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Book of abstracts

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Abstracts Plenary Lectures

Precision Fermentation for Nutritious and Sustainable Proteins

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Precision Fermentation is a technology that, from its recent buzzing interest may seem novel, but actually has been around for decades and has in fact led to numerous products that we use and consume every day. Earlier terminology to describe the same or similar technology includes genetic and metabolic engineering or synthetic biology.

Nine leading organizations in biotechnology, took an effort to define precision fermentation and reached consensus on: "Precision fermentation is a sub-category of fermentation, using biotechnological approaches and innovations for the controlled cultivation of selected and/or modified microbial cells, to produce specific substances. These fermentation-derived products can be a single molecule or multiple molecules purified from the fermentation broth or biomass."

Established products from precision fermentation with an impressive history of safe use of over decades and that we are all familiar with include vitamins, amino acids, enzymes, organic acids, tailored fats and oils, enzymes, pharmaceutical products and biobased chemicals. With the advancement of modern genetic techniques and our ability to steer protein synthesis and export more efficiently, macro-ingredients such as fermentative whey proteins that are made by precision fermentation are becoming a commercial reality.

Vivici is a scale-up company, based in Oegstgeest (NL) that originated from a collaboration between dsm-firmenich and Fonterra, combining decades of biotechnology experience with leading dairy food science. It is Vivici's mission to make the promise of dairy protein from precision fermentation a commercial reality to meet the world's growing need for nutritious, functional and sustainably-produced proteins.

Vivici has launched its first protein, Vivitein™ BLG (beta-Lactoglobulin) in the US and the development of our second protein Vivitein™ LF (Lactoferrin), is well underway. The development, characterization and scale up of BLG will be elaborated on as well as the development of compelling food applications. Fermentative BLG is lactose-free, cholesterol-free, free from antibiotics and is a vegan-friendly alternative to Whey Protein Isolate (WPI). It has 1.5x more branched chain amino acids - in particular Leucine - than WPI, which is of particular relevance for medical and sports nutrition applications.

Doubling our protein production, which is needed to feed 10b people in the future, cannot be accomplished within planetary boundaries by growing animal protein alone. Vivici is dedicated to develop great tasting sustainable protein solutions that complement other protein sources, to meet the need of a growing Global population.

miRADAR: a paper-based test to simplify MS diagnosis

Ivar Gruppen, Renske Verkuijlen and Mathilde van Nieuwenhuizen
iGEM team 2024 Wageningen

Multiple Sclerosis (MS) is a neurological disease that impacts nearly 3 million people worldwide. For 10% - 30% of patients in the Netherlands alone, proper diagnosis can take months or even years due to the lack of a specific MS biomarker. To simplify MS diagnosis, miRADAR designed an accessible diagnostic test based on dysregulated miRNA patterns in blood. To detect these miRNAs, our test consisted of miRNA amplification, an RNA threshold detection system, and output signal generation using toehold switch logic gate circuits. Through multiple models we found eight unique miRNA biomarkers distinguishing MS from similar diseases, and designed optimal logic gate circuits. As a proof-of-concept, we developed a cell-free paper-based test that successfully detected an MS-relevant miRNA. With our test, we provide a modular framework for miRNA-detection to improve diagnosis of MS and many other diseases.

Biocatalytic approaches to produce fine chemicals

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Catalysis drives the production of most modern chemicals, yet traditional processes often rely on scarce or toxic metals, harsh conditions, and generate substantial waste. Nature has evolved fascinating catalysts, enzymes, to perform diverse chemical reactions. These offer a sustainable alternative, as enzymes operate under mild conditions with exquisite selectivity, making them highly attractive for industrial manufacture of fine chemicals and pharmaceuticals.

In particular, nicotinamide adenine dinucleotide (NAD)-dependent oxidoreductase enzymes can catalyze an impressive array of redox reactions with selectivities rarely achieved by traditional catalysts. However, their industrial use is limited by the stability, cost, and efficiency of natural cofactors. To address this, we investigate synthetic nicotinamide analogues as alternative cofactors to improve economic viability and modulate reaction performance.[1]

In this lecture, I will present our recent advances in understanding how oxidoreductases such as alcohol dehydrogenases and ene reductases accept these cofactor analogues, and how this knowledge can be leveraged to expand their reactivity. Through repurposing enzyme mechanisms, we aim to unlock new biocatalytic routes toward valuable fine chemicals and pharmaceuticals.[2]

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Three Thoughts on Innovation, Collaboration, and the Bigger Picture

NBC September 23 2025

Dr Eva-karin Gidlund
CA&IO NorthX Biologics

Biotechnology today stands at a crossroads where scientific breakthroughs, business models, and societal needs do not always align. Lessons from biologics manufacturing and CDMO partnerships reveal that success depends not only on technical excellence but also on clarity of expectations, long-term planning, and a culture of data integrity. For an innovation owner, choosing the right CDMO is not simply about finding a factory to execute a recipe, but about engaging a partner who understands the "cake" you intend to bake and why. Just as chemistry matters in biologics, chemistry also matters in relationships, finding the right CDMO partner can be as decisive as finding a life partner. Relying on an organization that "just manufactures" often results in the black box syndrome, where innovators lose insight into and ownership of their own processes. To avoid this, knowledge sharing and mutual transparency are essential. In Swedish, the word *TILLIT* (trust) is a palindrome, read the same forwards and backwards, symbolizing a cultural perspective on trust as a reciprocal relationship. Building partnerships grounded in this kind of trust is crucial to ensuring that biotech innovation translates into lasting impact for patients and society.

Emerging technologies like AI offer the potential to rethink value creation across the biotech value chain, by modeling long-term patient benefits, identifying systemic cost savings, and supporting new approaches such as subscription models or pay-for-performance contracts. The reflections presented weave together themes of innovation, collaboration, and strategic foresight, with the aim of inspiring a discussion on how to align ethics with economics and ensure that biotech delivers not just innovation, but enduring impact.

The Netherlands Biotech Society represents a uniquely important audience in this dialogu, a community with the expertise, credibility, and collaborative spirit needed to lead the way in an era marked not only by rapid advances in AI, but also by challenges of fake news, eroding trust in science, and increasing societal skepticism. The role of this community will be pivotal in shaping models of innovation that are transparent, ethical, and trusted, anchoring progress that patients, policymakers, and the public can believe in.

Abstracts for Oral Presentation

Cell-free manufacturing of viral vector gene therapies

Benjamin Blaha (Fuse Vectors)

Adeno-associated virus (AAV) vectors remain central to gene therapy, yet current production methods are limited by cell-based systems that restrict scalability, throughput, and flexibility. Fuse Vectors has developed a cell-free AAV production platform that decouples vector generation from cellular machinery, enabling rapid, scalable, and high-quality output.
This work highlights the technical development journey of the platform, including the critical pivots, challenges, and lessons that shaped its design. Current applications demonstrate utility across discovery and R&D, with potential for seamless translation into clinical manufacturing. Beyond technical performance, the approach offers a path to faster development timelines, improved safety and consistency, and broader accessibility of AAV-based therapies. By advancing cell-free manufacturing, this platform seeks to catalyze a step-change in how the industry develops and delivers gene therapies.

COMPARISON OF SINGLE-USE AND STEEL FERMENTORS FOR MALARIA VACCINE EXPRESSION

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Abstract

The National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH) has developed a malaria transmission-blocking vaccine (TBV) candidate recombinant Pfs230D1M, which is currently in Phase I/II clinical trials and targeted to transition to Phase III. Successful technology transfer for manufacturing of Pfs230D1M is crucial to the success of clinical trials and is expected to facilitate manufacturing in affected areas of the world. NIH and Thermo Fisher Scientific have collaborated to conduct proof-of-concept studies assessing capabilities of the Thermo Scientific™ HyPerforma™ enhanced Single-Use Fermentor (eS.U.F.) to manufacture Pfs230D1M. The results show quality and quantity of recombinant Pfs230D1M protein expressed in the eS.U.F. were comparable to those achieved with a stainless-steel fermentor. The eS.U.F. enables rapid expansion of vaccine manufacturing with little infrastructure modification, facilitating vaccine delivery particularly to low resource settings around the world.

Introduction

Malaria is a Life-threatening disease caused by Plasmodium parasites, transmitted by the bite of infected Anopheles mosquitoes. Malaria remains a leading cause of morbidity and mortality worldwide. According to World Health Organization, there were 249 million cases and 608,000 deaths due to malaria in 2022 [1, 2].

Recent efforts by the NIH and collaborators have led to the development of a TBV candidate i.e., conjugated recombinant Pfs230D1M with a recombinant ExoProtein A (EPA) which enhances the immunogenicity [3, 4]. In a phase II safety and efficacy trial in Mali (NCT03917654), Pfs230D1-EPA/AS01 induced high levels of antibodies and conferred significant reduction in mosquito infections (>80%) which reduces the risk of subsequent human infections. Conjugated Pfs230D1-EPA is significantly more effective than previous TB malaria vaccine candidates [4]. Currently, a conjugated Pfs230D1 vaccine is in phase I / II clinical trials in progress to transition to a phase III trial as a combination vaccine.

By simplifying technology transfer for manufacturing recombinant Pfs230D1M, the active pharmaceutical ingredient, the vaccine can more easily be produced locally around the world, which may facilitate its delivery to at-risk populations.

Utilization of the eS.U.F. will allow potential production and manufacturing facilities to quickly setup and express the Pfs230D1M protein or other recombinant protein vaccines.

- S.U.F. requires less infrastructure and maintenance than traditional stainless-steel vessels.
- S.U.F. 6L-300L working volumes
- Rushton or enhanced impellers

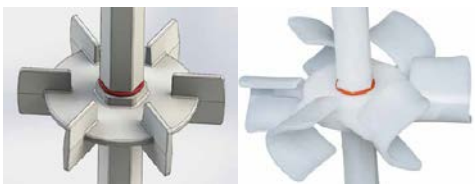

Figure 1. Original S.U.F. impeller design (left) next to the eS.U.F. impeller (right)

Materials and methods

- PTM4, trace salts, and solutions per *Pichia* Guidelines [5]
- Initial batch vol. 18L with 40 g/L Glycerol
- Batch conditions: 30°C, 30% DO, pH 5 (with 28% NH_4OH)
- *Pichia pastoris* Pfs230D1M
- Glycerol batch and feed up to EFT 22h
- Temperature shift 30 to 25°C and pH shift 5 to 3 at the initiation of induction.
- Methanol feed from EFT 24h to 70h
- Expression of recombinant Pfs230D1M protein.
- Final volume 30L
- Supernatant harvested in two 15L batches with CentriPAK™ BPCs using Bios16™ bioprocessing centrifuge at 5,345 x *g* 20 min.

Results

eS.U.F. 30L

- Final wet cell weight 442.6g/L
- Dry cell weight of 98.6g/L.
- Molecule titer and activity were similar to 60L stainless-steel fermentor.

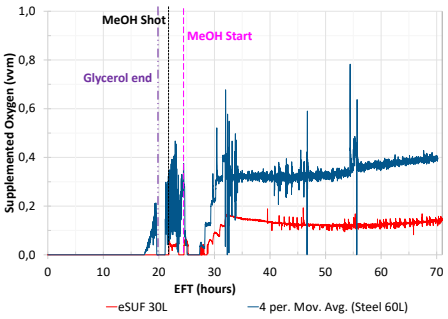

Figure 2. Culture data comparing supplemented oxygen gas flow in vessel volumes per minute (vvm) for the HyPerforma eS.U.F. in comparison to the 60 L working volume stainless-steel fermentor.

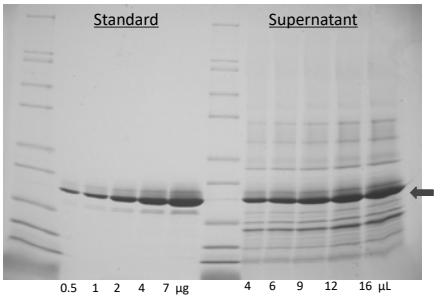

Figure 3. Image of Coomassie blue-stained gel for analysis of eS.U.F. fermentation, which shows good expression of the recombinant Pfs230D1M protein for vaccine use. Achieved with simple single-pass technology transfer.

Figure 4. HyPerforma eS.U.F. 30L and 300L Vessels with TruBio Controller. The volume used would depend on the site scale needs.

Conclusions

The HyPerforma eS.U.F. systems further enable cultivation of *P. pastoris* cultures to high density with similar performance to stainless-steel vessels. In reviewing previous P. pastoris fermentations conducted using a Rushton version S.U.F., the eS.U.F. reduced oxygen consumption by more than ~80% while reaching similar cell Mass [6,7].

Increasing access to this more effective TBV malaria vaccine, would be a step towards the goal of achieving herd immunity and eliminating Malaria.

- It is estimated that from each eS.U.F. 30L culture 30,000 vaccine doses can be produced.
- This technology transfer from 60L stainless-steel vessel to eS.U.F. was successful in first run.
- Stainless-steel 60L used twice as much O_2 / L of culture

As the eS.U.F. 30L only requires electricity and compressed gasses to operate, it is possible the system could produce vaccines closer to susceptible populations. This would help to reduce the costs of vaccine production while increasing availability [8].

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Cultivation of animal proteins as three-dimensional structures: modelling and simulation studies

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Abstract

Cultivated meat (CM) offers a sustainable alternative to conventional meat, while preserving the nutritional and organoleptic properties desired by consumers (Chen et al., 2022). To manufacture structured, whole-cut CM products, it is convenient to grow cells on scaffolds, successfully mimicking the cytoarchitecture of meat. However, such three-dimensional (3D) structures require an efficient and constant perfusion of oxygen- and nutrient-rich media, and the cells typically experience shear stress and mass transfer limitations (Jaques et al., 2021). These challenges limit process scalability, as well as applicable choices for scaffold materials and scaffold and bioreactor types, leading to reduced scaffolded tissue and cell titers to maintain cell viability. To accelerate the affordable production of 3D CM products, cost-effective scalable process configurations need to maximize cell growth while minimizing shear stress and mass transfer limitations.

This work investigated the physical limits for scaling up the production of 3D CM using scaffolding materials, aiming at the annual production of 1 kiloton structured product. Edible hollow fiber reactors have been chosen due to their technical feasibility. A mathematical model has been developed to optimize independent process parameters, and identify physical limits and trade-offs between reactor scale, yield, product quality, and costs. Preliminary results suggest that process scalability and costs are highly sensitive to fiber permeability and rupture tolerance. The maximum achievable cell titer is limited by the fiber wall thickness and oxygen penetration depth. Product quality depends on the fiber lumen diameter, since larger diameters lead to lower CM content, but may allow for larger bioreactors, denoting the evident trade-offs between product quality and process scalability. A comprehensive sensitivity analysis has provided further insights into favorable fiber material properties, cell kinetics and stoichiometry targets, and opportunities for process optimization, to serve as valuable guidance for future research within the cellular agriculture community.

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Acknowledgement

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How to improve photosynthesis to improve crop yield?

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Photosynthesis is the main driver of plant biomass production. While crop yields have been increased impressively through breeding the past century, there never has been a strong selection on high photosynthesis efficiency. With the growing demand for biobased materials, we are likely to soon need more high yielding crops. Over the past decade, several genetic engineering approaches have been proposed to improve the efficiency of photosynthesis, some of which with remarkable success. I will provide an evaluation of such approaches, but since genetic modification beyond gene editing still face considerable regulatory restrictions in the EU, I will also explore how such may be achieved by crop breeding. To provide a proof of principle for such, my group explores the opportunities to characterize the genetic variation for photosynthesis efficiency in the model species *Arabidopsis thaliana*. One of the challenges in investigating such genetic variation is the ability to adequately phenotype photosynthesis parameters. For this purpose, we use sophisticated phenotyping platforms designed for high-throughput imaging of light use efficiency of photosystem II electron transport (ΦPSII or Fq'/Fm') and related photosynthetic parameters through chlorophyll fluorescence measurements, such as available in the Netherlands Plant Eco-phenotyping Centre (www.npec.nl). It demonstrated to be very efficient and reliable in phenotyping several, large, genetically segregating Arabidopsis populations, diversity panels and cybrid panels, under different growing conditions. We used the observed genotypic variation to identify nuclear encoded quantitative trait loci (QTL) for photosynthesis parameters, as well as variation residing on the chloroplast genome.

From Structural Integrity to Thermoprotection: Functional Insights into the Biological Role of the polysaccharide schizophyllan in *Schizophyllum commune* for Biotechnological Applications

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Abstract

Schizophyllum commune produces the β-(1,3)(1,6)-glucan schizophyllan, which it incorporates into its cell wall and secretes into the culture medium. While schizophyllan is well-studied for its industrial applications, its biological role for the fungus itself remains largely unknown. Here, we show that medium buffered with 10-fold higher KH_2PO_4/K_2HPO_4 led to an 8.8-fold reduction in rigid cell wall schizophyllan and a 4.8-fold decrease in water-soluble schizophyllan. This also made the mycelium 2.7-fold less elastic. We further demonstrate that *S. commune* can utilize schizophyllan as a carbon source and that spores coated in schizophyllan exhibited ~2-fold increased shelf life and heat tolerance, and over 13.9-fold greater survival during freeze–thaw cycles. Additionally, schizophyllan may facilitate microbial interactions: it promoted the growth of *Pseudomonas putida* while inhibiting that of several other soil bacteria. Notably, *Escherichia coli* and *P. putida* cells coated in schizophyllan also showed enhanced survival under thermal and freezing stress. These findings reveal that schizophyllan may serve several roles in *S. commune* biology, contributing to stress resilience, structural integrity, and microbial interactions. This insight may inform new strategies for engineering fungal materials and thermo- or bioprotectants.

From Cell Factory Design to Bio-Based Chemicals: The potential of industrially attractive microalgae in modern biotechnology.

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Abstract.

Microalgae are emerging as powerful platforms for the sustainable production of high-value molecules, offering key solutions for the transition to a bio-based economy. These photosynthetic microorganisms possess unique biological traits, such as efficient solar energy conversion, carbon capture, and remarkable metabolic flexibility that make them highly attractive for biotechnological applications[1]. Over the past two decades, advances in genetic engineering have unlocked their potential for the production of a wide range of valuable compounds, including lipids, isoprenoids, and pharmaceutical ingredients. However, realizing their full commercial potential requires overcoming fundamental biological bottlenecks, particularly in enhancing photosynthetic efficiency, optimizing carbon utilization, and redirecting metabolic fluxes towards desired high-value products.

In this talk, we will focus on how microalgae can be strategically developed as versatile platforms for the production of lipids and other high-value molecules. Our work centers on industrially relevant species such as *Phaeodactylum tricornutum* and *Nannochloropsis oceanica*, which are distinguished by their natural accumulation of triacylglycerols (TAGs) and the omega-3 fatty acid eicosapentaenoic acid (EPA). Beyond lipids, these species are increasingly recognized for their capacity to support heterologous production of plant-derived isoprenoids, recombinant proteins, and enzymes.

We will share recent progress (both published and ongoing) in engineering these microalgae for the heterologous production of plant isoprenoids, such as lupeol, betulinic acid, and pinene for applications in pharmaceutical industry and food&flavouring, respectively[2]. These efforts include the expansion of their genetic toolboxes and the systematic identification of engineering targets to enhance lipid accumulation and customize lipid profiles[3,4]. Finally, we will highlight advances in tailoring *N. oceanica* for the production of specific medium-chain fatty acids, which hold promise for applications ranging from sustainable replacements for tropical oils to the development of novel nutritional and industrial products[5].

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Safe and Sustainable by Design

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Health and environmental damage from exposure to (unregulated) chemicals is a severe problem. Worldwide, 2 million deaths (3.6% of total) and 53 million Disability-Adjusted Life Years (2.1% of total) were attributable to exposure to chemicals in 2019 let alone the burden that it causes to the environment. To lower this burden, the EC launched the Chemicals Strategy for Sustainability (CSS) to create a healthy, sustainable, climate neutral and circular economy in 2050 by phasing out, minimizing the usage of, and substitution of existing and new harmful chemical products with safer and less harmful alternatives. A complete shift towards Safe and Sustainable by Design (SSbD) of chemicals, materials and technologies is the ultimate goal.

Safe and Sustainable by Design (SSbD) provides an opportunity to proactively design innovative products that not only provide solutions to societal challenges, such as the energy transition, but also ensure no harm is caused to humans or the environment. Achieving this delicate balance between performance, health, safety, sustainability, and costs necessitates an integrated approach during all phases of product innovation.

The concept of SSbD will be presented, along with its regulatory context. In addition, practical examples will be included to illustrate the implementation of SSbD principles.

Waste-2-Plastic:

Recycling of CO_2, nitrogen, phosphorus and water for the production of bioplastic

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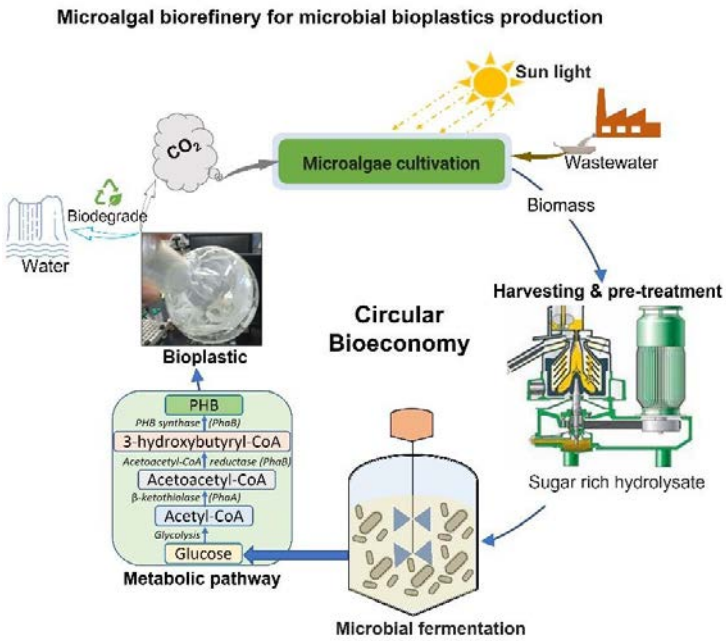
Abstract

Billions of tonnes of plastic materials derived from non-renewable resources have been accumulated over the past decades, with more than 85% remaining in the environment. Biodegradable plastic degrades into CO_2 and water without harmful by-products, and without leaving solid residues in the environment. Among the most promising materials for biodegradable plastics are microbial bioprocessed polyester belonging to the polyhydroxyalkanoate (PHA) family. PHA is produced by specialized bacteria, which feed on carbohydrates (usually from food crops), nitrogen (from the Haber-Bosch process) and phosphorus (from limited mineral sources). The major problem for commercialization of PHAs is its high production costs compared with plastics derived from petrochemicals, mainly due to the high costs (up to 50%) of the carbohydrates selected as feed for the bacteria. As such, the selection of economical and resource-efficient bacterial feed is a key aspect in order to facilitate a viable market total cost for the final product. Within Waste-2-Plastic we investigate the potential of microalgae as a feedstock for bacterial PHA production[1]. In this algae-based medium the carbohydrates are derived from CO2 in flue gases, nitrogen and phosphorus are derived from recycled waste water. Our sustainable PHA will be used by industrial partners to make furniture, packaging and to study biodegradability. In this circular, resource-effective material flow we therefore clean polluted water, counteract global warming and produce sustainable low-cost bioplastic in a CCU process.



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Multiparameter Sensors and DO Sensor Pills for Shake Flasks: Removing Black Boxes for Improved Bioprocess Development

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Abstract

Shake flasks are widely used in early-stage process development due to their increased cost- and space-efficiency compared to bioreactors, which grants the ability to test multiple conditions in parallel more easily. However, shake flasks differ significantly from industrial production bioreactors in their design, operation, and size, and their utility has been limited by a lack of compatible sensor technology for real-time, non-invasive monitoring. We aim to close the data gap between shake flasks and bioreactors. Therefore, we developed a novel Multiparameter Sensor (MPS) and Dissolved Oxygen (DO) Sensor Pills, which can be combined with our Liquid Injection System (LIS) and DOTS software for control. The current sensor-design enables seamless integration of future developments such as pH- and glucose-monitoring.

We introduced Pichia pastoris as an expression host in our lab for enzyme production for future sensor developments. We started working on production before the new sensors were available and the process optimization was performed via trial and error. The optimization used >200 LIS cartridges in 26 experiments with 4-8 variations over the course of approximately one year. The DO feedback-controlled methanol induction yielded the same results in one week with 6 parallel experiments. Volumetric yield was increased from 3.8 µg/ml to 34.7 µg/ml with 25 ml of culture in shake flasks. Overall, the combination of the MPS with DO Sensor Pills and the Liquid Injection System allowed much faster process development in small and intermediate scale.

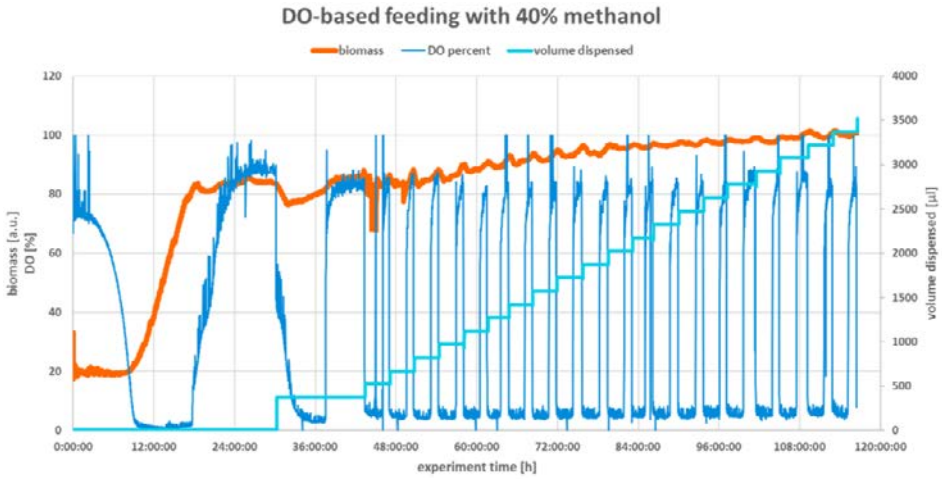

Figure 1 – Exemplary Measurement Data of Multiparameter Sensor (MPS) in combination with DO Sensor Pills for online Biomass and Dissolved Oxygen (DO) data. DO-controlled feeding allowed for optimized, automated addition of methanol into the shake flask culture.

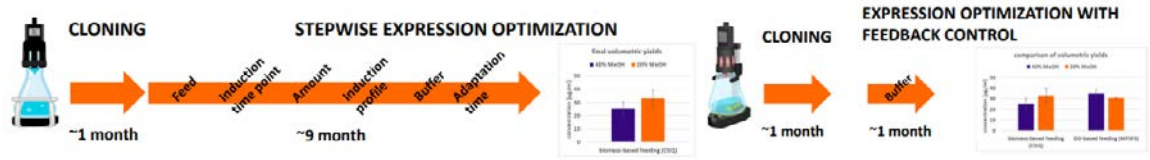

Figure 2 – Using automated DO-controlled methanol feeding, the volumetric yield increased from 3.8 µg/ml to 56.5 µg/ml in shake flasks. The combination of the MPS with DO Sensor Pills and the Liquid Injection System allowed much faster process development in small and intermediate scale

Towards a fungal-based colorful future: successful upscaling and dye application of a color-producing fungus

Bram Visscher[1], Thom de Jager[1], Ha Le Thi Thanh[1], Jasper Meijer [2], Laura Claret Fernández[1], Richèle Wind[1], Miaomiao Zhou[2]

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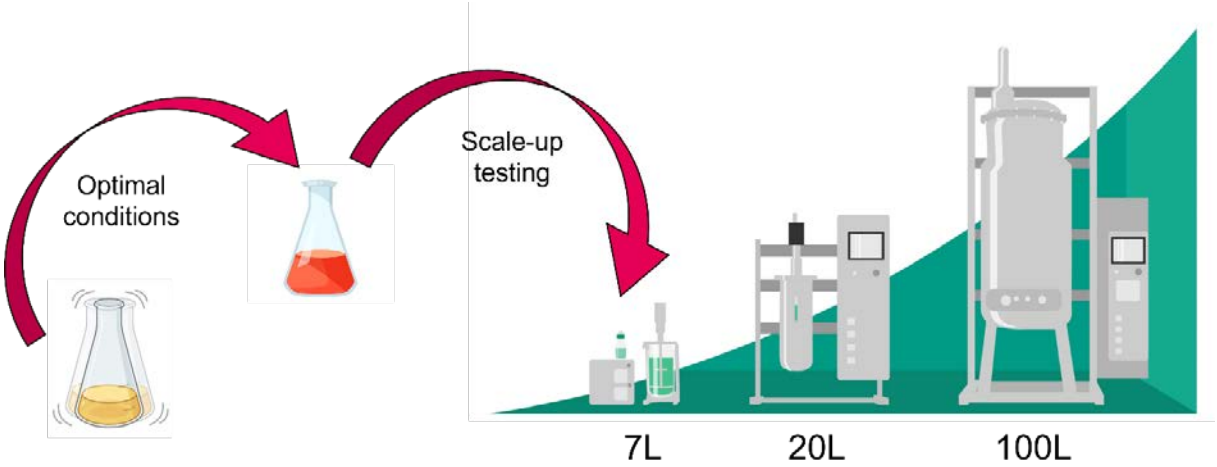
Abstract

Colorants and dyes have been part of human culture from its very beginning. From cave paintings to textile dyes, natural pigments have been ever present in society, either as a form of art, cultural expression or as a fashion statement. After the industrial revolution, many of these dyes and pigments started to be produced from petrochemicals, making them cheaper and more accessible than their natural counterparts. Because of their relevance, the global market for colorants is expected to reach 86,76 billion euro by 2030. However, the production of synthetic, non-biodegradable dyes is a contaminating affair, negatively affecting both the environment and human health. In order to replace these toxic dyes, we need to find a sustainable and healthy alternative which has a competitive yield and is cost-efficient compared to petrochemical-based dyes. A possible solution for the high-volume, cheap production of biobased dyes is the cultivation of colorful fungi in large-scale bioreactors. These fungi can easily be isolated from nature, providing a wide array of colors and shades. In this project, selected the best growing and coloring orange strain of two available. Subsequently, we successfully cultivated the selected orange color-producing fungus in shake flasks and 7L bioreactors. Although we faced challenges on the consistency of the cultures, the process could be optimized at these small scales. Once the process was optimized, we upscaled the color production to a 100L bioreactor, obtaining a high degree of biomass and colorant concentration. The colorant obtained from the upscaled run was extracted from the biomass and successfully applied to packaging material from the partner company Paperfoam. Other partners, including DuurzaamGoed and BIOMALab, are also testing the applicability and colorfastness of the colorant in textiles and vegan leather alternatives. As future research, we need to ensure continuous production of the colorants in an affordable, sustainable way. In all, this research represents a leap forward in the production, extraction and application of biobased fungal colorants for the industry, positively contributing to a biobased economy.



Managing biological complexity in an industrial setting

Cees Sagt[1],

[1] *Principal Scientist Strain Development, dsm-firmenich, Delft, the Netherlands)*

Abstract

Science holds the key to progress; our research unlocks the door. At dsm-firmenich, we continuously develop our unparalleled science and technology platforms, drawing on an R&D network spanning five continents. In the name of making a positive impact on billions of lives every single day, we are determined to keep pushing the boundaries of what is possible.

Our biotechnology competencies help us identify and develop new molecules, applications, and the strains and processes to produce them. However, for complex biological systems the functionality of the parts used is very context dependent. A biological part can lead to properties and effects which are difficult to predict since not all interactions are known. The inherent evolutionary pressure on microbes to select for the best adapted variant invokes genetic instability when a biological part creates a growth disadvantage for the cell. In addition, the complexity and evolving nature of biological systems lies at the heart of poor data quality. The poor signal to noise ratio of biological data makes it difficult to use this data in statistical-based, mathematical approaches (like ML and AI) for strain improvement.

By showcasing biotech innovations from lab bench to customer I will explain how this complexity is managed in an industrial setting. Three examples from the last decade will be presented: bio-based succinic acid, fermentative steviol glycosides, and bio-based vitamin A, using yeast as a cell factory. It will be illustrated how early process design, cutting-edge strain, and enzyme development go hand in hand with creativity and uncertainty to bring a new bioprocess to scale.

PHA production from organic waste: harnessing nature's way to recycle carbon

René Rozendal - Paques Biomaterials

Polyhydroxyalkanoates (PHAs) are a promising natural alternative to petrochemical plastics due to their superior biodegradability, particularly in applications that have a high risk of plastic leakage to the environment. PHAs are naturally produced by bacteria, where they act as a carbon and energy reserve. Hence, the accumulation of PHAs inside the cell helps bacteria to survive in changing environmental conditions, particularly when carbon and energy sources are scarce and intermittently available. Man made environments that are particularly dynamic with respect to substrate availability are wastewater treatment systems. This explains why PHA accumulating bacteria are abundantly present in these systems. By understanding the selective environments inside wastewater treatment systems and further optimizing them, Paques Biomaterials succeeded in the development of a highly effective technology for waste valorisation. Specifically, the technology produces PHBV (a type of PHA) with high HV content (10-50%) that can be tweaked for specific applications. The process has been validated in a wide variety of industries and Paques Biomaterials is now scaling up the technology.

Towards the ultimate process

Ruud A. Weusthuis & Mark M. M. Bisschops
Microbial Biotechnology at Bioprocess Engineering
Wageningen University & Research

This presentation explores strategies for realizing “the ultimate bioprocess” within microbial biotechnology and bioprocess engineering. It frames the development and combination of the ultimate cell factory and bioreactor. It emphasizes retentostat culture as the ideal cultivation mode for constant conditions and minimized downtime, in combination with halted growth and long-life span. Central themes include the integration of *in-situ* product removal (via advanced phase separation strategies), matching strain traits to downstream process conditions. It describes how the yield can be maximized using non-canonical redox cofactors by controlling metabolic electron flow. The talk considers how modulating energy requirements for product formation, respiration and maintenance can improve the efficiency and rate of the conversions. Specific challenges addressed include overcoming gradients, increasing product and solvent tolerance and replacing oxygen as a terminal electron acceptor. The presentation concludes with a list of ten foundational challenges essential for enabling the next generation of robust, high-yield, and industrially relevant microbial bioprocesses.

A New Dawn for Methanotroph Cultivation: Bioelectrochemical Systems yield almost pure cultures of Methanoperedens Archaea

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Abstract
Anaerobic methanotrophs, particularly '*Candidatus* Methanoperedens' species, play a critical role in mitigating methane emissions from anoxic environments. These archaea oxidize methane to CO_2 using various electron acceptors, thus mitigating the release of this potent greenhouse gas into the atmosphere. However, their "unculturable" nature has hindered in-depth studies of their physiology and metabolic capabilities.

This study showcases the power of bioelectrochemical systems (BES) to cultivate and characterize these elusive methanotrophs. By employing a poised electrode as an alternative electron acceptor, we achieved highly enriched cultures of '*Ca.* Methanoperedens', with their relative abundance increasing from 11% to 96%. Our BES setup allowed for the first experimental determination of growth rates for these archaea, revealing doubling times of an impressive 4.6 days at 30°C, compared to previous estimates of ~30 days doubling time in bioreactor enrichment cultures. We confirmed that 98% of the current is generated from methane.

This innovative approach not only unlocks the potential for cultivating previously "unculturable" methanotrophs but also provides a powerful tool for unraveling their unique metabolic capabilities and their role in greenhouse gas mitigation. Our findings pave the way for future research into the potential applications of these organisms in bioremediation and sustainable energy generation.

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Heterologous expression of bacteriocins in *Saccharomyces cerevisiae*

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Abstract

Antimicrobial peptides or bacteriocins are excellent candidates for alternative antimicrobials, but high manufacturing costs limit their applications. Recombinant gene expression offers the potential to produce these peptides more cost-effectively at a larger scale. *Saccharomyces cerevisiae* is a popular host for recombinant protein production, but with limited success reported on antimicrobial peptides. Individual recombinant *S. cerevisiae* strains were constructed to secrete two class IIa bacteriocins, plantaricin 423 (PlaX) and mundticin ST4SA (MunX). The native and codon-optimised variants of the *plaA* and *munST4SA* genes were cloned into episomal expression vectors containing either the *S. cerevisiae* alpha mating factor (MFα1) or the *Trichoderma reesei* xylanase 2 (XYNSEC) secretion signal sequences. The recombinant peptides retained their activity and stability, with the MFα1 secretion signal superior to the XYNSEC secretion signal for both bacteriocins. An eight-fold increase in activity against *Listeria monocytogenes* was observed for MunX after codon optimisation, but not for PlaX-producing strains. After HPLC-purification, the codon-optimised genes yielded 20.9 mg/L of MunX and 18.4 mg/L of PlaX, which displayed minimum inhibitory concentrations (MICs) of 108.52 nM and 1.18 µM, respectively, against *L. monocytogenes*. The yields represent a marked improvement relative to an *Escherichia coli* expression system previously reported for PlaX and MunX. The results demonstrated that *S. cerevisiae* is a promising host for recombinant bacteriocin production that requires a simple purification process, but the efficacy is sensitive to codon usage and secretion signals.

Mining of Biocatalysts with AI: Science or Science Fiction?

Kristina Haslinger, University of Groningen, Groningen/ The Netherlands

Abstract

Engineering biosynthetic pathways for the production of valuable small molecules hinges on access to enzymes with tailored catalytic properties. Identifying suitable biocatalysts, however, remains a major bottleneck. In this talk, I present how we used a machine learning model trained on enzyme activity data to identify potential biocatalysts for substrates of interest and/or to predict the substrate scopes of uncharacterized enzymes. This work currently revolves around fungal type III polyketide synthases, catalyzing the decarboxylative condensation of various Coenzyme A activates substrates in an iterative fashion. Ongoing and future efforts on other enzyme families will reveal if the same approach is more broadly applicable.

Using Transaminases as Dehalogenases?

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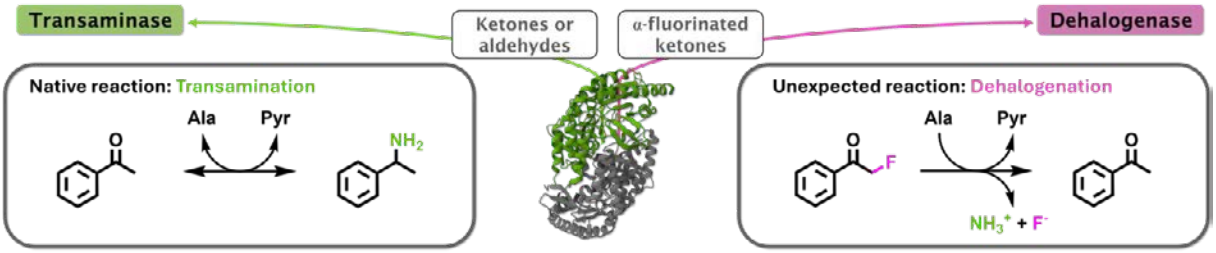
Abstract

Transaminases (TAs) are known for their ability to reversibly transfer amino groups from amino donors to amino acceptors with their cofactor PLP. This transamination is usually highly substrate- and enantioselective, enabling their industrial use for the production of amines as precursors. Recently, the groups of Kroutil, Grogan and Lavandera[1] discovered that TAs surprisingly catalyze the dehalogenation of 2-fluoroacetophenone (2-FA) to acetophenone instead of its reductive amination to the aminated halogenated counterpart. This dehalogenation was proposed to occur in the second half reaction, while the first half reaction was catalyzed as usual with amine donors.



Excited by these findings, we aimed to get a deeper knowledge of this unusual reactivity of TAs. Therefore, we set out to screen a large panel of known and recent TAs (40-50) for the hydrodehalogenation reaction of 2-FA to acetophenone. In the next step, TAs that catalyze the model reaction are compared to find structural similarities and thus requirements within the active site or tunnel to the active site, which enable this dehalogenation instead of the common reductive amination. In addition, a panel of halogenated substrates were screened to get a deeper understanding of structural requirements for the substrates that enable the dehalogenation.

Acknowledgements

The following researchers (including their research groups) are acknowledged for providing plasmids: Uwe Bornscheuer, Robert Kourist, Hans Renata, Wolfgang Kroutil, Francesca Paradisi.

This project has received funding from the European Union's Horizon Europe research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 101150523.

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Microbial and Protein-Based Strategies for Rare Earth Element Extraction and Separation

Peter-Leon Hagedoorn[1], Kristina Djanashvili[1], Rob Schoevaart[2], Mattijs Maris[3], Thomas Barends[4], Lena Daumann[5], Nunzia Picone[6], Mike S.M. Jetten[6], Arjan Pol[6], Huub J.M. Op den Camp[6], Robert A. Schmitz[1]

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Abstract

Rare earth elements (REEs), 17 metals including the lanthanides, scandium, and yttrium, are vital to technologies like electric vehicles, wind turbines, and medical imaging. Their extraction, however, is environmentally damaging, and recycling rates are low. Fascinatingly, some bacteria take up REEs from the environment, yet this emerging field remains largely unexplored. They produce proteins that specifically bind REEs, but the biochemical mechanisms are still poorly understood. We explored REE-protein interactions and protein-based strategies for sustainable REE recovery, focusing on gadolinium (Gd) and neodymium (Nd) as illustrative examples.

Gadolinium, in chelated form widely used in MRI contrast agents, is excreted through urine after use. Current wastewater systems cannot effectively remove and recycle it. To address this, we expressed and purified wildtype and engineered Lanmodulin (LanM) in *E. coli* BL21 cells. LanM, a natural REE-binding protein, showed strong affinity for Gd^{3+}, determined through spectrophotometric competition assays. We immobilized His-tagged LanM on a solid support and are testing its potential to selectively recover Gd from urine using a protein-based and sustainable approach.

Neodymium is a vital part of permanent magnets in electric motors and renewable energy systems. To assess whether this metal is taken up my microbes and interacts with proteins, we aerobically cultured *Methylacidimicrobium thermophilum* AP8 bacteria in chemostats on CH_4 as energy source and 0.5 µM Nd^{3+} (1). X-ray crystallography and ICP-MS revealed 94.5% ± 2.0% Nd^{3+} occupancy inside the methanol dehydrogenase XoxF1. Kinetic analysis confirmed efficient Nd^{3+}-dependent methanol oxidation with a V_{max} of 0.15 ± 0.01 µmol methanol · min^{-1} · mg^{-1} and a K_M of 1.4 ± 0.6 µM.

Together, these examples explore the fundamentals of microbial interactions with REEs and the broader potential of REE-utilizing microbes and their proteins for extraction and recycling of rare earth elements.

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Acknowledgement

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Abstracts for Poster Presentation

P01

Mycoprotein production on grass substrate; optimization and scale-up

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Abstract

The global production of meat is expected to more than double from 229 million tonnes in 1999 to 465 million tonnes in 2050. Considering the well-studied negative effects of livestock production on climate change and the environment, these numbers call for more efficient resource management. Grass is the most abundant crop in the Netherlands, with more than 50% of the Dutch arable land being used as grasslands. Despite the prevalence of grasslands, grass has limited applications beyond the use as ruminant feed given its low economic value. However, the chemical composition of grass, characterized by its high content of nutrients, renders it a suitable substrate for microbial fermentation. The goal of the current project is therefore to assess the performance of submerged fungal fermentation using grass as a substrate for the production of mycoprotein. Growth performance will be assessed in shake flasks before ultimately scaling up to 2L bioreactors. The effect of fungal fermentation on the availability of soluble fibre, soluble protein, and soluble carbohydrate in the grass will also be assessed.

P02

From pathogen to platform: elucidating a microalgae-virus infection for biotechnological solutions

Sofia Amendola[1], Ana Pozo Rodriguez[1], Richard Kormelink[2], Dirk Martens[1], Maria Barbosa[1], Sarah D'Adamo[1]

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Microalgae are a promising platform for industrial biotechnology due to their metabolic versatility and sustainability, as they can use light and CO2 to produce a diverse range of valuable compounds[1]. While microalgae-infecting viruses are commonly viewed as harmful pathogens that devastate production cultures, they are also highly specialized infective agents, capable of modulating their host's cellular and metabolic pathways to redirect cellular resources toward their own survival. This makes them powerful "natural engineers"[2].

Recently, a virus specific to the green marine microalga *Tetraselmis striata* was discovered[3], offering the opportunity to study the infection dynamics of an algal virus in an industrially relevant species. Our study investigates the molecular mechanisms of this viral infection, aiming at understanding how microalgal viruses rearrange the host metabolism and how this can be translated into the optimization of microalgae as cell factories.

Firstly, an infection pipeline was developed using flow cytometry to monitor the infection and a plaque assay to determine viral concentration. This allowed for a synchronized, high virus-to-cell ratio infection in T. striata, from which the transcriptomes of both the host and the virus were obtained. By analyzing the transcriptome, we can understand how the virus hijacks the host's metabolism and, conversely, how the host defends itself against the infection. This research offers a glimpse into the intricate microalgae-virus microcosm, providing valuable insights that can inspire new solutions for microalgae biotechnology. Ultimately, understanding the interplay between T. striata and its native virus can inspire the development of an original engineering strategy to transform this virus-host dual system into an innovative biotechnological platform for recombinant protein synthesis.

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P03

Bulky alkene reduction catalyzed by ene reductases

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Reducing double bonds in a selective manner is still a challenge in classical chemistry. While complex asymmetric catalysts are needed in organic synthesis, enzymes are already equipped to solve these stereoselectivity issues (Lonardi *et al.*, 2023), for example Ene-reductases (EREDs), which are a well-known and described enzyme class. These flavin-dependent enzymes can be deployed in the synthesis of fine chemicals by facilitating the production of key intermediates or products like dihydrocarvone, dihydrocinnalmaldehyde or butanol (from pyruvate)(Toogood and Scrutton, 2018; Kumar Roy *et al.*, 2022).

Usually, the substrates accepted by EREDs present a relatively accessible double bond – with at least one hydrogen and an electron withdrawing group. The introduction of one stereocenter is often reported (Parmeggiani *et al.*, 2022). Notably, (R/S)-carvone, 3-methyl-cyclohexenone and ketoisophorone are employed to characterize this enzyme class.

Our research focuses on investigating the selective reduction of bulky alkenes, enabling the introduction of two stereocenters. By “substrate walking” (Eger *et al.*, 2020), applying various cyclohexenone analogs against 22 different ene-reductases (EREDs), we provide initial insights into the reduction of tetra-substituted double bonds. Enzyme activity and selectivity towards the resulting diastereoisomers are thoroughly examined. Furthermore, an analysis of 3D enzyme models is conducted to identify key structural factors limiting reduction efficiency with bulky substrates, aiming to overcome these challenges. To investigate the potential for industrial application, co-immobilization experiments were performed to introduce an inherent cofactor regeneration system, examined in a large scale reaction setup.

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P04

Unlocking microalgae biotechnology: viruses as a path beyond model species

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2 Wageningen University and Research, Virology chair group, The Netherlands

Abstract

The establishment of microalgae as industrially robust protein production platform is hindered by low productivities and limited genetic engineering tools. Both are influenced by the few model species for which most of algal research is developed, compared to the enormous diversity within this group. In order to significantly advance efforts for transforming microalgae into next-generation cell factories for sustainable production of valuable compounds, we must push the boundaries beyond model species and take advantage of the huge cellular and metabolic biodiversity microalgae offer. Here, we investigate viruses infecting microalgae as a way to more easily expand the molecular toolbox for microalgae, by using their natural capability to efficiently infiltrate and control their hosts molecular machinery. To date, use of viral elements in recombinant applications in microalgae is very underexplored. We focused on a recently described virus infecting *Tetraselmis striata,* because of the small viral genome size and the industrial use of this microalga. Because of the novel status of this virus, we focused on identifying gene expression regulatory elements which can be used to expand the molecular toolbox of *T. striata* and potentially other microalgae. This was done by combining bioinformatic analysis of the viral genome with cloning tools to test the identified elements in vivo in the microalgal host. 24 intergenic regions (IGRs) were identified and analyzed for presence of promoter motifs. The most promising candidate promoter-containing IGRs were tested for gene expression-inducing activity in *T. striata.* At least two novel promoter regions were identified which could promote expression of a reporter gene in the microalgal cells, including that of the major capsid protein, and other candidate promoters are currently being characterized. The data shown here is the first step in harnessing the biotechnological potential of a novel microalgae-infecting virus and developing the first virus-based gene expression platform for microalgae.

P05

Release of fermentable carbohydrates and soluble protein through mild mechanical cell disruption of *Synechococcus sp.* cyanobacteria and subsequent enzymatic hydrolysis

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Abstract

A mild biorefinery approach to release fermentable sugars (glucose) and soluble protein from *Synechococcus sp.* cyanobacterial biomass was developed. Mechanical cell disruption strategies were evaluated and key process parameters, including biomass concentration, time and disruption method, were optimized using a Design of Experiment (DoE) approach. Furthermore, an enzymatic approach was optimized maximizing both glucose and soluble protein release, varying enzyme dosage, pH, temperature and time using a DoE approach. The disrupted and hydrolyzed biomass was used to produce lactic acid via anaerobic fermentation, which is an important chemical building block for bioplastics, food, pharmaceuticals and cosmetics [1]. By replacing conventional fermentation media with glucose and protein-rich cyanobacterial biomass, no arable land or expensive high purity nitrogen-sources are needed for the production of lactic acid anymore, leading to a circular production system, from efficient CO_2 capture to lactic acid.

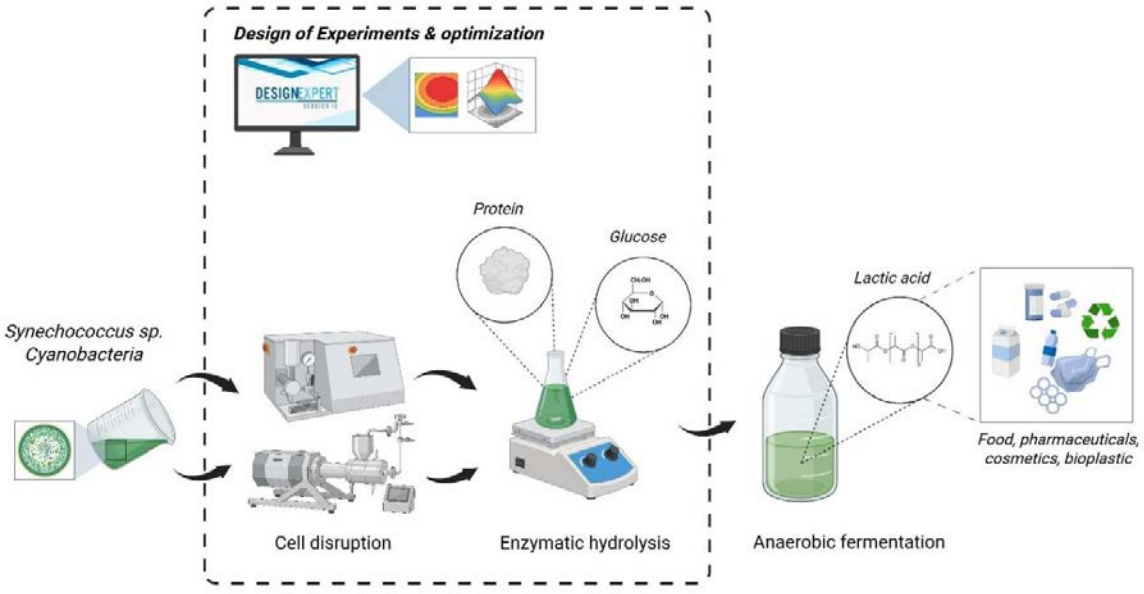

Figure 1: Graphical abstract

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P06

A whole genome sequencing workflow that can be routinely implemented to identify genomic rearrangements and mutations in genetically engineered strains

Claudia de Buck[1, 2], Martijn Melissen[1], Mark Bisschops[2], Ruud Weusthuis[2], Maria Suarez Diez[1]

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Abstract

Genome editing techniques are commonly used in the field of microbial engineering to implement gene knock-outs, knock-ins and other genomic alterations. Validation of the desired changes is a key step and genome edits are typically verified by PCR and gel electrophoresis or PCR-amplicon sequencing. However, these methods miss untargeted variation in other parts of the genome that could influence strain performance and phenotype. To this end, we have developed an organism-agnostic workflow that analyzes long-read sequencing data and reports any variation between a sample and a reference. Visualization of the output is supported and well-described to allow researchers with diverse (non-technical) backgrounds to interpret and analyze the results themselves. Using this workflow we can obtain *de novo* assembled complete bacterial genomes using a commercial sequencing service for a price that is reasonable for routine analyses. We subjected 11 wild-type and engineered prokaryotic strains to our workflow to uncover untargeted genome variation. We found that 8 of the sequenced strains contained undesired mutations (SNPs/frameshifts) or large structural variation including a transposon and large duplications. Moreover, in several strains with one or more plasmids we report plasmid multimerization (5/9 cases), resulting in mixed populations with plasmids up to 4x their original size. All in all, we show how routine whole-genome sequencing uncovers previously unknown genomic variation, which can inform strain selection for physiological experiments as well as accelerate biological interpretation of phenotypic behavior of a genetically edited strain.

P07

SUNPERFORM – Implementing a synthetic carbon fixation route in *Nannochloropsis oceanica*

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Abstract

This project aims to implement synthetic carbon fixation pathways in the industrially relevant microalga *Nannochloropsis oceanica*, with the ultimate goal to improve Photosynthetic efficiency and lay the groundwork for enhanced lipid production. This research exists within the broader ambition of the SUN-PERFORM consortium to engineer microalgae as robust and sustainable bioproduction platforms of solar-fuels. More specifically, this project aims to tackle inherent natural photosynthetic (in)efficiencies when relying in Calvin-Benson-Bassham (CBB) cycle and RuBisCO for CO_2 fixation. It proposes a strategy to overcome these limitations by implementing two synthetic carbon fixation pathways, relying in heterologous and engineered enzymes. These synthetic pathways use bicarbonate as a carbon source, bypassing the limitations of Rubisco, such as its low catalytic efficiency and susceptibility to oxygenation that leads to photorespiration. By avoiding these losses and directly generating acetyl-CoA that can feed lipid biosynthesis without net carbon loss, this approach has the potential to significantly improve photosynthetic efficiency and net carbon fixation. This work will attempt the first implementation of a complete synthetic carbon fixation pathway in microalgae, laying the groundwork for their development as efficient bioproduction platforms and offering a blueprint for future enhancements of photosynthesis in plants. Additionally, we are developing a modular genetic engineering strategy integrated with system-wide omics analyses to establish *Nannochloropsis* as a versatile and powerful photosynthetic chassis

Naduthodi, M.I.S., Claassens, N.J., D'Adamo, S., Van Der Oost, J., Barbosa, M.J., 2021. Synthetic Biology Approaches To Enhance Microalgal Productivity. Trends in Biotechnology 39, 1019–1036. https://doi.org/10.1016/j.tibtech.2020.12.010

Erb, T.J., 2024. Photosynthesis 2.0: Realizing New-to-Nature CO2-Fixation to Overcome the Limits of Natural Metabolism. Cold Spring Harb Perspect Biol 16, a041669. https://doi.org/10.1101/cshperspect.a041669

P08

Towards full scale PHA production on residual streams

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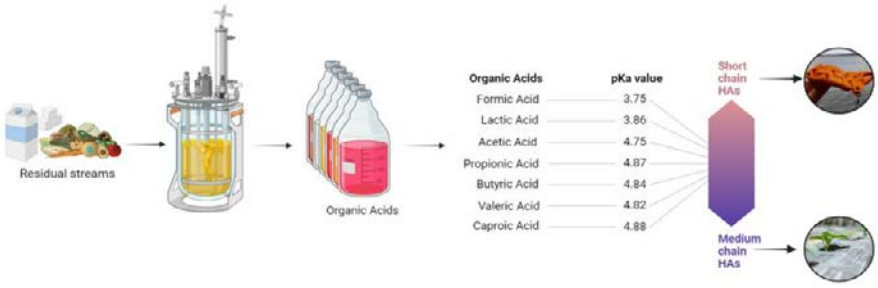
Abstract

The growing urgency of climate change underscores the need to replace conventional plastics with sustainable alternatives. Polyhydroxyalkanoates (PHAs), bioplastics produced by bacteria from volatile fatty acids (VFAs), are a promising option. Yet, large-scale implementation remains hindered by the variability of VFA profiles generated from industrial residual streams, which complicates process control and scalability. This research addresses this challenge by focusing on stabilizing and controlling VFA production from waste substrates to enable reliable, full-scale PHA production with consistent quality. Lab-scale experiments were conducted using different feedstocks under controlled conditions, applying a design of experiments approach to systematically evaluate process parameters. The results show that acclimated inoculum combined with tailored operational settings produce stable VFA profiles suitable for PHA synthesis. Furthermore, data analysis revealed the most effective conditions and confirmed their robustness. Collectively, these strategies provide a pathway towards consistent conversion of industrial residual streams into PHAs, advancing the feasibility of sustainable bioplastic production at scale.

Illustration



Acknowledgement

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P09

Design-Build-Test-Learn guided engineering of the transcription factor-based pyruvate biosensor

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(presenting author underlined)

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Abstract
Whole-cell biosensors are powerful tools for metabolite monitoring, yet challenges such as narrow dynamic range and high leaky expression limit their broader applications. Here, we presented a systematic two-cycle Design-Build-Test-Learn (DBTL) workflow to develop and optimize transcription factor-based pyruvate biosensor in *Escherichia coli*. In the first iteration of the cycle, we constructed a biosensor that exhibited response to intracellular pyruvate levels within 0.05-10mM range. In the second cycle, we implemented design of experiment (DoE) to systematically explore combinatorial effects of promoters and ribosome binding sites (RBSs). A round of fractional factorial design was used to identify factors with a significant effect on biosensor performance, revealing that RBS2 significantly influenced dynamic range by modulating basal and maximum expression, while RBS1 affected signal span. Guided by Akaike Information Criterion (AIC), we identified an optimal model incorporating main and interaction effects. The best-performing strain exhibited an 18.5-fold increase in dynamic range and a 37.2-fold reduction in leaky expression. Quantification of intracellular pyruvate confirmed an operational range of 1.23-6.81 µmol/g DCW. Our work demonstrates the power of DBTL cycles with statistical modelling for biosensor engineering, enabling more precise metabolic regulation and screening applications.

Illustration

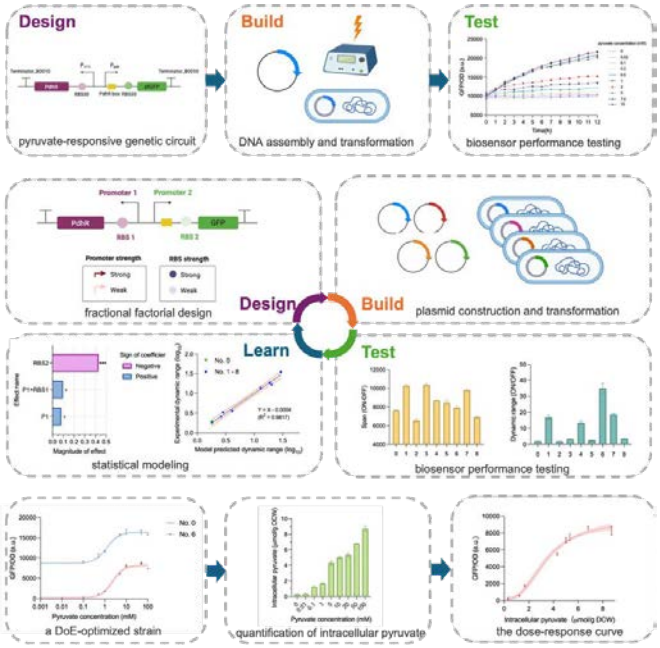

Figure 1. Overview of two rounds of DBTL cycle for the development and optimization of the pyruvate biosensor.

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Acknowledgement
Zihan Gao was supported by the China Scholarship Council (CSC) under Grant No.202304910065. Pieter Candry was supported by the Dutch Research Council (NWO) Veni Talent Programme (File no. 21027) and the Dutch Sectorplan Bèta-II - Biology.

P10

Engineering coexistence in a lignocellulose bioprocessing SynCom using spatial organization

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Abstract

Synthetic microbial communities (SynComs) could allow for greater bioprocessing efficiency compared to single species cultures. The division of labour, where specialist functions are distributed among community members, makes SynComs excellent targets of study for bioprocessing of complex feedstocks. However, challenges remain. The lack of tools to control community composition and long-term coexistence limits their potential in continuous or sequential batch cultivation systems. We aim to overcome these challenges by co-culturing *Pseudomonas putida* KT2440 - an obligately aerobic model organism for metabolic engineering - with *Clostridium phytofermentans* ISDg – an obligately anaerobic cellulose, hemicellulose and pectin fermenter. Encapsulation of both microbes inside spherical hydrogel aggregates provides structural rigidity, altered diffusion rates and potentially higher levels of biomass compared to planktonic culturing. The resulting spatial organization through the formation of an oxygen gradient enforces coexistence and could allow for control of community composition through controlling DO levels. We believe this could be applied for bioprocessing of lignocellulosic biomass into high-value building block chemicals. We demonstrate coexistence when grown on soluble substrates, with a final relative abundance of 57.6% *C. phytofermentans* & 42.3% *P. putida*. Through the spatial organization resulting from an oxygen gradient, this combination of microbes could allow for high-rate bioprocessing of lignocellulosic biomass into valuable platform chemicals, thereby significantly contributing to the establishment of a biobased economy.

Illustration:

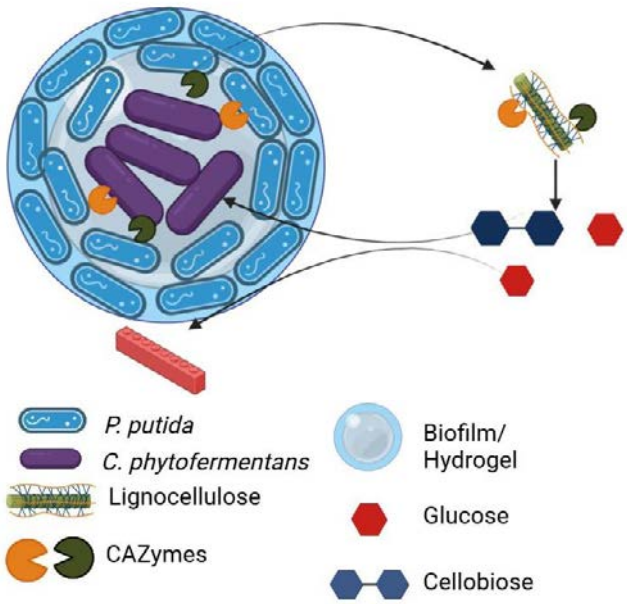

Figure 1: Schematic overview of the proposed spatially organized system. The lego block illustrates the various platform chemicals that could be produced by engineered strains of P. putida KT2440.

P11

Inducible Autolysis: A Platform for Microalgal Multiproduct Biorefineries

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Abstract

Microalgae biorefineries are transitioning from single-product extraction to integrated multiproduct platforms, creating a need for scalable and cost-efficient downstream processing. Enzyme-assisted extraction offers a selective and mild strategy for cell wall disruption, but its application is hindered by the high cost and single-use nature of commercial enzymes. Here, we present a genetic engineering strategy that enables inducible autolysis of microalgal cells, addressing the limitations of conventional enzyme-assisted extraction. This system is based on strain-specific enzymes tailored to the biochemical composition of their cell walls [1]. The enzymes are expressed when activated by a change in media composition once optimal biomass accumulation is achieved. Controlled induction of these enzymes achieves efficient cell wall hydrolysis. This strategy could significantly reduce the cost and complexity of downstream processing, enhance the efficiency of multiproduct extraction, and improve the overall process sustainability. The inducible autolysis platform represents a promising advance for next generation microalgal biorefineries and contributes to the development of circular bioeconomy strategies.

Keywords: Microalgae biorefineries, Cell walls, Enzymes, Optimization, Genetic engineering, Autolysis

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VLAG Africa Talent Programme, under project number 6152011180.

P12

BIOFASHIONTECH TURNING COTTON INTO COLOUR

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Abstract

The textile industry generates yearly 150 million ton of textile waste globally, of which 85% is burned or decomposed while only 15% is recycled. Besides its large carbon footprint, the textile industry is also responsible for 20% of the global clean water pollution. As that an fraction of the dyeing and finishing agents, mainly derived from fossil fuels, that are used during the dyeing process end up in the wastewater. This research validated the applicability of the BioFashionTech concept, see figure 1, through a proof-of-concept run which uses cellulolytic enzymes produced by organism 1, turning waste stream cotton into glucose through enzymatic hydrolysis. The obtained hydrolysate was then used as the carbon source for the cultivation of an extracellular microbial pigment producer(organism 2). The produced microbial pigment can then be used as a biobased dye in the textiles industry. The conditions for the enzymatic hydrolysis were initially optimised using the Box-Behnken Response Surface model, which predicted an optimum cellulose conversion rate over 75%. The highest cellulose conversion rate that was obtained during the enzymatic hydrolysis was less than the predicted optimum at less than 10.0%, revealing a bottleneck in the process. The pigment producer was successfully cultivated on the obtained hydrolysate in all three proof-of-concept runs in shake flasks, reaching a maximum absorbance$_{495\ nm}$ of 0.585±0.024 in 72 hours. Further investigation regarding the nutrient enrichment of the hydrolysate showed that pigment production decreases when nutrients and the biomass production increases under enriched conditions. The obtained results also showed that the biomass increase is not sugar dependent as that a statistically significant increase in biomass was obtained using the nutrient enriched hydrolysate containing only 5% of the optimal sugar concentration. While the obtained cellulose conversion rate during the enzymatic hydrolysis of cut/shredded lab coats was lower than predicted. The second organism was able to produce pigments using the glucose in the hydrolysate. Successfully validating the BioFashionTech concept and contributing to the improvement of the circularity and sustainability of the textile industry by upcycling textile waste into biobased pigments.

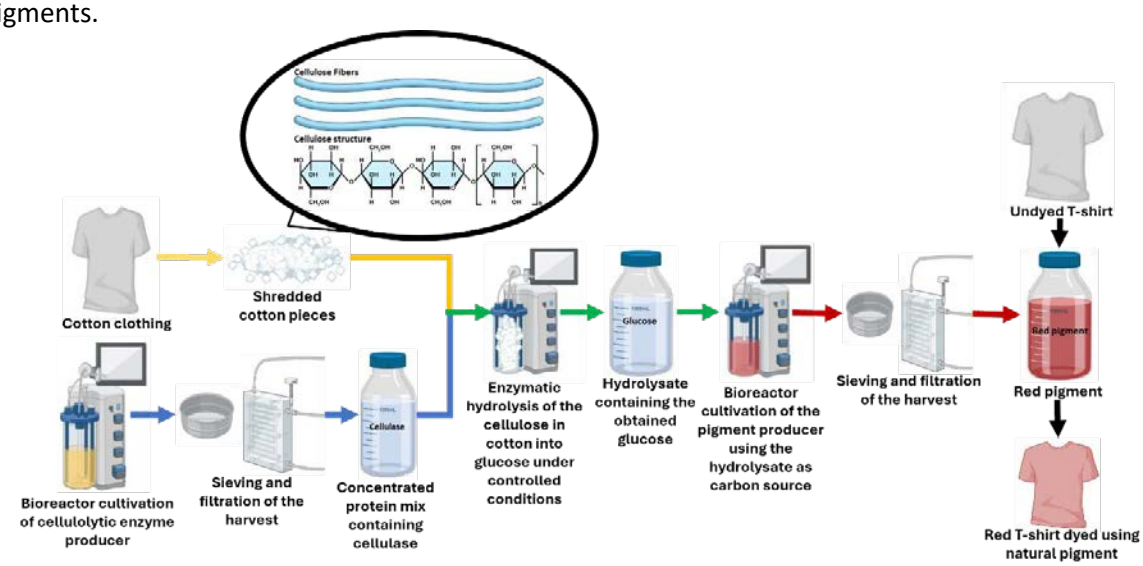

Figure 1: An overview of the BioFashionTech concept. 1) (Yellow) Shredding of cotton into smaller pieces to maximise surface area. 2) (Blue) Cultivation of the cellulolytic enzyme producer and its downstream processing. 3) (Green) Enzymatic hydrolysis of the cellulose present in the cotton fibres obtained in part 1 using the enzymes produced in part 2 to produce glucose. 4) (Red) Cultivation of the pigment producer using the obtain glucose from part 3 as its carbon source. (Figure made in BioRender.com)

COMPARISON OF SINGLE-USE AND STEEL FERMENTORS FOR MALARIA VACCINE EXPRESSION

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Vu Nguyen and David L. Narum, Laboratory of Malaria Immunology & Vaccinology, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892

Abstract

The National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH) has developed a malaria transmission-blocking vaccine (TBV) candidate recombinant Pfs230D1M, which is currently in Phase I/II clinical trials and targeted to transition to Phase III. Successful technology transfer for manufacturing of Pfs230D1M is crucial to the success of clinical trials and is expected to facilitate manufacturing in affected areas of the world. NIH and Thermo Fisher Scientific have collaborated to conduct proof-of-concept studies assessing capabilities of the Thermo Scientific™ HyPerforma™ enhanced Single-Use Fermentor (eS.U.F.) to manufacture Pfs230D1M. The results show quality and quantity of recombinant Pfs230D1M protein expressed in the eS.U.F. were comparable to those achieved with a stainless-steel fermentor. The eS.U.F. enables rapid expansion of vaccine manufacturing with little infrastructure modification, facilitating vaccine delivery particularly to low resource settings around the world.

Introduction

Malaria is a Life-threatening disease caused by Plasmodium parasites, transmitted by the bite of infected Anopheles mosquitoes. Malaria remains a leading cause of morbidity and mortality worldwide. According to World Health Organization, there were 249 million cases and 608,000 deaths due to malaria in 2022 [1, 2].

Recent efforts by the NIH and collaborators have led to the development of a TBV candidate i.e., conjugated recombinant Pfs230D1M with a recombinant ExoProtein A (EPA) which enhances the immunogenicity [3, 4]. In a phase II safety and efficacy trial in Mali (NCT03917654), Pfs230D1-EPA/AS01 induced high levels of antibodies and conferred significant reduction in mosquito infections (>80%) which reduces the risk of subsequent human infections. Conjugated Pfs230D1-EPA is significantly more effective than previous TB malaria vaccine candidates [4]. Currently, a conjugated Pfs230D1 vaccine is in phase I / II clinical trials in progress to transition to a phase III trial as a combination vaccine.

By simplifying technology transfer for manufacturing recombinant Pfs230D1M, the active pharmaceutical ingredient, the vaccine can more easily be produced locally around the world, which may facilitate its delivery to at-risk populations.

Utilization of the eS.U.F. will allow potential production and manufacturing facilities to quickly setup and express the Pfs230D1M protein or other recombinant protein vaccines.

- S.U.F. requires less infrastructure and maintenance than traditional stainless-steel vessels.
- S.U.F. 6L-300L working volumes
- Rushton or enhanced impellers

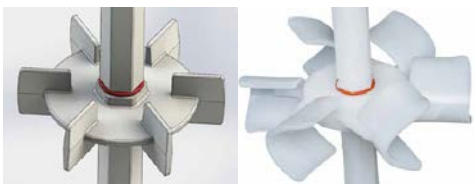

Figure 1. Original S.U.F. impeller design (left) next to the eS.U.F. impeller (right)

Materials and methods

- PTM4, trace salts, and solutions per *Pichia* Guidelines [5]
- Initial batch vol. 18L with 40 g/L Glycerol
- Batch conditions: 30°C, 30% DO, pH 5 (with 28% NH_4OH)
- *Pichia pastoris* Pfs230D1M
- Glycerol batch and feed up to EFT 22h
- Temperature shift 30 to 25°C and pH shift 5 to 3 at the initiation of induction.
- Methanol feed from EFT 24h to 70h
- Expression of recombinant Pfs230D1M protein.
- Final volume 30L
- Supernatant harvested in two 15L batches with CentriPAK™ BPCs using Bios16™ bioprocessing centrifuge at 5,345 x *g* 20 min.

Results

eS.U.F. 30L

- Final wet cell weight 442.6g/L
- Dry cell weight of 98.6g/L.
- Molecule titer and activity were similar to 60L stainless-steel fermentor.

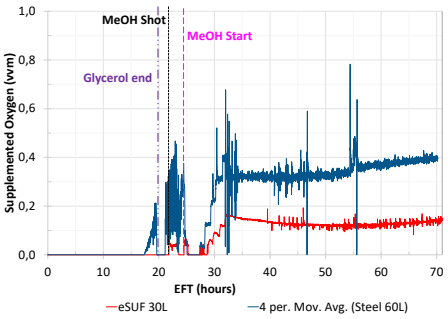

Figure 2. Culture data comparing supplemented oxygen gas flow in vessel volumes per minute (vvm) for the HyPerforma eS.U.F. in comparison to the 60 L working volume stainless-steel fermentor.

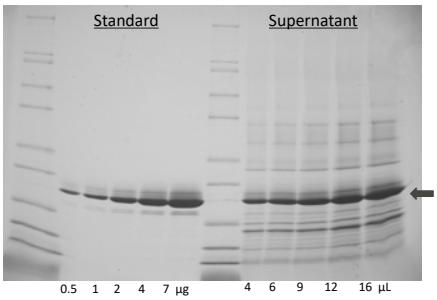

Figure 3. Image of Coomassie blue-stained gel for analysis of eS.U.F. fermentation, which shows good expression of the recombinant Pfs230D1M protein for vaccine use. Achieved with simple single-pass technology transfer.

Figure 4. HyPerforma eS.U.F. 30L and 300L Vessels with TruBio Controller. The volume used would depend on the site scale needs.

Conclusions

The HyPerforma eS.U.F. systems further enable cultivation of *P. pastoris* cultures to high density with similar performance to stainless-steel vessels. In reviewing previous P. pastoris fermentations conducted using a Rushton version S.U.F., the eS.U.F. reduced oxygen consumption by more than ~80% while reaching similar cell Mass [6,7].

Increasing access to this more effective TBV malaria vaccine, would be a step towards the goal of achieving herd immunity and eliminating Malaria.

- It is estimated that from each eS.U.F. 30L culture 30,000 vaccine doses can be produced.
- This technology transfer from 60L stainless-steel vessel to eS.U.F. was successful in first run.
- Stainless-steel 60L used twice as much O_2 / L of culture

As the eS.U.F. 30L only requires electricity and compressed gasses to operate, it is possible the system could produce vaccines closer to susceptible populations. This would help to reduce the costs of vaccine production while increasing availability [8].

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P14

The value of a Designer in Bioprocess Engineering

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Abstract

Delft University of Technology offers Engineering Doctorate (EngD) programs 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. 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.

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www.tudelft.nl/engd

P15

Fast Estimation of Growth Kinetic Factors of *E. coli* in a 96 Well Microtiter Plate Applying a New Launched Online Measuring Device

Mykhaylo Semenov Petrov[1], Laura Civil Ferrer[1], Ivan Schlembach[1], Andreas Schulte[2], Juan Camilo Porras Correa[2], Tibor Anderlei[3], Suresh Sudarsan[1].

[1]DTU-Biosustain, Denmark; [2] Kuhner Shaker GmbH., Germany; [3]Adolf Kühner AG, Switzerland.

Abstract

The rational development of aerobic cell factory-based processes necessitates a comprehensive understanding of substrate and oxygen uptake kinetics. Conventional methods, such as substrate-limited chemostats or constant-feed fed-batch experiments, can be employed to determine kinetic parameters like μ_max, K_s, Y_x/s, and Y_o/x. These methods are labor-intensive and thus require prompt assessment of such parameters from micro-scale cultivations.

In this study, we utilized the state-of-the-art μ-scale cultivation device (Kuhner microTOM), determining in each well of a 96 deepwell microtiterplates the oxygen transfer rate curves. Three distinct experiments were performed (i.e., with varying glucose concentrations, pulsing different glucose concentrations during starvation, and varying filling volumes) with this device to estimate the pertinent growth kinetics of tyrosine-producing *Escherichia coli* strain. From these experiments, we estimated the substrate affinity constant (Ks) value of 9.6 mg/L for the tyrosine-producing strain, while the background strain with and without the PTS glucose uptake system exhibited values of 3.7 mg/L and 2.3 mg/L, respectively. Estimating Ks values and other kinetic parameters, early in the bioprocess will be beneficial in predicting the diverse metabolic regimes that a cell would undergo in an industrial-scale bioreactor and perform appropriate scale-down experiments to develop a robust bioprocess.

P16

Multiparameter Sensors and DO Sensor Pills for Shake Flasks: Removing Black Boxes for Improved Bioprocess Development

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Abstract

Shake flasks are widely used in early-stage process development due to their increased cost- and space-efficiency compared to bioreactors, which grants the ability to test multiple conditions in parallel more easily. However, shake flasks differ significantly from industrial production bioreactors in their design, operation, and size, and their utility has been limited by a lack of compatible sensor technology for real-time, non-invasive monitoring. We aim to close the data gap between shake flasks and bioreactors. Therefore, we developed a novel Multiparameter Sensor (MPS) and Dissolved Oxygen (DO) Sensor Pills, which can be combined with our Liquid Injection System (LIS) and DOTS software for control. The current sensor-design enables seamless integration of future developments such as pH- and glucose-monitoring.

We introduced Pichia pastoris as an expression host in our lab for enzyme production for future sensor developments. We started working on production before the new sensors were available and the process optimization was performed via trial and error. The optimization used >200 LIS cartridges in 26 experiments with 4-8 variations over the course of approximately one year. The DO feedback-controlled methanol induction yielded the same results in one week with 6 parallel experiments. Volumetric yield was increased from 3.8 µg/ml to 34.7 µg/ml with 25 ml of culture in shake flasks. Overall, the combination of the MPS with DO Sensor Pills and the Liquid Injection System allowed much faster process development in small and intermediate scale.

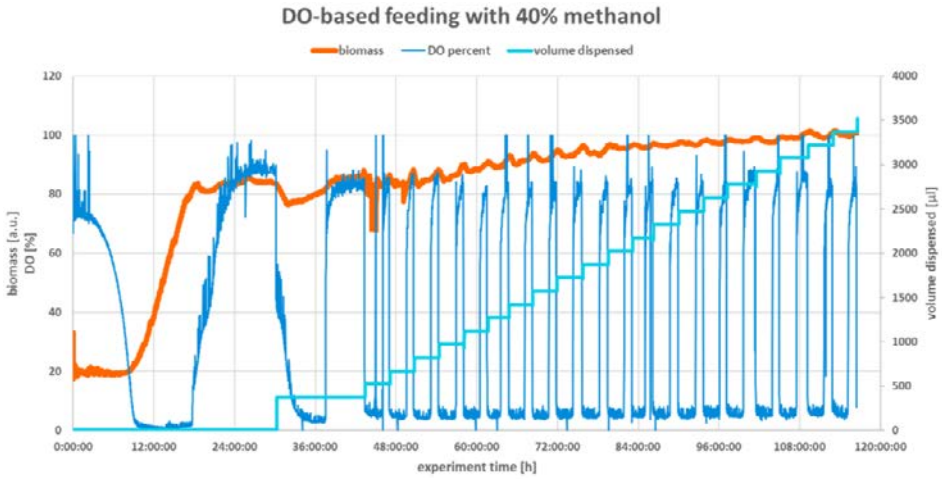

Figure 1 – Exemplary Measurement Data of Multiparameter Sensor (MPS) in combination with DO Sensor Pills for online Biomass and Dissolved Oxygen (DO) data. DO-controlled feeding allowed for optimized, automated addition of methanol into the shake flask culture.

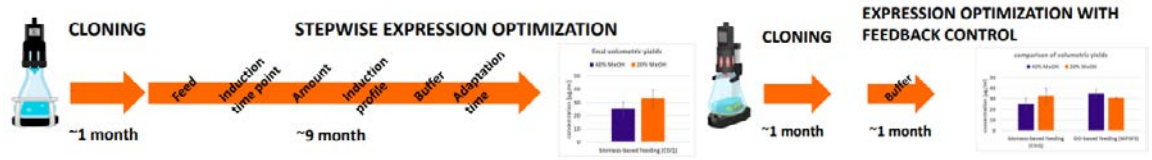

Figure 2 – Using automated DO-controlled methanol feeding, the volumetric yield increased from 3.8 µg/ml to 56.5 µg/ml in shake flasks. The combination of the MPS with DO Sensor Pills and the Liquid Injection System allowed much faster process development in small and intermediate scale

P17

Unveiling the Midpoint Potential of Chanoclavine Synthase: A Novel Superoxide-Driven Haem Enzyme

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Ergoline forms the core structure of ergot alkaloids, which are extracted from fungi and used to treat various diseases. It is structurally similar to several neurotransmitters, enabling their modulation of neurotransmitter receptors. The haem catalase chanoclavine synthase (EasC) catalyses the oxidative cyclization essential for ergoline biosynthesis. However, the exact mechanism of EasC biocatalysis remained unknown. Using cryo-electron microscopy, we resolved the structure of EasC from the fungus *Claviceps fusiformis*, revealing that the substrate prechanoclavine unexpectedly binds in the NADPH-binding pocket rather than the haem-binding site (Chen *et al.*, 2025). In contrast to typical haem enzymes, we showed that EasC uses superoxide instead of canonical transient haem iron–oxygen complexes. The superoxide generated at the haem consequently transforms the substrate at the distant NADPH-binding pocket to form the central C ring of ergoline. To assess the thermodynamic feasibility of this superoxide-mediated reaction, we determined the midpoint potential (E_{m7}) of EasC using a modified spectrophotometric xanthine oxidase reduction assay within an anaerobic glovebox, yielding an E_{m7} of -103 ± 5 mV. We propose that the superoxide-generating mechanism catalysed by EasC could be widespread in metalloenzyme-catalysed reactions. Additionally, we demonstrate that the xanthine oxidase assay is a valuable tool for determining the redox potential of novel haem enzymes.

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P18

Diversity of α-acetolactate decarboxylase in the Saccharomycotina yeast subphylum: From discovery to brewing application.

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Abstract

Diacetyl, a vicinal diketone with a low sensory threshold, is a prominent off-flavour in beer, necessitating extended lagering to allow its reduction to non-flavour-active compounds. In brewing, bacterial α-acetolactate decarboxylases are commonly used to mitigate diacetyl formation by converting its precursor, α-acetolactate, directly into acetoin. Here, we report the first discovery and characterization of functional α-acetolactate decarboxylases enzymes of eukaryotic origin, specifically from yeasts within the Saccharomycotina subphylum. Using a homology-based search against fungal genomic databases, 29 candidate genes were identified across 18 yeast species from only three genera (*Lipomyces*, *Dipodascus* and *Wickerhamiella*) and classified into distinct phylogenetic groups. Phylogenetic analysis revealed both fungal and possible bacterial origins, suggesting evolutionary conservation and horizontal gene transfer events. Seven genes were heterologously expressed in *Saccharomyces pastorianus* lager brewing strains. Fermentation trials in both lab-scale septum flasks and E.B.C. tall tubes demonstrated that yeast-derived α-acetolactate decarboxylases significantly reduced diacetyl levels, with some performing comparably or superior to the benchmark *Brevibacillus brevis* enzyme. These strains also showed normal fermentation kinetics and produced beers with diacetyl concentrations below sensory thresholds, effectively eliminating the need for extended lagering. Our findings uncover a previously unrecognized enzymatic activity in budding yeasts and present yeast α-acetolactate decarboxylases as promising non-bacterial alternatives to improve process efficiency and sustainability in lager beer production.

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P19

Monitoring and Analysis of a pH Optimization Approach for CHO Cell Cultivation via BioNsight® cloud

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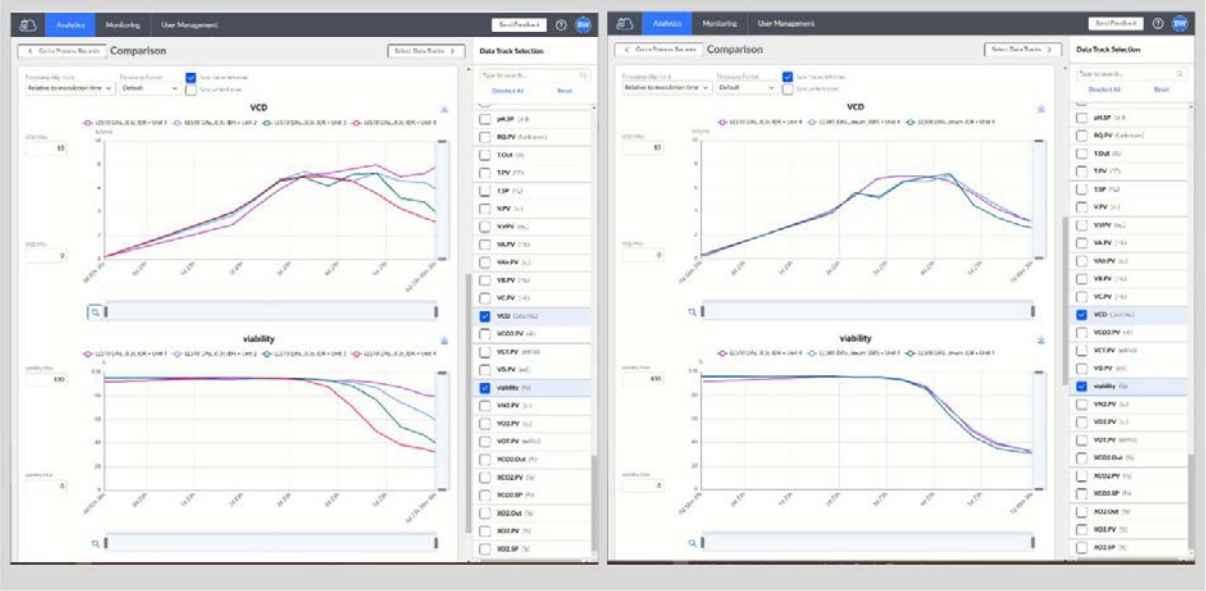
Abstract

In the competitive field of bioprocessing, efficient data management and analysis are essential for innovation and operational excellence. Eppendorf has developed a cutting-edge cloud solution that integrates real-time monitoring, secure data storage, and advanced analytics within a scalable and user-friendly platform. This solution, BioNsight cloud, enhances bioprocess workflows by providing immediate insights, ensuring data integrity, and supporting sophisticated analytical techniques. Designed to facilitate collaboration and adapt to growing needs, our cloud platform empowers bioprocessing professionals to achieve greater efficiency, accuracy, and innovation. In our example, BioNsight cloud was used to monitor and analyze CHO cell cultures growing under different pH conditions on a small scale using the DASbox® Mini Bioreactor System. The optimal pH for cell growth and high viability, as determined at small scale, was then applied at bench scale using the SciVario® twin bioprocess controller. BioNsight cloud simplified the pH optimization process in many ways, particularly through easy real-time monitoring of parallel reactors, data tracking, and data analysis.



BioNsight cloud analytics tab – overlay of data tracks for comparison of viable cell density and viability in 4 vessels with 4 different pH setpoints, allowing for quick evaluation of process performance within one experiment (left) or between biological replicates (right: 3 reactors cultivated at pH.SP 7.2).

P20

StrainoScope: A Streamlined Nanopore Metagenomics Pipeline for Strain-Level Classification

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Abstract

Metagenomic sequencing has become a cornerstone in modern biological research, with applications spanning medicine, biotechnology, and environmental sciences. One of its most promising uses is the analysis of complex microbial communities, where mixed cultures can be profiled to gain insights into their taxonomic composition, functional potential, community dynamics. Such knowledge is invaluable for understanding host-microbe composition in health and disease, optimizing microbial communities for industrial processes, and monitoring environmental systems. However, several technical and analytical challenges remain. Among the most critical is the accurate classification of microorganisms at the strain level, a task complicated by the high degree of genetic similarity between closely related organisms. In addition, the wide array of available bioinformatics tools can make it difficult for researchers, particularly those without advanced computational expertise, to select, configure, and integrate the most appropriate methods for their datasets.

To address these issues, we developed StrainoScope, a streamlined metagenomics pipeline specifically designed for strain-level classification and microbial community profiling. StrainoScope integrates a curated set of publicly available tools into a unified, user-friendly framework, lowering the barrier of entry for non-specialists while retaining flexibility for advanced users. By applying optimized parameters and tailoring outputs across tools, StrainoScope achieves high-resolution classification that closely reproduces the known composition of microbial mock communities. Moreover, its performance in continuous bioreactor experiments demonstrates its ability to track microbial dynamics over time, revealing clear patterns in the rise and decline of specific strains. Taken together, StrainoScope provides researchers with an accessible yet powerful solution for advancing strain-resolved metagenomic studies across diverse biological domains.

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P21

Implementing an ATP-battery in *N. oceanica* for improved photosynthesis

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This study addresses the challenge of decarbonizing aviation and shipping sectors by developing cost-effective microalgae-based biofuels. The biggest disadvantage of producing triacyl glycerols (TAGs) using microalgae as a production platform is the high cost compared to the production of fossil fuels or land-grown crops. Therefore, it is essential to increase efficiency and productivity of the process while bringing down costs. The SUN-PERFORM, an EU-Horizon project, aims to achieve exactly this by using several consecutive approaches. The first is the use of nanocrystals that are capable of down-converting light energy and therefore modulate the wavelength of sunlight to wavelengths that can be efficiently used by microalgae. The second approach pioneers the implementation of an ATP-battery that enzymatically converts ATP into high-energy storage molecules, such as arginine phosphate or creatine phosphate. These molecules will act as a reservoir for cellular energy and enhance metabolic efficiency. The third approach is to genetically engineer the microalgae to direct the metabolic flux to production of lipids.

This study will introduce the general concept of SUN-PERFORM, and its focus on the genetic implementation of an ATP-battery in *N. oceanica.* This was achieved by introducing heterologous arginine phosphate kinase enzymes into the microalga. These enzymes act as an ATP buffer by temporarily storing energy: they use ATP to convert arginine and phosphate into arginine phosphate, which can later be converted back to release ATP when energy demand increases. Preliminary growth experiments in light-saturating conditions show an improved growth rate for mutants containing the arginine kinase enzymes.

The ATP-battery system specifically aims to reduce the photo-inhibitory effect caused by excessive light, which normally triggers non-photochemical quenching (NPQ) and it reduces photosynthetic efficiency. While NPQ protects cells from photodamage, it diverts the absorbed energy away normally used from photochemical reactions, ultimately lowering the photosynthetic efficiency and reducing growth rates and lipid production of the microalgae. By alleviating these energy losses, the ATP-battery system can enhance photosynthetic performance, leading to improved microalgal growth and lipid production.

P22

HSF Next-Generation Sequencing: direct capturing methylation, single molecule ssRNA viruses and ultra-resolution microbiome MAGs

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Abstract

At the Hanze Sequencing Facility (HSF) of the Hanze University of Applied Science, we have established a versatile and high-performance Next-Generation Sequencing (NGS) platform with both Oxford Nanopore Technologies (ONT) MinION and illumina systems. Hereby, we present 3 representative case studies to illustrate our expertise in combining sequencing with data science supports innovative research in biomedical, agricultural, and environmental biotechnology.

Case study 1: Direct methylation measurement in Jurkat cancer cell lines

Making use of the single-molecule sequencing technology of ONT MinION, we evaluated the anti-cancer effects of natural bioactive compounds—such as betulinic acid from birch bark and curcumin from Curcuma longa—by analyzing apoptosis-related methylation patterns in Jurkat cancer (T-cell leukemia) cell lines. The directly measurement of methylation with MinION bypassed the error-prone and costly bisulfite reactions required by the Illumina system. Our results explicitly show that betulinic acid altered the overall methylation patterns by 16.7%, demethylated silenced tumor suppressor p53 gene and pro-apoptotic genes (like NOXA and PUMA), which could help restore apoptosis in cancer cells.

Case study 2: Direct sequencing of single molecule ssRNA viruses in bee blood

The global decline in biodiversity is accelerating, with *Apis mellifera* (Western honeybee) populations particularly affected by invasive species and viral pathogens. We developped a pipeline for viral diagnostics by directly sequencing single molecule ssRNA in bee blood (haemolymph). Using the MinION, a 11kb viral genome of Deformed Wing virus was characterized out of millions of host transcripts. These findings refine methodologies in agricultural virology and enhance virus surveillance by enabling the design of more accurate qPCR primers.

Case study 3: Ultra-resolution MAGs of soil microbiome

We have combined MinION and Illumina systems, and sequenced microbiomes collected from the Arctic Island of Spitsbergen at two time points spaced seven years apart. Integrating WGS data generated from both systems revealed ultra-resolution Metagenomic Assembly Genomes (MAGs) of all the species present in the sample, differentiating closely related strains, which is unachievable with solely MinION or Illumina system. The complete MAGs allowed for more accurate estimates of species abundance and sample diversity indexes giving a more comprehensive view of the Arctic microbiome evolution under climate changes and for mining of novel enzymes.

P23

De novo NMN overproduction in *Escherichia coli* as noncanonical redox cofactor

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Abstract

The production of bulk chemicals using microbial cell factories is a sustainable alternative for the petrochemical industry. Unfortunately, many microbial production processes are not yet efficient enough. Ideally, the product yield should approach the maximum theoretical yield in which all electrons of the substrate end up in the product. Thus, we need to be able to steer the electron flow. To direct electrons exclusively from substrate to product, Weusthuis et al. (2020) proposed to use noncanonical redox cofactors (NRC)[1]. As a potential NRC, NMN could be used. NMN is present in *Escherichia coli* as a degradation product of NAD^+. Unfortunately, cellular NMN levels are naturally too low to function as a redox cofactor in *E. coli*[2]. Therefore, cellular NMN levels need to be increased.

Until now, research mostly focused on NMN biosynthesis via routes requiring additional precursors in the medium, thereby increasing costs of these processes[3]. To reduce production costs, NMN should be produced *de novo*. To date, two alternative routes have been explored to increase NMN levels. One approach involves *E. coli*'s native nucleoside triphosphate diphosphatase MazG, the other the heterologous expression of NadE from *Francisella tularensis.* However, previously reported intracellular NMN levels are too low to function as redox cofactor.

Here, we show that cellular NMN levels can be greatly increased when we produce NAD^+ from NMN. To achieve this, we first constructed an NAD^+ auxotrophic *E. coli* strain. This strain was used to test alternative NadE enzymes from different microorganisms for NMN producing activity. Several NadE enzymes were found that restored growth in our NAD^+ auxotroph. Finally, deletion of the NMN amidohydrolase PncC reduced NMN degradation, resulting in significantly increased NMN levels in our *E. coli* strains.

Our results show that NMN can be produced to high intracellular levels *de novo*. Furthermore, this research gave more fundamental insights in $NAD(P)^+$ biosynthesis of *E. coli*.

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P24

Selective reduction of allenes with OYE enzymes

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Abstract

Old Yellow Enzyme (OYE) families are flavin-dependent enzymes that catalyse asymmetric hydrogenation reaction [1]. The conventional substrate range of OYEs includes activated alkenes with an electron withdrawing group (EWG) such as aldehyde, ketone, and similar functional groups. OYEs were recently shown to reduce also non-conventional substrates, such as oximes [2,3]. The ability of ene reductases to catalyse the reduction of other compounds, such as allenes, has not yet been investigated. In this study, a range of allene substrates with several different OYE enzymes are explored. Initial results show complete conversion within 4 hours of 10 mM ethyl-2,3-butadienoate to ethyl-3-butenoate using OYE enzymes OYE2 and OYE3 (Figure 1).

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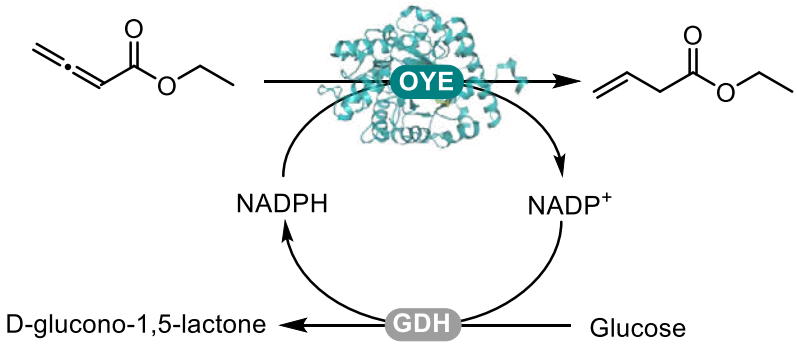

Figure 1. OYE-catalysed reduction of ethyl-2,3-butadienoate to ethyl-3-butenoate using NADPH as co-factor and GDH as recycling system for the co-factor.

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P25

From Waste to Value: A Systems Biology Investigation of *Pycnoporus sanguineus*

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Abstract

The valorization of agricultural and food industry by-products into high-value compounds represents a cornerstone of the burgeoning circular bioeconomy. Lignocellulosic waste, a recalcitrant material due to its high lignin content, is a prime candidate for this conversion. The use of microbial cell factories has emerged as a key strategy to facilitate this process.

Among the most promising of these biological agents is the white rot fungus *Pyncoporus sanguineus*. Known for its ability to degrade lignin, *P. sanguineus* also produces a variety of bioactive compounds with significant antimicrobial, antioxidant, and anticancer properties. Despite this potential, the full scope of its metabolic capabilities remains largely unexploited.

To address this, genome-scale metabolic models (GEMs) are being employed. These computational frameworks offer a comprehensive overview of an organism's metabolic network and have been instrumental in the rational design and optimization of microbial cell factories. Through methodologies like flux balance analysis (FBA) and its dynamic counterpart, dFBA, GEMs enable the simulation of microbial growth and metabolic activities, providing valuable insights for metabolic pathway optimization and strain engineering. This research, therefore, focuses on the construction of a GEM for *P. sanguineus* with the ultimate aim of developing novel bioprocesses for waste valorization and identifying new metabolic pathways with potential pharmaceutical and industrial applications.

Acknowledgement

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P26

Plant virus-derived VLPs for biotechnological applications

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Abstract
Promoting human health requires availability of affordable solutions to health problems. Viral epidemics or pandemic outbreaks, on top of chronic and emergent diseases, not only demand rapid pharmaceutical measures but also require developing novel therapeutics and alternative production and delivery approaches. In this context, nanoparticles have been revealed as high-value pharmaceutical tools, as they can act as nanocarriers of a variety of therapeutic cargoes, such as drugs, genes, antigens, and tissue-specific agents. However, currently developing nanoparticles are mostly based on synthetic platforms that involve complex production processes. As an alternative, virus-like particles (VLPs) are naturally occurring virus-shaped and genome-free nanoparticles that can be tailored to act as nanocarriers of a diverse range of cargoes in medicine. Plant virus-derived VLPs have advantages over other commonly used VLP platforms (e.g., based on mammalian viruses and mammalian cell production systems). For instance, plant viruses are unable to infect humans, lowering the risk of a pre-existing immunity during eventual application.

In this NWO-funded project, we aim to create a universal plug-and-play toolkit comprising various (enveloped/non-enveloped/icosahedral/helical) plant virus-derived VLPs for use in various biotechnological applications, such as by acting as scaffold for vaccines (Figure 1, WP1 Task 1), or by enabling encapsulation of cargoes (Figure 1, WP1 Task 2). Our recent data has demonstrated the limitations of using icosahedral VLPs for genetic fusion of a desired protein/peptide. For instance, CSDaV VLPs could be decorated with GFP using the SpyTag/SpyCatcher (ST/SC) system. However, genetic fusion of the CSDaV coat protein with either the ST peptide or SC domain prevented self-assembly into VLPs, likely due to steric hinderance, unless co-expressed with unfused coat protein thereby lowering the decoration efficiency. Future experiments will explore chemical conjugation as a way to improve the decoration of icosahedral VLPs.

Another aim in this project is to establish a microalgae-based production platform for the developed plug-and-play VLP toolkit (Figure 1, WP2). Microalgae are unicellular photosynthetic eukaryotes that can be easily cultivated in closed photobioreactors using water, light, and carbon dioxide, and show additional advantages over plant cell bioreactors, such as high growth rates, protein secretion, and fast genetic engineering that will boost production levels.

The outcome of this project will be a powerful platform that combines plant virus-derived VLPs and a microalgae production system to enable fast and customizable biopharmaceutical engineering.

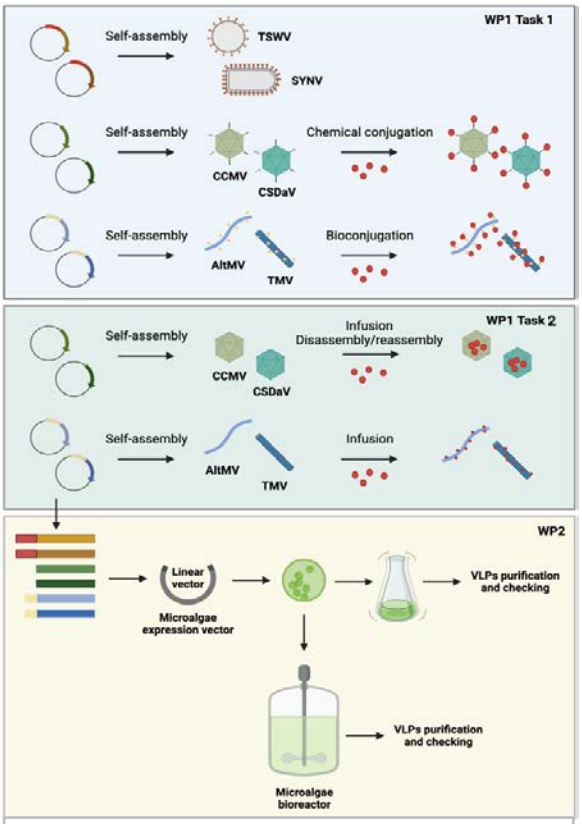

Figure 1: Overview of the workflow and output of the project. In workpackage (WP) 1 Task 1, VLPs derived from tomato spotted wilt virus (TSWV), sonchus yellow net virus (SYNV), cowpea chlorotic mottle virus (CCMV), citrus sudden death-associated virus (CSDaV), tobacco mosaic virus (TMV), alternanthera mosaic virus (AltMV) will be designed to act as vaccine scaffold while in WP1 Task 2, they will be as tailored for cargo encapsulation. In WP2, a microalgae-based production platform will be established.

P27

Hydrogen-driven recycling of NADPH for enzymatic chemical synthesis

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Abstract

Most modern chemicals are synthesized by toxic metal catalysts, which could be replaced by biodegradable biocatalysts. However, nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) cofactors are required for many biocatalytic reactions to proceed successfully. Since stoichiometric addition of NAD(P)H is not economically feasible, systems for NAD(P)H regeneration have been developed and implemented. The most commonly used are glucose dehydrogenases (GDH) or alcohol dehydrogenases (ADH) with glucose or isopropanol, respectively, as sacrificial electron donors [1]. High costs and accumulation of waste products of those molecules make the system less suitable for chemical manufacturing.

To overcome these disadvantages, we aim to develop and implement an NADPH recycling system that uses H_2, containing the *Desulfovibrio desulfuricans* [Fe-Fe]-hydrogenase (DdHydAB) and the *Thermosynechococcus vestitus* ferredoxin (PetF) and ferredoxin-NADPH reductase (PetH) (**Figure *1***). By using H_2 as a sacrificial electron donor, the recycling of NADPH becomes a greener process using a renewable electron source without producing any byproducts, making it 100 % atom efficient.

The [Fe-Fe]-hydrogenase is responsible for the heterolytic cleavage of H_2. The electrons will subsequently be transferred via the PetF to the PetH, which will then reduce $NADP^+$ to NADPH. We have successfully produced and purified both [FeFe]-hydrogenase and ferredoxin-NADPH reductase (FNR). Furthermore, we demonstrate enzymatic activity for the matured DdHydAB. Henceforth we are planning to produce the ferredoxin and make the system work in solution. Next steps will be the immobilization of DdHydAB and PetH, which we can then be linked to NADPH-dependent biocatalytic reactions.

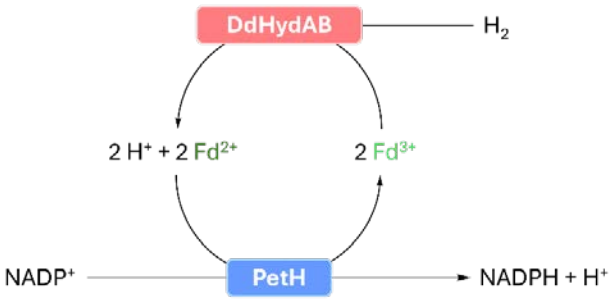

Figure 1: Hydrogen-driven enzymatic recycling of NADPH.

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P28

Online Monitoring and CO2 Control Fusion: Improving Microalgae Cultivation in Pilot-Scale Photobioreactors

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Abstract

Microalgae's ability to fix carbon photosynthetically and accumulate bio-compounds makes them attractive to the market while also promising allies for the sustainable future transition. However, large-scale microalgae cultivation is limited by the value of the target product produced to cover the production costs. The industrial photobioreactors in which they are grown present more challenges than the lab-scale ones, such as gradients, transport phenomena and higher retention times. Monitoring and control systems are responsible for operating and maintaining the culture in the desired conditions. Currently, basic pH control by sparging CO2 is the most common, but there is much room for improvement to achieve resource-efficient processes. In this study, an improved interconnexion between the CO2 monitored signal and control system is pursued. To address that, a novel two-PID strategy together with digital filters was developed and validated in a pilot-scale tubular photobioreactor. A digital model of the real system was designed by identifying the transfer functions from CO2 injectors, mimicking the behaviour of *Tetradesmus obliquus*. The validation was performed during growth and accumulation phase, triggered by nitrogen-depletion. A reduction of the 60% of the CO2 integrated absolute error, together with a reduction of 46 minutes in settling time were achieved. Signal noise was also reduced substantially.