samples were collected and filtered to investigate the change of P release/uptake and nitrification/denitrification rates during the cycle studies across the three phases.

This study supports the UQ Biosustainability Hub's vision by enhancing EBPR resilience under carbon-limited conditions, contributing to sustainable wastewater treatment. By optimizing bioprocesses for resource efficiency, it aligns with the Hub's mission to drive net-zero transitions through innovative, industry-focused biotechnological solutions.

The benefits of immobilized sulfate-reducing bacteria in mining-influenced water treatment

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Xinting, a second year PhD student at the University of Queensland in Brisbane Australia with a background in chemical engineering and focusing on the treatment of mining influenced water using biological processes. She has played a crucial role in designing and conducting experiments to evaluate the performance of microbial entrapment technology (MET) in synthetic mining wastewater in her prior research. Therefore, she provides great insight on experimental setups and will actively contribute to advancing technical development of MET bead performance testing in real-mining wastewater.

Mining activities often generate acid mine drainage (AMD), characterized by high acidity and heavy metal content. Sulphate-reducing bacteria (SRB) can treat AMD by reducing sulphate, with the resultant sulphide precipitating heavy metals, potentially aiding in the recovery of rare earth elements. However, SRB treatment faces challenges like microbial instability from process disturbances and toxic shocks, and generally requires a large process footprint if applied in a constructed wetlands. Entrapped SRB (ESRB) technology offers an alternative by entrapping microbes in a porous hydrogel matrix, promoting biomass growth and nutrient diffusion while maintaining a stable microenvironment. This application of ESRB can increase cell density, protect against inflow shocks, enhance performance stability, and offer cost savings in a bioreactor setup. To compare ESRB and non-entrapped SRB with respect to sulphate reduction performance and stability when exposed to process disturbances. The strength and lifespan of ESRB beads will also be assessed after application. The results will evaluate the potential for ESRB technology to add value to AMD treatment and metal recovery.

This study develops a method for sulfate removal from acid mine drainage (AMD) utilizing immobilized sulfate-reducing bacteria (SRB) in a hydrogel matrix. In long-term observation of two parallel sequencing batch reactors (SBR) – one with non-encapsulated SRB in a free cell suspension and the other with ESRB, it was found that ESRB exhibited superior sulfate removal efficiency with less organic carbon consumption required. The sulfate removal ability of ESRB showed significant improvement across six-months of reactor operation, achieving a high increase in sulfate removal rate at 1.29g SO 4/g cell/day compared to non-encapsulated SRB at 0.37g SO4/g cell/day. The ESRB bioreactor also showed greater stability, with reduced susceptibility to disturbances such as temperature fluctuations. Therefore, experimental results suggest that ESRB technology has the potential to offer an innovative solution for mining wastewater treatment and bioremediation of AMD affected lands. In addition to wastewater treatment, ESRBs have the potential for valuable recovery of rare earth metals, through sulfate reduction and metal precipitation. Through their metabolic processes, SRB converts sulfate into hydrogen sulfide (H2S) which subsequently reacts with metals, forming insoluble metal sulfides. The ESRB technology benefits mining corporations to align with their ESG goals, commitments and responsibilities towards the environment, capacity for increased recovery of rare earth elements and reduced risks associated with mining waste pollution. The integration of biological solutions to tackle AMD treatment and land remediation lowers costly chemical additions that also pose potential environmental consequences.

Other abstracts - Biologics

Abstracts

Aiden Beauglehole

Jose Alejandro Rodriguez-Siza

Thornwit Chavalparit

Veronica S Martinez

Xining (Stella) Yang

Zizhang Liu

Systems Biology To Understand Clonal Variation In Chinese Hamster Ovary Cells

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AIBN, Brisbane, Australia

Focus Area: Bioengineering, Bioprocess Optimisation, Computational Biology, Multi-omics Analysis, Precision Fermentation, Translational / bioeconomy research

Area of Expertise: Bioinformatics, Data Scientist, Bioprocess engineering/optimisation, Fermentation Services, Molecular Cloning, Omics - Metabolomics, Proteomics, etc., Recombinant Protein Production, Strain Engineering, Systems Biology

Favorite Organisms: *Chinese Hamster Ovary, Escherichia coli, Pichia pastoris*

Advances in biopharmaceutical production have underscored the importance of optimising recombinant protein expression processes to ensure supply and accessibility. Chinese Hamster Ovary (CHO) cells are widely used for producing recombinant Factor IX, an essential therapeutic for Haemophilia B. However, complex post-translational modifications required by Factor IX pose significant challenges, necessitating advanced strategies to enhance productivity and stability. Traditional methods involving random genomic integration result in considerable clonal variability, complicating both production and analytical interpretation.

In this study, we utilised cell line development technologies, including Beacon, CRISPR, and recombinase-mediated cassette exchange, to achieve site-specific transgene insertion aimed at reducing clonal variation. Adopting a systems biology approach integrating proteomics and transcriptomics, we examined eighteen CHO cell lines and confirmed extensive clonal heterogeneity. Despite this variability, applying statistical and integrative analyses, such as bootstrapping and Data Integration Analysis for Biomarker Discovery Using Latent components (DIABLO), enabled identification of specific proteomic and transcriptomic signatures associated with clonal variability.

Our findings provide crucial insights into the metabolic and molecular shifts within CHO cells during cell line development. This research underscores persistent challenges posed by clonal variation and suggests a shift in focus toward comprehensive biological modelling of cell line behaviour. Ultimately, this integrated systems biology framework offers promising directions for improving predictability, efficiency, and stability in CHO-based recombinant protein production, with broader implications for the biopharmaceutical industry.

Carbon Redirection Through Operational Parameters in a Monoclonal Antibody-Producing CHO Cell Line

Jose Alejandro Rodriguez-Siza[1], Verónica S. Martinez[2], Maria C. Molina[3], Claudia V. Altamirano[1,4]

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Focus Area: Bioprocess Optimisation, Multiomics Analysis, Precision Fermentation

Area of Expertise: Bioprocess engineering/optimisation, Molecular Cloning, Recombinant Protein Production

Favorite Organisms: *Chinese Hamster Ovary*

Carbon flux in mammalian cell cultures is critically important, as it influences both culture behavior and the quality attributes of biopharmaceuticals. In CHO cells, prolonged cultures exhibit reduced galactosylation index, leading to antibodies with immature glycosylation patterns. This is due to decreased carbon flux for the synthesis of nucleotide-sugar activated (NSA), particularly UDP-Gal. Dissolved oxygen (DO) is a key parameter affecting carbon flux and, consequently, antibody glycosylation.

This study, we investigated the metabolic behavior of cultures of an Antibody-producer CHO cell line, under varying DO conditions and compared itto cultures with attenuated phosphofructokinase (PFK) activity, a key enzyme in redirecting carbon toward NSA synthesis. We observed that low oxygen availability have a significant impacted on ammonium production but did not on specific glucose consumption. However, when cultures were exposed to phenylalanine, an indirect PFK inhibitor, specific glucose consumption doubled without affecting lactate or ammonium production. Suggesting that glucose was diverted to alternative pathways, such as the pentose phosphate pathway, for activated sugar synthesis.

In summary, carbon flux plays a pivotal role in glycosylation and culture performance, with PFK activity being critical modulators. These findings highlight the importance of optimizing culture conditions to maintain biopharmaceuticals quality.

Alternative Method for Recombinant Protein Expression: Monoclonal Antibodies Synthesis via mRNA Transient Transfection

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Focus Area: Bioengineering, Bioprocess Optimisation

Area of Expertise: Bioprocess engineering/optimisation, Recombinant Protein Production

Favorite Organisms: *Chinese Hamster Ovary*

Recombinant proteins have been widely utilized in biopharmaceutical research, therapies, and industry for decades. The most remarkable therapeutic, monoclonal antibodies (mAbs), has exhibited potential growth as market demand increased due to their application in highly specific targeting of detrimental pathogens in the human immune system. Recombinant mAbs are generally expressed in vitro with mammalian cell lines, such as Chinese hamster ovary (CHO) or Human Embryonic Kidney (HEK) 293. Consequently, the elevated demand has driven the need for process optimization to enhance both the quality and quantity of protein production, yet the process itself requires intensive labor and time consumption through cell line development. Thereby, in this research topic, we aimed to propose an alternative upstream recombinant protein expression platform using mRNA transient transfection, which offers high efficacy and rapid protein production through a general CHO-S wild-type (WT) cell line.

We studied the temporal dynamics of protein expression using enhanced green fluorescent protein (eGFP) by transiently transfecting mRNA encoding eGFP sequence into CHO-S WT. Shortly, green fluorescence was detectable within 15 minutes post-transfection, emphasizing the rapidity of the proposed novel method. For the next phase, we co-transfected Bevacizumab (Avastin) heavy and light chain mRNAs and observed protein levels extracellularly and intracellularly with surface plasmon resonance (SPR, Biacore™) and mass spectrometry, respectively. Although intracellular Avastin was detected rapidly, secretion was found at the earliest 12 hours post-transfection, suggesting a vital period for the recombinant protein to be expressed through intracellular processing. Additionally, intracellular Avastin was found to peak at 12 hours before gradually decreasing, contrary to accumulated extracellular Avastin, which rose and plateaued after 4 days. Avastin quality was preliminarily measured via western blots. The supernatant harvested on the 4th day suggested a full combination of heavy and light chain subunits with noticeably higher protein purity in completely formed Avastin compared to the continuous production from either plasmid DNA transient transfection or a stable cell line. Overall, this study illuminated an alternative path for modern biopharmaceutical manufacturing, elucidating the crucial timelines of mAb formation. This knowledge is essential for enhancing future mRNA-based protein expression systems, especially in applications that demand precise regulation of protein production and timing.

Designing cell factories for difficult to express proteins

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Focus Area: Bioengineering, Bioprocess Optimisation, Computational Biology, Multiomics Analysis

Area of Expertise: Bioprocess engineering/optimisation, Modelling, Molecular Cloning, Strain Engineering, Omics - Metabolomics, Proteomics, etc., Recombinant Protein Production, Systems Biology

Favorite Organisms: *Chinese Hamster Ovary*

Mammalian cell-based bio-production is the key technology underpinning the Biotechnology Industry. Efficiency of this process is a major contributor to the type and scale of the facilities required as well as the overall cost to produce bulk drug substance. The industry is now transitioning from monoclonal antibodies toward novel recombinant proteins, such as fusion proteins and multi-specific antibodies. While industry can produce monoclonal antibodies at high production titres and process yields, this remains a major challenge for many other engineered proteins which are often characterized as “difficult-to-express”. Lower yields create significant manufacturing complexity and drive increased capital and manufacturing costs. Here we developed a novel workflow based on innovative automated systems to elucidate the features of a robust cell expression system for difficult-to-express proteins, leading to higher yields, therefore decreased production costs. The workflow consists of four steps: (1) generation of a cell library with overexpression of genes that could lead to better yield of difficult-to-express proteins, (2) generation of producer cell lines, (3) selection of good producer cell lines, and (4) reverse engineering to identify the proteins that improved production. We utilized the Beacon® Optofluidic System, which has the capacity to screen hundreds of cell lines. Initially, we tested the workflow using a small library of genetically modified cells, aiming to increase the production yield of a bispecific antibody. Resulting in a 30-fold increase in the bispecific antibody titre, and the factors driving the increased productivity were analysed by proteomics.

This works aims to generate cell lines that are able to generate higher titters of difficult to express proteins, resulting in the reduction on the size of the reactor needed to produce the same amount of proteins. Therefore, less consumables will be required, including water and energy and less waste will be produced.

Comparative Evaluation of Random vs. Site-Specific Integration Strategies in CHO Cells for Stable Therapeutic Protein Production

Xining (Stella) Yang

The University of Queensland, Brisbane, Australia

The production of therapeutic proteins using Chinese Hamster Ovary (CHO) cells is central to the modern biopharmaceutical industry. However, conventional cell line development using random genomic integration often results in variable productivity and limited long-term stability, creating inefficiencies in biomanufacturing workflows. To address this, our project compares the productivity and genetic stability of Bispecific Antibody producer CHO cells generated via random integration and site-specific integration using recombinase-mediated cassette exchange (RMCE). We evaluated stability and yield across multiple generations (Gen20, Gen40, Gen60, Gen80) using fed-batch culture, fluorescence tracking, and ddPCR-based gene copy number analysis. Initial results suggest that while random integration provides higher early expression levels, site-specific integration offers enhanced long-term stability and consistency in gene expression.

This work aligns with the Biosustainability Hub's mission by exploring scalable, stable, and more predictable expression systems for biologics manufacturing, reducing waste and batch failure risk. Improving expression reliability in CHO cells contributes directly to economically viable, sustainable bioproduction pipelines.

This research supports the Hub's vision by laying groundwork for efficient biomanufacturing processes that reduce resource consumption and improve product quality—key requirements for transitioning towards a global bioeconomy

Enhancing BsAb productivity by overexpression of key genes in CHO cells

Zizhang Liu

Brisbane, Brisbane, Australia

Focus Area: Bioengineering

Area of Expertise: Molecular Cloning, Recombinant Protein Production, Strain Engineering

Favorite Organisms: *Chinese Hamster Ovary, Escherichia coli*

Bispecific antibodies (BsAbs) represent a significant advancement in biopharmaceuticals, enabling enhanced therapeutic efficacy by simultaneously targeting two distinct antigens. However, their production in Chinese Hamster Ovary (CHO) cells face challenges such as low yield, which compromise manufacturing scalability and cost-effectiveness. This study aims to enhance BsAb productivity by overexpressing key molecular chaperones and metabolic regulators to improve CHO cell performance.

We constructed and validated expression plasmids using Gibson Assembly and restriction enzyme digestion, followed by colony PCR, sequencing, and miniprep/midiprep purification. These plasmids were integrated into CHO-H7 cells using Recombinase-Mediated Cassette Exchange (RMCE), a cell line that was specifically designed for precise gene integration, containing two landing pads, expressing either GFP and mCherry, each with a different recombinase recognition sites. Each CHO-H7 cell was engineered to overexpress a single target gene, allowing a systematic evaluation of individual molecular chaperones and metabolic regulators on BsAb production. The GFP cassette was replaced with the genes of interest (GOIs) to enhance cellular function, while the mCherry cassette will be replaced with the bispecific antibody (BsAb) gene, ensuring stable and controlled therapeutic protein expression. Aiming to enhance protein folding, alleviate endoplasmic reticulum (ER) stress, and optimize lipid metabolism, thereby improving antibody secretion. Future experiments will evaluate transgene expression and protein yield to assess the impact of gene overexpression on BsAb production.

This study aligns with the Biosustainability Hub's mission by advancing economically viable biological solutions for sustainable biomanufacturing. Enhancing CHO cell efficiency enables resource-efficient and scalable therapeutic protein production, minimizing energy use, costs, and overall environmental impact.

Conference Closing Remarks

You've networked, you've presented, you've caffeinated—
Mission accomplished.

This week we've built more bridges than plasmids—
and that's saying something. Let's keep those collaborations thriving!

May our microbes be productive, our promoters be strong,
our pathways be anything but linear,
our yields ever exponential,
and our funding always renewable.

Here's to transforming waste into wonder,
and building a truly circular, sustainable bioeconomy—
because even our science knows the world can't go in a straight line forever.

Sincerely,
UQ Biosustainability Hub

End of Program Book