Book of abstracts

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Abstracts Plenary Lectures

Sustainability: An Innovation Engine

Diana Visser (Corbion)

Abstract:

In today's dynamic world, sustainability has become a defining force shaping businesses globally. Corporations are recognizing the urgency to align their strategies with the United Nations' Sustainable Development Goals (SDGs) to address global challenges. In this keynote lecture, we delve into the journey of Corbion, a leading sustainable ingredient solutions provider, that aligned its strategy with SDGs 2 (Zero Hunger), 3 (Good Health and Well-being), and 12 (Responsible Consumption and Production) while using a science-based approach for target setting.

By embracing sustainability as a driver for innovation, Corbion has established its Incubator, a dedicated hub for nurturing and accelerating transformative ideas aligned with the SDGs and the company's core competencies.

The success stories that have emerged from Corbion's Incubator serve as compelling examples of sustainable innovation. Corbion has developed PLA bioplastics, a circular technology for lactic acid production, and omega-3 derived from algae. These groundbreaking achievements underscore the potential of sustainability-driven innovation to shape a brighter future for our planet.

During this keynote lecture, Diana Visser, VP Sustainability at Corbion will elaborate on the strategies that enabled the company's alignment with the SDGs and on it's science-based approach to target setting. Furthermore, Diana will share the methods employed by Corbion to ensure that every innovation fostered within its Incubator aligns seamlessly with the overarching sustainability goals.

Pitch: BiotechBooster

Nettie Buitelaar
Biotech Booster

Abstract:
Lost in translation: our country is highly recognized as a top producer of biotechnology science, and patents as well. As soon as we look at the number of products reaching the market, this view changes. So how can we make biotechnology work in our country? A recent visit to Boston by the Dutch life science sector demonstrated the importance of a vibrant, closely-knit ecosystem. Furthermore, the National Growth Fund has awarded the Biotech Booster program. This program offers mentorship, financial support and networking opportunities, to guide founders from the idea stage to developing an investable/commercial proposition. Biotech Booster is an excellent opportunity for scientists and entrepreneurs in the biotech sector to valorize their ideas and grow a successful business. The ultimate goal is to make sure that the benefits of biotechnology will have more and faster impact on society.

iGem team TU Eindhoven

Designing a modular and personalized autoimmune cell therapy for ANCA-associated vasculitis

Worldwide 400 million people suffer from autoimmune diseases, of which 1 million are affected by the inflammatory disorder ANCA-associated vasculitis (AAV). Current therapies against AAV are based on unspecific immunosuppressive drugs causing burdensome side effects, high healthcare expenditures and workloads, while not excluding disease relapses. To overcome these shortcomings, we designed !MPACT: a Modular and Personalized Autoimmune Cell Therapy. The design was made together with relevant stakeholders, a proof-of-concept was developed in the laboratory, and a kinetic model was built. We engineered mammalian cells that detect the autoantibodies (ANCAs) and subsequently produce the anti-inflammatory cytokine interleukin-10, which results in suppression of the autoimmune response. The activity of !MPACT depends on the ANCA concentration, hence, it adjusts to the disease activity, leading to fewer side effects than current therapies, preventing relapses, and reducing healthcare expenditures and workloads. The modularity of the used technology offers great potential to treat many autoimmune diseases.

Industrial biotechnology: a key pillar in the transition towards the bioeconomy?

Olivier Rolland

Abstract
Considering global warming and its consequences, Mankind is engaged into a radical transformation, involving the transitioning from a petro-based towards a bio-based economy. This implies a shift of paradigm related to the access and availability of carbon and the subsequent value chains. In this context, the recent emergence of synthetic biology opened up new avenues for the development of bio-sourced products and put industrial biotechnologies (known forever) in the forefront as a promising way to accelerate such transition. From the large-scale deployment of ethanol production in the 20th century to the "bio-revolution" that is expected to happen in the next decades, we will explore through concrete industrial examples (plastics, sustainable aviation fuels,…) the potential of industrial biotechnology to actually deliver a commercial product and share the learnings from those product developments. We will also investigate how recent and to-come breakthroughs may foster this new value chain development and attempt to foresee which risks might impede it.

Making (fungal) biology easier to engineer

Jean-Paul Meijnen

Biology – as kids we are obsessed with it. Plants, bugs, dinosaurs! But then we are told to grow up. But why? Biology is amazing. It grows itself, it repairs itself, it's alive. And now it is programmable. The past two decades have seen technological developments in the fields of genomics, transcriptomics, proteomics and metabolomics. And the past year, AI had its big breakthrough and also entered the field of biotechnology. At Ginkgo Bioworks, we are leveraging all these technologies to build a platform to make biology easier to engineer. Our research teams are using advanced high-throughput technology like genome editing, smart biological screens and lab automation to solve the global challenges we are facing today. And we combine that with experience from a large bacterial and yeast codebase, unique fungal host strains and a state-of-the-art foundry instrumentation. In this century, the most important thing to program isn't computers, it's DNA. So ask yourself: what could a world be, if you could grow anything?

Abstracts for Oral Presentation

How uniQure's modular platform approach drives continuous innovation in AAV gene therapy development and manufacturing

Mathieu Streefland, Erich Ehlert, Pierre Caloz

uniQure's BEVS platform for AAV manufacture has recently been approved for commercial supply in the US and EU. Having such an approved platform is a great asset. However, the process and analytical technology used is inherently several years old, since processes and methods typically don't evolve much after clinical Phase 3 supply.

In order to ensure that the platform can keep evolving by inclusion of state-of-the-art technology or increase regulatory requirements, uniQure has developed a modular approach towards its manufacturing platform.

The gene therapy field is rapidly maturing. As a result, we see more advanced technologies becoming available specifically for AAV products. We also see regulators raising the bar, for instance on characterization of full and empty capsids. This requires an approach that is flexible and allows rapid introduction across subsequent pipeline products, but also for subsequent stages of development within existing programs.

This talk will provide an overview of uniQure's platform approach for continuous innovation and gives examples of how new technologies are introduced and implemented in pipeline products.

Cell Therapy Scale-up: Bioprocess Development for the Production of Hematopoietic Cells

Brenda Juarez Garza[1], Eszter Varga[2], Tom van Arragon[3], Marcel Ottens[1], Emile van den Akker[2], and Marieke Klijn[1]

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Abstract

Blood transfusion is currently the most common cell therapy applied worldwide. According to the World Health Organization (WHO), roughly 118.5 million blood donations are collected globally every year for various medical purposes [1]. One of the purposes is the treatment of severe anemias, as these diseases can only be treated with blood transfusions and stem cell transplantations. However, these therapies come with risks involved due to donor-derived transfusion products, such as immune reactions and the transfer of blood-borne diseases [2]. An attractive therapy that could overcome these risks is *in vitro*-produced red blood cells (RBCs) or the corresponding stem cells, namely hematopoietic stem cells (HSCs) [3].

In vitro RBCs can be produced from induced pluripotent stem cells (iPSCs), which are pluripotent cells, comparable to embryonic stem cells, created by reprogramming human somatic cells. iPSCs have the potential to produce patient-specific progenitor or functional effector cells [4]. This reprogramming technique started a new era in regenerative medicine due to the self-renewing properties and multilineage differentiation potential of iPSCs [5]. Furthermore, the availability of patient-derived hematopoietic cells allows the study of erythropoiesis (i.e., RBC production) at the molecular and cellular level to develop new therapies to treat a broad range of hereditary anemias.

However, hematopoietic cells and RBCs from iPSC are mostly produced via multiple differentiation steps using static adherent protocols which hinders scalability towards clinically relevant volumes. A dynamic shake flask cultivation was developed to produce hematopoietic stem/progenitor cells (HSPCs), which can further be differentiated into functional RBCs. The dynamic cultivation led to a ~16,000 fold increase in cell number compared to static cultivation. This project aims to transfer the developed protocol to a suspended stirred-tank bioreactor-based process to produce HSPCs from iPSCs. Bioreactors allow tight process control based on online measurements and higher mass transfer rates compared to adherent cultures and shake flasks. Employing bioreactors will improve reproducibility and facilitate upscaling towards the desired volumes, as mini-transfusions (10^{11} RBC, required in phase I trial) could be generated with 3 to 4 three-litre bioreactors. The main challenges to be optimized are specific nutrient and oxygen requirements for each differentiation step, as well as shear stress effects.

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CyclomicsSeq: Advancing genomics through high-quality DNA sequencing

Dàmi Rebergen[1], Alessio Marcozzi[1], Jeroen de Ridder[1, 2] and Wigard Klooserman[1]

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Abstract

The field of cancer genomics is witnessing a paradigm shift with the advent of CyclomicsSeq, a novel technology designed to revolutionize cancer follow-up procedures. CyclomicsSeq harnesses the power of nanopore sequencing to detect and quantify circulating tumor DNA (ctDNA), offering a highly sensitive, non-invasive method for monitoring cancer evolution and response to treatment.

A unique feature of CyclomicsSeq is its ability to analyze cfDNA-derived PCR amplicons with unprecedented depth. This is achieved through a two-step process: the circularization of ctDNA fragments, followed by rolling circle amplification. The result is tandem repeats of the original ctDNA sequence, which significantly enhances the detection sensitivity of low-frequency variants.

The high-depth analysis of cfDNA-derived PCR amplicons allows for the tracking of specific mutations over time, providing real-time insights into tumor evolution. This capability is particularly valuable in the context of personalized medicine, as it enables the timely adjustment of treatment strategies based on the patient's unique genetic profile.

CyclomicsSeq's compatibility with Oxford Nanopore's long-read sequencing platforms further extends its utility. It allows for the detection of complex genetic alterations, including structural variants and fusion genes, which are often overlooked by conventional short-read sequencing technologies.

The application of CyclomicsSeq in cancer follow-up has the potential to transform patient care. By providing a non-invasive method for monitoring tumor dynamics, it reduces the need for invasive biopsies, improving patient comfort and compliance.

In conclusion, CyclomicsSeq represents a significant advancement in cancer genomics, offering a powerful tool for high-depth analysis of cfDNA-derived PCR amplicons in cancer follow-up. We look forward to discussing its potential and sharing our latest findings at the Netherlands Biotechnology Congress.

The application of enzymes in the valorization of sugar beet pulp

Harry W.C. Raaijmakers, Adeline Ranoux, Wouter J.J. Huijgen [1], Marco W. Fraaije, Alessandro Boverio[2] Nikola Lončar, Hugo L. van Beek, Simone Savino [3]

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Abstract

Sugar beet pulp is a major residual stream from the sugar beet industry, which is currently valorized as low value feed and/or green gas. In Europe sugar beet pulp accounts for a production volume of approx. 13 million tons per year. Sugar beet pulp consists mainly of cell wall polysaccharides. There is a trend towards biobased and more sustainable products as replacement of oil based products. The valorization of sugar beet pulp can meet these demands. A multi-product biorefinery processes for the production of micro cellulosic fibers, pectin, D-galacturonic acid and derivatives thereof has been developed. These products can be used in non-food applications a.o. as rheology modifiers, mild surfactants, anti-corrosion agents, complexing agents and chemical building blocks. The use of enzymes in the biorefinery of sugar beet pulp and its further conversions to functional biobased molecules will be presented. In particular the use of pectinases and cellulases for sugar beet pulp hydrolysis and the enzymatic conversion of D-galacturonic acid to meso-galactaric acid will be presented.

Production of infant food ingredients by precision fermentation

Evelien Vaessen, PhD[1], Edwin van der Pol, PhD[1] and Wouter Kuit, PhD[1]

[1] *FrieslandCampina Ingredients, Amersfoort, the Netherlands*

Abstract

Current developments in infant nutrition ingredients include human milk oligosaccharides and bioactive proteins. Human milk contains many specific ingredients that play an important role in the development of the immune system, microbiota development and other health functions in a newborn (Bode, 2015; Lönnerdal, 2016). Nowadays biotechnology can play a major role in producing ingredients that are specific for human milk and not, or only limited, present in cow's milk. FrieslandCampina Ingredients uses precision fermentation techniques for producing specific carbohydrates and bioactive proteins. The carbohydrates are so called human milk oligossacharides (HMOs) such as 2'-fucosyllactose (2'-FL), lacto-*N*-tetraose (LNT) and 3'-sialyllactose (3'-SL). These HMOs are produced during an aerobic fermentation process with a modified *E. coli* K12 strain. For example for 2'-FL, a fucosyltransferase is transformed into the *E. coli* strain in order to produce 2'-fucosyllactose using lactose and GDP-fucose (which is produced from glucose) as substrates. Upon having a suitable strain, the fermentation process is optimized to maximize 2'-FL production. Challenges in the full scale fermentation process include heat production and oxygen distribution in the large scale fermenter. Process implementation and subsequent optimization involves testing strain robustness against a range of fermentation settings in small scale bioreactors. After the fermentation process, the HMO product is purified during a DSP-process. Bringing these ingredients to the market does not only involve the production process, but also requires registration procedures in different countries or regions. All in all, the market for infant nutrition ingredients produced by precision fermentation is growing and is expected to further increase in the coming years.

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Transparently forward: Clarified fermented tomato plant stem juice as a biostimulant in greenhouses

Laura Claret Fernández[1], Chiel van Amstel, Puk Baas, Carlien Verberne-van de Laak[1] , Julie de Groot[2], Richèle Wind[1]

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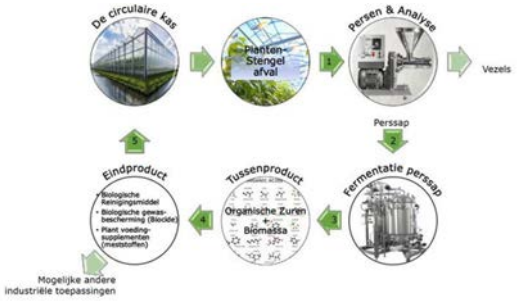
Abstract

Dutch greenhouse vegetable cultivation has shown an enormous increase in scale over the past 20 years, which has also largely increased the waste produced. The sector annually produces 300,000 tons of vegetable residual streams which have to be disposed of at cost. Since the Netherlands strives to become completely circular by 2050, upcycling side streams would offer both an economical and environmental advantage to the sector. As the largest crop, Dutch greenhouse tomato cultivation produces about 92,000 tons of side streams, including 72,000 tons of tomato stem and leaf waste as residual waste (1). The stems and leaves can be pressed, obtaining both fibers, which already have an application in packaging, and plant juice, for which application is largely unexplored. Due to its composition, the tomato plant juice could be used as a biostimulant in greenhouse cultivation, replacing chemical fertilizers and reducing costs. In this study, two different batches of tomato stem plant juice were obtained at different times (September and December 2022). In order to process the plant juice into a biostimulant, the material underwent a wild fermentation, which changed the composition of the juice. The leftover material that could not be utilized at the moment was frozen, thawed and fermented at a later time. A comparison between the frozen and fresh material was performed, observing that frozen material had lost part of its carbon sources. Samples of both raw and fermented plant juice were used in a tomato seedling growth experiment. Tomato plants grew as well with the plant juice as they did with a commercial fertilizer, showing that the material can be used as a biostimulant in greenhouse vegetable cultivation. Biostimulants stimulate, independently of the nutrient content of the product, the efficiency of nutrient use, tolerance against abiotic stress and/or crop quality (2,3,4). In order to apply the plant juice as a biostimulant, it would need to be diluted in water and supplied through the greenhouse irrigation system. However, the plant juice is turbid with plant material and microorganisms, which would easily clog the farming irrigation systems. Therefore, the plant juice should be clarified for its application. Different clarification procedures were applied, and it was discovered that pH changes, centrifugation and filtration are the most effective. This study has contributed to the future circularity of our greenhouses, allowing for plant waste material to be upcycled into a biostimulant after simple transformations that can be carried out on location, reducing both waste and costs for our farmers and our greenhouse system.



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Process intensification using continuous extractive fermentation technology FAST

Kirsten Steinbusch[1], Arjan Oudshoorn[1] and Pieter Nelisse[1]

[1] *DAB.bio, the Netherlands*

Abstract

Cost-effective biomanufacturing of large-volume chemicals would be the ultimate realization of biotech's full potential to decarbonise. With that end in mind, DAB.bio developed the next-generation bioreactor technology to run continuous and intensified processes in steady-state, where the product is concentrated an separated from the water phase by continuous extraction and in situ product removal, and DSP is reduced to a well proven and cost-effective extractant recovery process. Through a development journey of 15 years, DAB.bio's FAST technology now operates successfully at 500 l demo-scale, with stellar cost reductions proven on two presented products among 2-phenyl ethanol. The roadmap to full-scale deployment of this robust technology and the corresponding cost levels demonstrate that cost-effective biomanufacturing of large-volume compounds is within reach. An integrated collaborative approach is needed, driven by the advances of biotech and the need to abandon fossil feedstocks in the traditional chemicals industry.

References

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Acknowledgement

J.M. Daran of TU Delft, Biobase Europe Pilot Plant

Model-driven design of *Yarrowia lipolytica* as a cell factory for improved microbial oil production

Zeynep Efsun Duman-Özdamar[1-2], Jeroen Hugenholtz[3], Vitor A.P, Martins dos Santos[1], Maria Suarez-Diez[2]

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Abstract

Consumption of plant-based oils, especially palm oil, is increasing at an alarming rate. Fatty acids and oils derived from palm trees are used in food, feed and cosmetics. *Yarrowia lipolytica* is an oleaginous yeast with great potential as a sustainable alternative to palm as it can accumulate lipids around 40 % of its biomass under nitrogen-limiting conditions. Additionally, *Y. lipolytica* is non-pathogenic and regarded as food-grade yeast, thus its oil can be used for food-related applications. Due to these advantages, this oleaginous yeast is flagged as an attractive microbial-cell factory to sustain a bio-based circular economy for industrial implementation. However, replacing palm oil with microbial oil is not feasible yet and it is strongly dependent on the lipid accumulation capacity of the microbe. Therefore, obtaining enhanced lipid productivity via a systematic approach is essential. Herein we intertwined the analysis on the constrained-based genome scale metabolic model (GEM) of *Y. lipolytica* (iYali4, v4.1.2)[2] with the experimental validations on shake flask level to establish *Y. lipolytica* as a sustainable fatty acid production platform. In the design step, we performed an analysis on the GEM and evaluated the flux distributions for each reaction by simulating maximum production, maximum growth, and slow growth as a control. The analysis highlighted reactions from the glycolysis pathway, pentose phosphate pathway, fatty acid synthesis/elongation pathway, and amino acid metabolism. Among the overexpression targets key reactions for lipid synthesis such as pyruvate dehydrogenase and acetyl-CoA carboxylase providing acetyl-CoA and malonyl-CoA respectively were predicted. Furthermore, methionine synthase and glutamate synthase from amino acid metabolism was suggested as overexpression targets. Subsequently, the predictions from our analysis and from previous research on *Y. lipolytica* GEM model were experimentally validated through fractional factorial experimental design by supplementing amino acids (methionine, threonine, leucine, glutamate) into nitrogen-limited cultivation medium (C/N140) at shake flask level. While these additions did not alter the lipid accumulation, total lipid production was increased significantly for the addition of single and double amino acids due to higher biomass concentration. Among all tested conditions, methionine addition provided the utmost improvement, which is around 65%, in biomass concentration, total lipid, $Y_{X/S}$, and $Y_{P/S}$. Additionally, supplemented amino acids resulted in a shift from polyunsaturated fatty acids to monounsaturated fatty acids. Amino acid metabolism has been reported as a potential regulatory mechanism on the lipid synthesis pathway of *Y. lipolytica* in N-limiting environment. Efforts have been made to elucidate this mechanism via transcriptome analysis and simulations of flux distributions on GEM models. In this study, many genes related to this pathway were enriched and experimental validations resulted in improvement especially carbon utilization ability and total produced lipid. Moreover, we demonstrated that supplementing different types of amino acids can steer the fatty acid composition of *Y. lipolytica*.

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A Roadmap to lipids from light: Improving lipid production in the microalga *Nannochloropsis* through Genome-scale Metabolic Modeling

Sabine van Oossanen[1,2], Sarah D'Adamo [1], Maria Suarez-Diez[2], Vitor A.P. Martins Dos Santos[1], Maria J. Barbosa[1]

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Microalgae show great potential for replacing fossil fuels in the near future. Microalgae harness energy from light, which they use to fixate captured CO_2 into valuable biochemicals. However, strains and technologies must be improved in order to reach an economically feasible bulk production. As of recent, genetic engineering tools exist to improve industrial microalgal strains by metabolic engineering[1–3]. Still, limited understanding of the metabolism complicates selection of impactful genetic targets. Our research focusses on developing and testing a novel genome-scale constraint-based metabolic model (GEM) for the oleaginous microalga *Nannochloropsis oceanica*. Metabolic modeling is key to understanding carbon partitioning mechanisms underlying growth and lipid accumulation phenotypes. Moreover, the GEM provides insights for strain engineering to further advance *Nannochloropsis* as photosynthetic cell factory.

We constructed the first GEM of *N. oceanica* in an orthology-based approach, using well-curated scaffold GEMs, recent genomic data of *N. oceanica*[4] and physiological data such as biochemical compositions, growth rates and gas exchange rates to train and curate the GEM. Through flux sampling of the novel GEM in high lipid, high growth and low growth phenotypes, we predicted enzymatic targets for overexpression that result in lipid accumulation while ensuring growth.

From the predicted targets, we selected two enzymes involved in the lipid metabolism. Of these, overexpression mutants have been generated using a novel gene expression system[2]. The mutant phenotypes were characterized to validate the GEM predictions in nutrient replete and deplete conditions. Current targets show an initial increased lipid production rates (g/gDW/d) of 149% and 123% compared to wildtype, in nutrient replete conditions.

This study allowed for the selection of novel targets involved in lipid accumulation and increased the understanding on lipid metabolism, in *N. oceanica* using metabolic modeling. Ongoing investigation focusses on GEM refinement and further exploitation.

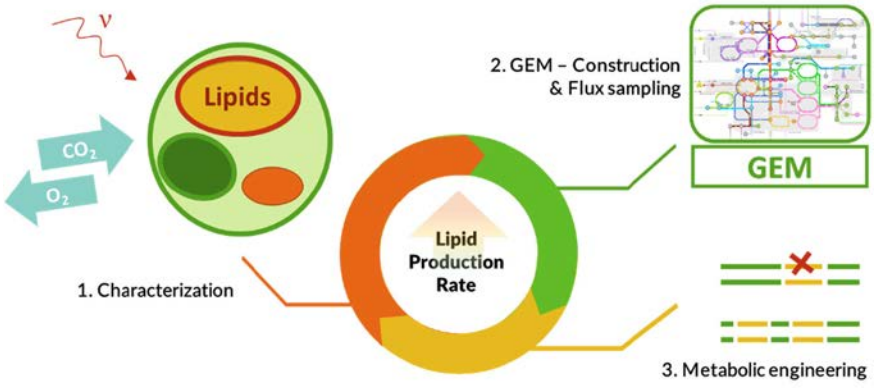

Illustration: Model-based approach for improving photosynthetic carbon fixation to lipids.

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Inclusion and sustainability in a data-centric bioeconomy

Lotte Asveld
TU Delft

Abstract
Data-driven biobased production has great potential to contribute to a sustainable society. Inclusive innovation is an important condition to realise this potential, in order to increase societal acceptance and to ensure that benefits of biotechnological products are distributed fairly and effectively. In this talk I will explore what inclusive innovation can imply in current datacentric bioeconomy by zooming in on two elements of the bioeconomy: value chains and use of data.

Value chains refer to the feedstocks for products such as biofuels or biochemicals that are often derived from agricultural residues. Including producers of these feedstocks in early stages of developing the production process can reduce uncertainties about efficient production and about sustainability impacts. Additionally, sharing benefits can increase security of supply.

With regard to the use of data; this refers to the wealth of genetic information that is contained in existing biodiversity that can be unlocked for the use of innovative, biotechnological applications. This unlocking becomes more and more accessible because of ever cheaper sequencing technologies that give rise to rich and novel datasets. How this data is subsequently applied and by whom can have considerable impacts on biodiversity. I will argue that researchers and companies that use digital sequence information have a responsibility to invest in equal distribution of access and benefit sharing deriving from genetic resources, in order to protect biodiversity and stimulate sustainable use of innovative biotechnologies.

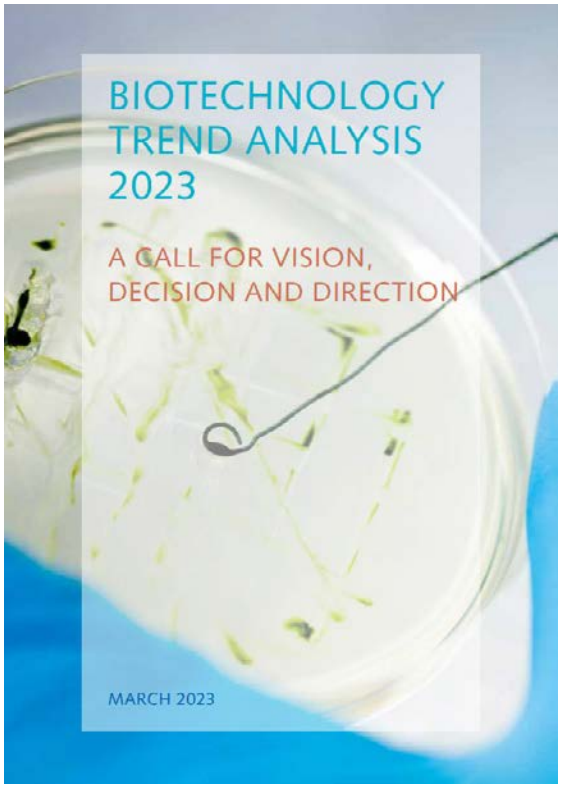
Biotechnology Trend Analysis 2023: A call for vision, decision and direction

Virgil Rerimassie[1]

[1] *Health Council of the Netherlands*

Abstract

The Netherlands is ill prepared for the rapid developments taking place in biotechnology and is missing out on opportunities to make full use of its economic and social potentials. But as well as major opportunities, biotechnology also brings risks to people and the environment. And for that reason, the government should prepare an integrated vision and carry it through. This is the central message of the Biotechnology Trend Analysis 2023 (https://cogem.net/app/uploads/2023/03/Biotechnology_Trend_Analysis_2023.pdf), presented to the Dutch Government by the Commission on Genetic Modification the Health Council of the Netherlands. During the NBC Virgil Rerimassie, part of the writing staff of the Trend Analysis, will present the report and its core findings and recommendations.



Acknowledgement: The members of the project committee of the Trend Analysis 2023 are: Marianne de Visser (Chair), Martina Cornel, Susana Chuva de Sousa Lopes, Ellen Moors, Jack Pronk and Paul Struik. Frank van der Wilk (COGEM) and Harrie van Dijk (Gezondheidsraad) were part of the writing staff.

Landfill gas becomes sustainable plastic

Nardy Kip[1], Bram Visscher[1] and Richèle Wind[1]

[1] *HAN University; Netherlands*

Abstract

Our project aims to address methane emissions from a source not yet used for biobased or circular purposes, namely landfill gas released from waste landfills. We are investigating whether methane-containing landfill gas, which would normally be emitted into the atmosphere, can be used for the production of bioplastics. This project is in collaboration with waste-management companies such as Afvalzorg and Sweco. We use methanotrophic bacteria to convert methane into a biopolymer, polyhydroxybutyrate (PHB), which can be used as a raw material for bioplastics. This bioplastics are biologically degradable and perfect to substitute current non-sustainable and not degradable single use-plastics. We have shown on a laboratory scale that methanotrophs can grow on landfill gas and that they can produce PHB. Production still needs to be optimized and scaled up to achieve good biofilter design.

In this way we recycle carbon, reduce methane emissions and reduce global plastic pollution and contribute to a circular waste to value system.

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Selecting next-generation biocatalysts by complementing recoded bacteria

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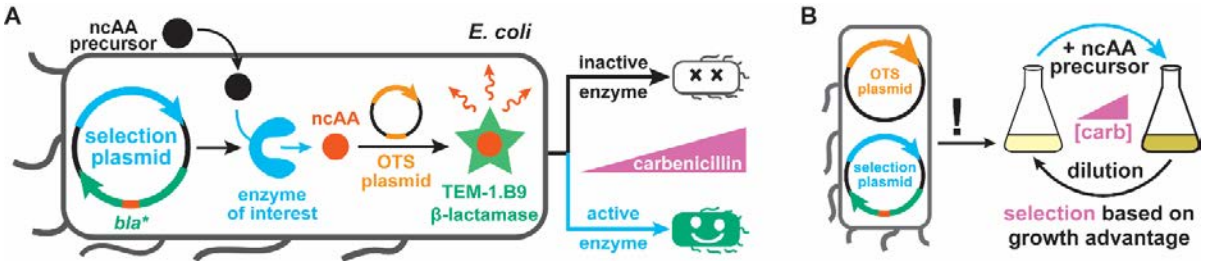
Abstract

Assaying enzymatic activities often proves a critical bottleneck for the directed evolution of useful biocatalysts. Commonly-employed, multi-well screens that evaluate enzyme variants one-by-one are laborious and slow when compared to nature's approach to select improved variants from large populations through adaptation to a selection pressure. To apply analogous selections for the directed evolution of industrially-useful biocatalysts, I will present an in vivo directed evolution platform that leverages recoded organisms addicted to non-canonical amino acids (ncAAs) to evolve biocatalysts that can provide these building blocks from synthetic precursors.[1] Repurposing recoded organisms to link enzymatic activities to bacterial proliferation requires the introduction of three readily-available genetic components into Escherichia coli (see Figure 1A): (1) an enzyme able to convert an appropriate precursor to a ncAA (=input); (2) an orthogonal translation system that enables the site-selective incorporation of this ncAA (=sensor); and (3) a β-lactamase featuring an in-frame stop-codon, whose activity to degrade the hydrolytically-stable penicillin derivative, carbenicillin, is strictly dependent on the incorporation of the same ncAA. Critically, in our selection platform, the growth rates of bacteria in presence of carbenicillin and the synthetic precursor correlate with the activities of the enzyme they produce. As such, improved biocatalysts are readily identified by subjecting bacteria harboring vast enzyme libraries to continuous growth-dilution cycles in presence of increasing carbenicillin concentrations (=selection pressure, Figure 1B). In my talk, I will showcase how our selection platform can be employe for the directed evolution of hydrolases as well as biocatalysts that catalyze C-H activation or C-C-bond-forming reactions. Lastly, I will discuss how our platform that requires minimal human intervention and no specialized equipment will enable the autonomous exploration of many evolutionary trajectories in a continuous fashion.



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Flavonoid production in engineered *Penicillium rubens*

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Abstract

Flavonoids are a diverse group of plant secondary metabolites with various health benefits for humans. Flavonoids typically occur as complex mixtures of similar compounds that are difficult to separate, which limits their availability as nutraceuticals or pharmaceuticals. Targeted, bottom-up biosynthesis in microbial hosts such as *Escherichia coli* and *Saccharomyces cerevisiae* has been shown to be an interesting alternative source for simple flavonoids such as naringenin. However in both organisms the crucial substrate malonyl-CoA has been seen to be yield limiting. Its production is strictly regulated and is directly routed into fatty acid biosynthesis. Therefore, extensive metabolic engineering is necessary to overcome this bottleneck. Since industrial strains of *Penicillium rubens* have been optimized to accumulate secondary metabolites such as the peptide penicillin and the polyketide lovastatin, we decided to explore its capacity to produce flavonoids from cinnamic acids.

Here, we present our engineering strategy to convert the secondary metabolite deficient *P. rubens* strain, *P. rubens* 4KO, into a microbial cell factory for flavonoids. By integrating two plant enzymes into the genome of this strain, we achieved a high naringenin titer in flask fermentations 36 h after substrate feeding. The high substrate conversion rate of 80% suggests that the availability of malonyl-CoA in this *P. rubens* strain is good without further manipulation. However, we also observed rapid degradation of naringenin over the course of the fermentation. Based on high-resolution mass spectrometric analysis, we propose a naringenin degradation pathway that is markedly different from previously reported flavonoid conversions in fungi. Further investigation and manipulation of this degradation pathway will pave the way to more efficient and sustainable production of flavonoids in *P. rubens* and may enable the valorization of plant biomass with engineered *P. rubens* to generate aromatic building blocks for the chemical industry.

Investigation of ethylene glycol metabolism in *Paracoccus denitrificans*

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Abstract

Plastics are produced daily in ever-increasing amounts, leading to a significant challenge in plastic waste degradation, recycling, and upcycling. Since the discovery of a PET-degrading bacterium in 2016[1], microbiological studies exploring environmentally friendly degradation approaches for polyethylene terephthalate (PET) have hugely increased. However, there is a notable research gap concerning the downstream processes of PET degradation, especially the degradation of the monomer ethylene glycol (EG). Also given the widespread usage of EG as an antifreeze agent, the understanding of its natural assimilation pathways by microorganisms and potential environmental impact remains limited. Our study elucidates an efficient metabolic pathway for EG assimilation in the soil bacterium *Paracoccus denitrificans*. In comparison to previously identified PQQ (pyrroloquinoline quinone)-dependent EG dehydrogenases in *Escherichia coli*[2] and *Pseudomonas putida*[3], EG dehydrogenase in *P. denitrificans* functions as a NAD (nicotinamide adenine dinucleotide)-dependent dehydrogenase. The cofactor NAD offers many advantages due to its ubiquitous presence and involvement in diverse cellular processes. The EG dehydrogenase and glycolaldehyde dehydrogenase were successfully purified and characterized in this study, as well as a transcriptional regulator that controls the EG assimilation pathway in *P. denitrificans*. We conducted *in vitro* enzyme kinetic measurements with six different alcohol and aldehyde substrates. Additionally, we performed cell extract enzyme assays using *P. denitrificans* cultures grown exclusively on EG to measure enzyme activities during growth. Our findings reveal that EG dehydrogenase exhibits the highest affinity for EG among the known NAD-dependent alcohol dehydrogenases. Furthermore, we identified the first EG-binding transcriptional regulator, which could be applied as a biosensor in the future. This study contributes to the understanding of the potential upcycling of PET by involving highly efficient EG-converting enzymes.

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Engineering Bacillus without genetic engineering

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Abstract

Genetic engineering of microbes offers a straightforward method to improve biotechnology processes based on a priory knowledge of the underlying pathways. In addition, adaptive laboratory evolution offers an auxiliary approach for strain improvement. In my talk, I will present our efforts to enhance plant colonization by *Bacilli* using experimental evolution [1-4]. These experiments at single isolate- and population-level revealed which genetic pathways are important for efficient plant root colonization and therefore provide a new list of genetic markers that can be either used for specific genetic engineering or used for screening of isolate libraries. Additionally, adaptive laboratory evolution generates strains without genetic engineering that allows application of the evolved isolates in agricultural settings for plant growth promotion.

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A synthetic co-culture approach for the conversion of carbon monoxide to bioplastics

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Abstract

Polyhydroxybutyrate (PHB) has been proposed as a potential biobased alternative to petrochemical plastics since the 1960s. However, the high cost of substrates required for its production, mainly sugars, has limited large scale implementation. Recently, the superior biodegradability of PHB compared to other bio-plastics has reignited the interest in this compound. C1 substrates have been proposed as a cheap alternative substrate for PHB production. One C1 source of particular interest is carbon monoxide (CO). This gas can be derived from gasification of wastes, or as an off-gas produced during various industrial processes.

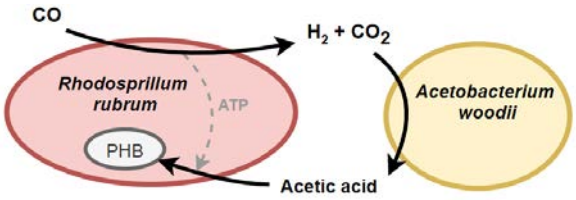

Figure 1, schematic overview of the co-culture.

In this work we describe a novel method for the anaerobic production of PHB from CO as sole substrate, using a co-culture of *Rhodospirillum rubrum* and *Acetobacterium woodii*. *R. rubrum* is a known PHB producer and has the ability to harness the energy derived from CO oxidation via the biological water-gas-shift. Meanwhile, the by-products of the water-gas-shift metabolism, namely H_2 and CO_2, are directly consumed by *A. woodii* through the Wood-Ljungdahl pathway, resulting in the production of acetic acid. The acetic acid produced by *A. woodii* circulates back to *R. rubrum*, which uses it as organic carbon source to produce PHB (driven by the energy generated during CO oxidation, figure 1). To unlock the potential of the co-culture, three cultivation strategies were tested. While continuous cultivation resulted in unstable growth and only temporary PHB accumulation, batch and fed-batch cultivations were successful in anaerobically converting CO into PHB in a single reactor vessel. During fed-batch mode, a PHB production rate of 58 ± 11 $mg.L^{-1}.day^{-1}$ was achieved with a final PHB content of 38 ± 5% of dry weight. A mono-culture of *R. rubrum* was not able to produce PHB when fed solely with CO, showing the significance of the co-cultivation approach.

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

Unlocking the potential of synthetic co-cultures: Advancing sustainable biotechnology with in-line Raman spectroscopy

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Abstract

Fuelled by the pressing need to address its environmental impact, the utilisation of CO_2 for the sustainable production of building-block chemicals such as ethanol has become a rapidly expanding research area. Recent years have witnessed the emergence of numerous CO_2 conversion pathways, highlighting the growing interest and active exploration in this research area [1]. However, producing complex products from simple feedstock such as CO_2, is pushing the limits of current biotechnological capabilities. The prevailing approach, genetic engineering of a single species to facilitate the desired conversions, may impose metabolic burdens which lead to restricted productivity and reduced economic viability. A promising solution lies in employing synthetic co-cultures, where the chain of biochemical conversion from substrate to product is split between two or more defined species. This alternative approach balances the metabolic burden, optimizing productivity and enhancing the economic potential of these sustainable processes [2].

Nonetheless, broad application of synthetic co-cultures is hindered by challenges such as the lack of a natural population stability. To obtain and control population stability in a bioreactor, advanced analytical technology is needed. In-line Raman spectroscopy, a real-time and continuous monitoring analytical technique, offers potential solutions to monitor and control population instability. Raman spectroscopy holds particular advantages over other analytical methods due to its non-invasiveness, the ability to quantify multiple relevant culture parameters in parallel, minimal interference from water, lack of sample preparation, and obviates the need for manual handling. Although successful cultivation control has been demonstrated for monocultures, there are limited examples in literature where Raman spectroscopy-based synthetic co-culture population monitoring and control has been reported [3].

This work explores the application of in-line Raman spectroscopy as a monitoring and control tool for a defined co-culture (*S. cerevisiae* and *L. cremoris*) to open avenues for the development of novel bioprocesses. The goal is to obtain correlation models that require a single signal to identify each population individually without fluorescent tagging, manual sampling, or sample manipulation. These models are a stepping stone towards automated control strategies to maintain constant population compositions. As industry is increasingly shifting from traditional petrochemical processes to more sustainable biochemical alternatives, synthetic co-culture-based bioprocesses hold to potential to drive sustainability efforts and contribute to the transition towards a carbon-neutral society.

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Abstracts for Poster Presentation

Cultivated Meat Production: debottlenecking scale-up challenges

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Abstract

Cultivated meat is animal meat produced by culture of animal cells in vitro. In recent years, it has been gaining attention for its potential to alleviate the environmental impact, food security, human health, and animal welfare problems associated with industrial farming.

To make cultivated meat, animal stem cells are first isolated from the animal and used to create cell banks. To initiate the production process, cells are first multiplied in a seed train and then transferred to an agitated bioreactor for proliferation in suspension. Once the target cell density has been achieved, cells are differentiated into either muscle or fat tissue.

To produce cultivated meat at a competitive price, a large-scale process with short processing times and high cell densities is needed. Processing times are mostly impacted by the time it takes for a stem cell to become fully differentiated, which can take several weeks. The opti-ox™ technology allows for more efficient and faster differentiation.

In this work, stem cells with integrated opti-ox™ technology were cultured in a bench-top reactor working in perfusion. Cell densities and metabolites were monitored during the process. Cell densities of 80 million cells/ mL were achieved in 8 days.

Achieving high productivities is crucial to reducing the volume and/or number of large-scale reactors, alleviating the capital investment required at a scale of hundred tons per year. This milestone represents a step forward in the quest to achieve a scalable and cost-effective cultivated meat process.

Unlocking the Power of Haplotype-Based Molecular Breeding using Long Read DNA Sequencing

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2. Presenting author

KeyGene®, the leading plant research company, specializes in developing cutting-edge technology for crop improvement. The availability of high-quality, contiguous reference genomes is essential for highly effective and innovative marker development and crop trait-relevant gene discovery . KeyGene's innovation power is driven in part by early adoption of new technologies such as long read sequencing (e.g. Oxford Nanopore Technologies). KeyGene's on-going efforts to improve this technology together with development of proprietary genome assembly software generated genome sequences of unprecedented quality and contiguity for a variety of crops. However, whole genome sequencing of large populations is still not cost effective, especially when studying large and complex genomes. Therefore, KeyGene has developed and applied innovations to reduce genome complexity in a random as well as targeted manner. These technologies are genome and context agnostic, can be tuned in relation to genome complexity and can therefore be broadly applied. Because these long read sequencing applications are based on native DNA, they can comprehensively detect variation, from single nucleotide polymorphisms to structural variation and base modifications in the context of genetic linkage. This opens the possibility to perform more precise haplotype-based molecular breeding contributing to crop improvement, also amenable for large and complex genomes. Examples of discovery and detection of random and targeted haplotypes in polyploid and large genomes will be shown. In this context, the most recent Oxford Nanopore Technologies platform innovations will be presented, with which we demonstrate haplotype-base genotyping in a breeding population and germplasm samples.

From Stress to Survival: Insights into the Adaptation and Proliferation of Wall-deficient *Escherichia coli*

Marjolein Crooijmans

The cell wall is a fundamental structure that protects bacterial cells from external stress factors and environmental changes. However after exposure to chemical or enzymatic cell wall targeting agents, certain bacteria are capable of shedding their cell wall and proliferating in osmoprotective conditions. This ability may have important implications for their survival in industrial environments, medical applications, but also for the development of antibiotic resistance. The mechanisms underlying the proliferation of Gram-negative wall-less bacteria remain poorly understood. Recent studies suggest that the accumulation of oxidative damage can inhibit the proliferation of wall-deficient cells. This can be overcome through mutations that reduce reactive oxygen species production and increase membrane blebbing.

Our research has established that the lack of a cell wall can have both positive and negative effects on the growth and survival of *E. coli* under conditions caused by antibiotics. We engineered an *E. coli* strain that can proliferate with or without a wall in order to further investigate this extraordinary growth mechanism. This strain enabled us to study the membranes of Gram-negative bacteria, the transition between walled and wall-less state, and the various types of stress involved in becoming L-forms. Notably, certain pathways related to the wall, such as peptidoglycan biosynthesis and biofilm formation, were found to be upregulated. These findings provide insight into the mechanism of cell growth without a wall.

Engineering *Streptomyces peucetius* for diversification of anthracycline production

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Anthracyclines are natural products with antiproliferative activities that are produced by Actinobacteria. The anthracycline doxorubicin is widely applied as anticancer drug, but its use is restricted by severe side effects. Recently, a potent doxorubicin derivative with reduced side effects was synthesized. This compound, *N*,*N*-dimethyldoxorubicin, contains a dimethylated amino group on the sugar moiety (*L*-rhodosamine). The aim of this study is to engineer the doxorubicin producer *Streptomyces peucetius* for production of *N,N*-dimethylated anthracyclines. Rhodosamine occurs in other natural anthracyclines, such as aclarubicin and rhodomycin. Several genes from the aclarubicin and rhodomycin biosynthetic gene clusters were introduced to *S. peucetius*, which resulted in the successful incorporation of rhodosamine and production of *N,N*-dimethyldaunorubicin. A major challenge in this pathway are the tailoring reactions required to obtain the doxorubicin backbone. Additionally, product toxicity limits high productivity, and we therefore make steps to improve drug tolerance of the recombinant production host. Therefore, a model *Streptomyces* strain was cultivated with increasing concentrations of *N*,*N*-dimethyldoxorubicin. The evolved strain was studied by genome sequencing and transcriptomics to identify the resistance mechanism. The most recent data and results of the pathway engineering will be presented.

Optimizing Metagenomics: A Pipeline for Targeted and Agnostic Analysis with High Sensitivity and Specificity

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Abstract

Metagenomics, the genetic analysis of genomes within environmental samples, holds significant potential for microbiome studies and pathogen identification investigations. Ensuring high sensitivity and specificity in metagenomics pipelines is critical to avoid false positives and false negatives. This poster presentation outlines the development of a metagenomics pipeline designed for both targeted and agnostic analyses.

Several read classifiers including Kraken2, Kaiju, Centrifuge, and KMA were assessed during the pipeline's development. Kraken2 emerged as the top performer, demonstrating exceptional speed and memory efficiency, outpacing the least performing tool, Kaiju, by a factor of 13 in speed and 8 times less memory usage.

To mitigate false positives following classification, a thresholding approach was implemented. Results on a test dataset revealed a sensitivity of 100% and a specificity of 98.74%. For the agnostic approach, the pipeline was benchmarked against expectations established by a previous study, which reported sensitivities ranging from 83% to 100% and specificities of 90% to 99%. However, the pipeline's validation yielded a sensitivity of 73.3% and a specificity of 87.5% for the agnostic approach. Notably, an unfiltered assessment achieved 100% sensitivity. Conversely, the targeted approach harmonized notably well with expectations.

For the targeted approach, the pipeline's performance was evaluated against the Twist Comprehensive Viral Research panel, a validated virus identifier. In comparison with results from the Twist panel, which utilized the One Codex analysis pipeline, our metagenomics pipeline successfully detected all viruses. Excess false positives were further curtailed through additional filtering with the k-mer aligner KMA.

The culmination of this work is the generation of an interactive HTML report that empowers customers to intuitively interpret the findings. This metagenomics pipeline promises a robust and efficient tool for researchers aiming to conduct both targeted and agnostic analyses while minimizing false positives.

Acknowledgement

We would like to thank Prof. Dr. J. de Vries (Medical Microbiology; LUMC) for collaborating on this project.

A *Candida glabrata* CRISPRi platform to study the mode of action of novel antifungals

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Abstract

Pathogenic fungi pose a threat to human health, agriculture and wildlife as they kill an excess of 1.5 million people each year and cause 20% of worldwide perennial yield loses[1,2].

Due to their high reproduction rate and genome plasticity fungi evolve rapidly, and are able to acquire resistance to antifungals[3]. Fungal resistance against all major antifungals has been observed with resistant fungi spreading quickly due to globalization. To study the mode of action of novel antifungals a CRISPR interference library targeting all genes in the opportunistic pathogen *Candida glabrata* was created[4]. First a *C.glabrata* ATCC 2001 htl$^-$ genome assembly was created for our in house strain using nanopore and Ilumina data. Assembly showed 504 snp's and the deletion of three auxotrophic markers compared to reference strain *C.glabrata* ATCC 2001.

Using the newly created assembly a set of single guide RNAs targeting essential genes was designed in *C.glabrata.* sgRNAs were designed to study the effective targeting region and other parameters derived from literature.

gRNAs were ordered as oligos and transformed into a gRNA expressing vector through golden gate cloning. sgRNA vector pools were transformed into a set of competent *C.glabrata* cells carrying a MET3-dCAS9. To study the effect of each sgRNA competitive growth assays were performed in a chemostat setup. Samples were grown for 3 days with samples being taken every 24 hours. sgRNA populations were compared using amplicon sequencing. As all sgRNAs target essential genes relative fitness of strains carrying a particular guide indicates sgRNA efficacy. Through this principle new sgRNA design principles were derived. These principles were used to design a genome wide CRISPR-i library in *C.glabrata.*

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Evolution of the catalytic mechanism at the emergence of the Baeyer-Villiger Monooxygenases

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Abstract

BVMOs are class B flavoprotein monooxygenases that catalyze the Baeyer-Villiger oxidation of ketones to esters. These enzymes showcase a relatively complicated mechanism which requires a tightly bound FAD, an electron donor, dioxygen and the substrate[1,2]. Because of the high activity and selectivity on a broad range of substrates, they have attracted a lot attention for their potential to produce high-valued chemicals. To make them applicable in industry, many studies have been carried out to better understand the nature of this kind of enzymes. In this work, we have used a historical biochemistry approach to understand the key steps in the establishment of the catalytic mechanism during the course of evolution. We performed ancestral sequence reconstruction on a representative dataset form the whole BVMOs family and resurrected three ancestral BVMOs (A2.0 → A2.1 → A2.2) to disclose how the catalytic mechanism evolved and the activity of extant BVMOs was defined. Upon the experimental characterization of these ancestors, we found BVMOs evolved from a non-catalytic FAD-containing protein (A2.0). Later, A2.1 was found to be reduced by NADH and NADPH inefficiently, however still unable to perform BV oxidations. Then, the usage of the electron donor changed dramatically from NADH to NADPH in A2.2, resembling the preference of modern enzymes. At the same time, A2.2 started to accept some typical BVMO substrates and became a *bona fide*, albeit poorly efficient, BVMO. Finally, from A2.2 to extant BVMOs, all those properties were further optimized to give the efficient biocatalysts we found nowadays in nature. We also observed that the capacity of using dioxygen improved in a step by step manner. In conclusion, the multi-component catalytic mechanism of BVMOs was established gradually over time from a non-catalytic protein, by recruiting a hydride donor (NADPH), an acceptor (O_2) and specific substitutions in the active site.

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Maximizing biomass and lipid productivity in various microalgal species from the genera *Nannochloropsis* and *Microchloropsis*

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Abstract

Establishing an economically feasible microalgal lipid industry depends on selecting suitable and robust strains with the highest lipid content and productivity. *Nannochloropsis* and *Microchloropsis* are attractive oleaginous microalgae genera and are considered promising cell factories for lipid production due to their high biomass accumulation rate and lipid content. In microalgae, lipid accumulation is triggered by nitrogen starvation, with a consequent decrease in photosynthetic efficiency, thereby limiting growth and fatty acid production over time. In addition, light is an essential and indispensable environmental factor significantly affecting microalgae's photosynthetic and metabolic pathways. Nonetheless, the different species within these genera, even within the same genus, exhibit varied growth and lipid accumulation responses to nitrogen starvation and high light intensities. These studies are often done in different cultivation systems and conditions, making the comparison difficult.

For this reason, a comprehensive and systematic study was conducted to study the differences in growth and lipid accumulation of different oleaginous species within the same genus and between two different genera to be able to choose the best microalgal strain for lipid production. In this study, we investigated the growth response and lipid accumulation of 8 microalgal species from the genera *Nannochloropsis* (*N*) and *Microchloropsis* (*M*) (*Nannochloropsis oceanica* Necton, *Nannochloropsis oceanica* IMET1, *Nannochloropsis sp.* CCAP211/78, *Nannochloropsis oculata, Microchloropsis gaditana* CCFM-01, *Microchloropsis gaditana* CCMP526, *Microchloropsis salina* SAG40.85, and *Nannochloropsis limnetica*) to the combined effect of light stress and nitrogen availability.

All strains were cultivated under controlled laboratory conditions at two different light intensities; 150 µmol. m^{-2} s^{-1} (LL) and 600 µmol. m^{-2} s^{-1} (HL) under nitrogen (N) starvation and N-replete conditions; the latter was used as a control. The growth rates of microalgae were measured using optical density, cell number, and biomass concentration; the photosynthetic efficiency was monitored by quantum yield, while lipid accumulation was quantified through lipid content analysis.

Our data shows that, in the presence of nitrogen, increasing light intensity strongly increased the growth rate and biomass productivity of all strains; at the same time, it did not affect the total fatty acid (FA) content significantly. The opposite was observed without nitrogen; although the cell concentration increased under High Light, it was lower than half the concentration in repleted conditions. In addition, the combined effect of nitrogen starvation and high light stimulated the accumulation of a notable amount of lipids, mostly Palmitic acid C16 and Stearic acid C18, affecting EPA production negatively. The results showed apparent differences between species; *N. sp* had the maximum growth rate and biomass productivity (0.290 ± 0.016 g/l/d) under nitrogen-repleted conditions and the highest lipid content (59%DW) and productivity (0.069 g_{lipid}/l/d ± 0.004) in nitrogen-depleted conditions at 600 µmol m^{-2} s^{-1}. The highest EPA content was achieved by *M. gaditana* CCFM01 (4.69%DW) under 150 µmol m^{-2} s^{-1} and repleted nitrogen conditions, while *N. oceanica* IMET1 showed the lowest EPA content with 2.94 %DW under the same conditions. The freshwater *N. limnetica* could not produce EPA, but more than 50% of the total FA was Oleic acid (C18:1), and the highest content of this FA was obtained in N-depleted condition at HL with 21.16% DW. Moreover, the photosynthetic efficiency of the different species underwent a visible decline under nitrogen starvation and high light.

Ultimately, our comparative study shows that the physiological response to cultivation conditions can vary significantly between different oleaginous microalgae species, even within the same genus. This knowledge is essential for selecting species with optimal traits for developing efficient microalgal lipid production strategies for industrial exploitation.

Keywords

Microalgae, *Nannochloropsis, Microchloropsis*, Lipid, TAG, Nitrogen starvation, Light intensity

Biocatalytic Deprotection of Aryl Ethers, Esters and Secondary Amines

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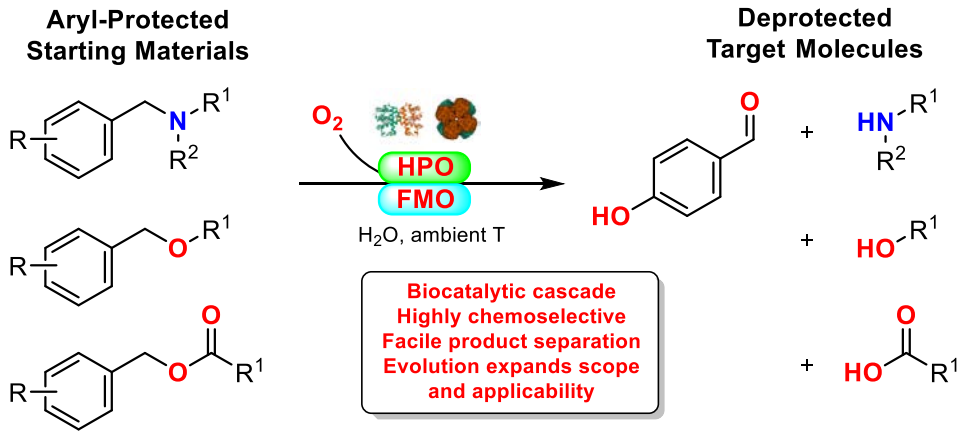
Abstract



Protecting groups (PGs) are of fundamental importance to synthetic organic chemistry.[1] The use of PGs, however, is strongly disfavoured by the chemical industry due to the inherent loss of process yield and efficiency. Aryl ethers are still reluctantly relied upon in industry,[2] due to their ease of addition and good chemical robustness. Their main drawback, however, is the stern reductive conditions required for their removal.[1] Several methods are available but none are green or very chemoselective, limiting applicability.

We aim to develop the first sustainable and efficient biocatalytic method for the deprotection of synthetically relevant aryl ethers. Our method exploits the oxygenation activity of a heme peroxygenase (HPOs) to arrive at *para*-phenolyl ethers from benzyl, *para*-methoxybenzyl (PMB), or other relevant ether starting materials.[3] These ethers are reactive substrates of a class of flavin-containing oxidases (FMOs), yielding *para*-OH benzaldehyde and deprotected target molecule, with consumption of H_2O and O_2 and release of H_2O_2. We obtain suitable biocatalyst partners for our cascade *via* library screening and subsequent directed evolution.

Our chemoselective deprotective cascade will improve reaction characteristics and sustainability of aryl ether/amine deprotection relative to current default chemicals methods. Simultaneously, synthetic applicability of aryl ethers in general will be expanded by rendering feasible the deprotection of molecules which are sensitive to hydrogenation.

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Shikimate pathway-dependent catabolism enables high-yield production of aromatics

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Abstract

Catabolism is a complex network of tightly regulated metabolic reactions that provides energy and biosynthetic precursors to all living organisms. Rewiring catabolism is essential for industrial biotechnology, but remains a great metabolic engineering challenge due to its high genetic stability acquired through evolution. In this study, by combining metabolic modeling, rational engineering, and adaptive laboratory evolution, we fundamentally redesigned bacterial catabolism for the sake of bioeconomy. We created a new-to-nature shikimate pathway-dependent catabolism (SDC) in *Pseudomonas putida* by reprogramming the shikimate pathway as the primary catabolic route. SDC supports growth by supplying the vast majority of the glycerol catabolic end-product pyruvate, enabling superior production of shikimate-derived molecules. Through SDC, aromatics production reached 89.2% of the pathway maximum theoretical yield, representing the highest ever yield for any aromatic molecule. Our study exemplifies the high metabolic plasticity of microbes, despite the immense catabolic regulation, and provides a bacterial *chassis* for the efficient production of high-added value compounds.

Silk fibroin production in tobacco for medical use

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ABSTRACT

Fibroin, the main component of silkworms silk, is a natural biocompatible and biodegradable material with exceptional mechanical properties. However, for the industrial exploitation of these proteins as base polymer materials for the production of biomedical products, the process of recovering fibroin from *Bombyx mori* silk has major drawbacks. From one side, it alters the proteins intrinsic physical and mechanical properties and results in an impure final product. From the other side, it involves killing large amounts of silk worms that poses animal welfare doubts especially for the cosmetic industry. Lastly, it results with the disposal of toxic and non-toxic waste in the environment that rises sustainability issues. Therefore, the necessity to develop a more sustainable production platform for recombinant vegan fibroin of improved quality.

The fact that fibroin is a large and repetitive protein –at both the nucleic acid and amino acid level- poses serious challenges to its recombinant production[1]. We propose an optimized plant-based expression system, as plants are flexible and versatile platforms for recombinant protein production and suited for the expression of large and highly repetitive constructs due to their genetic stability compared to microbes.

For the construction of the repetitive protein, we combine two strategies: on the DNA side, by generating a series of modular vectors to gradually assemble the fibroin gene based on the golden gate cloning procedure[2]; on the protein side, by exploiting an inteins mediated assembly[3] to ligate polypeptide fragments into larger fibroin proteins. The accumulation of fibroin will be tested in different subcellular compartments in tobacco.

Our results, so far, show the successful production of small fibroin polymers of 84kDa from tobacco plant leaves in various cell compartments. This leads to high potentials for the followed intein mediated assembly in order to obtain the full sized recombinant fibroin.

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Scale-up improvements of Photanol's photosynthetic glycolate production from lab to pilot

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Abstract

In the quest for sustainable and eco-friendly solutions to address the challenges posed by using fossil resources, Photanol operates at the intersection of biology, chemistry, and engineering, developing a groundbreaking technology that leverages the natural power of photosynthesis to produce valuable chemicals using engineered cyanobacteria. This enables the direct conversion of carbon dioxide and sunlight into valuable chemicals, offering an eco-friendly alternative to traditional petrochemical methods. At its core, Photanol employs synthetic biology techniques to reprogram cyanobacteria's metabolic pathways. By introducing specific enzymes and pathways, these microorganisms can be directed to produce a range of target chemicals, including biofuels, monomers for plastics, and specialty compounds. While this technology holds remarkable promise, challenges such as optimizing strains for efficiency, scaling up production, and ensuring economic viability must be addressed.

This abstract provides a summary of the recent developments for one of those challenges: scale up from lab to pilot to produce glycolate at kilogram-scale. Glycolate is currently mostly produced from fossil feedstocks, has a large potential for application in e.g., cosmetics and novel biodegradable plastics like poly-glycolic-lactic acid. For a successful scale up from lab (few liters) to pilot (hundreds of liters) a reliable inoculation train is of paramount importance. In this poster we will describe the main steps we took to improve the rate of our inoculation train by a factor 10 and bring the success rate to 100%. On top of that, the knowledge we gained in improving the inoculation train helped us to further improve the operational conditions of the pilot scale reactors. The improvements we made allowed us to make repeatable production batches of more than a month, building up product to kilogram scale. One of those batches was successfully used for validating the first steps in our downstream processing: successful conversion of glycolate to glycolic acid. The latter is a key step to get our product at the desired product quality.

The application of comammox bacteria in partial nitritation/anammox wastewater treatment systems

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Abstract

Nitrogen removal during tertiary treatment is an important step in wastewater treatment plants (WWTPs) to prevent eutrophication in receiving water bodies. Nitrogen is conventionally removed by the consecutive processes of nitrification and denitrification, but these processes are costly due to the requirement for intensive aeration and addition of carbon sources, respectively. An appealing alternative is combining partial oxidation of ammonia to nitrite (partial nitritation) by ammonia oxidizing bacteria with anaerobic ammonium oxidation (anammox), commonly referred to as PN/A. Benefits of PN/A are its hypoxic and fully autotrophic conditions, reducing oxygen demand and eliminating the need for externally supplied carbon. However, widespread application of PN/A currently faces challenges related to the successful suppression of nitrite oxidizing bacteria, the limited activity of anammox at ambient temperatures, and the final polishing of residual nitrogen compounds. Here, we explore the potential of the recently discovered complete ammonia oxidizing (comammox) bacteria to partially overcome these challenges. Both comammox and anammox bacteria were found to be present in the microbial community of a sequencing batch reactor (SBR) operated under hypoxic conditions and receiving ammonia, nitrite and nitrate via the medium. The removal of these nitrogen compounds was monitored through colorimetric assays, while the microbial community was characterized through metagenomic sequencing, 16S rRNA amplicon sequencing and fluorescence in-situ hybridization (FISH). A coculture of comammox and anammox bacteria was maintained in this SBR, resulting in the simultaneous removal of ammonia, nitrite, and nitrate. In parallel SBRs, nitrite was removed from the influent medium and/or additional oxygen was supplied, resulting in ammonia or nitrate accumulation, respectively, and substantial changes in the microbial community. The conversion mechanisms of ammonia, nitrite, and nitrate in these SBRs was studied using ^{15}N-labelled nitrogen compounds in batch incubations by measuring nitrogen gas production using gas chromatography-mass spectrometry measurements, and with membrane inlet mass spectrometry. Nitrogen isotope composition suggested nitrite production by comammox bacteria under oxygen limited conditions. This nitrite was subsequently consumed by anammox to oxidize ammonium. This potential to reduce nitrate was further investigated by pulsing $^{15}NO_3^-$ to an ammonia oxidizing pure culture of the comammox bacterium *Nitrospira inopinata* with simultaneous real-time determination of its oxygen consumption by a biological oxygen monitor (BOM). In summary, these findings indicate that comammox and anammox bacteria can coexist in oxygen-limited environments and potentially cooperate in the simultaneous removal of ammonia, nitrite, and nitrate. However, further enhancing the process in these SBRs towards full nitrogen removal and obtaining a deeper understanding of the mechanistic interaction between comammox and anammox bacteria is necessary for successful implementation in full-scale WWTPs.

Optimization of para-aminobenzoic acid production using Design of Experiments

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Abstract

Para aminobenzoic acid (PABA) is an aromatic compound used in the chemical industry for the production of dyes, plastics, fibers and pharmaceuticals (Averesch et al., 2016). The industrial production of this compound is petroleum-based having a great potential for biobased production (Kubota et al., 2016).

In this work, we explored the effect of different pathway genes expression levels over the production of para aminobenzoic acid using Design of Experiments. We focused on increasing the expression of the shikimate and para aminobenzoic acid pathway which form part of the main pathway enzymes for the production of this compound. Different factors including 7 shikimate and 2 para aminobenzoic pathway genes were considered. In addition, 2 levels (weak and strong gene expression) were assigned to each factor. This full factorial experiment would result in 512 strains to be constructed, which would be time-consuming and costly. Then, we decided to use a fractional factorial experiment which could allow us to explore the combinatorial space by constructing 15 strains in order to find optimal producers

We constructed 15 strains using a combinatorial design of genetic elements considering weak and strong promoters, Ribosome Binding Sites (RBS) and copy number of plasmids that regulate gene expression of the shikimate and para aminobenzoic biosynthetic genes in Pseudomonas putida. Without making any knockouts of native enzymes, the top strain from the first round of Design of Experiments reached approximately 2 mM (Fig. 2) in minimal media with glucose in 48h.

The value of a Designer in Bioprocess Engineering

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Abstract

Delft University of Technology offers Engineering Doctorate (EngD) programmes in Bioprocess Engineering, aiming at the translation of academic developments into real-life applications. These two-year salaried design traineeships offer an application-focused alternative to traditional PhD positions, and provide trainees with a solid basis for an accelerated start in an industrial career.

A very strong engineering background for the Designer in Bioprocess EngD programme is requested: mathematics, mass and heat transfer, and experience with modeling and simulation software tools.

In the first year of the programme, the EngD designers follow a dedicated curriculum involving relevant courses, interactive workshops, and group design assignments in close cooperation with industrial partners. In the second year, they perform their Individual Design Project, typically seconded within a company, working on real business cases covering from small start-ups to large multinational companies in the biobased, biopharmaceutical, food, cosmetics, bioplastics, and wastewater sectors, among others. In addition to technological topics, the participants acquire professional skills in areas such as stakeholder management, personal and project management, and communication.

Our industrial partners, on the other hand, benefit from fully supported collaborations delivering a fit-for-purpose (tailor-made) design project at a competitive cost, executed by one or more of our selected trainees under the supervision of TU Delft's principal investigators and experts, plus an experienced design coach. Examples of previous design assignments are:

- Downstream process development for a novel single cell protein production technology
- Raman-based chemometric model development for mammalian cell culture production
- Techno-economic feasibility of a chromatographic process to obtain a concentrated stream of antibodies from dairy products
- Evaluation of continuous manufacturing for enzyme production
- Continuous processing of food bio-chemicals: development of a CFD model for protein concentration via ultrafiltration
- Bio-based platform chemicals: sequential screening & selection of technical options

Besides promoting collaboration and fostering knowledge and technology transfer between academia and industry, our industrial partners additionally become part of the academic network of TU Delft and other TU Delft-wide initiatives, such as the Delft Process & Product Technology Institute and Delft Bioengineering Institute, furthering academic-industry initiatives for a sustainable future.

References

www.tudelft.nl/engd

Inheritable CRISPR based epigenetic modification in a fungus

Xiaoyi Chen, Juan P. Moran Torres, Yiling Li, Luis G. Lugones, Han A. B. Wösten*

ABSTRACT

The CRISPRoff system was recently introduced as a programmable epigenetic memory writer that can be used to silence genes in human cells. The system makes use of a dead Cas9 protein (dCas9) that is fused with the ZNF10 KRAB, Dnmt3A, and Dnmt3L protein domains. The DNA methylation resulting from the CRISPRoff system can be removed by the CRISPRon system that consists of dCas9 fused to the catalytic domain of Tet1. Here, the CRISPRoff and CRISPRon systems were applied for the first time in a fungus. The CRISPRoff system resulted in an inactivation up to 100% of the target genes *flbA* and *GFP* in *Aspergillus niger*. Phenotypes correlated with the degree of silencing in the transformants and were stable when going through a conidiation cycle, even when the CRISPRoff plasmid was removed from the *flbA* silenced strain. Introducing the CRISPRon system in a strain in which the CRISPRoff plasmid was removed fully reactivated *flbA* showing a phenotype similar to that of the wildtype. Together, the CRISPRoff and CRISPRon systems can be used to study gene function in *A. niger*.

Pre-steady state kinetic studies of glycosynthase uncover a general strategy to enhance activity

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Abstract

Glycosynthases are mutated glycoside hydrolases (GHs) in which the nucleophile is replaced with a small inert residue, usually glycine or alanine. In the presence of activated glycosyl fluorides of the opposite anomeric configuration to that of the original GH substrate, these enzymes can catalyze the formation of a glycosidic bond, thus producing oligo- and polysaccharides. This enzymatic synthesis of saccharides provides an attractive alternative to organic synthesis since it enables a complete control over the newly generated anomeric centers and allows the reaction to be carried out in aqueous environment under mild conditions. However, the structural elements that are required for improving the activity of glycosynthases remain mostly unclear.

In this study we characterized biochemically and structurally two glycosynthases: a GH52 β-xylosidase (XynB2s) and a GH10 xylanase (XT6s), both from *Geobacillus stearothermophilus*.

XynB2s catalyzes the condensation reaction of α-D-xylose fluoride, to produce α-D-xylobiose fluoride. By using directed evolution and rigorous kinetic analyses, an improved variant of XynB2s was isolated in which two crucial amino acid substitutions, F206L and T343P, led to a 100-fold increase in activity. Pre-steady state kinetics demonstrated unequivocally that product release is the rate-limiting step of the reaction. Based on these results we propose a general strategy to improve glycosynthases by introducing mutations that reduce the affinity of the enzyme to its synthesized product.

XT6s uses α-D-xylobiose fluoride to produce xylo- oligo- and polysaccharides. High resolution crystal structures of XT6s, with substrate and products, were obtained by soaking the crystals with α-D-xylobiose fluoride for short periods of time before freezing. These crystal structures allowed introducing rational mutations that reduce the affinity to the synthesized product and improve the turnover of the enzyme. In addition, mutations in the +3 binding site of the enzyme provided a variant which preferably produce short xylo-oligosaccharides.

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Withdrawn

Alcohols in high-throughput microdroplet screens – a cautionary tale

Authors: Tobias Fecker, Tom de Kanter, Jack Pronk and Rinke van Tatenhove-Pel

Biotechnological processes generate emissions, which can be reduced by selecting low-carbon footprint substrates. Microbial gas fermentation and electrobiocatalytic reduction enable the production of simple carbon molecules (e.g. C1 or C2-alcohols) from CO_2, yielding a low-emission alternative to conventional substrates like glucose.

Water-in-oil emulsions can be used for high-throughput screening of microbial cells. These pico-liter sized droplets are closed systems with a defined amount of substrate. They are separated by an oil phase, which allows for compartmentalization and screening of thousands of strains, for example for increased yield. This compartmentalization is crucial to preserve the conditions in each droplet.

So far, most emulsion studies have focused on glucose as a substrate as it does not transfer between microdroplets, preserving the cell and substrate compartmentalization. To expand the range of substrates to low-emission alternatives, we studied if simple alcohols could be used as a substrate in microdroplets studies. We tested their diffusion into fluorinated oil in simple biphasic systems and realistic microdroplet screening experiments. Contrary to the notion that a fluorinated oil phase should prevent organic molecules from transferring between droplets, we found that alcohols, and particularly ethanol, transferred between droplets and disrupted the substrate compartmentalization. Efforts to inhibit transfer were unsuccessful. Other substrates, like acetic acid, did not transfer and may present an alternative to alcohols in these setups.

Enabling simple carbon molecules as substrates in microdroplets would facilitate screening cells for new products and increased yield in low-emission biotechnology. However, it is crucial to assess if the chosen substrate complies with the strict criterium of non-transfer between microdroplets. Our findings demonstrate that simple alcohols can diffuse into the oil even without the presence of surfactant, hindering their use as substrates in microdroplet emulsion studies until new oils are engineered to prevent transfer. Other low-weight substrates will be more suitable.

Title: **High-throughput screening of microbial consortia using microdroplet sorting**

Authors: Sagarika B Govindaraju and Rinke J van Tatenhove-Pel

Defined microbial consortia are rarely used in industrial biotechnology. However, in nature, microorganisms co-exist and interact with each other, suggesting that cooperation provides a fitness advantage. By identifying beneficial microbial interactions in nature, we could design novel synthetic microbial consortia for industrial processes. We hypothesize that by using high-throughput screening and selection systems we can decipher interactions between microbes.

Water-in-oil emulsions are a promising high-throughput cultivation tool, as a milliliter of emulsion contains millions of microdroplets. Due to the absence of diffusion between microdroplets, each microdroplet acts as an individual cultivation system which can be inoculated by a single cell or combinations of cells. After inoculation, the cells grow and interact with each other. Beneficial interactions result in higher cell concentrations than competitive or inhibitory interactions. Using microdroplet sorting, we could identify consortia with beneficial interactions and selectively enrich for them.

Microdroplet cultivation coupled with sorting could drastically improve the throughput of screening compared to conventional cultivation systems such as shake-flasks and microtiter plates. For a 10-member consortium, to screen all possible combinations of consortium members, we would need 1023 individual cultivation systems. Using a few water-in-oil emulsions, we could in-parallel screen all 1023 combinations of a 10-member consortium and enrich for sub-consortia with beneficial interactions by sorting.

Before we can sort microdroplets with consortia of interest, we first need to develop a workflow to sort microdroplets using FACS. We inoculated microdroplets with a mixture of *Lactococcus cremoris* MG1363 (92.58%) and *L. cremoris* MG1363-GFP (7.42%). With 1 hour of sorting, we isolated 59 cells of interest from a starting population of $2 \cdot 10^6$ cells. Using FACS sorting, we enriched *L. cremoris* MG1363-GFP from 7.42% to 69.41%. This shows that, with our workflow, we can enrich for populations of interest that are present in low fractions in the inoculum.

Title: Binding of micro-nutrients to the cell wall of the fungus *Schizophyllum commune*

Fleur Kleijburg

Abstract

The cell wall fulfils several functions in the biology of fungi. For instance, it provides mechanical strength, interacts with the (a)biotic environment, and acts as a molecular sieve. Recently, it was shown that proteins and β-glucans in the cell wall of *Schizophyllum commune* bind Cu^{2+}. We here show that the cell wall of this mushroom forming fungus also binds other (micro-)nutrients. Ca^{2+}, Mg^{2+}, Mn^{2+}, NO_3^-, PO_4^{3-}, and SO_4^{2-} bound at levels > 1 mg per gram dry weight cell wall, while binding of BO_3^-, Cu^{2+}, Zn^{2+} and MoO_4^{2-} was lower. Sorption of Ca^{2+}, Mn^{2+}, Zn^{2+} and PO_4^{3-} was promoted at alkaline pH. These compounds as well as BO_3^{3-}, Cu^{2+}, Mg^{2+}, NO_3^-, and SO_4^{2-} that had bound at pH 4, 6, or 8 could be released from the cell wall at pH 4 with a maximum efficiency of 46-93 %. Solid-state NMR spectroscopy showed that the metals had the same binding sites as Cu^{2+} when a low concentration of this ion is used. Moreover, data indicate that anions bind to the cell wall as well as to the metal ions. Together, it is shown that the cell wall of *S. commune* binds various (micro-)nutrients. This binding may be used as a storage mechanism or may reduce availability of these molecules to competitors or prevent toxic influx in the cytoplasm. Moreover, it makes *S. commune* cell walls suitable for bioremediation of heavy metal or anion polluted environments.

Continuous extractive fermentation at scale improves productivity and economics of de-novo 2-phenylethanol production

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Abstract

Novel biomolecules are rapidly being introduced to the scientific world through innovations in synthetic biology in the shift to a more sustainable bioeconomy. However, product toxicity and related microbial inhibition phenomena frequently hamper the maximum product level that can be achieved, limiting productivity and economic attractiveness.

In situ product recovery (ISPR) is a means to overcome this limitation by removing the product from the fermentation broth. Conventional two-phase fermentations apply an organic overlay to withdraw and concentrate the product in the overlay. Although an improvement, the capacity of a batch overlay is intrinsically limited and the achieved extension of the production phase is often insufficient. To overcome this limitation, DAB.bio has developed a reactor concept to continuously add and remove organic overlay, eliminating the limitation of conventional batch overlay fermentations and enabling indefinite extension of production processes. This FAST™ bioreactor platform (Fermentation Accelerated by Separation Technology) integrates conventional (fed)-batch fermentation with continuous extraction and in-situ product removal in one reactor without the use of membranes or mechanical aids and allows precise control of the product concentration in the aqueous phase. This robust design is easily scalable to industrial scale and can be seamlessly integrated with existing down-stream processing, dramatically improving the productivity of bioprocesses.

We present the case study on a fed-batch process for de-novo production of 2-phenylethanol with Saccharomyces cerevisiae. The performance of a conventional process with batch overlay was compared with continuous overlay with ISPR in a 500L FAST™ reactor. As expected, continuous extraction with FAST™ extended the production phase and dramatically increased the volumetric productivity of 2-phenylethanol production. By decreasing the cost of bioprocesses, continuous extraction and product removal with FAST™ has enormous potential to accelerate market entry of specialty chemicals.

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CFSA: Comparative Flux Sampling Analysis as a guide for strain design

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Abstract

Metabolic engineering is often needed to obtain microbial cell factories with sufficient titer, rate and yield for industrial implementation. However, finding appropriate targets for genetic manipulation is often challenging. Genome-scale metabolic models of microbial metabolism have extensively been used to guide the design of microbial cell factories, still, many of the available strain design algorithms often fail to produce a reduced list of targets for improved performance that can be implemented and validated *in vivo* in a step-wise manner.

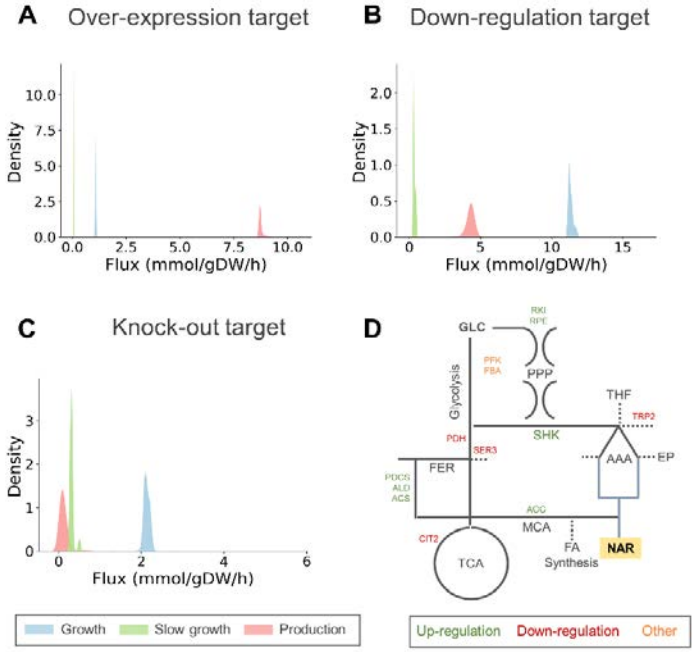

Figure 1. A-C. Example of over-expression, down-regulation and knock-out targets' flux distributions, where possible flux values are represented in the x-axis and their frequency (normalized to an area of 1) is shown on the y-axis. **D.** Summary of identified targets for improved naringenin production in *S. cerevisiae.*

We present Comparative Flux Sampling Analysis (CFSA), a strain design method based on the extensive comparison of complete metabolic spaces corresponding to maximal or near maximal growth and production phenotypes. Reaction fluxes are sampled from the metabolic solution space in three scenarios: growth, slow growth and production. Flux distributions are statistically compared resulting on the identification of potential down-regulation, knock-out and over-expression targets leading to growth-uncoupled increased production. Figure 1A shows genes classified as over-expression targets as the absolute flux through the corresponding reaction is higher in the production scenario regardless of the growth rate represented by the growth and slow growth scenarios. Similarly, genes are classified as down-regulation targets when the absolute flux through the corresponding reactions is lower in the production scenario compared to the growth scenario and does not overlap with the slow growth scenario (Figure 1B). The most extreme case of a down-regulation, a knock-out, is obtained when, for a down-regulation target, the flux in the production scenario is zero and the gene is not classified as essential (Figure 1C). Growth and production are competing objectives and often low fluxes obtained in the production scenario are not related to an increased production but to a decreased growth rate (e.g. fluxes through reactions for biomass components). Therefore, reactions where the production and slow growth distributions overlap are considered false positives. We validated CFSA applying it to the production of lipids by *Cutaneotrichosporon oleaginosus* and naringenin by *Saccharomyces cerevisiae*. A summary of the targets identified in the naringenin case study is shown in Figure 1C. In both cases we identified engineering targets including evident up-regulations belonging to the product synthesis pathway, distant targets that improve precursor availability, as well new engineering strategies that show the potential of this method.

Acknowledgement

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The Circular Greenhouse: production of organic acids, during wild fermentation of tomato plant juice for bio-stimulant purposes.

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Abstract

The Netherlands wants to be completely circular by 2050, and with the greenhouse tomato industry producing yearly ±72.000 tons of plant waste, upcycling of plant waste becomes a clear possibility to improve sustainability and circularity in this sector. The plant waste contains mostly fibres and juice that can be separated by pressing. The fibres are mainly used for making paper and building materials, but the possibilities for the tomato plant juice have not yet been explored. The tomato plant juice could be used as a feed source for the next generation of tomato plants. A viable way to enhance the tomato plant juice, and transform it into a biostimulant, is by fermenting it with micro-organisms, changing the composition of the juice and producing organic acids [1]. A biostimulant is a substance or micro-organism that, when given to the plant, stimulates natural processes to boost nutrient uptake, nutrient use efficiency and crop quality and yield[2]. Sequencing of extracted DNA from fermented tomato plant juice, in collaboration with Orvion, showed that the juice mostly contains lactic acid bacteria (LAB). LAB are a group of gram-positive bacteria able to produce a wide range of organic acids during fermentation with sugars such as glucose and fructose as feed source[3,4]. In turn, the organic acids have a bio-stimulant effect on the growth of plants that can be exploited[5,6].

To optimize the production of organic acids, the sugar concentration in the tomato plant juice should be as high as possible. Nanobubbling is a technique in which nanobubbles, which are stable in liquid, are generated. With the use of shear forces, the generated nanobubbles will collapse, leading to the generation of OH radicals, which are known to be one of the strongest oxidizers in nature [7]. The collapse of nanobubbles could lead to oxidation of plant cells still present in the pressed juice, liberating further sugars than currently present. In this project, tomato plant juice was nanobubbled for 5 and 30 minutes in collaboration with Aquamar. Sonication was performed after nanobubbling to collapse the nanobubbles and generate OH radicals, and Dynamic Light Scattering (DLS) was performed, in collaboration with Ardena, to ensure nanobubble collapse. Wild fermentations were performed with nanobubbled and sonicated tomato plant juice, which were analysed for organic acids with the use of High Performance Liquid Chromatography (HPLC).

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Crystallographic analysis and engineering of a plant chalcone synthase for methylated flavonoid biosynthesis

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Abstract

O-methylated flavonoids are naturally occurring compounds with many health-promoting properties. Compared to their unmethylated counterparts, methylated flavonoids possess improved bioactivities. They are produced in plants in low abundance and as complex mixtures of similar compounds that are difficult to separate. Synthetic biology offers the opportunity to produce various flavonoids in a targeted, bottom-up approach in engineered microbes with high product titers. However, the production of O-methylated flavonoids is currently still highly inefficient.

In this study, we investigated and engineered a combination of enzymes that had previously been shown to support homoeriodictyol and hesperetin production in *Escherichia coli* from fed cinnamic acid precursors. We determined the crystal structures of the enzyme catalyzing the first committed step of the pathway, chalcone synthase from *Hordeum vulgare*, in three ligand-bound states. Based on these structures and a multiple sequence alignment with other chalcone synthases, we constructed mutant variants and assessed their performance in *E. coli* towards producing methylated flavonoids. With our best mutant variant, HvCHS (Q232P, D234V), we were able to produce homoeriodictyol and hesperetin at 2 times and 10 times higher titers than previously reported. Our findings will facilitate the further engineering of this enzyme towards higher production of methylated flavonoids.

Acknowledgement

The authors acknowledge DESY (Hamburg, Germany) and EMBL Hamburg for the provision of the PETRA III beamline (P11 and P13). KH, BP, SH and WJQ thank Serj Koshian for designing the initial set of mutant enzymes during his summer internship, Dr. Robbert Hans Cool for providing training in protein purification, and Nika Sokolova for help with troubleshooting the steady-state kinetics.

CFD-CRD in Syngas Fermentation: What can we learn?

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Syngas fermentation is a promising commercial process for converting gaseous waste streams with significant CO and H_2 content into added-value chemicals. Currently, it is commercialized by a handful of companies; amongst them, *LanzaTech* employs an external-loop gas lift reactor (EL-GLR) for converting off-gases into ethanol. The typical gas and liquid flow pattern in such reactors was found to lead to frequent oscillations in dissolved gas concentrations, from the microbial point of view.
A two-way coupled Euler-Lagrangian CFD model was deployed to study how the oscillations in the dissolved gas concentration (Figure 1a) would affect individual cells and the global reactor performance. A recently developed lumped (linlog-based) metabolic kinetic model was coupled with a multiphase CFD model, to obtain results with a high spatio-temporal resolution from both the reactor and the microbial perspective. The dynamic kinetic model describes 12 compounds and 11 intracellular reactions, while the CFD model describes the fluid flow, turbulence and mass transfer in the industrial-scale EL-GLR.

This model was used to describe how large-scale dynamics would affect the biomass-specific ethanol productivity, cellular CO and H_2 uptake rate, and the intracellular metabolite concentrations. The model predicts that there is a typical delay in the seconds range between gas uptake and ethanol production. This time period is mainly used for the conservation of energy, i.e., orderly: harvesting electrons from the gas substrates, generation of the proton motive force and secretion of products.
In the large-scale reactor simulation, these mechanisms led to specific zones with high metabolic activity wherein high intracellular concentrations of reduced ferredoxin were predicted (Figure 1b), which is used as an intracellular storage pool for electrons originating from the substrate gases. When the cells are rich in ferredoxin, the excess electrons are being used for the production of ethanol. This happens at the top section of the reactor, where the reduced ferredoxin is oxidized and where high biomass-specific ethanol production rates were observed (Figure 1b,c). We also revealed zones with low metabolic activity, such as the end of the downcomer, due to depletion of intra- and extracellular electron sources.
This two-way coupled CFD-CRD model for syngas fermentation offers new fundamental insights into the microbial behaviour in large-scale syngas fermentation that can inspire further experimental investigation, and opens the way towards rational optimization of operating conditions and reactor design, for increased ethanol productivity in industrial syngas fermentation processes.

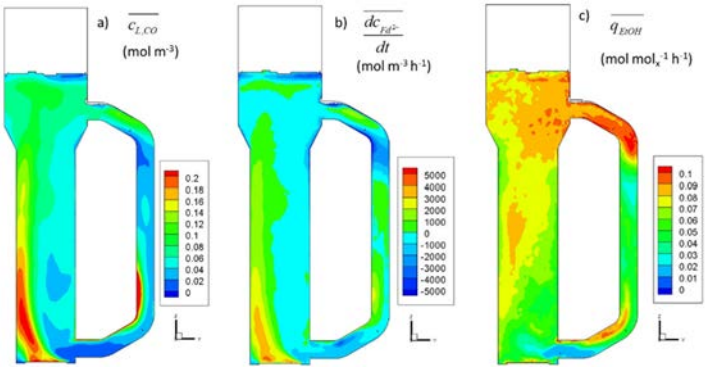

Figure 1. Surface plots in the *zy*-plane of the industrial-scale EL-GLR of a) the time-averaged dissolved CO concentration, b) the derivative of the intracellular reduced ferredoxin concentration, and c) the biomass-specific ethanol production rate.

Microbes for future: investigating and harnessing environmental bacteria for a greener tomorrow

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Abstract

Microbes can make a living everywhere: in hot springs and in the deep sea as well as on plant leaves and in our guts. They can even degrade petroleum, plastics, and toxic pollutants. These feats are made possible by the astounding diversity of microbial metabolism. Enzymes and metabolic pathways enable the synthesis, degradation, and conversion of thousands of molecules.

Our lab investigates and applies microbial metabolism across all scales of biological organization, reaching from enzyme biochemistry and protein crystal structures over laboratory cultures of model organisms to field work and the bioinformatical analysis of databases. By bridging the scales of microbial metabolism, we generate novel insights that are applied towards sustainable biotechnological approaches.

Here, we present insights into the characterization of novel enzymes for PET monomer assimilation and the engineering of marine microbes for PET degradation. Furthermore, we characterized a metabolic route for the assimilation of 6-aminohexanoate, the monomer of nylon, and we created engineered bacterial strains that convert methanol into value-added molecules, such as polyketides. Finally, we have studied the interactions of algae and bacteria in marine environments and described the global distribution of a novel metabolic route for carbon dioxide conservation in this context.

Wood treated with fungal biofinish as example of sustainable Engineered Living Materials (ELMs)

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Abstract

Challenges as accommodating the rising global population and limiting human-induced global warming asks for more sustainable, innovative, functional and longevity building materials. Such materials can contribute to reduce the negative environmental impact of human construction activities. Nature is a great inspiration source for the development of advanced and innovative building materials. Engineered Living Materials (ELMs) consist of living cells and polymeric matrices that are designed to be active and responsive, for instance to environmental conditions. *Aureobasidium pullulans* coated materials could be such an ELM. Biofilms of this fungus can coat wood that is impregnated with linseed oil. The biofilm together with the linseed oil extends the outdoor service life of wood by protecting the wood against different factors such as UV radiation and wood decay. In addition, the biofilm potentially has self-repairing abilities. We are studying the biofilm formation of *Aureobasidium pullulans* to be able to further optimize the wood coating and improve the functionality of building materials.

Microbial N cycling potential in sediment from a marine, seasonally hypoxic lake

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Abstract

Coastal ecosystems show increased eutrophication and deoxygenation due to anthropogenic activities. As an essential and limiting nutrient, nitrogen controls productivity in many ecosystems, and nitrous oxide (N_2O), an extremely potent greenhouse gas, is an intermediate of various nitrogen cycle processes. Therefore, a predictive understanding of the impact of nitrogen eutrophication and deoxygenation on marine microbial nitrogen cycling is crucial. Sediment was collected from the seasonally hypoxic, saline Lake Grevelingen in March 2023 while the bottom waters were oxygenated. Chemical profiling of the sediment and water column showed diffusion of ammonium to the water column. Because the chemical composition of the sediment changes with depth, the nitrogen cycling potential was studied for different sediment depths (0-2 and 25-30 cm). Batch incubations were set up using endogenous energy and carbon sources or supplementation of monomethylamine as electron donor and carbon source. The batch incubations showed immediate nitrate, nitrite and N_2O reduction for both sediment depths, in both absence and presence of monomethylamine. Oxidation of ammonium was observed in batch incubations set up with sediment from 25-30 cm depth but not in those set up with the surface sediment. Potential rates have been calculated and 16S rRNA gene sequencing (V3-V4 region) will reveal the shifts in the microbial community in the incubations over time. All in all, these findings indicate that there is potential for microbial nitrification, denitrification and dissimilatory nitrate reduction to ammonium (DNRA) in the sediment from Lake Grevelingen while its bottom waters are oxygenated. Combining the *in situ* geochemical data, microbial community composition and the *in vitro* potential for nitrogen cycling reactions is important information needed to make better predictive models of the future state of our coastal waters.

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Synthesis of Pharmaceutically Relevant Arylamines Enabled by a Novel Nitroreductase from *Bacillus tequilensis*

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Arylamines are essential building blocks for the production of high-value pharmaceuticals, therefore strategies for their efficient and cost-effective synthesis are of great interest[1]. Current industrial methodologies, while well-established, involve the use of costly reagents and energy intensive techniques with a significant environmental impact. As a result, flavin-dependent nitroreductases (NRs) have received increasing attention as greener and more selective biocatalysts for arylamine synthesis[2]. Herein, we assessed a novel NR from *Bacillus tequilensis*, named BtNTR, for the synthesis of pharmaceutically relevant arylamines, including valuable synthons used in the manufacture of blockbuster drugs such as Vismodegib, Sonidegib, Linezolid, and Sildenafil. Remarkably, BtNTR showed high conversion to the final arylamine (>98%) and good product yields (up to 56%) with the entire panel of screened nitroarenes. The enzyme displays a broad range of pH stability (5-10) and a melting temperature of 59°C, which makes it very appealing for industrial applications. Our results indicate that BtNTR has a broad substrate scope, including bulky nitro benzenes, nitro pyrazoles, and nitro pyridines. In addition, we report the crystal structure of BtNTR and some preliminary steady-state and pre-steady-state kinetic data, as well as some docking studies aimed at understanding the enzymatic reaction mechanism. This study suggests that BtNTR could be an interesting biocatalyst for the synthesis of pharmaceutically relevant amine-functionalized benzenes and heterocyclic molecules, providing an attractive alternative to traditional chemical synthesis methodologies.

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Discovery by cell-free protein synthesis and biochemical characterization of thermostable glycerol oxidases

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Key words: Alditol oxidases, flavin, glycerol, cell-free expression, in silico bioprospecting, enzyme engineering.

Abstract

Alditol oxidases are promising tools for the biocatalytic oxidation of glycerol to more valuable chemicals[1]. By integrating in silico bioprospecting with cell-free protein synthesis and activity screening, an effective pipeline was developed to rapidly identify enzymes that are active on glycerol. Three new thermostable alditol oxidases from *Actinobacteria bacterium*, *Streptomyces thermoviolaceus* and *Thermostaphylospora chromogena* active on glycerol were discovered. The characterization of these three flavoenzymes demonstrated their glycerol oxidation activities, preference for alkaline conditions, and excellent thermostabilities with melting temperatures higher than 75 ºC. Structural elucidation of the alditol oxidase from *Actinobacteria bacterium* highlighted a constellation of side chains that engage the substrate through several hydrogen-bonds, a histidine residue covalently bound to the FAD prosthetic group, and a tunnel leading to the active site. Upon computational simulations of substrate binding, a double mutant targeting a residue pair at the tunnel entrance was created and found to display an improved thermal stability and catalytic efficiency for glycerol oxidation. The hereby described alditol oxidases form a valuable panel of oxidative biocatalysts that can perform regioselective oxidation of glycerol and other polyols.

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General stress response: a mechanism to save energy and express heterologous proteins in slow-growing yeast cultures

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Slow growing yeast cells in fed-batch or continuous cultures are highly relevant for industrial biotechnology as they allow uncoupling of growth from product formation to increase yields. To effectively apply slow-growing cultures, most of the metabolic energy and substrate should be directed to product formation, e.g., heterologous protein expression. When its specific growth rate decreases, yeast gradually activates the conserved General Stress Response (GSR). The GSR confers resistance against multiple stresses, but we hypothesize that it also contributes to energy saving. For example, the GSR results in increased levels of heat shock proteins that act as chaperons that can reduce expensive protein turnover. To test this hypothesis, we manipulated the main transcription factors that activate the expression of stress response genes in *Saccharomyces cerevisiae*. This resulted in only minor changes of the maximum specific growth rate. Stress resistance was however strongly affected, which correlated well with observed changes in stress-responsive promoter driven protein expression. To study the impact on cellular energetics, the cellular maintenance energy requirements of mutant and parental strains were estimated using chemostat and fed-batch cultures. A reduction of the GSR strongly increased the maintenance energy requirement, which amongst others translated into reduced biomass yields and protein titers in fed-batch cultures. These findings strongly suggest that GSR not only plays a role in actual stress resistance, but also in energy saving in yeast. The GSR is hence a relevant target to further improve yeasts as microbial cell factories.

Key words: Slow growing yeast cultures, general stress response (GSR), energy saving, stress resistance, maintenance requirement, industrial biotechnology

Characterization of gas phase dynamics in bubble columns for bioprocesses

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Abstract

The ever growing global population and increasing awareness of climate change leads to an increased interest in replacing polluting production paths for, for example, fuels, plastics and animal protein with more sustainable alternatives. A promising process for replacing production paths is gas fermentation, in which gasses containing a combination of CO_2, CO, H_2 and O_2 are converted into valuable product streams. For optimization of the mass transfer rate in such processes, an accurate description of the gas phase behavior is required. Past experimental work with similar aim has focused on air-water systems, which are not representative of fermentation broths. This project aims to understand the impact of common fermentation broth components on bubble size distribution and local gas holdup, leading to improved estimates of the interfacial area. This is achieved through local bubble size measurements with a single fiber optical probe, and tomography from X-ray transmission data from three simultaneously recorded angles.

Antimicrobial compounds as a diagnostic tool to quantify and disentangle denitrifying anaerobic methane oxidation for potential use in wastewater treatment

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Abstract

Methane is the second most prevalent greenhouse gas contributing to 16% of global climate change and is commonly emitted from various natural and engineered aquatic environments. Denitrifying anaerobic methane oxidation (DAMO) has been proposed as a process with potential for application in wastewater treatment plants (WWTPs) [1–3]. However, our understanding of DAMO is primarily based on studies conducted with environmental samples or enrichment cultures. To develop diagnostic tools and gain deeper insight into the process of DAMO, we used antimicrobial compounds, including inhibitors and antibiotics, to study the physiology and community dynamics of a DAMO enrichment culture containing the nitrate-reducing, methyl-coenzyme M (MCR) containing methanotrophic '*Ca*. Methanoperedens' archaea and the nitrite-reducing, particulate methane monooxygenase (pMMO) containing '*Ca*. Methylomirabilis' bacteria. Additionally, we explored the potential application of DAMO in wastewater contaminated with high concentrations of ammonium and heavy metals.

Our results demonstrated that we could selectively inhibit one of the two individual methanotrophs. This resulted in a 70% reduction in the methane oxidation rate in both cases. Specifically, '*Ca*. Methanoperedens' showed susceptibility to puromycin and the MCR inhibitor 2-bromoethane-sulfonate (BES), while the novel MCR inhibitor 3-nitrooxypropanol (3-NOP) did not inhibit DAMO. '*Ca.* Methylomirabilis' exhibited susceptibility to the pMMO inhibitor 1,7-octadiene and a bacteria-suppressing antibiotics cocktail containing streptomycin, vancomycin, ampicillin, and kanamycin. Furthermore, we observed that DAMO was sensitive to ammonium at concentrations above 10 mM (half max. inhibitory concentration (IC50) of 52 mM). The impact of heavy metal pollution on DAMO was evaluated by testing lead, nickel, and cadmium at concentrations ranging from 10 to 1000 µM. The DAMO community showed a remarkable resistance to lead (IC50 > 1000 µM), while the inhibitory effect of nickel and cadmium was more pronounced (IC50 of 232 µM and <10 µM, respectively). However, these IC50 values exceeded the heavy metal concentrations typically found in highly polluted wastewaters.

Ultimately, this study provides valuable insights into the microbial interactions involving '*Ca.* Methanoperedenaceae' and '*Ca.* Methylomirabilota' within DAMO communities, enabling further investigations into their potential application in methanogenic, nitrogen-polluted water systems, particularly in WWTPs. The findings contribute to the development of diagnostic tools for wastewater treatment strategies aimed at mitigating methane emissions and addressing environmental challenges.

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Cyanobacterial cell factory development for CO_2-based chemicals

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Abstract
At Photanol, we have developed cyanobacterial cell factories for various compounds, such as biofuels, sweeteners and organic acids. The company started in 2012 and since then Photanol has been expanding its technology platform by continuously developing and adding more products to its portfolio. Cyanobacteria can capture solar energy via oxygenic photosynthesis and create their biomass from fixation of atmospheric CO_2 and water. This ability of cyanobacteria can be redirected towards production of chemical commodities by genetic engineering. Resulting biosolar cell factories present an opportunity for global concerns such as rising atmospheric CO_2 levels and (future) uncertain/fluctuating availability of sugar and oil as raw material. Moreover, direct conversion of CO2 into chemical commodities is more efficient than first fixing CO_2 by plants into sugar as feedstock for such commodities.

As an example, Photanol's proprietary technology has resulted in efficient organic acids production, such as lactate and glycolate, but also olefins such as ethylene. Each compound requires different metabolic pathway optimization to acquire fully optimized strains. In this poster, we highlight the challenges and opportunities in cyanobacterial strain engineering to obtain commercially relevant strains.