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Book of Abstracts

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Rectorate of Universidade NOVA de Lisboa

Lisbon, Portugal

NOVA SCHOOL OF SCIENCE & TECHNOLOGY

Dear Participants,

We are thrilled to host you for an exciting gathering of experts, researchers, and industry professionals from around the world to participate in the **12th European Symposium on Biopolymers (ESBP 2025)**.

ESBP2025 is dedicated to advancing knowledge and fostering collaboration in the field of biopolymers. Throughout the conference, you'll have the opportunity to engage in insightful lectures, workshops, poster presentations, and scientific discussions, while also enjoying networking opportunities.

We hope you have a rewarding and inspiring experience. We also encourage you to take some time to explore the beautiful city of Lisbon, rich in culture and history.

Thank you for joining us, and we look forward to making ESBP2025 a memorable and impactful event together!

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PL1 KANEKA Biodegradable Polymer Green Planet ® From CO_2 as a Carbon Source

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We have been developing biobased and biodegradable polymer " KANEKA Biodegradable Polymer Green Planet ® " for creating circular bio-economy for over 30 years and has already been put on the market in a variety of applications such as straws, cutlery, coffee capsules, bags, cushioning materials and paper coatings. Green Planet is a random copolymer consisting of (*R*)-3-hydroxybutyrate and (*R*)-3-hydroxyhexanoate as monomers and called PHBH (Fig.1). This polymer is polymerized by enzyme and accumulated in microbial cell body from renewable carbon sources such as vegetable oils and sugars. From 2023, we started the new project to realize carbon recycle polymer economy by using CO2 as carbon source of Green Planet. In this project, we will develop PHBH production pathway on hydrogen oxidizing bacteria and gas fermentation process for industrial production (Fig. 2).

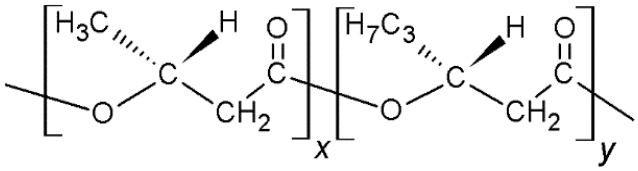

Fig 1. PHBH

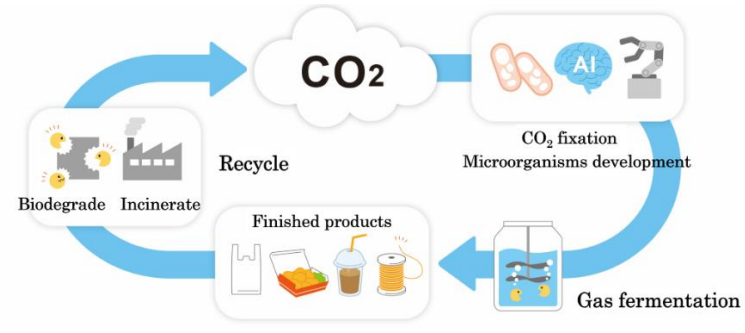

Fig 2. CO2 based carbon recycle polymer economy

PL2 How Biopolymer Design, Development and Production Can Benefit from Machine-Learning.

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Many consider the convergence of synthetic biology, metabolic engineering and machine-learning to be at a pivotal moment. With met seem to render possible the rational design of biopolymers (as opposed to trail and error), the engineering of the production pathways and host as well as more cost-efficient process development and manufacturing.

Starting from a short introduction into machine-learning, this contribution will revisit machine-learning applications for biopolymer design and process development. Opportunities for machine-learning in pathway and host engineering as well as biopolymer production will be highlighted. Examples from biopharma for on-the-fly process development and knowledge transfer will be shared aiming at an across sector exchange of visions and opportunities. In particular, it will be shown how process behavior can be learned by a machine-learning model while the process is running and how the model in turn can be used to derive more information by tuning the process conditions. Also, it will be shown how machine-learning models can be used to transfer knowledge across scales, providing an understanding of how changes in the process conditions at the larger scale impact on process performance. Lastly, the opportunity to use these models for advanced process control will be discussed.

PL3 Assessing Biotechnological Pathways for Biopolymer Production, Application, and Recycling

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Biotechnology applied to manufacturing bio-based products can be part of the solution to address many societal and environmental challenges.[1] Yet, despite significant advancements, biopolymers from renewable feedstocks remain less competitive than conventional plastics in terms of technology readiness level, scale, and market adoption. Besides the development of circular-by-design polymers and sustainable bioprocesses, their application and product performance are critical to increased market acceptance. From an end-of-life perspective, the use of enzymes and microorganisms as biocatalysts in waste management may facilitate recycling of post-consumer plastic waste, for example.

This presentation will explore different biotechnological pathways to produce, apply and recycle biopolymers and products. Specific examples of biopolymers and building blocks will be presented, which include fermentation of biomass and greenhouse gases (GHGs), enzyme technology, and bio-hybrid processes. The discussion will integrate environmental and techno-economic assessments to identify hotspots across product lifecycles and technology pathways as well as the conditions under which biopolymers and biotechnologies could achieve competitiveness.

PL4 Bacterial Cellulose: State of the Art

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Bacterial cellulose (BC) is a unique exopolysaccharide synthesized by certain bacteria, most notably *Komagataeibacter* species, forming a three-dimensional nanofibrillar network [1]. The exceptional characteristics of BC have positioned it as a highly sought-after biomaterial with a vast and expanding range of potential applications across diverse sectors [2]. However, despite its promise, BC has yet to reach mainstream. Many attempts have been made towards economic BC production, mainly by optimizing culture media and fermentation systems using agitated, airlift, membrane and horizontal bioreactors, strain selection. These efforts had limited success due to the low productivity rates, high capital investment and high operating costs [3]. In this presentation, recent efforts to improve the production of BC through genetic engineering and bioreactors design innovation will be addressed. The development of applications in cosmetics, biomedicine, food packaging and textiles will also be reviewed. Finally, perspectives on the sustainability and technoeconomical assessment will be discussed [4,5,6].

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PL5 Bio-Based Biodegradable Plastics in a Circular Economy

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Most plastics we use in our everyday lives are derived from fossil-based resources such as gas and oil. The plastic economy has been predominantly linear since its emergence in the mid 20th century with the majority plastics not re-used or recycled after they have served their purpose and end up in landfill or the environment [1].

Plastics have been identified as a key material within the circular economy which is best represented in the Ellen MacArthur Foundation diagram [2]. Plastic circularity is about retaining value in plastics as materials and thus keeping them in the material cycle for as long as possible. However, the circular economy is not just about re-use and recycling but also responsible consumption and production [3]. Circularity has resource implications (re-use and recycling require use of resources such as water and energy to keep a material in the economy) and so consumption must be balanced with the preservation of the resources that we have. The circular economy is not business as usual and so we must build in prevention and minimisation as part of responsible consumption and production for plastic e.g. the R principles of the circular economy (e.g. Refuse, rethink, reduce, reuse, repair, refurbish, remanufacture, repurpose, recycle, recover [4].

The bioeconomy is the use of natural resources to produce biobased materials such as bioplastics (be they biodegradable or not). The bioeconomy is a construction of humankind and is not inherently circular i.e. the bioeconomy can be constructed in a not sustainable way to directly replace the linear fossil economy (take, make, dispose). However, the bioeconomy can be designed/engineered to be circular and be the biological part of the circular economy. Indeed, as society transitions away from fossil-based resources, the vast majority of the circular economy will be biobased.

However circular economy policy is focused on fossil-based plastics and bioplastics are seen as a contaminant in recycling streams. Furthermore, entry into the market of biobased plastics is hindered by the lack of evidence of the recyclability of these plastics. Biobased biodegradable plastics are seen, incorrectly, as not recyclable as they are biodegradable. This presentation will look at bioplastics in a circular economy through the lens of policy development in Europe and scale up of technologies to address the recycling of biobased biodegradable plastics.

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KL1 Production of Biopolymers from Wastewater Opportunities and Bottlenecks

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Mixed microbial cultures (MMCs) enable the conversion of waste organic carbon into biopolymers, specifically polyhydroxyalkanoates (PHAs) and extracellular polymers (EPS). Both polymers have a different ecology role for the bacteria, have different polymer properties and applications. But they share the problem of developing a value chain from wastewater facilities to consumer products.

PHA's play a role in the fitness of certain microorganisms in dynamic ecosystems, like wastewater treatment plants. PHA can be produced by bacteria naturally present in activated sludge of in special enrichment cultures. EPS is produced to immobilises and protects bacteria in wastewater treatment, and is an intrinsic part of the biomass produced. Up to 30 % of the produced sludge can consist of this polymer. Where PHA is well characterised, EPS is still largely a molecular undefined compound.

PHA is a bioplastic and usually presented as an alternative for oil-based plastics. EPS is a hydrogel with potential a wider array of applications. It can be used a hydrogel, but also as binder in composite material and thereby also forms an alternative for traditional oil based plastics.

The similarity between both biopolymers is that both are produced within the environmental engineering sector in, for chemical industry, small production volumes. Linking the biopolymer production with the chemical/materials sector has proven a bottleneck in the upscaling and marketing of biopolymers derived from waste organic carbon.

KL2 Biopolymer Production: Harnessing the Structural Diversity of Microbial Exopolysaccharides for Various Applications

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Microbial polysaccharides represent a highly versatile class of biopolymers with immense potential for industrial innovation. Unlike plant-derived polymers, their chemical structures and functional properties can be precisely tailored through microbial production and advanced biotechnological modification. This confers distinct advantages, including controllable production, high purity, independence from agricultural and seasonal variability, and the ability to design targeted structural modifications. Together, these features open pathways to customized solutions that extend far beyond the limitations of traditional plant-based polysaccharides.

Synthetic biology and genetic engineering, combined with process optimization, now enable applications spanning food technology, pharmacy, medicine, and materials science, and are even unlocking opportunities in emerging fields such as energy storage, including lithium-ion batteries. This keynote will highlight both established microbial exopolysaccharides, such as xanthan, alginate, dextran, and levan, as well as novel, highly promising candidates like paenan, kozan, and fucosan. In addition, strategies for creating entirely new derivatives through biotechnological engineering will be presented, outlining a future in which in vivo biopolymer engineering provides access to next-generation materials.

KL3 Advanced Tools on Biopolymers

Manfred Zinn*[,1], Véronique Amstutz[1], Tim Börner[1,2], Hugo Fleuriot-Blitman[1], Alexander Kettner[1,3], Florian Miserez[1,4], and Julien Pott[1]

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Novel biopolymers must master the well-established triangular relationship of "material – performance – application", which is essential to ensure their success in their specific market of application. Despite decades of research and development, the growth of the biobased polymer market has been relatively slow, primarily due to the late onset of large-scale production, and a slower learning curve compared to petroleum-based plastics [1,2]. Thus, replacing fossil-based plastics remains a significant challenge.
To meet this challenge, it is essential to develop policy frameworks that support innovation, scale-up of sustainable technologies, and the establishment of circular business models—key steps towards defossilization and circularity.
Today, a range of advanced tools is available to aid in the design and optimization of biopolymers to meet specific application requirements. These include:

- Regulatory (norms) and sustainability assessment tools (LCA)
- (Bio-)Process design and production techniques
- Biopolymer analytical tools
- Synthetic and modification techniques
- Computational and modeling tools
- Application-oriented tools

Many of these tools are interconnected within feedback loops and are often applied in parallel.
In this presentation, we will explore each of these tools, with particular emphasis on bioprocess production techniques, illustrated through the example of polyhydroxyalkanoate (PHA) production.

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KL4 Engineering Bioinspired Functional Materials from Natural Biopolymers

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Modern systems biotechnology approaches are being applied to engineer enhanced microbial strains capable of processing a wide range of waste substrates and producing biopolymers. Polyhydroxyalkanoates (PHAs) and bacterial cellulose (BC) are some of the examples that are being used to replace fossil based synthetic materials. These biopolymers are synthesized through microbial fermentation by model organisms such as *Pseudomonas putida*, *Cupriavidus necator*, and *Komagataeibacter* spp (for BC).

Diversification in the structure and functionality of natural bacterial polymers can be achieved through several strategies, such as: i) Metabolic engineering combined with synthetic biology, which often focuses on engineering bacteria to produce novel polymers with customized chemical structures and better properties. Recombinant microorganisms have also been designed to produce materials naturally generated by other organisms, such as spider silk[1]. Fibres produced from recombinant spider silk proteins are particularly notable for their exceptional mechanical performance, combining strength and elongation at break to achieve a remarkable toughness; ii) Anchoring new functionalities through customized peptide tags based on the PHA synthesis mechanism, such as phasins, which play an essential role in granule formation and PHA metabolism and have emerged as tools for functionalizing PHA surfaces. Minimized versions of those proteins have been developed for in vitro association to hydrophobic materials[2]; iii) Compatibilizing polymers to generate new bio-based blends. In this sense, living PHA producers can be used as drivers to improve the barrier properties of cellulosic materials by depositing PHA in the inner pores of hydro- and aerogels[3]. This innovative technology allows the compatibilization of these immiscible biopolymers. Compared to traditional coating methods, this cell-mediated approach not only allows PHA to be distributed on the surface of cellulosic materials, but also ensures polymer penetration throughout the gel due to bacterial self-movement. Using this methodology, we have also developed biohybrid films combining BC and recombinant spider silk to overcome dry BC's mechanical limitations (inherent brittleness and stiffness). All these examples open the door to the creation of biohybrid materials with tunable functionalities through the use of biotechnological approaches.

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KL5 Enzymatic Biodegradation of Rubber and Fossil Hydrocarbon Polymers

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All biologically produced polymers such as natural rubber (polyisoprene), polyhydroxyalkanoates (PHAs), polysaccharides and others include functional groups in their molecule backbone. These biopolymers can be more or less easily attacked by microbial enzymes that oxidatively or hydrolytically cleave the polymer at their functional groups. In contrast, many fossil hydrocarbon polymers (e. g. PE, PS) don´t have functional groups and are not suitable substrates by currently known enzymes. Therefore, these polymers are not biodegradable without physical or chemical pretreatments. I will provide an update of our recent advances in understanding the substrate binding and cleavage mechanism of rubber oxygenases and PHA depolymerases and comment on reports claiming that hydrocarbon polymers such as PE and PS are biodegradable by free living bacteria or by proteins and/or intestinal bacteria of insect larvae.

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KL6 Natural and Sustainable Polymers of Bacterial Origin and their Biomedical Applications

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In this work we have focused on the production and use of bacteria-derived sustainable biomaterials for use in biomedical applications. Two main types of biomaterials have been focused on, including Polyhydroxyalkanoates (PHAs)[1] and bacterial cellulose (BC)[2]. PHAs are polyesters produced by a range of bacteria including *Ralstonia eutropha*, *Psuedomonas sp.*and *Bacillus subtilis*. These polymers are biodegradable in the soil and in the sea. In addition, they are also resorbable in the human body and are highly biocompatible. Hence the PHAs can be used for biomedical applications such as the development of scaffolds for hard and soft tissue engineering, medical devices, and drug delivery. BC can also be produced by a range of bacteria including *Gluconobacter xylinus and Sarcinia ventriculi.* BC is also a green polymer, is sustainable and degradable in the soil. It is also highy biocompatible and can be used in biomedical applications.

Polyhydroxyalkanaotes are polyesters with monomer chain length ranging between C_4 - C_{16}. They are divided in to two main types, short chain length PHAs (scl-PHAs) with monomer chain length C_4-C_5 and medium chain length PHAs (mcl-PHAs) with monomer chain length C_6-C_{16}.The scl-PHAs are normally hard and brittle whereas the mcl-PHAs are soft and elastomeric in nature. Hence, we have mainly used the scl-PHA, Poly(3-hydroxybutyrate), P(3HB), for bone tissue engineering[3], drug delivery[4] and medical devices development such as coronary artery stents, and the mcl-PHAs for cardiac, nerve, pancreas, kidney and skin regeneration. For bone tissue engineering we have used neat P(3HB) and composites of P(3HB) with Bioglass®[3], hydroxyapatite[8] and carbon-based materials[7]. The mcl-PHAs have been used for the development of cardiac patches[6], nerve guidance conduits[5], wound healing patch, bioartificial pancreas and bioartificial kidney. Processing techniques used include additive manufacturing, electrospinning and melt electrowriting.

Bacterial cellulose has also been produced under static culture conditions using *G. xylinus*. This is a highly nano-fibrillated structure and hence is an excellent substrate for cell attachment and growth. We have surface modified bacterial cellulose to create antibacterial bacterial cellulose[9]. We have also used BC as a filler for P(3HB) based composites since BC is one of the stiffest known materials. In addition, we have electrospun BC for a range of applications.

In conclusion, we have successfully used bacteria-derived sustainable biobased materials for a variety of biomedical applications. Both PHAs and bacterial cellulose have a lot of potential in the future as sustainable biomedical materials of choice.

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KL7 Shaping Up' Bacterial Biopolymers for Biomedical and Food Applications

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Bacterial biopolymers are increasingly valued as sustainable, versatile materials with transformative potential in both biomedical and food industries. Advanced but also simple engineering and processing strategies are being used to "shape up" these natural polymers—tailoring their physical, chemical, and functional properties for specific applications. From biofilms and hydrogels to nanofibers and edible coatings, bacterial-derived materials such as polyhydroxyalkanoates (PHAs), bacterial cellulose, and exopolysaccharides are being reimagined as scaffolds for tissue regeneration, vehicles for drug delivery, and barriers for food preservation. Integrating materials science, bioprocessing, and innovative fabrication techniques allow molecular customization, broadening the performance and application range of these biopolymers. Case studies will highlight recent developments in achieving tunable mechanical properties, enhanced biocompatibility, and functional integration (antimicrobial, antioxidant, and functional food applications). By shaping up bacterial biopolymers—both literally and conceptually—we can unlock new pathways toward safer, smarter, and more sustainable materials for human health and nutrition.

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KL8 From Biowaste to Bioplastics: a Roadmap of Challenges, Constraints, and Opportunities

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Biowaste is commonly defined as the biodegradable waste of plant, vegetable, and animal origin, with urban areas being among its main production hotspots [1,2]. It represents a promising feedstock for the production of high-value bioproducts [3]. Among these, bioplastics that are both biobased and biodegradable (such as polyhydroxyalkanoates, PHA) offer a sustainable alternative to conventional fossil-based plastics. The latter present high chemical stability, making them suitable for a wide range of applications while simultaneously degrading extremely slowly in the environment. Consequently, the lifespan of plastic products is up to about 10 years while the time it takes to decompose is up about 500 years [4]. As a result, plastic waste accumulation in the environment is expected to reach 1.2 billion tons by 2050, posing serious pollution concerns [5].

Coupling biowaste management with bioplastic production not only addresses waste disposal and plastic pollution challenges, but also contributes to reducing environmental impacts and advancing the principles of the circular bioeconomy concept. Most biowastes is suitable for PHA production, especially when dealing with mixed microbial cultures (MMC)-based processes. However, several technological, economic, and regulatory constraints slow down the large-scale implementation of biowaste exploitation for PHA production. Biowaste management in the EU is regulated under Directive 2008/98/EC (known as Waste Framework Directive) of the European Parliament and of the Council on waste of 19 November 2008, which was consolidated in 2018 and repealed certain previous Directives [6].

This talk will explore the current state of the art, recent advances, and critical bottlenecks in the biowaste-to-bioplastics value chain, with a particular focus on MMC-PHA production, feedstock variability, market uptake, and current policies including criteria for biowaste reuse and the end-of-waste status. An overview of relevant European legislation for bioplastics will also be discussed.

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KL9 Mixed Microbial Culture Poly(3-Hydroxybutyrate-co-3-Hydroxyvalerate) from Municipal Wastewater – Defining Quality, Dispelling Myths, and Dancing with the Elephant in the Room

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Technology feasibility for mixed microbial cultures (MMCs) to produce polyhydroxyalkanoates (PHAs), and especially poly(3-hydroxybutyrate-co-3-hydroxyvalerate), has been repeatedly validated for over twenty years and up to demonstration scales. It is shown that MMC PHA-rich biomass can be produced in different ways and process configurations while using a plethora of substrates including those derived from (waste) organic matter - industrial process waters, wastewaters, and sludge residues.
So, bioplastics can be made as a renewable resource from waste – an essential element of a circular economy (CE). Nevertheless, MMCs are ugly ducklings in a world of pure culture and white biotechnology advancements that similarly, and necessarily, lift methods and processes of PHA production also from residual (waste) organic matter. A popular underlying misconception is that commercial PHA quality is a bridge too far for the ugly duckling. Notwithstanding, ideas and needs of quality and quality control are not always specifically well addressed, nor well-defined, in the research literature. They go well beyond questions of PHA average monomer content, average molecular weight, and purity.
Thus, research reporting PHA production outcomes commonly misses key context. It is not enough to motivate CE by the fact that a *" PHA "* can be produced with a particular organic waste – either by MMC or pure culture methods. Industrial relevance of the research requires knowledge in details of the specific type of PHA being produced, its property specifications, and a clear vision to property specification quality control at industrial scale. *That* PHA must somehow become realizable at meaningful industrial production volumes. Large production volumes can be furnished by an ensemble of smaller foreseeably stable, but collectively large, supply chains. Such supply chains offer inherent significant potential for general *glocal* growth by regional replicated hubs. These hubs can become increasingly established over many years following a roadmap to bring CE to a fact-of-life around the world.
This presentation addresses developments from the past 8 years that have targeted a specific PHA supply chain context alongside tackling real-world challenges and doubts while demonstrating technological feasibility in a *glocal* vision of a significant global capacity for MMC PHA production using municipal wastewater. The goal has been to understand PHA value and quality in supply chains that emanate PHAs as renewable resources - by-products from essential services of municipal wastewater treatment. Fate of unwanted impurities is predictable for green solvent recovery methods. This enables managed risks for policy and governance of the end-of-waste. End-of-waste is an elephant in the room.

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KL10 Degradation of Bioplastics by Mealworms

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Several studies have reported the biodegradation of commodity plastics by mealworms and other insects [1, 2]. In this study, the degradation of polyhydroxyalkanoate (PHA) was compared with that of several commodity plastics. The mealworms' preference for different plastic types and their effect on larval growth were examined. Additionally, the resulting feces were quantified and analyzed for the presence of microplastics. Mealworms were fed 9 cm diameter plastic films of low-density polyethylene (LDPE) (trash bin liner and single-use plastic bag), high-density polyethylene (HDPE) (shopping bag), starch-based single-use plastic bags, and three different PHAs: poly(3-hydroxybutyrate) [P(3HB)], poly(3HB-*co*-11 mol% 3-hydroxyhexanoate) [11 mol% HHx], poly(3HB-*co*-18 mol% 3-hydroxyhexanoate) [18 mol% HHx], and poly(3HB-*co*-27 mol% 3-hydroxyhexanoate) [27 mol% HHx]. These plastics were provided as the sole diet for 21 days. The oat diet served as a control. To detect the presence of PHA-degrading bacteria, the mealworm gut contents were plated on PHA agar plates. The mealworms' ingestion capability was significantly influenced by the type of plastic. The mealworms fully ingested the starch-based film, 11 mol% HHx, 18 mol% HHx, and 27 mol% HHx films within 6 h, 7 days, 7 days, and 15 days, respectively. The trash bin liner, P(3HB), and single-use plastic bags were steadily ingested at 68%, 83%, and 85%, respectively, while the shopping bag was not consumed. A decline in larval weight from day one suggests that a long-term sole plastic diet is detrimental to mealworm growth. Plastics excreted in the larval feces were visible under an optical microscope, and gas chromatography analysis revealed 100% recovery of the ingested PHA. Gel permeation chromatography analysis showed a 13% to 35% decrease in molecular weight (M_w). Additionally, no PHA-degrading bacteria were detected in the mealworm gut.

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L1.1 Polyhydroxyalkanoates with Controlled Monomer Composition and Distribution for the Development of Medical Devices

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To address future challenges in the development of less invasive and more personalized medical devices, a wide range of biocompatible materials with diverse mechanical properties is required. In this context, polyhydroxyalkanoates (PHA) are biocompatible, non-immunogenic, and non-carcinogenic polymers. To date, studies reported in the literature regarding the use of PHA in the medical field rely on PHA such as poly(3-hydroxybutyrate) (PHB), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBHV), poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P3HB4HB), and poly(4-hydroxybutyrate) (P4HB), with limited possibilities. [1-4]

Pseudomonas putida KT2440 was used to biosynthesize original PHA. By controlling the concentration and timing of substrate additions in flasks or bioreactors, the monomer units and their distribution along the polymer chain were targeted [5,6]. Substrates of interest included fatty acids with 5 to 8 carbon atoms containing phenyl or cyclohexyl rings, and 11-carbon fatty acids with unsaturation (alkene or alkyne), halogens (bromine), or other functional groups (amine, thiol, *etc*.). This enabled control over both monomer ratios and distribution along the chain (block or statistical).

Biosynthetic pathways were studied to indirectly regulate the activation of *de novo* synthesis. It was possible to better understand the biosynthetic mechanisms involved which differ from those of more widely studied aliphatic PHA [7].

This study led to the development of PHA with controlled compositions that are rarely explored. As a result, a broad range of PHA with reactive functions is now available—for example, enabling the avoidance of a propargylation step by directly grafting azide-functionalized PEG with various functional groups or molecular weights *via* click chemistry onto alkenes and alkynes.

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L1.2 Isolation and Screening for Purple Phototrophic Bacteria with Increased Polyhydroxyalkanoates Productivity

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Polyhydroxyalkanoates (PHA) are biodegradable polymers produced by certain microorganisms as intracellular carbon and energy reserves and are feasible substitutes of conventional plastics[1]. Purple phototrophic bacteria (PPB) are a group of anoxygenic bacteria capable of producing PHA in anaerobic conditions using light as energy source to uptake external carbon. The use of PPB mixed cultures (PMC) to produce PHA using wastes as feedstock has been gaining relevance, however, the achieved productivities are still low when compared to aerobic cultures [2,3]. The identification and isolation of PPB with high PHA productivity capacity for application in either single-cultures or as dominant PPB in open cultures could make the use of PPB technology for PHA production more feasible.

In this work, 7 PPB isolates, 5 identified as *Rhodobacter sphaeroides*, 1 as *Rhodopseudomonas palustris* and 1 *Afifella marina* were firstly isolated from either different PHA producing PMC or from soil and water samples from different locations in Portugal. Culture growth characterization in flasks, under anaerobic conditions and a light intensity of 1 W/L, using acetate and CO2 as the main carbon sources, revealed *R. sphaeroide*s strains as the best PHA producers with polyhydroxybutyrate (PHB) contents of > 30% cdw, while A. marina reached a maximum of 24% and R. palustris attained 18% PHB content. In 8 day fed-batch assays with acetate, one *R. sphaeroides* isolate achieved a maximum PHB content of 63.7±2.20% cdw with a PHB concentration of 2.71±0.10 gPHB/L, resulting in a productivity of up to 0.34±0.02 gPHB/(L.day), out besting previously reported productivities for *R. sphaeroides* while using alow light intensity of 1 W/L. Fed-batch assays under osmotic shock (30 gNaCl/L), caused a 30% decrease of biomass concentration when compared to the non-saline medium. Nonetheless, the *R. sphaeroides* strain reached higher maximum PHA contents of up to 73% cdw reaching up to 82% of the final PHA concentration of the assay without the osmotic shock. This result opens the possibility of using this isolate in non-sterile saline environments (enabling lower operation costs), since high NaCl concentrations will impair contamination. Fed-bacth assays with a strategy of increasing the light intensity throughout the assay (from 1 W/L to 2 W/L and 4W/L) reached higher final PHA concentrations of 3.32±0.10 gPHB/L and productivities of 0.42±0.01 gPHB/(L.day) proving to be a successful strategy. The strategy of isolating PPB strains from PHA producing PMC proved to be a successful one, with the isolated *R. sphaeroides* strain being a promising candidate for scaling-up PHA production as a single culture or in open culture operation.

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L1.3 Some Like it Hot: Exploring Thermophiles for PHA Biosynthesis

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Polyhydroxyalkanoates (PHA) are microbial polyesters recognized as sustainable alternatives to conventional synthetic polymers. Despite these advantages, their widespread industrial adoption remains limited by high production costs. One promising strategy to overcome this barrier is the use of extremophilic microorganisms as production hosts.

In this study, we aimed to identify and isolate extremophilic bacteria capable of PHA biosynthesis. Our initial screening confirmed that many halophilic bacteria are effective PHA producers. However, the high salt concentrations required for their cultivation present significant technical challenges, particularly in downstream processing. To address these limitations, we shifted our focus to thermophilic microorganisms, an underexplored group in the context of PHA production.

We developed a novel isolation protocol tailored to enrich PHA-accumulating thermophiles from various environmental sources, including compost and activated sludge. This approach led to the identification of several thermophilic strains with industrial potential for PHA production. Among these, isolates from the genus *Aneurinibacillus* demonstrated a remarkable capacity to incorporate diverse monomer units into PHA polymers, expanding the range of possible material properties.

In addition to environmental isolation, we systematically screened thermophilic strains from public microbial collections to identify robust PHA producers. Notably, *Caldimonas thermodepolymerans* demonstrated considerable potential for PHA synthesis from a variety of waste substrates, further underscoring its biotechnological relevance. To unlock the full potential of this thermophilic producer, we are actively developing and already applying tailored synthetic biology and metabolic engineering tools as well as metabolic modeling based on omics- data. These efforts aim to enhance PHA yield, broaden substrate utilization, and fine-tune polymer composition, thereby paving the way for the industrial application of thermophilic extremophiles in sustainable biopolymer production.

Finally, we employed *C. thermodepolymerans* and its engineered mutants with deletions in *phaC* and *phaZ* genes as model systems to investigate the physiological role of PHA in thermophiles, particularly in relation to stress resilience and thermal adaptation. Our findings suggest that, unlike in other stressors, PHA accumulation is not a predominant strategy for coping with high-temperature stress in thermophilic bacteria.

Overall, this study highlights the untapped potential of thermophilic bacteria for sustainable PHA production and lays the groundwork for further biotechnological development of extremophile-based bioprocesses.

L1.4 Preservation and Reactivation of a Stored Mixed Microbial Culture for PHA Production

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Polyhydroxyalkanoates (PHA) production by mixed microbial cultures (MMC) and used cooking oil (UCO) as substrate is challenging. For this reason, once the MMC enrichment is achieved, storing a fraction under adequate conditions for later reactivation can be of great value to PHA-accumulation research. Storing cultures using glycerol is a common practice in biotechnology research. However, the research about PHA enriched MMC conservation and reactivation is scarce. This study aims to address that gap by evaluating the reactivation of a MMC enriched in PHAstoring bacteria (24.7 wt. % PHA) from a 24 L reactor fed with UCO (R1). MMC was preserved at 5 °C in glycerol (20 % v/v) for 87 days. Then, it was centrifuged (3500 rpm), washed 3 times with water (to eliminate glycerol), and used to inoculate other enrichment unit (R2, 4 L). Both units (R1 and R2) operated as sequential batch reactors (SBRs) with a feast/famine (F/F) regime, where the withdrawal occurred at the end of the feast. R2 was started with a F/F regime of 6/6 h (compared to 12/12 h in R1). During the first 5 cycles, the withdrawal was centrifuged, and the solids returned to the reactor. With this strategy, after only 7 days the PHA accumulated at the end of the feast was 11.3 wt. %, although the active biomass (X) was low (0.2 g /L). On day 15, an accumulation assay (just UCO addition), showed that the MMC could reach 18.6 wt. % PHA in 12 h. Thus, the F/F regime was adjusted to 8/4 h, increasing the PHA content to 18.5 wt. % and the active biomass to 0.7 g/L (Table 1).

Table 1. Summary of the enrichment units (R1 and R2) operational conditions and results.

Enrichment reactor	Stages	Operational conditions		Results	
		Loading rate	F/F	X start feast	PHA final feast
		g UCO/(L·h_{feast})	h/h	g/L	% (wt.)
Before storage (R1)		0.104	12/12	0.7 ± 0.36	24.7 ± 3.7
Reactivation (R2)	I (0-21 d)	0.087	6/6	0.2 ± 0.04	9.5 ± 2.4
	II (22-45 d)	0.133	8/4	0.7 ± 0.02	18.5 ± 2.3

To further optimize enrichment on R2, on day 23, accumulation assays (16 h) were carried out using the MMC collected at the end of the feast phase, with different UCO loading rates (Figure 1). The better results in terms of PHA accumulated (38 wt.%) and substrate yield (0.58 g PHA/g UCO) were obtained at the load already applied in R2 (0.133 g UCO/(L·h)). These results suggest that while the loading rate applied in R2 was adequate, extending the feast length may further enhance intracellular PHA accumulation. It is remarkable that at the end of these assays, the solids concentration was around 5 g VSS/L (X» 2.4 g/L), which is a high value compared to R2 operation and most of the PHA production studies with MMC. Based on the obtained results,

the reactivation of the biomass can be considered successful, and the strategy implemented useful for future conservation of a PHA-storing population.

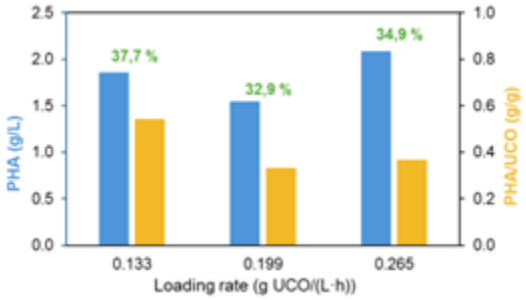

Figure 1. Results of the accumulation assays with the MMC of the day 23 of R2 at different UCO loading rates. PHA accumulated in g/L (■), with the wt. % on top (■); PHA accumulated by gram of UCO used in g/g (■).

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L1.5 Exopolysaccharides of polyextremophiles: Adaptation to Multiple Extremes

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Polyextremophiles—organisms that thrive under multiple extreme environmental conditions such as extreme temperatures, pH, salinity, pressure, and radiation—exhibit unique adaptive mechanisms that enable their survival [1]. One critical adaptation is the production of exopolysaccharides (EPSs) through cell wall-associated or extracellular glycosyltransferases (GTs) [2]. EPSs serve multifaceted roles, including protection against environmental stresses, desiccation, phage attacks, phagocytosis, and antibiotics, as well as contributing to microbial communication, quorum sensing, virulence, energy storage, and biofilm formation. While EPS production has been studied in various extremophiles, research remains limited in the context of polyextremophiles, particularly among psychrophiles, halophiles, and piezophiles. This study gives an overview of current knowledge on the adaptive strategies of polyextremophiles, with an emphasis on the functional significance of EPS production under combined stress conditions. By integrating insights across microbial systems, this work underscores the pivotal role of EPSs in polyextremophile resilience and explores their promising potential in biotechnological applications [3].

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L1.6 *Ralstonia eutropha' s* Phar - A Transcriptional Factor with Ambivalent Role

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Approximately 40 % of all prokaryotic strains – including both bacteria and archaea – naturally produce polyhydroxyalkanoates (PHAs) as intracellular carbon and energy storage compounds [1]. PHAs are stored as water-insoluble, granule-like inclusions consisting of a polymer core surrounded by a surface layer of several PHA granule-associated proteins (PGAPs) [2]. Among PGAPs, phasins – amphiphilic, low-molecular-weight proteins – are the most abundant ones and play a critical role in regulating the surface-to-volume ratio of PHA granules [2]. In the best studied PHA producer, *Ralstonia eutropha*, eight phasins (PhaP1-PhaP8) have been identified [2]. Expression of the major phasin gene *phaP1* is controlled by the transcriptional repressor PhaR, which binds to the *phaP1* promoter region under non-PHA-accumulating conditions. Upon PHA synthesis, PhaR binds to the nascent granule, thereby relieving repression of *phaP1*, whereas, towards the end of PHA synthesis, the PHA granule, that is covered with phasins, can no longer accommodate PhaR and the repression is again restituted [3-4]. Until now, an analogous repressive mechanism was also assumed for the phasin gene *phaP3* [5-6]. However, various studies have reported distinct expression patterns for *phaP1* and *phaP3* [7-8], suggesting that their transcription may be differentially regulated.

In this study, *in vivo* β-galactosidase reporter-gene assays revealed that PhaR functions as an activator of *phaP3* expression while simultaneously serving as a repressor of *phaP1.* Palindromic PhaR binding sites were found *in silico* at both the *phaP1* and the *phaP3* gene. The identification of the previously unknown transcription start site (TSS) of *phaP3* shows that the location of the PhaR binding sites differs relative to the promoter. While PhaR binds to P_{phaP1} at the transcription start and directly upstream of the -35 box, PhaR binding sites at P_{phaP3} are located about 70 and 200 bp upstream and 40 bp downstream of the TSS, respectively. Supporting this, *in vitro* electrophoretic mobility shift assays (EMSAs) confirmed PhaR binding to various regions of *phaP3.* Together, these findings establish that PhaR does not act solely as a repressor but rather plays a more complex regulatory role in phasin gene expression, contributing to the fine-tuned control of PHA granule formation in *R. eutropha*.

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L1.7 Genes and Genomes Coding PHA Synthases

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PolyHydroxyAlkanoates (PHA) are microbially produced polymers that could represent a sustainable alternative to conventional synthetic polymers. Although they are relatively widely studied, much less is known on particular genes coding enzymes for their synthesis, i.e., PHA synthases. So far, four different classes of PHA synthases have been defined. Not only has this classification become obsolete, but computational resources and databases like COG and KEGG do not respect it. Therefore, their correct identification is cumbersome. Moreover, additional knowledge on their transcriptional and posttranscriptional regulation or epigenetic modification is generally missing.

During the last several years, we sequenced, assembled, and analysed various genomes of PHA-producing bacteria, e.g. *Caldimonas thermodepolymerans*, *Caldimonas aquatica*, *Tepidimonas taiwanensis*, *Aneurinibacillus thermoaerophilus*, and others [1-3]. In this study, we utilized third-generation sequencing data, particularly PacBio and Oxford Nanopore Technologies, to reveal genome-wide DNA methylation patterns in selected PHA-producing strains. Moreover, we identified differentially methylated positions in several strains under different cultivation conditions and matched them with PHA production gene machinery. In addition, we utilized our genome-wide transcriptomic studies performed with RNA-Seq, to infer gene regulatory mechanisms around PHA synthase in *C. thermodepolymerans* and predicted its possible posttranscriptional regulation by hitherto undefined small RNA. Similarly, we predicted many regulatory non-coding elements in *Rhodospirillum rubrum*, another potent and versatile PHA producer.

Finally, we shifted our focus to bioinformatics database mining to understand how correct and unambiguous identification of PHA synthases could be performed. We analysed publicly available resources for functional annotation, particularly KEGG and COG, and proposed our initial dataset to correct limitations of current resources. As we show, this resource can be continuously updated while screening all currently available microbial genomes.

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L2.1 Deep Hybrid Modelling and Control of Microbiome Evolution

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Hybrid modeling combining First-Principles with Machine Learning (ML) is becoming a pivotal methodology for Industry 4.0 enactment. The combination of ML with prior knowledge generally improves the model predictive power and transparency while reducing the amount of data for process development. There is, however, a research gap between emerging machine learning methods and the current practice in bioprocess development [1].

In this work, we present an automatic control system that makes use of Physics Informed Neural Networks (PINNs) [2] to act as a digital twin of a natural microbiome in a Sequencing Batch Reactor (SBR) in order to maximize Polyhydroxyalkanoates (PHA) production through the control of 3 different feeding solutions: An Acetate/culture medium solution, an Ammonia/culture medium solution and a culture medium solution.

First, the PINN model was trained with historical data from a Sequencing Batch Reactor (SBR). During this step, deep learning centric approaches, such as the Adaptive Moment Estimation Algorithm (ADAM) were used. After the PINN was trained, a Model Predictive Control (PC) control step was carried out. The PINN model acted as a digital twin of the process to forecast the dynamics of the SBR based on process measurements and 3 decision variables: Feeding rate of acetate, feeding rate of ammonia and feeding rate of medium. Afterwards, based on the forecast, the MPC selects the parameters that maximize the productivity of the next feast and famine cycle (defined as the amount of biomass (VSS) multiplied by the amount of PHA at the end of the feast).

After a total of 14 cycles, corresponding to 3 hydraulic retention times, the PHA has increased from the starting 5 mg/gVSS in 14 g/L of VSS to 226 mg/gVSS in 6.5 g/L of VSS. This was further confirmed via a dedicated accumulation experiment, which resulted in 528 mg/gVSS in 10 g/L of VSS.

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L2.2 Towards Modelling Aided Accelerated PHA Material Design

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Polyhydroxyalkanoates (PHAs) are a large group of microbially produced polyesters, which have potential to replace many current fossil-based materials. The polymers can exist as homopolymer, random or block co-polymer structures consisting of over 150 different monomers. The extensive number of combinations, where the monomer composition, ratio and polymer length among other details affect the material properties, offers possibilities to identify novel materials beyond the commercial PHA grades currently available. A challenge to overcome is the high amount of time and resources needed to experimentally synthesize and characterize each polymer variant.

To accelerate the novel material design, physics and AI based modelling can be used to predict some of the most important material properties prior to any experimental steps taken. First, we have developed a method to predict the glass transition temperature (Tg) of polymers. Molecular dynamics simulations for amorphous homopolymers polyhydroxybutyrate (PHB), polylactic acid (PLA), and polyglycolic acid (PGA) and random copolymers P(LA-3HB) were able to predict correctly the linear correlation between Tg and lactic acid fraction in P(LA-3HB) copolymer. Natural PHA producing and engineered strains are used to produce experimental copolymer series to validate the model with additional data, both for Tg values and further property predictions.

Another challenge in exploring the possibilities of novel polymer materials biosynthetically is the synthesis of polymers consisting of uncommon monomers. The substrate specificity and/or limited activity of the PHA synthase can hinder the synthesis of some copolymers. Incomplete characterization and the dimer structure of the synthase add a level of complexity for protein design. A conditional variational autoencoder was used to design a library of 10 000 new-to-nature PHA synthases, that was filtered down to 16 sequences and experimentally screened [1]. Two of the novel PHA synthases were confirmed active and were used to produce polyhydroxybutyrate (PHB) when expressed *in vivo*. Training the model with different methods could enable the design of synthases for specific purposes.

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L2.3 Novel Continuous Multi-Reactors Approach for Polyhydroxyalkanoates Production with Mixed Microbial Cultures

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Environmental pollution due to the intensive use of plastic materials, caused a particular interest towards the production of biodegradable plastics, such as polyhydroxyalkanoates (PHA). Current industrial PHA production involves the use of pure or recombinant microbial strains which typically require ad hoc formulated substrates and sterile operating conditions, driving up the market price of PHA (up to – 6.26 €/kg) and limiting the widespread availability of this biopolymer [1]. The Mixed Microbial Culture (MMC)-PHA production (which is an alternative to pure cultures) is typically operated in a multi-stage process wherein the microbial selection step plays a pivotal role, and it is generally conducted in a Sequenced Batch Reactor (SBR), in which the feast/famine regime is temporarily applied [1]. However, at large scale the use of SBR leads to the integration of powerful pumps and oversized liquid/solid separation units to handle large quantities of liquid streams in a short period, resulting in further raising the investment costs. This work presents a novel continuous multi-reactors approach in which the Feast phase takes place in the first tubular reactor (1L working volume), fed with nutrient-rich feedstock, while the Famine phase is operated in the second continuously stirred tank reactor (CSTR, 5L working volume), with both reactors connected through a recirculation stream imposed by a recirculation factor (R_C) [2]. The effluent of the Famine reactor is in part continuously sent to a third accumulation reactor (CSTR, 2L working volume), fed by a nutrient-free feedstock. A synthetic mixture of acetic and propionic acids was used as feedstock for both the Feast reactor, operated at two organic load rates (OLR 2.12 and 4.25 gCOD/Ld), with C/N ratio of 35 and RC of 8; and the accumulation reactor operated at higher OLRs of 5.04 and 6.12 gCOD/Ld. A further continuous step of biomass inactivation was tested consisting of thermal (at 4°C) or acid (pH 2 with sulphuric acid) quenching, followed by PHA extraction using a combination of NaOH (0.2 M) and H_2O_2 (1.5%, wt/wt) as extraction reagents. Overall, during the experimentation, an average intracellular PHA content of 64 ± 1 % (wt/wt) was obtained and, with the acid quenching, the extracted polymer resulted in a purity grade as high as 96% (wt/wt) with a molecular weight varying between 600 and 2.000 kDa. In conclusion, data obtained in this study highlight the strong potential of the continuous-flow process to achieve both high intracellular PHA content and high purity of the extracted polymer. Further work will be focused on evaluating the process performance using real organic waste streams as feedstock.

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L2.4 Engineering of *Pseudomonas fluorescens* SBW25 Towards Efficient Production of Tailored Alginates

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Alginate is a linear, extracellular polysaccharide composed of varying ratios of β-D-mannuronic acid (M) and its C-5 epimer, α-L-guluronic acid (G), widely utilized in the food, pharmaceutical, and biotechnological sectors [1,2]. While typically derived from seaweeds, two bacterial species, Azotobacter and Pseudomonas, encode the biosynthetic pathway for alginate, and by that have the potential to produce alginates with a more tailored composition and without the seasonal variations found in alginates produced by seaweeds [1,3].

This project focuses on genetically engineering *Pseudomonas fluorescens* SBW25 to enable efficient and tailored alginate production. *P. fluorescens* SBW25 does not naturally produce alginate under laboratory conditions. However, by introducing a premature stop codon in the *mucA* gene, using CRISPR/Cas9 base editing, alginate production was successfully activated, as *mucA* encodes a negative regulator of the biosynthesis pathway. Three truncated MucA mutants were constructed and cultivated in a bioreactor parallel system, yielding alginates with differing titers, M/G ratios, and molecular weights, ranging from approximately 80 kDa to 200 kDa. To further modulate the alginate structure, the role of epimerases in catalyzing the conversion of M to G was examined. Deleting *algG*, the gene encoding the sole alginate epimerase in *P. fluorescens*, abolished alginate production. This supports its essential role within the cell envelope spanning biosynthesis complex and its protective function for the interaction with the lyase AlgL. However, the integration of mutated epimerase variants restored alginate synthesis, potentially without full catalytic function. Four specific amino acid substitutions near conserved M-binding motifs were introduced, affecting the epimerase binding affinity and activity. Additionally, heterologous expression of different epimerases provided further insights into alginate epimerization patterns. Overall, this research provides a very promising base for the industrial production of tailored bacterial alginates, providing polymers with more homogeneous and tuneable M/G ratios, including different molecular weights, which renders them highly attractive for various applications.

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L2.5 Upcycling Depolimerised Plastic Fractions: Novel Bioprocesses for Biopolymer Production Using *Pseudomonas putida*

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Global plastic consumption, projected to rise from 460 million tonnes in 2019 to 1,231 million tonnes annually by 2060 [1], necessitates sustainable recycling within planetary boundaries. Traditional methods like mechanical and chemical recycling are limited by material degradation, raw material constraints, and high energy consumption. Depolymerisation of end-of-life plastics offers a promising alternative, but the resulting monomer blends contain diverse additives, impeding direct reuse [2,3]. Biological valorisation of these monomer blends poses a promising alternative. Microbial growth on plastic monomers and subsequent conversion into biodegradable polymers has been demonstrated using engineered strains of *Pseudomonas putida* [4-6], a reported natural producer of polyhydroxyalkanoates (PHAs). While robust growth and monomer conversion has been shown, these processes still face limitations in obtained biomass concentrations, product titres, and overall process efficiency.

To investigate growth behaviour on different plastic monomers, *P. putida* was grown on carbon-equimolar substrate monomers in microtiter plates. Metabolic activity was tracked using online monitoring of the oxygen transfer rate in 96-well plates [7]. Microbial growth was enhanced by optimizing carbon concentrations and implementing co-substrate supplementation. Possible feeding strategies were investigated in shaken systems, yielding improved substrate provision for a knowledge-based scale-up of the process to 2-L scale. Upon sufficient growth in larger scale, PHA production optimization will be pursued. Furthermore, the transfer of these processes to real depolymerized plastic fractions is investigated, addressing the handling of precipitated monomers and potentially toxic additives.

This work presents the development of a fed-batch strategy for efficient plastic monomer provision and utilisation where the application for real depolymerised plastic is kept in mind. The demonstration and optimization of microbial growth on mixed monomers and subsequent conversion into PHAs contributes to a sustainable plastic economy and advances the development of bio-based materials. This research aligns with the growing demand for biodegradable alternatives and highlights the role of bioprocesses in addressing plastic waste.

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L2.6. High-Throughput Screening of Cyanobacterial PHB Production Using Fluorescence-Based Detection

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Different cyanobacterial cultures isolated from distinct environments—*Synechocystis sp.*, *Leptolyngbya sp.*, and a mixture of *Synechocystis sp.* and *Synechococcus sp.*—were tested for their polyhydroxybutyrate (PHB) production potential. The use of cyanobacteria is a breakthrough in PHB production on the grounds of producing this polymer from biomass that has been harvested in photoautotrophic conditions which consume CO_2. The aim was to evaluate these cultures under a wide range of conditions, including varying nitrogen and acetate concentrations, different temperatures, and glycogen accumulation enhancement. Given that, these factors are widely described in literature to play a key role in PHB synthesis [1]. An innovative PHB quantification technique was applied, enabling high-throughput screening over 80 different conditions in less than a month – using only 25 mL of culture in total. This approach was significantly faster than traditional chromatography methods. All experiments were carried out in multiwell plates, where samples were stained with Nile Blue, and fluorescence was measured to quantify PHB content. Experiments followed a two-stage cultivation approach: in the first stage, cultures were first grown in 3L photobioreactors until nutrients depletion, the resulting biomass was transferred to multiwell plates containing the different test conditions for PHB accumulation. Nitrogen concentrations ranging from 100 to 0 mg/L and phosphorus from 15 to 0 mg/L were assessed. The optimal timing for transferring cultures to the PHB accumulation stage was determined by monitoring glycogen storage during the growth phase. Additionally, acetate concentrations and temperatures were also tested. Once optimal conditions were identified per strain, cultures were grown under these conditions in 3L photobioreactors for final PHB quantification. After nutrient depletion, inorganic carbon (50 mg/L) was added to promote glycogen accumulation. On the fourth day, cultures were shifted to PHB production by placing them in darkness and supplementing with strain-specific acetate doses. Daily samples were taken to analyze biomass, acetate consumption, PHB, and glycogen content. Acetate and glycogen were quantified by liquid chromatography, while PHB was measured by gas chromatography [2]. Fluorescence screening revealed that the best PHB accumulation occurred under nitrogen starvation, in darkness, at 30ºC, with acetate supplementation after 4 days of glycogen accumulation. Maximum PHB levels reached 7% of dry cell weight (dcw) for *Synechocystis sp.* and 12.9% dcw for *Leptolyngbya sp.*

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L2.7 Poly-β-hydroxybutyrate Production from Bread Waste *via* Sequential Dark Fermentation and Photofermentation

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The growing demand for sustainable alternatives to petroleum-based plastics and the urgency of food waste reduction have increased interest in microbial bioplastic production from organic residues [1]. Poly-β-hydroxybutyrate (PHB) is a biodegradable polyester produced by bacteria, archaea, and yeasts as an energy reserve under nutrient-limiting conditions [2]. Purple non-sulfur bacteria (PNSB) can synthesize PHB under photoheterotrophic conditions by using organic acids as carbon and electron sources and harvesting light as energy through photofermentation (PF) [1]. Coupling dark fermentation (DF) with PF represents a sustainable and efficient approach. In this two-step process, heterotrophic bacteria first convert food-derived carbohydrates into organic acids during DF, which are then utilized by PNSB to produce PHB [3].
Despite its promise, PHB production from organic waste streams using PNSB remains underexplored, particularly concerning light quality, a key factor affecting bacterial performance. Since bacteriochlorophylls (BChls) absorb light at 590 and 800–880 nm, tailoring the light spectrum can greatly enhance PF efficiency [1,4].
This study aimed to evaluate six PNSB strains for their capacity to grow and accumulate PHB using bread waste as substrate. The system comprised a DF step to enrich the waste with organic acids, followed by PF. The best-performing strain was further tested in a 5 L photobioreactor with LED lighting at 593 nm (yellow) and 860 nm (infrared), corresponding to BChl absorption peaks.
DF was carried out using *Lactobacillus amylovorus* DSM 20532 on a 7.5% (w/v) bread/water substrate at 37 °C for 120 h, resulting in a lactate- and acetate-rich broth. The fermented bread broth (FBB) was supplemented with anaerobic digestate and phosphate buffer to provide essential nutrients, eliminating the need for synthetic media, then sterilized and pH-adjusted to 6.8.
The six PNSB strains were screened in sealed 100 mL serum bottles under batch conditions at 25 °C with white LED light (150 µmol photons m^{-2} s^{-1}). *Cereibacter johrii* Pisa7 showed the highest PHB accumulation (50.73% w/w) and biomass gain (+1.26 g L^{-1}) over 336 h and was selected for scale-up.
In the photobioreactor, *C. johrii* Pisa7 reached PHB contents of 15.17% and 11.51% (w/w) in two trials, with productivities of 2.03 and 0.89 mg L^{-1} h^{-1}. These results confirm process scalability and demonstrate the potential of bread waste as a low-cost carbon source for PHB production within a circular bioeconomy framework.

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L3.1 Quality Control of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) Properties by Engineering Copolymer Blends

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Plastic waste and its negative environmental impacts motivate the development of products and applications from renewable and biodegradable polymers such as polyhydroxyalkanoates (PHA) [1,2]. Quality control and the ability to tune the polymer properties toward specific application requirements are essential for industrial development. Independent of the PHA production method, purification, and recovery of PHA from biomass is always necessary [3]. Therefore, in this presentation, a non-halogenated solvent, dimethyl carbonate (DMC), used for PHA extraction was applied in solution blending to engineer PHA properties by tailoring poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) copolymer blends. The goal is to demonstrate the possibility of combining PHA property modulation with industrial steps of PHA recovery from PHA-rich biomass.

Homogeneous solution blends were formulated in DMC by tailoring the weight ratio of more crystalline poly(3-hydroxybutyrate) (PHB) with a more amorphous pre-eutectic PHBV copolymer blend. The effect of blend composition on thermo-mechanical properties and microstructure phase morphologies was investigated. The blends exhibited an immiscible nature with two T_gs and phase-separated microstructure. Depending on the weight ratio, the morphology ranged from drop-in-matrix to co-continuous domain. The inherent compatibility of PHB and PHBV allowed an interpenetration of harder and softer phases, leading to an enhanced thermo-mechanical property. These biphasic microstructures of copolymer blends enabled the modulation of elongation at break (3 to >100%) and stiffness (1500 to 250 MPa) by increasing weight fraction of pre-eutectic PHBV. With this approach, it is shown that combining various PHBV grades with more and less crystallinity can be a scalable route of quality control towards application-specific PHBV bioplastics.

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L3.2 Harnessing the Predatory Power of *Bdellovibrio bacteriovorus* HD100 for High-Efficiency PHA Recovery: Insights from Microbial Community Dynamics

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Bdellovibrio bacteriovorus HD100, an obligate predatory bacterium, has garnered significant attention as a biological tool in medical and industrial biotechnology. Its unique life cycle occurs within the periplasm of prey cells, where it exploits the host cytoplasm as a nutrient and energy source. Among these applications, the use of *B. bacteriovorus* as a lytic agent for the extraction of polyhydroxyalkanoates (PHAs), biodegradable polymers with growing relevance in the bioplastics sector, offers an environmentally friendly alternative to conventional chemical extraction methods and thereby improves the cost-effectiveness of the production process [1]. Moreover, using mixed microbial cultures (MMCs) for PHA production can reduce operational costs by operating under non-sterile conditions. Additionally, PHA-accumulating biomass can be treated to prevent polymer hydrolysis and dried for storage, enabling the decoupling of biomass production from downstream PHA recovery processes [2].

In this context, integrating *B. bacteriovorus* as a biological recovery tool in MMC systems presents a powerful alternative to traditional chemical and mechanical extraction methods. Nonetheless, the obligate intracellular lifestyle of *B. bacteriovorus* poses challenges in fully elucidating predator-prey interactions, prey specificity, and the influence of community complexity on predation dynamics. To overcome these challenges, a quantitative real-time approach based on flow cytometry microscopy was developed to enable high-throughput, single-cell resolution analysis of predatory activity. Additionally, we conducted a systematic study into the predation efficiency of pre-treated biomass, determining the importance of the membrane prey integrity in the predation event. To further enhance polymer release, we investigated the prey preference of the predator in both pure and mixed cultures, as well as the direct relationship between the prey/predator ratio and predation yield. Our findings also reveal that *B. bacteriovorus* exhibits prey preference, even among closely related strains, and that selecting an appropriate prey-to-predator ratio can significantly boost predation efficiency, achieving up to an 80% reduction in prey biomass compared to initial concentrations.

These insights deepen our understanding of the ecological factors governing *B. bacteriovorus* predation, highlighting its potential as a novel microbial chassis for sustainable PHA recovery and bioplastic production.

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L3.3 Approaches for Efficient and Eco-Friendly PHA Recovery

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Polyhydroxyalkanoates (PHAs) are bio-based and biodegradable polymers considered promising alternatives to conventional fossil-based plastics. However, the economic and environmental sustainability of their production remains limited, primarily due to the complexity and inefficiency of downstream processing. Traditional solvent-based extraction methods typically rely on halogenated solvents and generate significant waste, while chemical digestion approaches can degrade polymer quality and molecular weight. In this context, this study explores two environmentally friendlier and cost-effective downstream processing strategies for PHA recovery.

First, a solvent-based approach using non-halogenated acetone for extracting poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) [P(HB-*co*-HHx)] from wet biomass was developed, with water employed as a green precipitant. Optimal recovery was achieved with 22.5 wt% biomass loading and 20 wt% final water content, yielding purities above 95 %. The HHx content (> 20 mol%) was found to be a critical parameter influencing extraction efficiency, while molecular weight had minimal impact. Pretreatments such as water removal or high-pressure homogenization (HPH) slightly enhanced performance. Importantly, the process eliminated the need for biomass drying, reducing energy consumption and minimizing solvent waste.

In parallel, a mechanical downstream method based on high pressure homogenization was applied to recover both poly(3-hydroxybutyrate) (PHB) and P(HB-*co*-HHx). This technique achieved near-complete yields with purities exceeding 95 %, particularly after optimized purification steps. Even in its basic form, the method provided > 85 % purity, demonstrating its viability as a standalone recovery option or a pretreatment step. Monitoring protein content in the supernatant served as an effective measure of cell disruption, and no signs of polymer micronization were observed (Thiele et al., 2025).

Together, these approaches offer complementary, scalable solutions for sustainable PHA downstream processing. Acetone-water extraction provides high-purity recovery with reduced environmental impact, while high pressure homogenization presents a solvent-free alternative suitable for integration into streamlined bioplastic production workflows.

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L3.4 Films Based on Supercritical Carbon Dioxide Treated Polyhydroxyalkanoates-Rich Microbial Biomass

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Petroleum-based plastics significantly harm the environment, which encourage the development of sustainable alternatives [1]. Bio-based materials from natural sources such as fungi, algae, insects, or bacteria, offer eco-friendly solutions [2]. Materials like cellulose, starch, polyhydroxyalkanoates (PHA), chitosan, polylactic acid (PLA), and proteins are biodegradable, non-toxic, and compatible with biological systems [3,4] . Many also have properties comparable to conventional plastics, making them suitable for a wide range of applications. Polyhydroxyalkanoates (PHA) are one of the most interesting biopolymers produced by bacteria, as they can be tailored to have specific properties to suit diverse applications [5]. Although versatile, these polymers are expensive to produce and by themselves sometimes have poor mechanical properties and low chemical resistance [6] . Blending them with other materials or processing them in other ways may lead to materials with more interesting properties and lower production costs.

In this work, microbial biomass, with PHA content of 53wt% and protein content of 21.5wt%, was evaluated for the preparation of films by hot pressing. The biomass underwent different processing treatments: ultra-turrax homogeneization and supercritical CO_2. Hot-pressed films were evaluated for their thermal, mechanical, optical and barrier properties. Films from the ultrahomogenized and scCO_2 biomass showed the best mechanical properties, with Young Modulus 132.6 ± 62.0 MPa and a tension at break of 5.23 ± 0.70 MPa, they were translucent and presented high oxygen barrier properties. This work demonstrates the direct use of PHA-rich biomass for bioplastic films' fabrication, avoiding the need for PHA extraction.

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L3.5 Design of Crosslinked Networks with Tunable Hydrophilicity Based on PHA

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Polyhydroxyalkanoates (PHAs) are bio-based, biodegradable, and biocompatible polymers. However, their intrinsic hydrophobicity limits their use in certain biomedical applications. To overcome this drawback, chemico-enzymatic approaches combining microbial fermentation with chemical modifications or copolymerization have been explored [1]. An alternative strategy involves the development of crosslinked structures, which can enhance mechanical properties and incorporate more hydrophilic components.

This research aims to design novel crosslinked networks based on PHBHHx with tunable hydrophilicity by incorporating hydrophilic polymers. Among the promising candidates, polyethylene glycol (PEG) and poly(2-oxazoline)s (POx) are widely studied for biomedical applications. While PEG has long been favored for its biocompatibility, solubility, and stealth properties in drug delivery, its repeated administration can induce anti-PEG antibodies. Conversely, POx exhibits minimal immunogenicity and superior chemical stability while maintaining PEG-like properties, making it a compelling alternative, particularly for applications requiring low immunogenicity and high in vivo stability. Since POx lacks reactive functional groups for coupling reactions, it was partially hydrolyzed to introduce primary amines [2]. Polyethyleneimine (PEI), a cationic polymer commonly used in cell transfection, was also employed due to its chemical reactivity and functional versatility.

We synthesized three types of networks: two chemically crosslinked and one physically crosslinked. The first network comprises PEI and polyethylene glycol diacrylate (PEGDA), forming via aza-Michael addition. The second is based on poly(oxazoline-co-PEI) and poly(hexamethylene diisocyanate) (PHMDI), in which urea bonds are formed through condensation reactions. These networks differ both in their hydrophilic components and crosslinking chemistry.

PHAs were embedded within both types of networks, and their thermomechanical and structural properties were characterized. Additionally, co-networks were synthesized using telechelic PHA oligomers bearing terminal isocyanate groups, allowing their integration into the network structure. The resulting materials were analyzed in terms of structure, mechanical behavior, and solvent interaction in environments of varying polarity. The material $PHA_{26}POx_{71}PHMDI_3$ stood out for its homogeneity, high swelling ratios in various solvents (250% in water, 220% in DMSO, 110% in acetone, and 260% in an isopropanol/isooctane/acetone mixture), as well as its good mechanical properties. These performances are attributed to a low crosslinking density and the ability of free PHA chains to crystallize, thereby acting as reinforcing domains [3].

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L3.6 Evaluation of Mechanical Properties and Biodegradation Behaviour of Polyhydroxyalkonoate (PHA) Based Blends

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This study evaluates the effects of blending poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (PX), on the mechanical and physical properties, dynamic mechanical properties, warping, and shrinkage behavior of 3D printed and injection molded samples. Additionally, the influence of different blending ratios on biodegradation in water and sediments from a lake was investigated.

These copolymers belong to the PHA family of biopolyesters, which are synthesized by several prokaryotic microorganisms [1]. The blends' viscoelastic properties were investigated through dynamic mechanical analysis (DMA) through the storage modulus (G') and tan delta (tan(δ)). The results showed that increasing the PX content in the blend reduced the storage modulus, indicating a decrease in stiffness when PX was added [2]. The tan delta curves revealed a greater damping capacity in blends with a higher PX content, indicating improved energy dissipation. The warping coefficient analysis revealed that blends with a higher PX content had significantly lower warpages than pure PHBV, suggesting that PX blends have the potential to reduce warping during additive manufacturing [3].

Shrinkage analyses revealed that a higher PX content was associated with less shrinkage, especially in the injection flow direction. This study clearly showed that the amorphous nature of PX plays a role in shrinkage reduction [4].

Biodegradation in water and lake sediments showed that within 20 weeks, most samples were completely degraded, with samples having a higher PX content presenting better degradation rates. This reveals a sustainable material that can quickly degrade after use. However, in water, the degradation was demonstrated at a slower rate compared to the degradation in sediments; variation happened due to the lack of microbial diversity in freshwater ecosystems [5].

This research shows how to tailor the mechanical performance of PHA-based materials by blending, for applications requiring different stiffnesses and characteristics, thereby reducing shrinkage and warping. The results suggest an improved quality of 3D printed objects, biodegradable in water and sediments, leading to a wider and more sustainable range of applications.

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L3.7 Adaptation of *Pseudomonas putida* KT2440 to 6-Acetylthiohexanoic Acid (6-ATH) and Its Implications in PHA Metabolism

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The increasing emergence of antibiotic-resistant pathogens is a growing global health threat and efforts are needed to study and develop novel antimicrobial strategies. Polyhydroxyalkanoates (PHAs) are biodegradable and biocompatible polymers with promising biomedical and environmental applications. Among them, PHACOS, a functionalized PHA containing thioester groups, has shown antimicrobial activity against *Staphylococcus aureus* [1], offering a potential tool to design infection-preventing biomaterials. This polymer is synthesized by *Pseudomonas putida* KT2440 using fatty acids and the precursor 6-acetylthiohexanoic acid (6-ATH) [2]. In this work, we explored sugar-based carbon sources to enhance PHACOS production, aiming to improve sustainability and economic feasibility.

During our studies, *P. putida* KT2440 growth was inhibited when cultured with fructose and 6-ATH. However, after extended incubation, a spontaneously adapted strain (*P. putida* A1) emerged, achieving OD_{600} = 1.30 after 24 hours. PHA content dropped from 39% of CDW without 6-ATH, to 7% with the precursor. Genome sequencing of the A1 strain revealed a single nucleotide deletion in *fadDI* gene, encoding a long-chain fatty acid acyl-CoA synthetase involved in β-oxidation and PHA metabolism [3]. Structural predictions suggested C-terminal domain disruption, potentially altering enzymatic function. To elucidate the role of *fadDI* and its interaction with 6-ATH, we constructed the *P. putida* Δ*fadDI* mutant. Both Δf*adDI* and A1 strains showed similar phenotypes when co-fed with fructose and 6-ATH (OD_{600} = 1.2; PHA = 7%). Given the role of FadDI enzyme in fatty acid metabolism, we tested growth and biopolymer synthesis in cultures supplemented with octanoic and decanoic acids. Co-feeding with octanoate and 6-ATH led major reductions in growth and PHA accumulation in the Δf*adDI* mutant strain (OD_{600} = 1.5, PHA = 20%) compared to KT2440 wild-type strain (OD_{600} = 8.7, PHA = 75%). Regarding decanoate cultures, both growth and PHA synthesis were markedly inhibited in the Δ*fadDI* mutant strain.

Our findings provide new insights into FadDI fatty acid acyl-CoA synthetase function in *P. putida* KT2440 and its interplay with 6-ATH. The metabolic shifts suggest compensatory activities by other acyl-CoA synthetases and possible inhibitory effects of 6-ATH on PHA biosynthesis. Further studies will clarify the regulatory mechanisms and support optimized PHACOS production in engineered strains.

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L.4.1 Transforming Bacterial Cellulose into Microparticles for Biomedical Applications

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Bacterial cellulose has emerged as a sustainable biopolymer with remarkable potential in a wide range of applications [1], including in the design of sustainable and efficient drug delivery systems, which still remain a significant challenge in pharmaceutical research. This study reports a novel approach for producing bacterial cellulose (BC)-alginate composite microparticles utilizing blow-spinning technology and an eco-friendly solvent Nmethylmorpholine-N-oxide (NMMO). These microparticles were designed to overcome the intrinsic constraints of pure BC particles, including inadequate size homogeneity and poor stability, while also mitigating the rapid deterioration linked to alginate-based systems. The integration of the BC and alginate system resulted in an average particle diameter of around 50 µm, as validated by particle size distribution measurements. The produced microparticles exhibited exceptional stability and uniformity. Rheological studies revealed that the addition of alginate significantly affects the viscosity and shear-thinning properties of polymer solutions, facilitating the creation of smaller and more stable particles. Studies on drug encapsulation, using aspirin as a model drug, revealed loading efficiencies of 45% for pure BC particles, 53% for BC:alginate (9:1) composites, and 58% for BC:alginate (8:2) systems. Fluorescence spectroscopy validated effective drug loading, while drug release studies demonstrated a well controlled and steady release profile over 120 hours, illustrating improved diffusion control for prolonged therapeutic delivery. This study demonstrates the potential of BC-alginate composites as a scalable, environmentally sustainable system for controlled drug administration. It also highlights the enhanced stability, encapsulation efficiency, and adjustable release profile achieved with these composites. The integration of rheological optimization and ecological solvent processing represents a significant advancement in polysaccharide-based drug delivery systems.

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L 4.2 Biofertilizers Reinvented: when Plant Growth-Promoting Bacteria Build their Own Fertilizer Capsule

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The rising global demand for sustainable agriculture necessitates innovative approaches that maintain high crop yields while reducing reliance on conventional chemical fertilizers. Biofertilizers containing plant growth-promoting rhizobacteria (PGPR) represent a promising alternative, as they enhance soil fertility through mechanisms such as nitrogen fixation, production of growth regulators, and soil microbiome enhancement. However, the widespread adoption of bioinoculants is currently hindered by economic constraints, primarily stemming from traditional encapsulation processes that require external hydrogel carriers and complex gelation techniques.

This study introduces an advanced biofertilizer production concept based on the encapsulation of beneficial rhizobacteria within self-produced exopolysaccharide gels. Specifically, *Azotobacter vinelandii*, known for its capacity to naturally synthesize alginate, was utilized to form hydrogel matrices directly during bacterial cultivation. This innovative in-situ encapsulation eliminates the need for externally added gelation agents, thereby simplifying bioinoculant production, reducing manufacturing costs, and enhancing overall economic feasibility [1].

A comprehensive systematic investigation was conducted to elucidate essential parameters affecting biofertilizer development, including optimal bacterial cultivation conditions, gelation efficiency, and final product stability. Multiple *Azotobacter vinelandii* strains were screened, with strain CCM 289 exhibiting superior alginate production, robust gelation properties, and effective synthesis of plant growth-promoting metabolites such as indole-3-acetic acid and siderophores. Beyond demonstrating basic proof-of-concept, this research also provides fundamental insights into the relationships between the encapsulation methodology, structural characteristics of alginate hydrogels, and resultant biofertilizer performance. By revealing critical connections between gel structure and bioactivity, this study paves the way for rational optimization of encapsulation procedures, allowing tailored design of next-generation biofertilizers.

In summary, this innovative encapsulation approach, which utilizes self-generated exopolysaccharide gels, represents a promising advancement in biofertilizer technology. It provides considerable environmental advantages by lessening reliance on conventional fertilizers, while concurrently being economically viable through streamlined production processes. The outcomes underscore the substantial potential of self-produced alginate-based PGPR formulations as robust, environmentally sustainable solutions for contemporary agricultural challenges, thereby significantly contributing to enhanced soil fertility, improved plant growth, and long-term agricultural sustainability.

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L4.3 Dissolving Microneedle Arrays Technology for Efficient and Painless Drug Delivery

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Microneedles (MNs) are an emerging technology in transdermal drug delivery, offering significant advantages over traditional oral and injectable routes, including pain-free administration, reduced infection risks, improved drug bioavailability and patient compliance [1]. Among the various MNs types, dissolving microneedles (DMNs), fabricated from biocompatible polymers, dissolve upon insertion into the skin, releasing encapsulated drugs in a controlled rate [2]. In this study, FucoPol, a fucose-rich exopolysaccharide produced by Enterobacter A47 [3], was explored as a novel matrix material for DMNs fabrication due to its inherent bioactive properties, including anti-inflammatory, antioxidant, and potential immunomodulatory effects [4].

DMNs arrays were fabricated by casting various FucoPol concentrations (2, 5, and 10wt%) into polydimethylsiloxane (PDMS) moulds, followed by multiple vacuum cycles (~20 mbar) to ensure complete cavity filling, and dried at room temperature. Two different polymers, microbial-derived medium-chain-length polyhydroxyalkanoate (mcl-PHA) or polycaprolactone (PCL), were tested as potential materials to form a supportive backlayer. Model drugs such as sodium ibuprofen and sodium diclofenac were incorporated into the polymeric matrix before DMNs fabrication to assess drug loading capability and release behavior.

DMNs array fabrication was highly affected by fabrication parameters, such as solution viscosity, number of vacuum cycles, applied pressure, and backlayer material. The resulting DMNs exhibited a pyramidal shape with an average height of 600 µm. Dissolution studies in agarose gel demonstrated complete DMNs dissolution in under 1 min. Preliminary *in vitro* skin insertion tests showed that adequate mechanical strength and successful penetration, supported by histological imaging. Initial drug release profiles indicate a rapid release within a few minutes post-insertion.

These findings highlight FucoPol as a promising natural polymer for the development of DMNs systems for transdermal drug delivery.

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L4.4 Polyhydroxyalkanoates: Tailoring Electrospun Membranes for Skin Repair

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Polyhydroxyalkanoates (PHAs) are a family of biopolymers with diverse properties, enabling their application in a wide range of fields. In biomedicine, they stand out as promising alternatives to conventional materials for tissue engineering. In the context of skin repair, traditional dressings such as gauze provide only passive protection, often resulting in suboptimal wound healing. Electrospinning has emerged as a powerful technique to tailor the morphology and structure of PHAs, allowing the fabrication of biocompatible membranes that offer not only structural support and barrier function but also a favorable environment for cell regeneration [1–3]. This work aimed to optimize the electrospinning parameters for producing porous, fibrous membranes based on two different PHA copolymers and to characterize them in terms of morphology, structure, and thermo-mechanical properties, as well as assess their potential use as wound dressings. AQUI The copolymers differed in composition and molecular weight, requiring specific adjustments in the electrospinning process to achieve comparable fiber diameters (~1.5 µm). Ceftriaxone, an antibiotic, was incorporated into the membranes to confer antibacterial properties. The drug release profile and antibacterial activity against Staphylococcus aureus were evaluated. The resulting membranes exhibited suitable wettability and water vapor transmission rate (WVTR) for wound dressing applications, in addition to showing no cytotoxic effects. Preliminary results demonstrated antibacterial activity; however, the influence of zinc oxide on drug release modulation is still under investigation. Overall, initial in vivo studies revealed that PHA-based electrospun membranes outperformed a commercial dressing in promoting wound healing, highlighting the beneficial effect of the nanofibrous structure in mimicking the extracellular matrix and supporting tissue regeneration.

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L4.5 Enrichment and Application of Bacterial Sialic Acids Containing Polymers from the Extracelular Polymeric substances of "Candidatus accumulibacter"

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Pseudaminic and legionaminic acids are a subgroup of nonulosonic acids (NulOs) unique to bacterial species [1]. There is a lack of advances in the study of these NulOs due to their complex synthesis and production. Recently, it was seen that "*Candidatus Accumulibacter*" can produce Pse or Leg analogues as part of its extracellular polymeric substances (EPS). In order to employ a "*Ca. Accumulibacter*" enrichment as production platform for bacterial sialic acids, it is necessary to determine which fractions of the EPS of "*Ca. Accumulibacter*" contain NulOs and how to enrich and/or isolate them. We extracted the EPS from granules enriched with "*Ca. Accumulibcater*" and used size-exclusion chromatography (SEC) to separate them into different molecular weight (MW) fractions. This separation resulted in two high molecular weight (> 5500 kDa) fractions dominated by polysaccharides, with a NulO content up to 4 times higher than the extracted EPS. This suggests that NulOs in "*Ca. Accumulibacter*" are likely located in high molecular weight polysaccharides. Additionally, it was seen that the extracted EPS and the NulO-rich fractions can bind and neutralize histones. This opens the possibility of EPS and NulO-rich fractions as potential source for sepsis treatment drugs.

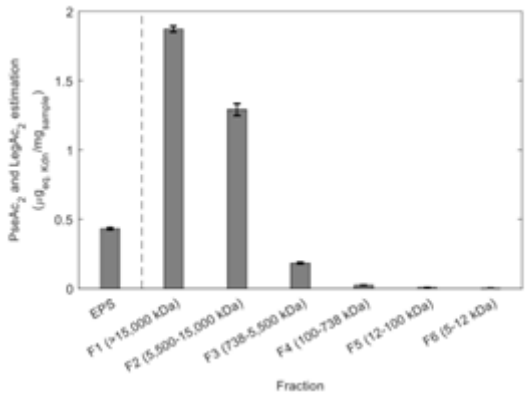

Fig. 1 Relative quantification of NulOs in the extracted EPS And in the different MW fractions obtained by SEC. The detected NulO is LegAc2 or PseAc2, which could not be distinguished as the have the same molecular weight. The amount of NulOs was estimated based on the relative area of a spiked standard of Kdn

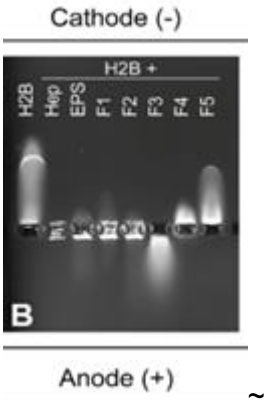

Fig. 2 Histone binding assay.Histones were incubated with
different amounts of the fractions of the EPS.

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L4.6 Tailoring Double-Shelled Hollow Microparticles from Polyester-Modified Bacterial Cellulose for Efficient Microbial Encapsulation and Release

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The development of advanced encapsulation systems capable of protecting and delivering living microorganisms is of growing interest for applications in health, agriculture, and environmental biotechnology. Key challenges include achieving biocompatibility, mechanical robustness, and precise control over release profiles using fully biodegradable materials.

In this work, we present the design and fabrication of double-shelled hollow microparticles based on bacterial cellulose (BC) chemically modified with polyhydroxyalkanoates (PHAs). BC, a highly crystalline and hydrophilic biopolymer, was covalently grafted with several PHAs—poly(3-hydroxybutyrate) (PHB), poly(3-hydroxyoctanoate-co-3-hydroxyhexanoate) (PHOHx), and poly(3-hydroxy-acetylthioalkanoate-co-3-hydroxyalkanoate) (PHACOS)—to improve compatibility with hydrophobic matrices and tune the physicochemical properties of the system. The grafting reactions, carried out via acyl chloride intermediates in ionic liquid media, yielded hybrid materials that retained the key attributes of BC while incorporating the barrier and functional properties of PHAs.

These modified biopolymers were used to formulate microparticles through coaxial electrospray technology. Several shell materials, including PHB, PBS, and PBAT, were evaluated. The optimized formulation resulted in reproducible double-shelled hollow microparticles composed of a PHB outer shell and a BC-PHB inner layer. The particles displayed excellent spherical morphology, high mechanical integrity, and a hollow core suitable for the encapsulation of viable bacteria at densities up to 10^8 CFU/mL. Viability and release assays confirmed prolonged bacterial survival and a gradual, sustained release over time.

These results highlight the versatility of the developed system for the encapsulation and protection of diverse bioactive elements, offering a biodegradable and scalable platform for future applications in targeted microbial delivery, bioremediation, and smart bioactive packaging.

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L4.7 Chitosan/Carboxymethyl Cellulose Aerogels for Controlled Release Applications in Inflammatory Bowel Disease Treatment

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In recent years, biopolymer-based aerogels have gained considerable attention in the field of targeted drug delivery due to their unique combination of high surface area, low density, and highly porous structure [1, 2]. These characteristics allow for efficient drug loading, sustained release, and responsiveness to environmental triggers such as pH, temperature, or enzymes. Among various biopolymers, chitosan (CTS) and carboxymethyl cellulose (CMC) stand out owing to their complementary physicochemical properties [3, 4]. CTS, a naturally derived polysaccharide, is well-known for its biocompatibility, biodegradability, and mucoadhesive nature, as well as its pH-sensitive behaviour, which makes it particularly suitable for colon-specific delivery systems. CMC, on the other hand, is a water-soluble cellulose derivative that enhances hydrophilicity, structural integrity, and gel-forming capacity of the matrix. When they were combined, CTS and CMC form a stable polyelectrolyte complex that can be tailored to achieve site-specific drug delivery with improved functional performance. This synergy makes them highly attractive for applications requiring localized therapeutic action, such as the treatment of inflammatory bowel diseases (IBD).

In this study, a CTS/CMC-based aerogel system were developed and evaluated as a delivery platform for 5-aminosalicylic acid (5-ASA), a commonly used therapeutic agent in IBD treatment. The chemical structure of the produced aerogels was characterized using Fourier Transform Infrared Spectroscopy (FTIR), and morphological analysis was performed via Scanning Electron Microscopy (SEM). Critical physical properties such as apparent density and porosity were also determined to evaluate the suitability of aerogels for biomedical applications. Swelling studies and *in vitro* drug release experiments were carried out under simulated gastrointestinal conditions to mimic the intended site of action. Through these analyses, the release behaviour of 5-ASA from the aerogel matrix was investigated, with special attention to release patterns at colonic pH levels.

All findings proved that developed CTS/CMC aerogel system could hold significant potential for targeted drug delivery applications, particularly in diseases needs localized intestinal therapy. The outcomes of this work are expected to provide a foundation for future studies aimed at optimizing such delivery systems and also advancing their clinical translation.

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L5.1 Bioplastics from the Field: Valorizing Agricultural by-Products through Sugars365

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The search for sustainable feedstocks for the future of chemical and plastic production is a top priority for industry stakeholders. Major manufacturers, consumer brands, and European regulatory bodies have set ambitious sustainability targets and are actively seeking innovative approaches to reduce carbon footprints across the value chain. As a result, there is an increasing urgency to transition to renewable resources and sustainable feedstocks. A major challenge in this transition lies in identifying and processing alternative biomass sources. Fibers365 addresses this challenge through a circular concept that leverages breakthrough technologies to convert agricultural by-products into valuable outputs such as biogas, fibers, fertilizers, and feedstocks for biomolecule and biopolymer biosynthesis. Its patented steam explosion process can handle a broad range of non-food, annually renewable plants without the use of added chemicals—enhancing both sustainability and circularity by closing the CO_2 cycle and contributing to soil fertility within 365 days. Until now, the liquid phase generated during the steam explosion of agricultural fibers has been primarily used for biogas production. However, its full potential remained unexplored. Fibers365 recently developed a second-generation (2G) bio-feedstock named Sugars365, derived from this liquid stream. Sugars365 contains up to 35% (w/w) free sugars and less than 2% solid content. It has already demonstrated successful lipid production through yeast fermentation. This study investigates the potential of Sugars365 as a substrate for producing biodegradable bioplastics, specifically polyhydroxyalkanoates (PHAs). The feedstock presented a carbon-to-nitrogen ratio of approximately 40, with glucose, xylose, acetic acid, and formic acid identified as the main carbon sources. Importantly, only low levels of bacterial growth inhibitors were detected. When tested with three bacterial strains known to synthesize polyhydroxybutyric acid (PHB) and other medium-chain PHAs, Sugars365 supported both microbial growth and biopolymer accumulation.

Our results confirm that both C6 and C5 sugars, as well as the present organic acids, were effectively utilized as carbon sources. PHA accumulation reached up to 60% of cell dry weight (CDW). These findings highlight Sugars365 as a promising sustainable 2G-feedstock for the biotechnological production of biodegradable biopolymers—offering a high-value route to further valorize side streams from the rapid steam explosion pretreatment of agricultural by-products.

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L5.2 Single-Cell Protein Bioplastic Films from Recovered Nitrogen and Carbon: a Circular Approach with High Anaerobic Biodegradability

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The increasing environmental burden of fossil-derived plastics and their poor degradability under real-world conditions drive the need for innovative, biodegradable materials from renewable resources [1]. This study presents a novel protein-based bioplastic film derived from single-cell protein (SCP) produced by upcycling nitrogen from anaerobic digestate and carbon from cheese whey via food-grade microbial cultures (Figure 1). The SCP was plasticized with glycerol and processed into flexible films through compression molding. Morphological and mechanical analyses revealed that the films retained intact microbial cell structures, resulting in porous matrices with moderate mechanical strength (modulus: 5–10 MPa; strength: 0.12–0.22 MPa). Despite relatively low cohesion, the materials demonstrated desirable translucency and moisture uptake properties suitable for edible film or coating applications. In view of the application in the food sector, the biodegradability of the SCP films was evaluated under mesophilic anaerobic digestion conditions through biochemical methane potential tests. The SCP films achieved complete biodegradability (100%) and a high biomethane yield (471 ± 8 mL $CH_4 \cdot g^{-1}$ VS) after 34 days, outperforming conventional biodegradable polymers such as PLA and starch-based films. This high degradation rate is attributed to the high biodegradability of the raw material, including SCP and glycerol. These findings underscore the potential of SCP films as sustainable alternatives to petroleum-based and even conventional bio-based plastics in food packaging and coatings. Furthermore, their compatibility with anaerobic digestion offers a unique end-of-life valorization pathway, closing the loop on nitrogen and energy recovery within circular bioeconomy systems.

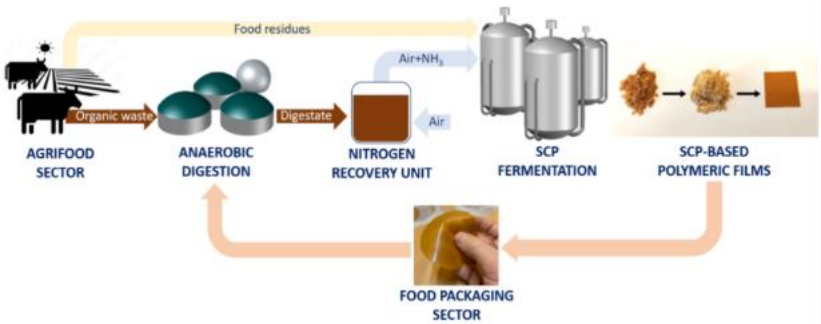

Figure 1. Integrated biorefinery scheme valorizing food residues and waste nitrogen into SCP-based biopolymers. The latter biopolymers can be used for food packaging applications and eventually be biodegraded and valorized through anaerobic digestion, targeting circular nutrient and energy recovery.

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L5.3 From Breakdown to Biocycle: Modeling PHB Depolymerization for Circular Use by introducing novel probability function

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Current analytical and mathematical fail in predicting the molecular mass distribution of polymers following chemical degradation. We present a simulation-assisted kinetics model to elucidate the depolymerization dynamics of PHB, overcoming current limitations.

Our simulation replicates the random scission process of PHB hydrolysis allowing us to fit GPC data and calculate the yield of water-soluble oligomers. A key assumption—validated by HPLC-MS measurements—is that oligomers smaller than seven repeating units are water-soluble and represent the effective depolymerization yield. The simulation provides the fraction of these oligomers, which follows an exponential function of the conversion and is integrated into a pseudo second order kinetics model to derive a time-dependent yield prediction. Using this model, we accurately predicted the yield of our own PHB hydrolysis experiments in the melt. Furthermore, by integrating the Arrhenius equation, the model was extended to capture temperature dependence and successfully reproduced experimental data from Li and Stratman [1], providing strong validation of its predictive power.

Complementary to the modeling, carbon source screening confirmed that all water-soluble depolymerization products are suitable substrates for microbial fermentation to re-synthesize PHB, enabling a closed-loop biopolymer cycle.

We are developing a new probability mass function, analogous to the Schulz-Flory distribution, to replace the simulation component of our model. By integrating this function over the desired range of repeating units (specifically, chains of length 1–7), we can directly calculate the probability of short-chain formation and thereby determine the depolymerization yield. This probability mass function has the potential to generalize beyond PHB, offering a versatile tool for predicting molecular mass distributions resulting from the chemical degradation of both linear and branched polymers [2].

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L5.4 Biobased vs. Conventional Microplastics: The Impact on Gilthead Seabream (*Sparus aurata*) Brain Health and Behaviour

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In recent years, the packaging industry has strongly invested in the development of new biopolymers (e.g. PBS, PPF, PLA) that can be used as suitable and more sustainable alternatives to conventional petroleum-based plastics (e.g. PET), widely acknowledged as environmentally hazardous [1]. However, recent evidence has showed that biopolymers may not be as “eco-friendly” as they are assumed to be, as their higher environmental degradability may facilitate the breakdown and leaching of monomers and/or additives for which the toxicological attributes to biota are still not well established [2,3]. In order to ensure biopolymers are truly safer and more sustainable than conventional polymers, it is essential to keep assessing the physiological consequences of various biomicroplastics exposure in marine species. To this end, this study aimed to compare the behavioural and brain toxicological responses of the commercially- and ecologically-important gilthead seabream (*Sparus aurata*) exposed to one conventional and widely-used petroleum-based polymer (polyethylene terephthalate, PET), and two biopolymers (polybutylene succinate, PBS, already commonly used, and polypropylene fumarate, PPF, which shows promising applications in the packaging industry and in biomedicine) [4]. *S. aurata* juvenile specimens were orally exposed to microplastic-contaminated feeds (500 µm, 60 particles day^{-1}, representing environmentally relevant concentrations) for 28 days, followed by a 7 day depuration period to ascertain recovery responses. Biomarkers indicative of oxidative stress (catalase, glutathione S-transferase and superoxide dismutase activities), cell damage (ubiquitin content and lipid peroxidation) and neurotransmission (acetylcholinesterase activity), as well as histopathological alterations, animal condition and behavioural performance (swimming activity and antagonistic interactions), were analysed. Data analysis is ongoing. However, preliminary results show that microplastic exposure had no effect on animal condition and neurotoxicity. Nonetheless, PET and PBS exposure moderately disrupted the antioxidant defense system, while PPF inflicted no significant effects. Additionally, PET contributed to the increase of antagonistic interactions amongst fish, but no impacts in swimming behaviour were found.

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L5.5 Characterization of Exopolysaccharide-Based Biostimulants Obtained from Agri-Food Byproducts to Enhance Plant Tolerance to Salinity

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Soil degradation, climate change, and salinity stress increasingly compromise the sustainability of agricultural systems. In response to these growing concerns, biostimulants appear as viable alternatives to reduce conventional fertilizers' use, offering enhanced plant stress tolerance and promoting soil health while reducing negative environmental impacts [1]. Within this context, bacterial exopolysaccharides (EPS) stand out for their functional roles in water retention, soil aggregation, and abiotic stress alleviation. However, high production costs, primarily attributed to the need for expensive substrates, limit their agronomic use [2]. Solid-state fermentation (SSF) of agro-industrial residues emerges as a sustainable strategy to tackle such a challenge while aligning with circular bioeconomy principles [3]. This approach supports EPS production and addresses another pressing issue in the agri-food sector: a suitable management strategy for valorising solid organic waste and by-products.

This study focuses on the molecular and rheological properties of EPS produced by *Burkholderia cepacia* through SSF using solid by-products from the juice industry, namely Ginger Juice Waste (GJW) [4]. These EPS have been tested in agronomic bioassays for their potential application as biostimulants, aiming to enhance plant physiological responses and improve tolerance mechanisms under saline stress conditions. To better understand their physiological action mechanism and the specific role in promoting plant resilience to extreme conditions, a detailed physicochemical EPS characterization was performed.

Two fermented GJW containing EPS were studied, one processed under sterilized conditions, and a second without sterilization, and both were compared to unfermented GJW material. After a solid-liquid extraction of the samples, three different purification strategies, namely ethanol precipitation of crude EPS, dialysis of the crude EPS using a 12-14 kDa membrane, and ultrafiltration at 30 kDa. EPS fractions of varying purity were analysed and compared. EPS characterization included monosaccharide sugar composition, volatile fatty acids content, molecular weight distribution, FTIR spectroscopy, thermogravimetric analysis, rheological behaviour, and elemental composition. Results show clear differences in composition and structure based on the processing typology, and an increase in the sugar content of the fermented material, confirming the presence of EPS in the biostimulant formulations.

These insights contribute to a better understanding of SSF-derived EPS and their role in enhancing plant tolerance to saline stress. Overall, this work supports the

development of sustainable biostimulants from agro-industrial by-products to enhance plant resilience, aligning with circular bioeconomy strategies.

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L5.6 Fit-for-purpose PHBV Production from Lignocellulosic Biomass: Coupling an Artificial Rumen and Sequencing Batch Reactor

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The transition to a circular bioeconomy requires biotechnological pathways to convert renewable, low value feedstocks into high-value bioproducts, including functional biopolymers. Plant-derived renewable resources are typically rich in fibrous material, predominantly in lignocellulosic biomass, where cellulose and hemicellulose are closely associated with lignin in a complex structural matrix. We present the use of an Artificial Rumen Reactor (ARR) for the continuous conversion of lignocellulosic biomass into volatile fatty acids (VFAs), which in turn serve as precursors for microbial synthesis of polyhydroxyalkanoates (PHAs), specifically poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). This process is part of the JTF-funded project *"Market-Driven Synthesis of Fit-for-Purpose Bioplastics"*, which optimises fermentation to produce PHAs and opens possibilities for producing application specific bioplastics with unique properties**.**

Inspired by the rumen of ruminants, the ARR mimics this complex microbial ecosystem under controlled conditions. By decoupling Solid Retention Time (SRT, 10 days) from Liquid Retention Time (LRT, 2 days), the reactor sustains high hydrolytic activity while continuously removing VFAs via membrane filtration, preventing product accumulation. Operated under anaerobic (and microaerobic) conditions, the ARR demonstrates long-term stability, providing a reliable platform for continuously converting lignin-rich biomass into ~360 mg VFAs per gram of dry biomass. The resulting VFA profile from roadside grass is dominated by acetic (~68%) and propionic acid (~19%), with minor amounts of butyric-, isobutyric-, and valeric acid [1].

The VFAs produced serve as a direct feedstock for PHBV-accumulating mixed microbial cultures cultivated under a feast-and-famine regime in a Sequencing Batch Reactor (SBR) system. Modulating the VFA composition in the feed is known to influence the hydroxyvalerate (HV) content and the molecular weight of the resulting PHBV [2]. Current research is focused on influencing the VFA profiles generated by ARR, e.g., using alternative substrates or bioaugmentation and coupling the ARR with the PHBV-producing SBR to achieve precise control over HV content. Depending on the intended application, this strategy enables fit-for-purpose PHBV production, yielding polymers with custom made properties such as flexibility, tensile strength, or biodegradation rate. Our findings demonstrate that this integrative technological approach positions the ARR as a plug-and-play module within bioplastic production chains.

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L5.7 Enabling a Circular Bioeconomy: Process Innovations for PHA Bioplastics

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Polyhydroxyalkanoates (PHAs) are fully bio-based and biodegradable polyesters offering significant potential as sustainable alternatives to conventional plastics. However, their commercial viability remains challenged by high production costs, particularly concerning feedstocks and downstream processing.

This overview presents integrated strategies along the entire PHA value chain — from substrate selection to product recovery — aimed at improving the economic and environmental performance of microbial PHA production. Various carbon sources, including animal by-products [1,2], and plant oils such as canola oil [3], were evaluated for their suitability in high-cell-density fed-batch fermentations using *Ralstonia eutropha* Re2058/pCB113.

Optimized feeding protocols enabled biomass concentrations above 300 g/L and volumetric PHA productivities exceeding 3 g/(L·h). Targeted feeding strategies also allowed for the adjustment of the monomer composition of P(HB-*co*-HHx) copolymers between 0 and >30 mol% HHx, tailoring material properties to specific application needs. Further optimization focused on nutrient limitation strategies (nitrogen and phosphorus) and the systematic adjustment of the carbon-to-phosphorus ratio, which significantly influenced both biomass formation and PHA accumulation.

Downstream processing was addressed through environmentally benign and scalable methods, including solvent extraction using acetone/water systems and mechanical disruption via high-pressure homogenization. Both approaches achieved PHA purities above 95 % without the need for biomass drying or halogenated solvents, offering viable alternatives to conventional recovery techniques. [4-5]

The results demonstrate a comprehensive and modular bioprocess platform for the sustainable and cost-effective production of tailor-made PHAs, supporting their broader implementation in a circular bioeconomy.

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L6.1 Extracellular mcl-PHA Depolymerases in *Pseudomonas*: Enzymatic Drivers of Bioplastic Degradation

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Increasing environmental concerns over plastic pollution have accelerated the global shift toward a circular economy, where bioplastics offer a promising and sustainable alternative, supporting a more eco-friendly plastic life cycle. In this context, composting and biological recycling stand out as key strategies, relying on microorganisms and their enzymatic capabilities to break down polymers into their monomeric components. This approach is particularly relevant for the degradation of bioplastics such as polyhydroxyalkanoates (PHAs), a widely studied family of biodegradable and biocompatible biopolymers that are naturally produced and accumulated by microorganisms. Their environmental degradation is mainly mediated by extracellular PHA depolymerases (ePhaZs), specialized enzymes secreted by a limited group of microbial species. One such example is *Pseudomonas solani* GK13, which accumulates PHA and secretes a prototypical ePhaZ that specifically degrades mcl-PHA [1]. In this study, a comprehensive characterization of strain GK13 PHA metabolism and its genomic features was performed [2]. This in-depth analysis provided the basis for broadening the scope of our investigation to other bacteria belonging to the *Pseudomonas* genus. The (meta)genomic analysis performed revealed a close correlation between the ePhaZ production capacity and the ability to synthesize mcl-PHA within this genus. This finding suggests that such microorganisms may contribute to the carbon economy of microbial communities by storing PHA in carbon-rich times and sharing it with the rest of the population during times of carbon scarcity. Building on these insights, we predicted and experimentally validated the potential of other microorganisms, such as *P. benzenivorans* 1477, to both produce and degrade PHA. Moreover, the *in silico* screening conducted led to the identification of a series of novel putative ePhaZ enzymes. Selected candidates were subsequently cloned, purified, and tested, confirming their enzymatic activity against PHA substrates. This work lays the groundwork for future biotechnological applications, including the engineering of microbial consortia for efficient bioplastic recycling and the development of enzyme-based systems to accelerate PHA degradation under controlled conditions.

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Economy (PTI-Susplast+). This study was supported by an FPU (Ayudas para la Formación de Profesorado Universitario 2021) fellowship (FPU21/04158) from the Spanish Ministry of Universities.

L6.2 Monitoring Microplastic Generation from PHA Degradation in Soils Using Fluorescence-Based Detection Methods

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Soil pollution is currently a major environmental issue, with its extent continuing to grow largely due to the widespread use of plastics [1]. To help reduce this contamination, many initiatives are promoting the use of biodegradable plastics such as polyhydroxyalkanoates (PHA). However, current knowledge about PHA degradation remains incomplete, particularly regarding their behavior in soils. Yet, elucidating the biodegradation mechanisms in terrestrial environments is crucial, especially to assess the potential formation of microplastics during this process [2]. Although methods for extracting and analyzing microplastics are now well established [3], several recent studies have highlighted the benefits of combining these approaches with fluorescence-based staining techniques to improve the detection and quantification of such particles in complex matrices like soils [4,5]. In this work, we developed and optimized new experimental protocols to monitor the formation of microplastics generated from the degradation of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P34HB) under controlled soil incubation conditions. By combining controlled degradation tests with a custom-designed fluorescence detection system and dye-based staining methods, we aimed to better characterize the fragmentation pathway, quantify the number of microplastics produced, and evaluate how their generation evolves in relation to the degradation rate of the PHA material. Preliminary results demonstrate the efficiency of this approach in detecting micrometer-sized fragments with high sensitivity, allowing for a precise characterization of the properties of the generated microplastics, including their size, thickness, and shape. These results offer valuable insights into the fragmentation mechanisms of PHA during the degradation process. Overall, these methodological developments provide powerful tools to advance our understanding of the behavior of biodegradable polymers in soils, moving beyond the sole concept of biodegradability toward a more comprehensive evaluation of their environmental impact.

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L6.3 Identification and Functional Characterization of Enzymes Involved in Poly(*cis*-1,4-isoprene) Degradation in *Rhodococcus*

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The microbial degradation of synthetic and natural poly(*cis*-1,4-isoprene) rubber is expected to become an alternative treatment technique for waste from poly(*cis*-1,4-isoprene) products, such as scrap tires. In this study, we aimed to develop a biodegradation technology for natural rubber (NR) waste by utilizing NR-degrading bacteria and their enzymatic functions. Specifically, we investigated the identification of genes, and the enzymatic functions involved in the degradation of oligo-isoprenoid aldehydes (OIA) generated during the poly(*cis*-1,4-isoprene) metabolism of a gram-positive rubber-degrading bacterium, *Rhodococcus* sp. strain RDE2 [1], which was isolated from the waste of a rubber-processing factory.

Affinity analysis toward cofactors and deproteinized NR (DPNR) induction assays based on OIA oxidation activity in the cell extracts suggested that the OIA degradation in strain RDE2 is primarily mediated by aldehyde dehydrogenases (ALDH) with high affinity for NAD^+ and without significant induction by DPNR. Molecular and phylogenetic analysis of ALDH genes predicted from the genome data of strain RDE2 identified six candidate ALDH genes (RDE2_08210, RDE2_16440, RDE2_35730, RDE2_42360, RDE2_43470, and RDE2_46120) that are phylogenetically close to known OIA-degrading ALDHs [2, 3]. Transcriptional analysis of these genes under DPNR-supplemented growth conditions indicated that ALDH genes without significant transcriptional induction are likely involved in OIA degradation.

Purified recombinant proteins of the ALDH candidates were tested for OIA oxidation activity. Among them, RDE2_16440 and RDE2_35730 showed high activity with NAD^+, while RDE2_42360 exhibited high activity with $NADP^+$. Gene disruption mutants were constructed, and their growth on DPNR as the sole carbon source was evaluated. The mutants of RDE2_16440, RDE2_35730, and RDE2_42360 showed reduced growth compared to the wild-type strain. Additionally, OIA oxidation assays using cell extracts from the mutants revealed that RDE2_16440 and RDE2_35730 function predominantly with NAD^+, whereas RDE2_42360 functions with $NADP^+$. Collectively, these results indicate that RDE2_16440, RDE2_35730, and RDE2_42360 are key ALDHs involved in OIA degradation in *Rhodococcus* sp. strain RDE2. Further analyses using multiple ALDH gene deletion mutants are currently in progress to elucidate the OIA degradation mechanism in this strain.

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L6.4 Cracking Bioplastics: Reprogramming PHA Depolymerases for a Greener Future

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The growing demand for sustainable alternatives to fossil-based plastics has positioned biodegradable polyhydroxyalkanoates (PHAs) at the forefront of bioplastic research and development]. These microbial polyesters are uniquely suited for a circular economy, as they are the only bioplastics both synthesized and biodegraded entirely by bacteria. Their intrinsic circularity, if properly harnessed, offers a compelling model for sustainable materials design. However, as the large-scale use of bioplastics begins to expand, it is essential to implement effective end-of-life strategies from the outset[1]. Biodegradability alone does not ensure a truly sustainable solution: comprehensive waste management and recycling tools must be in place to support not only environmental goals but also the economic viability and scalability of bioplastic applications[2]. Enzymatic recycling, which uses depolymerases to break down polymers into reusable building blocks, offers a highly selective, mild, and eco-friendly approach to closing the material loop. Within this context, PHA depolymerases are key enzymes, enabling the recovery and valorization of PHA-derived monomers or oligomers under controlled conditions. To fully realize their industrial potential, it is essential to tailor these enzymes to the specific requirements of each recycling process, ensuring optimal efficiency and applicability at scale. In this study, we aimed to enhance the activity of the intracellular medium-chain-length PHA (mcl-PHA) depolymerase PhaZ from *Pseudomonas putida* KT2440 (PhaZ_KT), a promising candidate for industrial enzymatic recycling processes. A random mutant library of PhaZ_KT was generated via error-prone PCR, and high-throughput screening led to the identification of two improved variants. These, PhaZ88 and PhaZ32, were heterologously expressed in *E. coli* with N-terminal His-tags and purified in soluble form. Biochemical assays showed that PhaZ88 displayed enhanced activity on p-nitrophenyl esters, indicating increased esterase-like functionality, while PhaZ32 exhibited superior degradation of PHA nanoparticle suspensions, highlighting its potential in polymer breakdown. Molecular dynamics simulations of PhaZ88 suggested that the S184F mutation, located near the lid-core interface, modulates the flexibility of the catalytic lid, likely facilitating access to the active site and influencing substrate preference. These findings demonstrate how rational protein engineering can boost the efficiency of PHA depolymerases and support the translation of PHA natural circularity into scalable industrial recycling solutions. By tailoring enzymes for specific degradation contexts, we move closer to realizing a genuinely circular bioplastic economy, designed to be sustainable from the start.

Keywords: PHA depolymerase; random mutagenesis; bioplastic recycling; enzyme engineering; molecular dynamics.

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L6.5 Improving Degradation Yields of Poly(*Cis*-1,4-Isoprene) Rubber: A Study of the Enzymatic Kinetics of Latex Clearing Protein

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Latex clearing protein (Lcp) is a crucial enzyme in the oxidative degradation of poly(*cis*-1,4-isoprene), the main component of natural rubber (NR). Despite significant biochemical advances, to date, the kinetic behavior of Lcp from *Streptomyces* sp. K30 (LcpK30) has not been characterized, limiting the efficiency of NR conversion. In this work, LcpK30 was produced in *Escherichia coli* BL21 (DE3) + p4782.1::*lcp*K30 with L-rhamnose as the inducer, yielding 6.05 mg/L of purified protein. Kinetic assays demonstrated a positive correlation between the initial reaction rate and poly(*cis*-1,4-isoprene) concentration, reaching a maximum rate of 7.05 nmol O_2/min at the enzyme's saturation point, corresponding to 5 µg Lcp/mg NR. The Michaelis–Menten constant (K_m) was determined to be 308.3 mg/mL, with the Hill model providing the best fit for the data. NR-to-oligoisoprenoid conversion reached 12.9 mg in 24 hours, exceeding previously reported yields, while gel permeation chromatography analysis indicated conversion efficiencies over 80%, far exceeding the reports of previous studies where only 30–40% conversions were achieved. Furthermore, Fukui function analysis suggested that the aldehyde terminal groups of the oligoisoprenoids may be less susceptible to enzymatic degradation, which would explain the mass distribution of the degradation products. This study presents new insights into the catalytic efficiency of LcpK30, laying the groundwork for future applications in bioconversion processes of rubber-based materials.

Keywords: Latex clearing protein, Oligoisoprenoids, Poly(cis-1,4-isoprene), Rubber biodegradation

P1 (FLASH). Cyanobacteria Microbiomes for Bioplastics Long-Term Production

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The market for bioplastics is experiencing steady growth due to their ability to provide sustainable alternatives to traditional plastics. Polyhydroxyalkanoates (PHA) are the only bioplastics directly synthetized from microorganisms, but the high cost and the negative impacts of current production based on heterotrophs limit their industrial use [1]. In this work we propose a novel approach to bioplastics production using photoautotrophic cyanobacteria in the form of microbiomes obtained from environmental samples. This approach eliminates the need for aeration and high substrate demands of heterotrophs, and instead utilizes light, captures CO_2 and produces O_2 [2,3]. Specifically, we will introduce a production methodology consisting on a dual-phase photobioreactor operation which involves cycles of alternating 7-days cell growth and subsequent 7-days biopolymer accumulation [4]. Initially, at the beginning of the growth phase, nutrients are added to the culture to allow biomass growth. In the accumulation phase, the PBRs are enclosed to prevent light penetration, and a supplement of acetate is added to enhance PHB production. At the end of this phase, a portion of the reactor content is purged and replaced with fresh medium with nutrients to begin a new repetition (growth/accumulation).

In this presentation, we share results from four distinct microbiomes cultured over 11 cycles, spanning more than 160 days of operation. Initially, PHA yields were relatively low across all cultures, with less than 10% of dry cell weight (dcw) being PHA. However, as the experiment progressed, PHA production increased significantly. Our strategic production methodology consistently achieved yields of 25–28% dcw PHA for much of the 160-day period. These values are among the highest reported for the cyanobacterial strains present in the studied microbiomes, including *Synechocystis* sp. and *Synechococcus* sp. Remarkably, one microbiome reached levels as high as 40% dcw PHA. The detailed analysis revealed that the microbiome with the highest PHB production exhibited significant overexpression of the *phaC* gene, which is directly involved in PHB synthesis. In contrast, microbiomes with lower PHB accumulation showed overexpression of *gltA*, a gene associated with the tricarboxylic acid (TCA) cycle. This suggests a shift in metabolic focus between energy production and storage. Furthermore, maintaining a dark environment during the PHA accumulation phase, which reduces dissolved oxygen levels, proved crucial for enhancing PHB accumulation. Lower oxygen availability likely inhibits the TCA cycle, directing resources towards storage compound biosynthesis, such as PHB. This research signifies a novel approach towards maximizing the potential of cyanobacteria microbiomes for sustainable bio-based product generation.

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P2. Potential Sustained Production of PHBV Copolymers in Cyanobacteria Microbiomes

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Polyhydroxyalkanoates (PHAs), produced by various microorganisms, offer a promising alternative to petroleum-based plastics [1]. Among them, the copolymer poly(3- hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) is considered one of the most promising candidates, exhibiting superior physical properties to the classical homopolymer poly-3- hydroxybutyrate (PHB) [2].

Cyanobacteria are a ubiquitous group of photoautotrophic bacteria, emerging as potential novel PHBV producers that require no specific sugar-based substrates, relying solely on atmospheric CO_2, H_2O, and readily available, cost-free, solar energy. we presented the first successful production of PHBV, under non-sterile conditions using a cyanobacteria-enriched microbiome dominated by *Synechocystis sp.* and *Synechococcus sp.*. A semi-continuous cultivation approach, alternated between growth and accumulation phase, lasted a long period of time (56 days), was employed with valerate supplementation as an external carbon precursor, expanded working volumes to 2.5 L. The approach incorporated multiple repetitions (4 repetitions) of PHBV production and recovery. These key factors provide important guidance for successfully building and expanding future smart manufacturing facilities. The maximum PHBV content reached 10.7%dcw, the highest proportion of PHBV achieved was 57.4%. Moreover, fluorescence microscopy images staining with Nile Blue positive validated that PHBV biosynthesis in cyanobacterial cells. However, excessive valerate concentrations may induced cellular toxicity, impairing both biomass productivity and nitrogen assimilation. Thus, optimizing valerate dosage is critical to balancing microbial metabolism and maximizing PHBV yield.

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P3. EPS Smash Predicts Exopolysaccharides in Environmental and Human Microbial Communities

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Polysaccharides perform a myriad of essential functions in the extracellular space surrounding most bacteria. Capsular polysaccharides envelop bacterial cells, aiding in aggregation, adhesion, and protection against harmful substances [1]. Polysaccharides secreted into the extracellular space, without attachment to the cell surface, provide the producer with vital functions, including structural integrity, toxin adsorption, and water retention [2]. The biosynthesis of these extracellular polysaccharides (exoPS) is often complex and highly diverse, with significant differences even between bacterial strains. Nevertheless, exoPS biosynthesis can generally be grouped into four major pathways, each typically organized within biosynthetic gene clusters (BGCs) [3].

Despite their prevalence, identifying the genomic potential for exoPS production remains challenging. To address this, we developed epsSMASH, a bioinformatic tool for predicting known and novel exoPS BGCs in bacterial genomes. epsSMASH identifies 23 specific exoPS BGC types using stringent criteria adapted from the antiSMASH framework and predicts novel BGCs using more flexible rules [4]. The tool includes a user-friendly web interface and a command-line version for advanced analyses, empowering researchers to study exoPS production in diverse microbial genomes.

We here introduce epsSMASH and used it to examine the distribution of exoPS in high quality metagenome assembled genomes from several wastewater treatment plants, globally distributed, soil, and the human microbiome. Given its comprehensive analysis capabilities and ease of use, we believe epsSMASH will serve as an essential tool for microbiologists investigating exoPS production in various microbial species.

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P4. From Wild Type to PHA Factory: Engineering *Caldimonas Thermodepolymerans*

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Polyhydroxyalkanoates (PHA) are a diverse family of natural, biodegradable polymers that have gained increasing attention as sustainable alternatives to conventional plastics in certain industrial areas. Thermophilic bacteria fitting into the Next Generation Industrial Biotechnology (NGIB) concept represent promising microbial platforms for efficient PHA production. One notable example is the Gram-negative bacterium *Caldimonas thermodepolymerans* DSM 15344, capable of synthesizing poly(3-hydroxybutyrate) (PHB) on 20 g/L xylose at levels reaching up to 87 wt. % of CDM.

To further enhance its industrial applicability, genetic engineering approaches following principles of synthetic biology have been employed. Using homologous recombination, targeted gene deletions were performed to control monomer incorporation and improve the overall PHA extensibility. In this work, we compare the biotechnological characteristics along with stress robustness testing of four deletion mutants derived from the wild-type *C. thermodepolymerans* DSM 15344, providing insights into strain improvement strategies for sustainable biopolymer manufacturing.

The genetically modified strains analyzed in this study include: *C. thermodepolymerans ΔphaC* (derivative of DSM 15344 with deleted gene of PHA synthase IS481_08630); *C. thermodepolymerans AI01* (derivative of DSM 15344, deleted genes of restriction endonucleases: ΔIS481_08585, ΔIS481_14855, ΔIS481_14025); *C. thermodepolymerans ΔphaZ* (derivative of *C. thermodepolymerans AI01*, deleted gene of PHA intracellular depolymerase IS481_07130); and *C. thermodepolymerans KS01* (derivative of *C. thermodepolymerans ΔphaZ* with deleted gene of 2-methylcitrate synthase IS481_11870).

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P5. Click Me If You Can: Wild-Type *Aneurinibacillus* Bacteria as Unlikely Architects of Functional Pha Copolymers

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Polyhydroxyalkanoates (PHAs) are promising biodegradable alternatives to conventional plastics, yet their material properties are often limited by the narrow range of monomers incorporated by most natural producers. In this study, we demonstrate the remarkable capacity of wild-type *Aneurinibacillus* spp., isolated from composts and activated sludge, to biosynthesize a wide array of functional and structurally unique PHA copolymers without any genetic modification.

When cultivated on glucose (4 g/L) in combination with structurally distinct co-substrates — particularly lactones and diols (4 g/L) — these thermophilic strains incorporated a broad range of PHA monomers, including 3-hydroxyvalerate, 4-hydroxyvalerate, 5-hydroxyvalerate, 4-hydroxybutyrate, and 4-hydroxyhexanoate, demonstrating exceptional substrate flexibility of their native PHA synthases.

Further experiments with α-methyl-γ-butyrolactone led to the production of poly(3HB-*co*-4-hydroxy-2-methylbutyrate), a copolymer containing the rare α-branched monomer. Its structure was confirmed by gas chromatography and nuclear magnetic resonance spectroscopy.

Exploiting the intrinsic alcoholytic activity of class IV PHA synthases, we also employed terminal alkyne-functionalized alcohols to achieve end-group modification of the PHA chains. The resulting clickable polymers bear alkyne moieties suitable for post-synthetic derivatization via click chemistry. Their incorporation was indirectly supported by size-exclusion chromatography coupled with multi-angle light scattering (SEC-MALS), which revealed a significant decrease in molecular weight consistent with chain scission and end-capping.

Material characterization of the resulting copolymers using SEC-MALS, Fourier Transform Infrared Spectroscopy, and Differential Scanning Calorimetry revealed altered thermal properties, reduced crystallinity, and enhanced elasticity compared to poly(3-hydroxybutyrate), further underlining the functional potential of these naturally derived biopolymers.

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P6. Life Under Pressure: the Osmotic Challenge for *Rhodospirillum rubrum*

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In recent years, significant industrial advancements have not only enhanced the quality of daily life but they have also led to severe environmental pollution, such as plastic waste. One potential solution to this issue is replacing petroleum-based plastics with biodegradable materials, for example polyhydroxyalkanoates (PHAs). These materials can be produced various microorganisms such as the purple non-sulfur bacterium *Rhodospirillum rubrum*. A key advantage of *R. rubrum* as a PHAs producer is its metabolic flexibility, allowing it to thrive under diverse conditions, including light, dark, anaerobic, and aerobic environments.

This study was focused on the adaptation of *R. rubrum* to osmotic stress through evolutionary engineering to enhance PHAs production. Initially, the bacterial strain was exposed to various salt concentrations to identify the optimal concentration for subsequent adaptation experiments. During these evolutionary trials, PHA content in the adapted strains was measured using GC-FID, and total dry biomass was also quantified. After the evolutionary experiments, the cultures were subjected to selected stressors, including high temperatures, osmotic shock, and freezing-thawing cycles, followed by viability assessment using flow cytometry. In addition, a series of experiments were performed to verify the effect of increased NaCl content on PHA production in a wild-type R. rubrum strain, a knock-out PHA synthase (*phaC1* and *phaC2*) mutant strain and a knock-out PHA depolymerase (*phaZ2*) mutant strain.

Our results confirmed that continuous exposure to 40 g/l NaCl osmotic stress positively impacted PHA production in *R. rubrum*, with concentrations ranging from 15–30% throughout the experiments. The evolutionary engineering approach proved effective for boosting PHA production in *R. rubrum*. The *phaZ2* mutant strain did not show significantly increased PHA production when compared to the wild-type strain, however, follow-up experiments will need to be performed to confirm or refute this.

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P7. Animals Degrade the Biopolymer Polyhydroxyalkanoate

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The biopolymer polyhydroxyalkanoate (PHA) is a valuable carbon and energy storage compound synthesized by many bacteria and halophilic archaea[1-3]. However, only bacteria and fungi are currently known to degrade microbial PHAs (high molecular weight PHA), using an enzyme called PHA depolymerase (PHAD)[4]. Here, we show that animals also have a PHAD that can degrade microbial PHAs. We discovered a PHAD in the gutless oligochaete, *Olavius algarvensis*. These marine worms gain their nutrition by digesting their bacterial symbionts, with PHA making up to 42% of the carbon in their dominant symbiont[5, 6, 7]. Enzyme assays with a heterologously expressed *O. algarvensis* PHAD confirmed that it degraded PHA to its hydroxyalkanoate monomers. Imaging of mRNA showed that PHAD was expressed in the oligochaete's epidermis, the site of symbiont digestion, suggesting that the host can degrade the PHA produced by its symbionts. Through database comparisons, we discovered PHADs in over 66 gut-bearing animal species from nine phyla and 19 protist species from three major supergroups. Parallel enzyme assays using animal PHADs from the sponge *Amphimedon queenslandica* and the springtail *Folsomia candida* demonstrated that the newly discovered animal homologs also degrade PHA. Given how widespread PHADs are in protists and animals, and the ubiquity of microbially produced PHA in terrestrial and aquatic environments, the ability of protists and animals to degrade PHA could play a previously unrecognized role in carbon cycling.

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P8 (FLASH). Engineering *Paenibacillus polymyxa* for High-Yield Production of Levan and Dextran

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Bacterial polysaccharides are produced by many different species and have a large variability in terms of structure, composition and properties. Generally, they are divided into hetero- and homopolysaccharides, meaning they are composed of different or the same monomers. The structural differences of polysaccharides are rooted in the underlying synthesis processes.

Levan and dextran are homopolysaccharides produced by various bacterial species by specific synthase enzymes that are secreted and/or membrane-associated. Both require sucrose as a substrate, which is hydrolyzed into glucose and fructose, which are then polymerized, respectively.

Dextran is an α-glucan which consists of α-(1→6)-linked D-glucose with possible α-(1→2)-, α-(1→3)- or α-(1→4)- branches. Dextran synthesis is carried out by dextransucrases, which are mostly found in lactic acid bacteria of the species *Leuconostoc, Streptococcus*, and *Weissellia.* Levan synthesis is carried out by levansucrases, which are secreted enzymes that catalyze the transfer of fructose from sucrose hydrolysis to form β-(2→6)-linked fructose polymers and can be found in the species of *Bacillus*, *Lactobacillus* and also *Leuconostoc* [1]. Both levan and dextran are highly relevant for industrial use due to their unique physicochemical properties. Therefore, reaching high product titers and optimizing the production strains and processes is of high interest.

Paenibacillus polymyxa is Gram-positive a non-pathogenic spore-forming soil bacterium that has been studied extensively in the context of different industrial applications. It is a natural producer of EPS, namely paenan and levan and is genetically accessible [2]. In this work, we show the development of *P. polymyxa* as a host for the optimized production of levan and dextran polymers. By introducing a series of targeted deletions and integrations, as well as an optimized fed-batch process, high product titers of dextran and levan are achieved.

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P9. Microbial Production of Copolyester Containing 3-Hydroxyoctanoate from Sugar

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Polyhydroxyalkanoates (PHAs) are microbial polyesters synthesized from renewable resources and are widely studied as sustainable alternatives to conventional plastics. Among various strategies to expand their utility, fine-tuning their physical properties through controlled monomer composition and sequence is of particular interest.

In our previous study, we developed a biosynthetic route for producing a block copolyester, poly(3-hydroxybutyrate)-*block*-poly(2-hydroxybutyrate) [P(3HB)-*b*-P(2HB)], using an engineered polymerizing enzyme, $PhaC_{AR}$ constructed as a chimera of PhaCs derived from *Aeromonas caviae* and *Ralstonia eutropha* (*Cupriavidus necator*) [1]. The resulting P(3HB)-*b*-P(2HB) exhibited favorable mechanical properties, clearly distinct from that of the random copolyester. Notably, the block copolyester exhibited elastomer-like properties [2].

Recently, we introduced two-point mutations into $PhaC_{AR}$ to expand its substrate specificity toward lactate (LA) and medium-chain-length 3-hydroxyalkanoate (MCL3HA) monomers [3-4]. Using this engineered enzyme, we aimed to construct a biosynthetic system for simultaneously incorporating 3HB, LA, and MCL3HA monomers *de novo* from sugar. To supply MCL3HA from sugar, we employed PhaG, a 3-hydroxyacyl-ACP:CoA transacylase that diverts intermediates from the fatty acid biosynthesis into PHA biosynthesis. In addition, biosynthetic enzymes for 3HB and LA incorporation were co-expressed to enable the production of copolyesters. Recombinant *Escherichia coli* strains were cultivated at 30 °C for 48 h in sugar-supplemented media, and the resulting polyesters were extracted using chloroform. Monomer compositions were quantitatively analyzed by gas chromatography, and the polyester structures and linkage patterns were further characterized using nuclear magnetic resonance (NMR) spectroscopy.

As a result, a binary copolyester consisting of 3HB and MCL3HA monomer, identified as 3hydroxyoctanoate (3HO), was successfully synthesized. This indicates that 3HO, which was synthesized from sugar via the fatty acid biosynthetic pathway in *E. coli*, was successfully incorporated into the copolyester. However, a ternary copolyester containing 3HB, 3HO, and LA was not obtained, suggesting a possible metabolic competition between the 3HO and LA biosynthetic pathways. We are currently working on expanding this system to produce the ternary copolyester.

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P10. Hot Bacteria, Cool Bioplastics: Polyhydroxyalkanoate Production by *Aneurinibacillus*

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Polyhydroxyalkanoates (PHA) are biodegradable polyesters synthesized by various microorganisms as intracellular carbon and energy storage compounds. Due to their biocompatibility and environmental sustainability, PHA have gained increasing attention as an alternative to petrochemical-based plastics. In recent years, extremophilic bacteria, particularly thermophiles, have emerged as promising candidates for PHA production. Their ability to thrive at elevated temperatures offers multiple advantages, including reduced contamination risk, faster metabolic rates, and improved process efficiency. Exploring thermophilic genera such as *Aneurinibacillus* opens new avenues for cost-effective and robust biopolymer production under industrially relevant conditions.

Members of the thermophilic genus *Aneurinibacillus* represent a unique group of PHA-producing bacteria with several advantageous traits. One of their key features is the presence of a promiscuous PHA synthase, which enables the incorporation of a wide variety of unusual monomers into the polymer chain. This broad substrate specificity results in the synthesis of structurally diverse PHA with potentially enhanced material properties. Unlike many other PHA-producing strains, *Aneurinibacillus* species are capable of accumulating PHA even under nutrient-rich conditions, such as in complex media, which increases their applicability in industrial settings. In addition, these bacteria can utilize various substrates, including lactones and diols, although their metabolic response varies depending on the cultivation conditions. In our study, we also focus on the expression of genes involved in PHA biosynthesis, aiming to better understand the regulation of polymer production in this promising thermophilic genus.

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P11. Exploring Natural Biopolymer Production During Sulfide Removal by the Green Non-Sulfur Bacterium *Chloroflexus Aurantiacus*

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The production of biopolymers such as polyhydroxyalkanoates (PHA), synthesized by microorganisms from natural substrates under unbalanced nutrient conditions, is an increasingly relevant research line to mitigate the environmental impact of petroleum-based plastic production and pollution. In this context, the thermophilic green non-sulfur bacterium *Chloroflexus aurantiacus* exhibits unique metabolic versatility, including the ability to assimilate CO_2 via alternative pathways to the Calvin-Benson-Bassham cycle [1], as well as to remove sulfide while producing value-added compounds such as PHA and natural pigments in a single-stage process. This study explores the natural capability of four *C. aurantiacus* strains: J-10-fl (DSM 635*),* OK-70-fl (DSM 636), Y-400-fl (DSM 637), and 244-3 (DSM 638) to accumulate biopolymers and grow on organic and inorganic carbon sources under anaerobic conditions in laboratory-scale experiments.

Under photoheterotrophic conditions, all strains showed effective growth and sulfide removal (≥82 %). Among them, DSM 637 exhibited the highest biomass productivity (111.6 ± 17.0 mg L^{-1} d^{-1}), five times higher PHA content, and ~2.7 times higher pigment production than other strains, indicating a strong biotechnological potential.

In photoautotrophic assays using CO_2 as the sole carbon source, growth performance varied notably. DSM 638 showed the highest growth rate (0.20 d^{-1}) but a limited biomass yield, while DSM 637 achieved the highest biomass concentration (224.5 ± 0.0 mg VSS L^{-1}). Sulfide removal exceeded 90 % in strains DSM 635 and DSM 637, with growth ceasing upon sulfide depletion, suggesting sulfide as a limiting factor in CO_2 fixation.

Under mixotrophic conditions with sulfide-up shock, DSM 637 reached a peak productivity of 126.2 ± 30.9 mg L^{-1} d^{-1} following organic carbon addition. PHA and pigment synthesis were enhanced despite inorganic carbon accumulation, likely due to organic carbon metabolism. Maximum PHA content of 13.1 ± 1.7 % dcw occurred at 144 h under heterotrophic conditions, while total carotenoids and BChl *c* reached 1.7 ± 0.3 and 1.4 ± 0.0 %, respectively. These findings highlight the potential of *C. aurantiacus*, particularly DSM 637, as a promising candidate for sustainable biopolymer and pigment production under flexible metabolic conditions.

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P12. Investigation of the PHA Granule Proteome in the Heterologous Producer *Escherichia coli* W

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Polyhydroxyalkanoates (PHAs) present a promising and sustainable alternative to petroleum-derived plastics, with the potential to significantly support the transition toward a circular bioeconomy. A major challenge for their large-scale implementation is the optimization of production systems to achieve greater efficiency and economic viability. Although native PHA-producing bacteria such as *Pseudomonas putida* and *Cupriavidus necator* can reach high yields, their industrial application is often limited by complex metabolic regulation and reliance on costly substrates. To overcome these limitations, a biotechnological bottom-up strategy has been employed: transferring the PHA biosynthetic machinery into non-native hosts, thereby combining the advantages of both systems.

In this context, *Escherichia coli* has emerged as a widely used heterologous platform, owing to its rapid growth, extensive genetic toolbox, and well-characterized metabolic network. Among its strains, *E. coli* W stands out for its metabolic robustness, superior growth rate, and ability to grow on minimal medium with inexpensive carbon sources such as sucrose. These advantages have already enabled industrial production of 1,4-butanediol, L-valine, or PHA [1] in this host.

In this work, synthetic biology tools were employed to construct and express both scl- and mcl-PHA biosynthetic machinery in *E. coli* W. PHA granules were subsequently extracted, and a proteomic analysis was conducted to investigate the granule-associated proteins beyond those of the PHA machinery. Thus, *E. coli* W proved to be a versatile system for studying the role of native phasins that are amphipathic proteins essential for maintaining granule stability within the context of a heterologous granule interactome. Notably, in hosts lacking native phasins, certain endogenous chaperones appear to assume this stabilizing function [2,3]. These findings offer valuable insights into phasin functionality and support their potential application to enhance granule stability and improve overall PHA production in engineered microbial systems.

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P13. In Vivo Biopolymer Engineering Towards Synthetic Biopolymers – Demonstrated by Systematic Xanthan Engineering

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In addition to searching for new exopolysaccharides to replace fossil-based additives, producing exopolysaccharide variants with tailored structural features offers significant potential for improving their functionality in various industrial applications. In this study, a robust genetic framework for engineering *Xanthomonas campestris (LMG 8031* by combining the pK19*mobsacB*-based deletion system with the pSRK-plasmid-based expression system was established. This approach enabled precise gene knockouts and controlled expression of heterologous genes to modify the structure of Xanthan. As a proof of principle, the natural xanthan repeating unit by sequentially deleting native glycosyltransferase genes and complementing them with their homologs from *Kozakia baliensis SR-745 was reconstructed*. The successful biosynthesis of structurally authentic xanthan confirmed the functional compatibility of the *K. baliensis* glycosyltransferases within the *X. campestris* host. The reconstituted xanthans show identical properties in terms of molecular weight distribution, monomer composition, as well as rheological properties. This strategy lays the foundation for systematic glycoengineering of EPS producers and their resulting polysaccharides, allowing for the generation of novel bio-based materials or additives with customizable properties.

To expand this approach, this strategy was employed to engineer a truncated xanthan variant by utilizing two *X. campestris mutant* strains with a deletion of the *gumI* gene, and respective deletion of the acetyl transferase genes *gumFG*. The deletion of the *gumI* gene leads to the production of a truncated version of xanthan gum, lacking the terminal mannose, while the deletion of the *gumFG* genes results in an additional deacetylated inner mannose [1]. As this base structure is analogous to the core structure of the EPS produced by *K. baliensis,* its (putative) *galactosyltransferase* GT1, which is described to catalyze the transfer of UDP-Gal to the Glc-Glc*-Man-GlcA repeating unit [2], was overexpressed. The resulting novel polysaccharide showed a terminal galactose compared to the terminal mannose in native xanthan, while the product titer and molecular weight of the produced EPS remained unaffected.

Ultimately, all respective complementations or modifications did not affect growth behavior or product titer, allowing a simple translation of this approach into industrial production processes, as both the deletion system and expression system are easily transferable to other host strains.

This method highlights the potential for using a recombinant approach to enhance the properties of exopolysaccharides by introducing non-natural sugars into the chemical structure of the polysaccharides to enable a tailored design of synthetic biopolymers to widening the structural diversity of exopolysaccharides. In the future, additional modifications by employing engineered glycosyltransferases quickly become conceivable.

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P14. Valorisation of Used Cooking Oil into PHA with Mixed Culture

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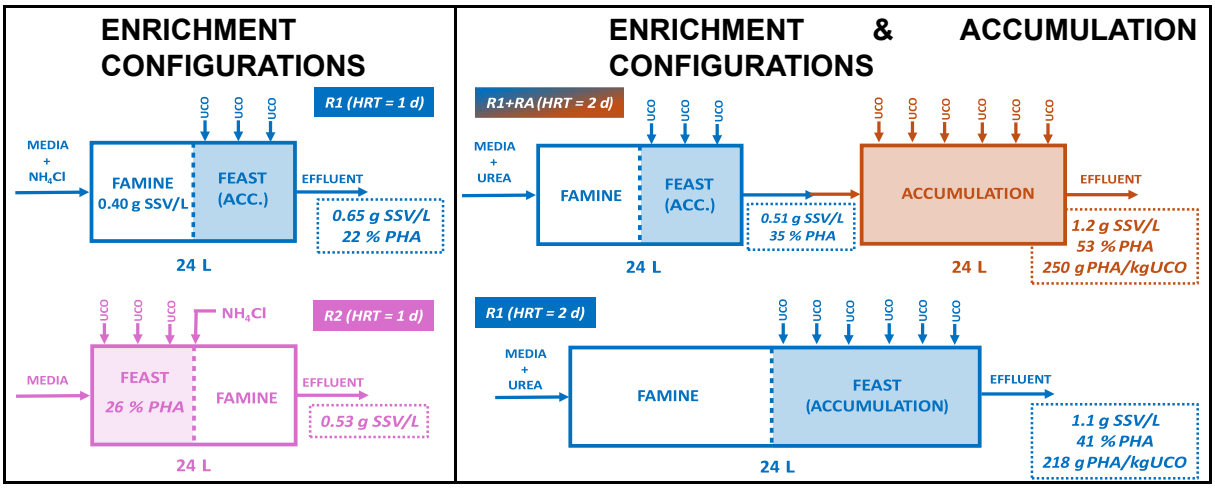
The production of polyhydroxyalkanoates (PHA) from vegetable oil using pure cultures is already implemented at industrial scale. However, the high operational costs hinder the competitiveness of PHA price compared to conventional plastic materials. So, developing technologies that use mixed microbial cultures (MMC) and waste as feedstock is of growing interest. Used cooking oil (UCO), produced in high volumes worldwide, represents a promising substrate. Although, the presence of compounds from Maillard reactions (caused by the high cooking temperatures) could make the MMC enrichment challenging.

In this research work, the MMC enrichment was achieved in aerobic conditions using sequencing batch reactors (SBRs) operated at a hydraulic retention time (HRT) of 1 d, under a feast/famine regime and the carbon (UCO) and nitrogen sources added uncoupled. Two cycle distributions were tested: in R1, the discharge occurred at the end of the feast phase, while in R2 at the end of the famine phase. In both cases similar active biomass concentrations (0.40-0.53 g VSS/L at the end of the famine) and percentage of PHA accumulated inside the cells (22-26 wt. % at the end of the feast) were reached. Enrichment stability was initially compromised by acidification episodes, which were prevented with the change of the nitrogen source from NH_4Cl to urea. Then, it was corroborated that R1 can be used for enrichment and accumulation as a single-reactor system, yielding results similar than a two-reactor system (R1 coupled to an extra SBR for accumulation) with an HRT=2 d. R1 demonstrated good PHA accumulation (41 wt. %) and substrate yield (218 g PHA/kg UCO) at HRT of 2 days.



The main microbial populations enriched in R1 at the end of the operation were *Aquabacter* (52.14%), *Massilia* (39.25%), *Chryseobacterium* (4.42%), *Dulcicalothrix* (0.66%) and *Bacillus* (0.54%). Furthermore, the PHA was extracted using NaOH, resulting in recovery of 87 % with a purity of 68 %. The characteristics of the extracted

material were analysed, and they resulted similar than polypropylene and low-density polyethylene in terms of crystallinity and thermal properties.

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P15. Cze vs Ger: Comparing Post-Transcriptional Regulation in Two Strains of PHA Producing *Caldimonas thermodepolymerans*

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Polyhydroxyalkanoates (PHAs) are biodegradable, biocompatible, and renewable polymers naturally produced by microorganisms as intracellular carbon and energy reserves. Thanks to their promising material properties, PHAs represent a sustainable alternative to conventional petroleum-based plastics. *Caldimonas thermodepolymerans*, a moderately thermophilic, non-sporulating, Gram-negative bacterium represent promising candidate for the Next Generation Industrial Biotechnology (NGIB) [1] approach which exploits its special properties, such as thriving in extreme conditions, for sustainable production of value-added chemicals.

The bacterial post-transcriptional regulation of gene expression by small RNAs (sRNAs) is a powerful mechanism how bacteria response to stress. These non-coding regulatory elements can modulate gene transcription levels, ensuring that the abundance of gene products is appropriate for the bacterial needs at a given time, whether that means stimulation or inhibition of the translation. Their investigation promises a more precise understanding of regulatory processes and offers additional genomic elements that can be targeted by synthetic biology in order to fine-tune bacteria used in biotechnology.

In this work, we compare the sRNA content in two strains of *C. thermodepolymerans* DSM 15344^T. The type strain was initially isolated from activated sludge in Germany, deposited in Leibniz Institute DSMZ in Germany (GER) and later in the Czech Collection of Microorganisms in Czechia (CZE). The aim was to evaluate whether variations in cultivation conditions and phenotypic characteristics of *C. thermodepolymerans* strains obtained from different public culture collections are associated with differential expression of sRNAs. We analysed RNA-Seq experiments in which both strains were cultured on xylose. Using state-of-the-art tools for the structural and functional annotation, we conducted a comparative analysis of sRNAs in these bacteria. Additionally, we explored expression profiles of sRNAs and performed a differential expression analysis. The focus was mainly on the PHA metabolism and related sRNAs addressing differences between these two strains.

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P16. Genome-Wide Changes in *Rhodospirillum rubrum* DSM 467t Induced by Knock-Out of PhaZ2 Depolymerase

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Rhodospirillum rubrum is a metabolically versatile, Gram-negative bacterium capable of thriving in diverse environments through aerobic respiration, anaerobic fermentation, and photosynthesis. Besides its significance in carbon and nitrogen cycling, *R. rubrum* is notable for its ability to synthesize and degrade polyhydroxyalkanoates (PHAs), which serve as intracellular carbon and energy reserves. PHA accumulation and mobilization are central to the organism's survival strategy, with PHA degradation mediated by depolymerases such as PhaZ1 and PhaZ2. While PhaZ1 is thought to be periplasmic, PhaZ2 is an intracellular depolymerase that degrades native PHB granules [1, 2].

In this study, we engineered a knock-out mutant of *R. rubrum* DSM 467^T lacking the main depolymerase gene *phaZ2* and performed whole-genome sequencing using Oxford Nanopore technology. Comparative genomic analysis with the published wild-type reference genome enabled us to confirm the targeted deletion and assess genome-wide structural variations. In addition, direct detection of DNA methylation patterns revealed broad epigenetic changes associated with the loss of the PhaZ2 depolymerase. By integrating RNA-Seq data from the wild-type strain [3], we identified correlations between specific methylation patterns and gene expression.

Our results demonstrate that knock-out of the intracellular depolymerase in *R. rubrum* DSM 467^T induces widespread genomic and epigenetic changes, affecting not only PHA catabolism but also the regulation of metabolic pathways and stress responses. This study highlights the interconnection of genetic, epigenetic, and transcriptional regulation in the adaptation of *R. rubrum* to environmental and metabolic challenges.

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P17 (Flash). Recovery of High Value-Added Co-Products in Mixed Microbial Culture-Polyhydroxyalkanoate Production

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Polyhydroxyalkanoates (PHA) production with mixed microbial cultures (MMC), currently investigated at both laboratory- and pilot- scale, is gaining significant attention as a promising cost-effective alternative to pure culture PHA production processes. The overall process economics could be further improved through the simultaneous recovery of high value-added by-products, such as extracellular polymeric substances (EPS) [1]. In this work, the recovery of EPS (mainly carbohydrates and proteins) is explored as a co-product of an innovative multireactor MMC-PHA production system. The reactors configuration consists of a continuously fed tubular Feast reactor connected, through a recirculation stream, to a continuously stirred tank (CSTR) Famine reactor, whose effluent is in part continuously sent to a CSTR for PHAAccumulation [2]. The PHA-rich biomass is harvested from the Accumulation reactor, centrifuged, resuspended in ultrapure water, and stabilized at pH 2 using concentrated sulfuric acid to preserve the PHA content. The acidified biomass is then centrifuged again: the pellet proceeds to PHA extraction, while the supernatant is collected for EPS recovery. EPS precipitation was carried out using two methods: 1) pH-precipitation, by adjusting the supernatant at pH 7 with NaOH (3 M); 2) Ethanol precipitation, by adding ethanol (96%) in a 3:1 (v/v) ratio and storing the mixture at 4 °C overnight [3]. Following precipitation, the mixtures were centrifuged, and the resulting pellets were oven-dried at 60 °C. Carbohydrates and proteins contents were determined using the phenol–sulfuric acid method [4] and the Bradford method [5], respectively. Results on EPS partial characterization are summarized in Table 1. FTIR analysis of the pellets revealed characteristic carbohydrates peaks in the 1100–1047 cm^{-1} region, the amide I (C=O stretching) at 1640 cm^{-1}, and broad signals for hydroxyl and amine groups in the 3500–3000 cm^{-1} range. In conclusion, EPS recovery can be integrated into the MMC-PHA production process, offering an opportunity to valorize side streams and improve the overall sustainability and economic feasibility of the process. Future works will focus on assessing potential applications of the extracted EPS, such as metal adsorption or use as a flocculating agent.

Table 1: Partial EPS characterization

Precipitation method	EPS recovery (mg EPS/g VSS)	Total carbohydrates (mg glucose-equiv./g EPS)	Total proteins (mg BSA-equiv./g EPS)	PN/PS
pH precipitation	129.6	79.8	60.6	0.76
Ethanol precipitation	201.6	330.0	76.0	0.23

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P18. Harnessing CO_2 For Sustainable PHB Production in *Cupriavidus necator*

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One of the grand societal and scientific challenges of today is finding alternatives to fossil and agriculture-based feedstocks. Carbon dioxide (CO_2) stands out as a promising alternative carbon source, due to it is abundance, and the fact that its increasing atmospheric concentration drives climate change. This makes it an attractive feedstock for polyhydroxyalkanoate (PHA) production. Hydrogen-oxidizing bacteria, such as *Cupriavidus necator*, can use hydrogen as their sole energy source to convert CO_2 into PHA. However, PHA production from CO_2 is not yet commercially viable due to several challenges, including low productivity from CO_2 and high production costs partly due to high hydrogen price.

This project explored ways to improve commercial feasibility of CO_2-derived PHA. We investigated two approaches: using unpurified CO_2 stocks to polyhydroxybutyrate (PHB) synthesis and improving the hydrogen consumption efficiency for CO_2 fixation in *C. necator*. These results represent a step toward more efficient and cost-effective PHB production directly from CO_2 and demonstrate the potential of metabolic engineering to overcome some of the current limitations.

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P19 (Flash). Unraveling Key Factors for BC Production by *K. medellinensis*

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Bacterial cellulose (BC) is an extracellular polymer produced by many bacterial species among them *Komagataeibacter* genus. Research has shown that bacteria species in the *Komagataeibacter* genus are highly effective in BC production. BC polymer is synthetized as a three-dimensional matrix consisting of an interconnected network of cellulose nanofibers with a highly organized, crystalline structure. The unique properties and purity confers significant economic importance due to its expanding range of biotechnological applications. The efficiency of BC production is strongly influenced by strain producers as well as the culturing conditions. Additionally, structural properties of BC are strain-specific reflecting variations in bacterial enzymatic pathways and regulatory mechanisms (1).

The genome analysis of *K. medellinensis* ID13844 showed four *bcs* clusters (*bcs1*, *bcs2*, *bcs3* and *bcs4*) with different genomic structures and expression levels (2). The *bcs1* operon comprises seven genes corresponding to *bcsZ*, *bcsH, bcsA, bcsB*, *bcsC*, *bcsD* and *bglX*. In other *Komagataeibacter* strains, the *bcs1* operon is responsible for crystalline cellulose synthesis. BcsA and BcsB constitute the bacterial cellulose synthase, BcsC is the outer-membrane porin and BcsD associated with BcsH, is related with BC crystallinity. The *bcsABCD* core genes are accompanied by accessory genes encoding BcsZ (endo-β-1,4-glucanase), and BglX (β-glucosidase). The *bcs2* cluster comprising five ORFs associated with cellulose biosynthesis. The core genes consist of a fused *bcsAB2*, and the *bcsC2* gene. Upstream of *bcsAB2*-N three genes, *kpsC*, *kpsS* and *rfaB* associated with extracellular matrix formation. The *bcs3* cluster contains two ORFs corresponding to a fused cellulose synthase subunits, *bcsAB3*, followed by the *bcsC3* gene. Finally, the *bcs4* cluster was detected as a stand-alone copy of the *bcsAB4* gene. Analysis of the expression level of this clusters indicated that *bcs1* and *bcs4* operons direct the synthesis of the cellulose in *K. medellinensis* ID13488 whereas the expression of *bcs2* and *bcs3* operons remains in a basal level.

In this work, we have investigated the role of the *bcs4* operon in bacterial cellulose (BC) production in *K. medellinensis* ID13844, highlighting that BC biosynthesis is abolished in *bcsAB4* mutants. The diversity of *bcs* operons among different species and their significance will be discussed.

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P20. Polyhydroxyalkanoates Production from Polyethylene Terephthalate Degradation Products

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Polyethylene terephthalate (PET) is a thermoplastic polyester widely used in packaging. PET's raw materials are of fossil origin and it is not biodegradable. Although it can be recycled, the high cost of the process, the lack of collection chains and the difficulties in sorting the waste collected mean that most used PET ends up in landfills, rubbish dumps or in the environment. In 2020, it is estimated that of the 5.1 million tonnes of PET used in Europe, only 1.3 million tonnes was recycled PET [1]. PHA are polyesters that are synthesized and stored within the cell by various microorganisms and can subsequently be extracted and transformed. As well as being a sustainable and biodegradable alternative, it was found that it can be produced from the degradation products of PET. In this study, strain DM-14 was isolated from a mixed microbial consortium (unpublished data) by plating in *CHROMagar*™. The new isolate displayed small, round and cream colonies. Strain DM-14 was tested for its ability to grow on PET degradation monomer terephthalic acid (TPA). Different sources of TPA-containing samples were evaluated as sole carbon sources for cultivation, in controlled conditions of pH (7±0.01), temperature (30±0.01 ºC) and dissolved oxygen (DO) concentration (30% of the air saturation) in 3 L batch bioreactor experiments. TPA obtained by chemical hydrolysis was tested at concentrations of 5, 10 and 15 g/L, using glycerol as co-substrate, resulting in PHA contents in the cells of 12-18wt%, within 19-27 h of cultivation. Following these promising results, TPA containing materials obtained by via reactive extrusion [2,3] of different mixed plastics' samples were tested to assess the culture's ability to cope with feedstock variability and the presence of different types of contaminants. The results showed that, although bioprocess requires optimization to attain higher PHA yields and maximize the volumetric productivity, strain DM-14 is a highly interesting strain for further exploitation for the upcycling of mixed plastics wastes.

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P21. Transcriptomic Analysis of Osmotic Stress Response in the Thermophilic PHA-Producing Bacterium *Caldimonas thermodepolymerans*

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Caldimonas thermodepolymerans DSM15344 is a moderate thermophilic bacterium that can accumulate and degrade PHA at high temperatures (50-55°C) [1]. It has been shown to accumulate high amounts of PHA by utilizing a variety of industrially significant sole carbon sources such as xylose and glycerol at 55°C [2]. In this study, osmotic stress by supplementing growth media with NaCl was found as an enhancer to accumulate more PHA, although at the cost of a decreased bacterial growth rate. This osmotic stress resistance gives this bacterium a dual advantage as an NGIB organism, able to tolerate high temperature and resist salinity. To further study the gene regulatory response of *C. thermodepolymerans* in osmotic stress, transcriptomic analysis by RNA-sequencing was performed upon exposure to 2% NaCl (in tryptic soy broth (TSB)). Here, we provide an overview of the main stress responses in the cell that enable it to resist hyperosmosis. Several genes that are encoded for stress response proteins were significantly upregulated such as BON domain-containing protein, tetratricopeptide repeat protein, entericidin A/B, peroxiredoxin, and Ku protein. It was also observed the upregulation of the genes responsible for secretion system 6 (T6SS). A significant downregulation of motility-related genes in the transcriptome analysis was furthermore validated at the phenotypical level as a significant increase in biofilm formation These results showed the interconnected pathways in *C. thermodepolymerans* which regulate its gene expression.

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P22 (Flash). Auxotrophic Gas Fermentation with *Cupriavidus necator* for Carbon-Negative Product Synthesis

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The transition towards sustainable and circular bioeconomies necessitates innovative biotechnological solutions for carbon capture and utilization.

Gas fermentation using *Cupriavidus necator*, a versatile chemolithoautotrophic bacterium, represents a promising platform for the direct conversion of CO_2 and H_2 under aerobic conditions into value-added products such as polyhydroxybutyrate (PHB) [1]. This work focuses on the safe and efficient scale-up of gas fermentation processes using recombinant *C. necator* strains with the aim of increasing CO_2 conversion and PHB productivity under auxotrophic conditions. However, there are still challenges in terms of process safety and scalability, particularly due to the use of potentially explosive gas mixtures and the solubility of the gases used.

C. *necator* was cultivated with a safe gas mixture and monitored online using an adapted version of the RAMOS technology [2]. The gas flow rates and the duration of the measurement phases were optimized to enable high-resolution online monitoring of the gas transfer rates for all relevant gases (OTR, CTR, HTR) [3]. This setup enables efficient screening of high performing strains and prediction of product formation without relying on laborious offline analyses. In addition, the online monitoring of all relevant gases makes a closed gas balance possible. Critical parameters for scale-up will be subsequently characterized, with a focus on gas-liquid mass transfer. Specific k_La values for CO_2, H_2, and O_2 were determined in shake flasks using novel sensors and measurement techniques [4, 5].

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P23. OPTIMISING FERMENTATION OF THERMOPHILIC *CALDIMONAS THERMODEPOLYMERANS* FOR ENHANCED BIOMASS AND TAILORED PHA PRODUCTION

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Polyhydroxyalkanoates (PHAs) are promising biobased and biodegradable polymers with the potential to reduce environmental plastic pollution, yet achieving a controlled and consistent output for industrial production remains a key challenge. Poly(3-hydroxybutyrate) (P3HB) is a brittle material with a narrow processing window. Introducing monomers like 3-hydroxyvalerate (3HV) can improve the characteristics of the polymer, as the ratio of 3HB:3HV monomers influences mechanical strength and flexibility [1]. Controlling this monomeric composition enables the production of PHA materials according to predetermined specifications, which is essential in industrial PHA synthesis.

Our research addresses this challenge using precision fermentation with *Caldimonas thermodepolymerans*, a thermophilic bacterium with potential advantages regarding contamination difficulties and PHA production yield [2]. *C. thermodepolymerans* produces poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (P3HB-co-3HV; P3HBV), incorporating both 3HB and 3HV monomers [3,4]. These findings offer a foundation for fine-tuning polymer composition and understanding the organism's thermophilic growth behaviour and PHA production.

We successfully produced P3HB and P3HBV polymers using *C. thermodepolymerans* under controlled conditions. Batch-phase production with nitrogen limitation is employed for PHA production while developing fed-batch approaches, which entail gradually adding nutrients to the culture to sustain optimal conditions. Bioreactor-based strategies are explored to enhance cell dry weight and regulate monomer composition. By systematically refining feeding strategies, we aim to achieve high-density cultures, resulting in a high yield of PHA production and a controlled, scalable process for P3HBV production. Looking ahead, this work paves the way for developing efficient, scalable, and cost-effective high-temperature fermentation processes.

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P24. Process Development of *Pseudomonas taiwanensis* for Microbial Aromatics Production for the Polymer Industry

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Phenol is a widely utilized platform chemical, which provides the basis for many engineering polymers that enable modern lives. These include phenolic resins, polyamides and others. Phenol may also be used as starting material for pharmaceuticals, preservatives and pesticides. Currently, phenol is derived from petroleum-based precursors, which are a finite resource. To shift production to renewable alternatives, microbial production of phenolic compounds is desired [1].

First steps in microbial phenol production were performed in *Pseudomonas putida* S12 with production reaching 5 mM in fed-batch [2]. Due to the high toxicity of phenol even at low concentrations of about 10 mM, growth and production is inhibited entirely, which can be lowered by the use of organic extractants [2]. In this work, *Pseudomonas taiwanensis* VLB-120 was used for phenol production. The strain was developed for optimal production from glucose and glycerol using solvent-tolerant *Pseudomonas* strains [3].

To create an understanding of the underlying principles during microbial production and to increase the phenol titer, the cultivation is investigated with a holistic approach. Starting with an initial optimization in shake-flask scale for determining optimal cultivation conditions to achieve high yield phenol production, followed by screening of different product removal strategies for biocompatibility and phenol extraction efficiency [2],[4]. Currently the organism endures concentrations of up to 10 mM phenol, which is inhibiting further phenol production reducing process efficiency and overall yield [5].

On one hand, shake-flask based process optimization revealed that *P. taiwanensis* is able to achieve a higher phenol production from glycerol compared to glucose. This however results in a prolonged lag phase of the organism. The addition of octanoic acid was proven to be advantageous for other *Pseudomonas spp*. in reducing the lag phase for growth on glycerol and was therefore investigated [6]. Furthermore, the influence of different media components was tested and the omission of $CoCl_2 \cdot 6H_2O$ in trace element solution resulted in a final phenol concentration of 8 mM in shake flask scale. This is an increase of the overall yield to 22 ± 0.4% (Cmol/Cmol), superceding the one achieved by Wynands et al. [3]. On the other hand, product removal strategies including the addition of dodecanol as a second phase to the culture medium were able to achieve higher phenol production with concentrations in the organic phase reaching 20 mM.

Overall, we were able to increase phenol production from *P. taiwanensis* via process optimization to achieve a yield of 22% (Cmol/Cmol), making microbial phenol production more economically viable. With further strain optimization to endure higher phenol concentrations and improved downstream processing the feasibility of the process might be further improved, reaching even higher yields and titers, thus

contributing to the establishment of economically viable bio-based production routes for phenol.

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P25. Effects of Dissolved Oxygen Concentration on Growth Kinetics of *Haloferax mediterranei* for Polyhydroxyalkanoate Production Using Sucrose

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While polyhydroxyalkanoates (PHAs) have emerged as a promising alternative to conventional plastics, their large-scale production is still limited by the need for sterile conditions and complex downstream processing [1]. Halophilic microorganisms, however, offer a practical solution. Their natural habitat in high-salinity environments reduces contamination risk and simplifies downstream processes [2]. For effective scale-up, the use of defined and renewable carbon sources such as sucrose, which is abundantly available in Queensland, Australia [3], can enable controlled fermentation and ensure consistent product quality across batches. Previous research has studied optimisation of operational parameters such as pH, temperature, and salinity for *Haloferax mediterranei*, a well-known PHA-producing halophilic archaeon [4,5,6]. Despite this, the effect of oxygen has not been sufficiently studied, especially in high-salinity systems where oxygen transfer is inherently limited, potentially making aeration energy-intensive if not optimised.

To fill this gap, this study investigates the effect of oxygen supply on the growth and PHA production of *H. mediterranei*. Bioreactors were run under identical conditions, with oxygen supplied through compressed air or pure oxygen, under either intermittent or continuous aeration. Sucrose served as the main carbon source. The results show that while high dissolved oxygen (DO) concentrations do not significantly improve growth, a minimum DO concentration is necessary to keep the cells growing. The highest specific growth rate (μ_{max}) of 0.12±0.05 h^{-1} was achieved at a DO setpoint of 2.1 mg-O_2/L, maintained by consistent compressed air and stirring. Growth declined at lower DO concentrations of 1.2 and 0.5 mg-O_2/L, both maintained by less frequent pulses of intermittent aeration, resulting in μ_{max} values of 0.09±0.07 h^{-1} and 0.02±0.01 h^{-1}, respectively. Remarkably, even at 1.2 mg O_2/L, cells still grew steadily, reaching 6.69±0.14 g-VS/L. This was comparable to the maximum values observed at 2.1 mg-O_2/L (6.50±0.14 g-VS/L) and 9.4 mg-O_2/L (6.05±0.17 g-VS/L), suggesting that lower oxygen supply can still support substantial cell growth if maintained consistently. These findings indicate that maintaining low but sufficient DO concentrations through intermittent aeration with compressed air is effective and could lead to energy savings. This offers practical guidance for scaling up *H. mediterranei* cultivation for PHA production, where optimising oxygen supply can help reduce energy use in large-scale systems.

Keywords: Polyhydroxyalkanoate, *Haloferax mediterranei*, dissolved oxygen, sucrose, compressed air, pure oxygen.

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P26 (FLASH). Impact Of Oxygen Transfer Rate in the Valorization of Used Cooking Olive Oil into Poly-3-Hydroxybutyrate by High-Cell Density Cultures of *Cupriavidus necator* DSM 545.

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The large-scale production of poly-3-hydroxybutyrate (PHB) is hindered by high operational costs, mainly due to raw material expenses and fermentation requirements [1]. The use of waste plant oils offers a cheaper and enhanced sustainable alternative as feedstocks [1, 2]. PHB is one of the most extensively studied and commercially available polyhydroxyalkanoates (PHAs), with applications in packaging, tissue engineering and drug delivery [1]. *Cupriavidus necator* is a commonly used strain for PHB production in high-cell density cultures, reaching intracellular polymer levels above 80% of cell dry weight from a broad range of substrates. Reported PHB yields from sugars (glucose, sucrose or fructose) range between 0.4-0.5 $g \cdot g^{-1}$, whereas plant oils yield higher values of 0.7-0.8 $g \cdot g^{-1}$ [2, 3]. Common plant-based feedstock for *C. necator* include pure or waste rapeseed, soybean, palm and sunflower oils [3]. Among these alternatives, used cooking oil (UCO) represents an attractive substrate due to its high availability and low cost.

This study investigates the production of PHB from used cooking olive oil (UCOO) as the sole carbon source by *C. necator* DSM 545. Pulse feeding fed batch fermentation in a 3 L stirred tank bioreactor yielded 56.6 $g \cdot L^{-1}$ of biomass and 45.3 $g \cdot L^{-1}$ of PHB in 72 h. Then, a continuous fed-batch fermentation regime enhanced bioprocess efficiency, reaching in 123.1 $g \cdot L^{-1}$ of total biomass and 92.7 g $PHB \cdot L^{-1}$, with a maximum productivity of 1.8 g $PHB \cdot L^{-1} \cdot h^{-1}$. A kinetic model was developed to predict residual biomass, oil consumption, oxygen dynamics and PHB accumulation, based on volumetric mass transfer coefficient (k_La) and culture dilution rate (D) profiles. Oxygen transfer rate (OTR) was identified as a modulator of carbon flux distribution: PHB biosynthesis was maximized at 0.064 mol $O_2 \cdot L^{-1} \cdot h^{-1}$, while lower OTR values favoured. Our results confirm UCOO as an effective alternative substrate for PHB production and emphasize the role of oxygen in optimizing fermentation strategies.

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P27. Advancing Towards Circular Economy: Animal By-Products for Polyhydroxyalkanoate Production

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Plastics are valued for their versatility and durability but increasing awareness of their environmental and health impacts is driving the search for alternatives. Bioplastics like polyhydroxyalkanoates (PHA) offer a sustainable option, though they remain economically uncompetitive. The primary cost-contributing factors include the feedstock and downstream processing. In efforts to achieve a more circular production system and to reduce potential costs at an industrial scale, animal by-product streams can be considered a promising alternative to first generation feedstocks with over 5 Mt of animal fats rendered annually in Europe alone [1].

Pre-treatment of animal materials is crucial to achieve pure substrate phases and high substrate yields. Through hydrolysis, pre-treatment, and phase separation, distinct fat, fat-protein, and protein phases were produced [2]. Previously, animal by-products have been used for the production of poly(hydroxybutyrate-*co*-hydroxyhexanoate) [P(HB-*co*-HHx)] at the laboratory scale, reaching cell densities up to 55 g L^{-1} with an intracellular PHA content of 80 wt% [3-4].

In this study, the use of animal derived protein phases was evaluated and compared to commercially available protein phases, either as sole nitrogen source or as a growth booster. *Ralstonia eutropha* was cultivated from well-plate to laboratory bioreactor scale, yielding 28 g L^{-1} of cells with 75 wt% P(HB-*co*-18mol%HHx) when grown on animal fats and animal protein as the sole nitrogen phase. Although this yield represents only about half of what can be achieved using urea alone, growth-boosting effects of the added complex nitrogen source were observed during the early stages of cultivation.

Furthermore, different animal fats were screened, and a high cell density protocol was developed. Feeding strategies for both urea and fat were optimized, resulting in cell densities exceeding 120 g L^{-1} with a P(HB-*co*-22mol%HHx) content of 70 wt%. Overall, animal by-product streams hold significant potential for enabling cost-effective and sustainable large-scale PHA production.

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P28. Biopolymers and Biochemicals Production from Biogenic CO_2 Using a Novel Hybrid Fermentation System in the Semprebio Project

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The SEMPREBIO project aims to develop innovative biotechnological processes for the conversion of biogenic CO_2 into value-added products, minimizing greenhouse gas emissions and fostering a circular bioeconomy. A key focus is the production of polyhydroxyalkanoates (PHAs) and biochemicals, such as succinic acid, from CO_2 derived from biogas upgrading. In this context, a novel hybrid fermentation system (50 L) has been designed and constructed, integrating an intensive mass-transfer unit (IMTU) for efficient H_2 transfer—essential for autotrophic growth—with a fermentation unit for the bioconversion process using selected microbial strains.

Initial lab-scale studies demonstrated the feasibility of producing PHAs using *Cupriavidus necator* [1] and succinic acid [2] using *Actinobacillus succinogenes* under autotrophic conditions with CO_2 and H_2. These strains were cultivated in both synthetic media and the liquid fraction of digestate, with the latter showing high potential for nutrient recovery and circular resource use. The hybrid system includes an advanced control platform and will be fed with liquefied CO_2 from the cryogenic upgrading of biogas and nutrients extracted from digestate [3]. Current pilot-scale experiments are being carried out to optimize process parameters and assess the performance of different IMTU configurations—such as capillary columns, venturi injectors, and high-pressure chambers—to enhance gas transfer, improve productivity, and evaluate the overall economic viability. This work represents a significant step toward scalable and sustainable production of biopolymers and biochemicals from renewable carbon and energy sources.

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P29. Carbon-To-Phosphorus Ratio Optimization for Poly(Hydroxybutyrate-co-Hydroxyhexanoate) Production by *Ralstonia eutropha* Using Oleaginous Feedstocks Under Phosphorus Limitation Conditions

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The class of polyhydroxyalkanoates (PHAs) comprises a group of naturally occurring biopolymers that are characterised by excellent biodegradability [1]. The biodegradability of these biopolymers renders them a sustainable alternative to conventional plastics. However, economic feasibility and scalability remain major obstacles to their commercial success [2]. In this study, optimization of the carbon-to-phosphorus (C/P) ratio was carried out for the production of the PHA copolymer poly(hydroxybutyrate-*co*-hydroxyhexanoate) [P(HB-*co*-HHx)] under phosphorus limiting conditions using the recombinant strain *Ralstonia eutropha* Re2058/pCB113 and canola oil as feedstock. Optimization was performed by systematically varying the C/P ratio to increase the P(HB-*co*-HHx) yield. Cultivations were performed in parallel bioreactor systems, allowing for efficient screening of multiple C/P ratios simultaneously under controlled conditions. The results demonstrated that phosphate limitation, when finely balanced with available carbon, significantly influenced both cell dry weight and P(HB-*co*-HHx) yield. This study highlights the importance of the C/P ratio, which influences the yield, substrate utilisation efficiency and ultimately the cost of production.

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P30. Gel of Encapsulated *Azotobacter vinelandii* Enhance Plant Growth and Modulate Soil Microbial Communities

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The increasing requirements for sustainable agricultural practices require the implementation of innovative methodologies to achieve yields like those achieved with conventional fertilizers, thus improving the environmental sustainability of agricultural processes and preventing irreversible degradation of arable land. Among these novel technologies is the development of biofertilizers that inoculate soil with plant growth promoting rhizobacteria (PGPR), a diverse group of microorganisms recognized for their beneficial contributions, including nitrogen fixation, phosphate solubilization, the production of siderophores and phytohormones [1], as well as the synthesis of protective exopolysaccharide alginate and intracellular polyhydroxyalkanoates that serve as a carbon source under stress conditions [2].

The research presents an innovative method for biofertilizer production that uses *Azotobacter vinelandii* for in situ self-encapsulation within a gel carrier through the crosslinking of alginate synthesized during the bacterial cultivation process. This novel approach streamlines the preparation protocol, presenting opportunities for cost reduction and enhancing the overall competitiveness of the process. To validate this concept, selected bacterial strains were subjected to gelation experiments under conditions promoting alginate gel formation, using 2% (w/w) $CaCl_2$ as a cross-linking agent. *Azotobacter vinelandii* CCM 289 was chosen based on its superior alginate production, efficient gelation performance, and demonstrated potential for synthesizing indole-3-acetic acid and siderophores.

A total of 3 cultivation experiments were conducted on a model plant *Lactuca sativa*, incorporating various carrier compositions (cells in PBS; gel excluding cells; gel including cells; freeze-dried gel with cells; negative control without any addition). The main variations among the cultivation conditions were attributed to the different quality of the soil and the variations in the irrigation rates. The growth experiment was carried out within a fume hood adapted to a cultivation box equipped with regulated irrigation and illumination, along with the control of temperature and humidity parameters. As soil quality and the frequency of irrigation decreased in the experimental trials, the differences among the various groups became more apparent, accompanied by a significant improvement in fundamental growth metrics (fresh/dry weight, lengths of various plant parts), in addition to favorable alterations in the composition of the soil microbiome.

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P31. Evaluation of Different Limitation Strategies for Poly(Hydroxybutyrate-*co*-Hydroxyhexanoate) Production by *Ralstonia eutropha* in High Cell Density Cultivation

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The class of polyhydroxyalkanoates (PHAs) comprises a group of naturally occurring biopolymers that are characterised by excellent biodegradability [1]. The biodegradability of these biopolymers renders them a sustainable alternative to conventional plastics. However, economic feasibility and scalability remain major obstacles to their commercial success [2]. The aim of this study was to investigate the efficiency of defined limitation strategies, namely nitrogen and phosphorus, for the high-yield production of the PHA copolymer poly(hydroxybutyrate-*co*-hydroxyhexanoate) [P(HB-*co*-HHx)] by the recombinant strain *Ralstonia eutropha* Re2058/pCB113 using canola oil as feedstock in a high cell density culture (1 L bioreactor). Using phosphorus limitation strategy cell dry weight (CDW) and P(HB-*co*-HHx) content of up to 300 g/L and up to 218 g/L were obtained. Although nitrogen limitation is widely applied in the production of PHA, nitrogen-limited cultivation had the lower CDW and P(HB-*co*-HHx) production for the given conditions. The results of the present study demonstrate the highest P(HB-*co*-HHx) yield using canola oil as the main carbon source ever reported in the literature to our knowledge.

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P32. Application Of Physics-Informed Neural Networks (PINNs) for the Evolution of a Natural Microbiome into an Enriched Polyhydroxyalkanoate (PHA)-Accumulating Consortium

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In nature, microorganisms live in complex ecosystems known as microbiomes, wherein the collective capabilities of microbial communities often exceed those of individual species. Through interspecies communication mechanisms, including metabolite exchange and molecular signaling, they enhance productivity and resilience to environmental fluctuations. This communal lifestyle contributes to the stability of microbial consortia, making them more adaptable to diverse environmental conditions [1]. By harnessing these characteristics, natural microbiomes from various ecosystems can be engineered to produce commercially valuable metabolites, such as biopolymers like polyhydroxyalkanoates (PHA) [2]. Despite the advantages of using mixed microbial communities for PHA production, industrial application faces challenges related to low process control and reproducibility [3]. Conversely, in nature, microbial consortia appear to be robust systems with the flexibility to self-stabilize under environmental changes. In this study systems biology tools were employed to better understand and develop strategies to control microbiome dynamics, aiming to enrich a microbiome (collected at a marshland) in PHA-producers. We applied Physics-Informed Neural Networks (PINNs) to a 2-liter sequencing batch reactor (SBR) over 3 months comprising 6 hydraulic retention times (HRT). The reactor underwent multiple feast and famine (F/F) cycles, with automatic C/N ratio manipulation to enrich in high-growth, PHA-storing organisms. Over time, we monitored biomass, nutrient profiles, PHA content, and microbial composition. Overall, the natural microbiome evolved, showing biomass concentrations between 4.00 and 7.00 g/L. PHA storage capacity increased from 5.13 to 283.50 mgPHA/gVSS after 4 HRTs. Two accumulation bioreactors (after 3 and 6 HRTs) showed a rise in PHA volumetric productivity from 1.75 to 2.04 g/(L d). The PINN-controller demonstrated strong potential to explore the design space and autonomously implement efficient strategies for natural microbiome evolution.

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P33. Polyhydroxyalkanoates Production from Agro-Wastes: a Sustainable Approach to Prevent Persistent Plastic Pollution

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In recent years, serious pollution issues from litter, plastics, and microplastics (MP) have been the subject of great concern, and thus, several alternative and more sustainable solutions have been proposed. To prevent the creation of MP, biobased and biodegradable materials can be produced as promising alternatives to the petroleum-based plastics [1]. Polyhydroxyalkanoates (PHA) are biobased and biodegradable polyesters produced by microorganisms as intracellular carbon and energy reserves in nutrient-limited environments. These biopolymers are gaining attention as eco-friendly substitutes for the conventional petrolderived thermoplastics [1,2]. PHA can be produced using mixed microbial cultures (MMCs) from inexpensive raw materials, thus lowering the production costs, improving the adaptability of the process to complex environments, and enhancing its scalability. PHA production with MMCs is carried out in three stages [3,4]: (I) fermentation of organic resources into fermentation products (FP), including volatile fatty acids (VFAs), which are the PHA precursors; (II) selection of PHA-accumulating organisms through selective pressure (feast/famine (F/f) strategy); (III) PHA production, where the PHA-accumulating MMCs are fed with VFAs to maximize PHA production.

A three-stage pilot-scale system successfully converted fruit waste into poly(3 hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-co-3HV)]. Fruit waste was first fermented in a 54 L upflow anaerobic sludge blanket (UASB) reactor, inoculated with anaerobic granules, where the organic loading rate (OLR) and pH were adjusted to maximize the production of a fermented stream enriched in PHA precursors. These FP served as substrate for synthesizing P(3HB-co-3HV) with a 3HV content of 10-20% (wt.) and were supplied to a 150 L aerobic sequential batch reactor (SBR) under an F/f regime to select PHA-accumulating bacteria, optimizing OLR for biomass productivity. Finally, PHA accumulation was carried out in a 50 L fed-batch reactor, where conditions were controlled to enhance intracellular polymer storage. The three-stage process achieved stable fermentation (>85% acidification), while the selected MMC maintained high biomass productivity (0.95 ± 0.1 gX/L.d). PHA accumulation reached 55% wt., with a monomer composition of 87:17% 3HB:3HV, tailored for the targeted applications. The produced PHA will be further incorporated in the formulation of biodegradable plastic products to prevent plastic and MP pollution.

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P34 (FLASH) Mechanistic Analysis of Block Copolymerization Catalyzed by Engineered Polyhydroxyalkanoate Synthase

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Polyhydroxyalkanoates (PHAs) are produced using various microorganisms and used as biodegradable plastics. PHAs are synthesized by PHA synthases, PhaCs, from hydroxyacyl-CoA. Naturally occurring PHAs are homopolymers or random copolymers. In contrast, the engineered sequence-regulating polyhydroxyalkanoate (PHA) synthase PhaCAR synthesizes block copolymer, poly(3-hydroxybutyrate)-block-poly(2-hydroxybutyrate) [P(3HB)-b-P(2HB)] in Escherichia coli [1]. The block sequence is spontaneously generated from a mixture of 3HB-CoA and 2HB-CoA. In this study, we attempted to elucidate the mechanism of block copolymerization catalysed by PhaCAR using an in vitro assay system. The polymers were synthesized using purified PhaCAR and both 3HB-CoA and 2HB-CoA. The time-course analysis of the reaction revealed that PhaCAR initially polymerized 3HB-CoA and synthesized P(3HB) segment [2]. PhaCAR initially exhibited very low activity toward 2HB-CoA. Therefore, to synthesize P(2HB) segment, the substrate specificity of PhaCAR might be altered during the polymerization. To test the hypothesis, we measured PhaCAR activity toward 2HB-CoA after the synthesis of P(3HB) polymer chain. Expectedly, the PhaCAR after the reaction with 3HB-CoA exhibited significantly higher activity toward 2HB-CoA compared to the initial state. Therefore, the synthesis of P(3HB) segment caused PhaCAR to transition to the mature state and exhibit the activity toward 2HB-CoA. Next, to determine the effect of P(3HB) chain length on the PhaCAR maturity, 2–5-mer substrate analogues (primers) that mimic 3HB oligomers were chemically synthesized and were bound to PhaCAR. As a result, an increase in the degree of polymerization of the primer bound to PhaCAR correlates with enhanced activity towards 2HB-CoA. These results suggest that the elongation of the P(3HB) chain induces a change in the substrate specificity of PhaCAR towards 2HB-CoA. In conclusion, our findings provide significant insights into the mechanism of block copolymerization through changes in substrate specificity of PhaCAR.

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P35. Production 0f Biopolymers from Denitrifying Microorganisms in Granular Sludge Batch Reactor

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Granular sequencing batch reactors allows aggregation of microorganisms through granule shape thanks to high selection pressure [1]. These granules are aggregates of microorganisms embedded in a network of highly cohesive exopolymers (EPS). The cohesive property of these polymers called structural EPS (sEPS) led to develop extraction protocols [2] and understand their properties [3] which allows to develop a new pathway for matter recycling inside wastewater treatment field. We aim to increase our knowledge in this field by studying the role of specific populations in the production of sEPS. The case of PAOs having been studied recently [4]and we focus here on denitrifying populations. This study aims to link sEPS production and characteristics to GSBR operating conditions and sludge properties.

Based on the work from a previous thesis, [5], a 17L GSBR was operated during 3 months with alternating anoxic/aerobic conditions and a 4 carbon sources synthetic effluent composed of volatile fatty acids (Acetate and propionate), glucose and peptones. Nitrates were added to ensure anoxic conditions during non-aerated phase. The reactor followed 3.5-hour operating cycles consisting of 25 minutes of anoxic feed, 20 minutes of additional anoxic phase under slow agitation, 155 minutes of aerobic phase under rapid agitation, 6.5 minutes of decantation and 3.5 minutes of purging. The Volume Exchange Ratio (VER) was 50%. Typical ratios encountered in urban wastewater were applied: COD/P and COD/NTK ratios of 48 and 8, respectively. After inoculating with an activated sludge, the denitrifying microorganisms were selected by injecting the substrate during the non-aerated phase, allowing maximum COD consumption by the denitrification reaction in the anoxic phase and only slow growth in the aerated phase.

In order to assess the activity and proportion of denitrifying populations, COD and nitrate balances were carried out in the anoxic and aerobic phases. The properties of the sludge in terms of settling and treatment of carbon, nitrogen and phosphorus were monitored dynamically. The stability and robustness of EPS production was assessed by quantifying the dynamics of polymer extraction yields, as well as their structures and rheological qualities. This made it possible to relate the structure of the aggregates to the production of sEPS.

Ultimately, we hope to compare the results of this study with those obtained from the selection of other dominant populations and pure cultures.

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P36. Physics-Informed Neural Network (PINN) Framework for Batch-to-Batch Control of PHA Production

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Bioreactors are central to industrial biotechnology, but their complex, nonlinear, and dynamic behaviour presents a significant challenge for effective digitalization and optimization [1]. This is especially true in producing of polyhydroxyalkanoates (PHA), a promising biodegradable bioplastic synthesized by microorganisms under nutrient-limited conditions. Traditional mechanistic models based on first-principles equations can capture the system's dynamics, but they suffer from high mathematical complexity, and heavy computational demands, particularly in highly dynamic, fed-batch, or semi-batch operations common in PHA production. To overcome these challenges hybrid modelling strategies have emerged as promising alternatives [2]. Among these, Physics-Informed Neural Networks (PINNs) provide a unified framework that combines the flexibility of deep learning with the robustness of first-principles modelling by embedding differential equations (ODEs or PDEs) into the neural network loss function [3]. This ensures that the neural network models not only fit the available data but also adhere to the underlying governing laws of the system. In our recent work, we proposed a dual-FFNN PINN architecture for generic bioreactor modelling [4]. The approach utilizes two coupled feedforward neural networks, the first network (FFNN-S) approximates the dynamic trajectories of state variables as functions of time and control inputs, while the second network (FFNN-R) learns the reaction kinetics as functions of state variables. This modular structure improves training stability and interpretability. The framework was validated on a range of bioprocess case studies from microbial growth logistic conditions to complex fed-batch operations. Notably, in one of the case studies the model was trained on single-batch data and evaluated across 14 unseen testing batches, with no volume measurements provided during training. The PINN achieved $R^2 = 0.99$ for biomass and $R^2 = 0.98$ for volume, highlighting its generalization ability and data efficiency. As a next step, this dual-FFNN PINN framework will be extended to enable batch-to-batch control of PHA production.

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P37. Hybrid Modelling Application for the Supercritical Carbon Dioxide Extraction Process

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Supercritical carbon dioxide ($ScCO_2$) extraction offers a sustainable alternative to traditional solvent-based methods for separating nonpolar solutes, such as lipids, from biomass. This process eliminates the need for toxic organic solvents and subsequent purification steps, making it safer and more cost-efficient. Carbon dioxide is especially advantageous due to its non-toxic nature, affordability, and critical point at a low temperature (304.25 K), allowing for the extraction of thermally sensitive compounds without degradation [1,2].

Modeling the ScCO2 extraction process typically involves a combination of intraparticle and macroscopic material balance equations alongside mass transfer laws. A significant challenge in these models lies in defining the relationships between mass transfer coefficients, flow conditions, ScCO2 properties, and the physicochemical characteristics of the target solute. The latter are typically empirical and less reliable, eventually compromising the model's predictive power. In this study, a hybrid neural network (HNN) model was developed [3] to simulate the ScCO2 extraction of lipids from biomass. The model integrates a feedforward neural network (FFNN) with material balance equations represented as partial differential equations (PDEs), where the FFNN estimates the solute transfer rate from porous biomass into the bulk based on microenvironmental conditions.

Experimental data covering a range of operational conditions—temperatures (313–335 K), pressures (200–500 bar), and $ScCO_2$ flow rates (0.00017–0.0025 kg/s)—were used to train (80%) and test (20%) the model. The extraction column was discretized into multiple levels (3–10), and simulations demonstrated that increasing the number of discretization layers significantly improves the model's predictive performance.

Table 1-Comparison of hybrid models with an FFNN 5x4x1 (NW=29 parameters) and different numbers of discretization elements (No Levels)

No. Levels	Training Error	Testing Error	NW	AICc*	CPU(s)**
10	0.916	0.433	29	109	5.17E+4
7	1.32	0.891	29	131	3.33E+4
5	1.31	0.932	29	131	2.48E+4
3	1.28	1.03	29	129	1.42E+3

*AICc: Corrected Akaike Information Criterion
**CPU(s): Central Processing Unit time in seconds

This mechanistic modeling and neural network integration provides a robust foundation for developing a digital twin of the $ScCO_2$ extraction process, enabling future optimization and real-time process control. The next phase will leverage the model to identify optimal extraction parameters for enhanced efficiency and scalability.

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P38. Breaking The Law: Development Of Hydrogels from Hydrophobic Biopolymers

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Hydrogels, defined by their hydrophilic polymeric networks capable of retaining significant water content, have become increasingly important in biomedical applications such as tissue engineering scaffolds, drug delivery systems, and implants. Their versatility and biocompatibility have driven ongoing research efforts aimed at developing advanced, customizable solutions for clinical treatments. Additive manufacturing, particularly 3D printing, has emerged as an invaluable technique allowing the fabrication of precise, patient-specific implants to address critical-sized defects in regenerative medicine.

Despite their attractive biodegradability, biocompatibility, and tunable mechanical properties, polyhydroxyalkanoates (PHAs)—naturally occurring hydrophobic biopolyesters produced by microorganisms—pose a fundamental challenge when employed for hydrogel formation due to their intrinsic hydrophobicity. Conventional methods to generate hydrogels typically require the use of hydrophilic polymers, making PHAs seemingly unsuitable without chemical modification. However, chemical modification can compromise the biocompatibility, biodegradability, or mechanical properties essential for biomedical applications.

In this contribution, we present an innovative strategy that breaks conventional polymer-hydrogel paradigms by successfully fabricating hydrogels directly from hydrophobic PHAs through a solvent-exchange process. This approach entails initially dissolving PHAs in a water-miscible organic solvent followed by controlled solvent replacement with water, resulting in stable hydrogel networks without the necessity of chemically altering the polymer backbone. Through systematic studies, the influence of various parameters—including the type and molecular weight of PHAs, additives, solvent choice, and specifics of the solvent-exchange protocol—on the mechanical and structural properties of resultant hydrogels was evaluated and optimized.

Furthermore, leveraging this novel methodology, we demonstrate for the first time the feasibility of utilizing PHAs in additive manufacturing processes, specifically 3D printing, to produce customizable hydrogels with defined microarchitectures. This breakthrough not only expands the utility of PHAs beyond traditional thermoplastic applications but also provides a pathway toward advanced biomedical devices capable of integrating biodegradability and tailored mechanical performance.

Overall, this study redefines the limitations previously associated with hydrophobic biopolymers, paving the way for new classes of bio-based hydrogels that could significantly impact regenerative medicine and personalized therapeutic applications.

P39. Enzymatic Synthesis of Functional Pegylated Adipate Copolymers

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Many new active pharmaceutical ingredients (APIs) demonstrate high hydrophobicity and low water-solubility issues. In this regard, polymeric nanoparticles (NPs) have been extensively used as drug delivery carriers for the encapsulation of such APIs. One commonly used polymer is polyethylene glycol (PEG), owing to its biocompatibility, high water solubility, and capacity to prolong the drug residence time. However, concerns have arisen regarding PEG's immunogenicity and limited biodegradability. In addition, inherent limitations, including limited chemical handles can restrict PEG's effectiveness in physiological conditions. For this reason, in the present study, we combine the advantages offered by PEG with the use of an enzymatic synthetic route to produce novel PEGylated polyesters. Furthermore, it has been proven that incorporation of a hydrophobic diols into the PEGylated backbone influences NPs formation, stability, and drug encapsulation, despite high chemical similarity. As a preliminary result, samples containing PEG and 1,6-hexanediol in a 50:50 ratio (PEGA-Hex 50%) and PEG and 2-hydroxyethyl disulfide in a 50:50 ratio (PEGA-SS 50%) have proved to be the most promising candidates in this small library analysed. Both samples exhibited sufficient NPs stability, biocompatibility and superior encapsulation efficiency compared to the other variants.

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P40. Utılızatıon of S-RGO Embedded Chıtosan Gelatın Bıopolymerıc Composıte Membranes for Engıneerıng Applıcatıons

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Membrane separation technique has become more and more popular because of its low energy consumption, ease of use, controllability, and scalability [1]. Biopolymeric composite membranes are used as biosensors and electrochemical sensors in sectors such as food and pharmaceuticals, as well as in wastewater treatment and energy storage areas [2, 3]. Highly selective membranes with high penetration rates and chemical/mechanical stability are needed for the quick development. For these reasons, the synthesis of a composite polymeric membrane and its development studies constitute the basis of this context. In recent decades, chitosan, a relatively inert biomaterial with the ability to form films, has gained quick recognition for its potential in separation and purification technology. Chitosan membranes have emerged as a potential option for a number of applications due to their hydrophilicity, biocompatibility, simplicity of modification, and exceptional affinity for dyes, metals and proteins [1, 4]. S-RGO (sulfurized reduced graphene oxide), was added to the structure in order to increase porosity, surface area as well as electron and proton conductivity [5, 6]. S-RGO was synthesized as previously described in literature [7]. Chitosan and gelatin solutions were blended at different volumetric ratios and different amounts of S-RGO was added in order to introduce sulfonic acid groups as well as obtaining a layered structure [7]. The membranes were oven dried at 40°C for 1 day. The effect of various parameters were investigated i.e the blending ratio of the polymers, amount and type of inorganic fillers, synthesis conditions. The produced membranes were examined for structural integrity, crystallinity, morphology, and thermal stability using SEM, FTIR, XRD, and TGA. In addition to these analyses, some more analyses such as electrochemical impedance spectroscopy (EIS), swelling and water uptake analyses was carried out. This work will ultimately contribute to the growing field of green materials science by highlighting a bio-based approach for advanced manufacturing methods in membrane development.

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P41 (FLASH). MOF Incorporated Chıtosan Gelatın Bıopolymerıc Membranes for Water Electrolysıs

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Biopolymers, which are eco-friendly alternatives to synthetic polymers, are polymers that come from biological sources and are employed in various energy and environmental applications [1]. In this context, the development of sustainable membrane technologies for green hydrogen production via water electrolysis presents a significant opportunity for the application of natural biopolymers [2]. As well as offering functional groups for chemical modification and interaction with inorganic fillers, these polymers have excellent biodegradability, biocompatibility, and film-forming properties [3-5]. This study focuses on the fabrication of chitosan-gelatin-based composite membranes enhanced with titanium-based metal-organic frameworks (MOFs), MIL-125 and its amino-functionalized counterpart NH_2-MIL-125. Chitosan and gelatin were chosen as the fundamental biopolymers. MIL-125 and NH_2-MIL-125 were synthesized via a solvothermal route by following the procedure given in the literature [6, 7]. MOFs were incorporated into the chitosan-gelatin matrix through a solvent casting method [8]. The prepared membranes were characterized using FTIR, XRD, SEM, and TGA to assess structural integrity, crystallinity, morphology, and thermal stability. Proton conductivity was measured using electrochemical impedance spectroscopy (EIS), while water uptake and swelling behavior will be evaluated gravimetrically. The NH_2-MIL-125 incorporated membranes showed an enhanced proton conductivity, mechanical robustness, and thermal resistance due to enhanced hydrogen bonding interactions between the amino-functional groups of the MOF and the biopolymer chains. So, the role of MOFs as both structural reinforcers and functional nanofillers was emphasized in the membrane design strategy. This work demonstrated the potential of integrating natural biopolymers with advanced porous materials such as MOFs to create high-performance, sustainable membranes for clean energy applications. The findings will contribute to the growing field of green materials science, highlighting a bio-based approach for next-generation electrolyzer membrane development.

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P42. Tailoring P(3HB-*co*-3HV) Properties by Post-Production Blending

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Polyhydroxyalkanoates (PHAs) are promising biopolymers for replacing less eco-friendly polymers, although their price is still higher than conventional ones [1]. PHAs can be produced via mixed microbial cultures (MMC) from carbon-rich waste to reduce costs, however, this process has challenges due to the variability in waste composition and seasonality [1]. The most common PHA copolymer produced from wastes by MMC is the poly(3-hydroxybutyrate-co-3-hydroxyvalerate) - P(3HB-*co*-3HV). The thermal, mechanical, and viscoelastic properties of these copolymers can be adjusted by varying their 3-HV monomer content [1]. However, controlling composition during production is difficult, especially when using variable waste substrates. This work aims to overcome this challenge by tailoring the P(3HB-*co*-3HV) properties after production by blending samples with different 3-HV grades to replicate the properties of the original polymers with the same composition as the formulated blends.

Five P(3HB-*co*-3HV) with different 3-HV content (5, 14, 24, 36, and 79 mol%) were produced from fruit waste at pilot scale using MMCs [2] and characterized. Blends with target 3-HV contents of 14, 24, and 36 mol% were formulated and compared with the polymers originally produced with the same monomeric composition of the blends. Blending strategies included mixing two PHA-enriched biomasses before extraction or solvent-mixing two extracted original polymers with opposite 3-HV grades. Both methods controlled the monomer composition almost perfectly, but the first was the least accurate, as it depends on the extraction yield and resulted in a lower 3-HV content. Compared to the corresponding biologically produced polymers, the blends showed some differences in their thermal and mechanical behaviour, including higher melting temperatures and increased stiffness with reduced ductility. Notably, the biomass-mixed blends also exhibited a higher enthalpy of fusion than polymer-mixed blends, which could be attributed to the lower 3-HV content of the biomass-mixed blends.

This work demonstrates that, despite some differences compared to the original polymers, post-production blending is a potential strategy to control the P(3HB-co-3HV) properties, offering a promising alternative to the challenges of controlling monomeric composition during biological production, as it allows for manipulation of the final material properties. However, predicting blend properties requires further study, and future work will also apply the blending concept to industrial-scale processes like extrusion, usually found in polymer engineering.

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P43. Exopolysaccharides And Polyhydroxyalkanoates from Wastefed Microalgae/Cyanobacteria: a Simultaneous Production Attempt

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This study explores the sustainable production of high-value biopolymers—exopolysaccharides (EPSs) and polyhydroxyalkanoates (PHAs)—through the cultivation of microalgae and cyanobacteria in nutrient-rich waste-derived media. By repurposing waste streams as alternative culture media, the process reduces fossil resource dependence while promoting circular bioeconomy principles.

The species *Anabaena* sp. PCC 7120, *Nostoc* sp. PCC 9202, and *Porphyridium purpureum* SAG 1380-1a were selected based on promising lab-scale results, showing up to 17-fold higher EPS yields compared to conventional media. Scale-up experiments were conducted in a 5 L pilot reactor under conditions replicating those of the lab. During cultivation, EPS and PHA production were tracked alongside nutrient consumption (N, P), pH, and optical density. Protein, pigment, and fatty acid methyl ester (FAME) profiles and content were also analyzed.

Maximum EPS production was observed after 8–10 days, reaching 335 mg/L for *Anabaena* sp., 490 mg/L for *Nostoc* sp., and 405 mg/L for *P. purpureum*, before declining. PHA synthesis began after 15 days, peaking between days 24–27 with 514 mg/L (7.7%), 1991 mg/L (30.8%), and 465 mg/L (8.7%) respectively. *Nostoc* sp. showed the highest performance, with day 24 identified as key, yielding both 30.8% PHA and 403 mg/L EPS. Its PHAs were composed of 42.2% HV and 57.8% HD monomers.

Ongoing characterization of EPSs and PHAs includes HPLC (monosaccharide profiling), FTIR, molecular weight analysis, elemental analysis, and thermogravimetric analysis (TGA), supporting their potential for industrial applications.

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P44. Design Of Porous and Hydrophilic PHA-Based Structures Incorporating Polyoxazoline Via Non-Solvent Induced Phase Separation (NIPS)

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To form porous crosslinked networks, we employed the Non-Solvent Induced Phase Separation (NIPS) process, previously used for preparing PHA-based networks [1–2]. This technique leverages differences in polymer affinity and solubility in various solvents and involves three key components: the target polymer, a solvent, and a non-solvent. PHA is first dissolved in a defined amount of chloroform, followed by the addition of an equivalent volume of THF, acting as the non-solvent. This addition leads to phase separation, yielding a polymer-rich phase with minimal solvent content and a polymer-poor phase containing the solvent and non-solvent.

Due to its semi-crystalline nature, the polymer crystallizes upon precipitation in the polymer-rich phase, forming crystalline domains that act as physical crosslinking points. This results in a three-dimensional PHA network. The polymer-rich phase constitutes the structural matrix, while the solvent-rich phase flows through this matrix, forming interconnected porous channels within the material.

To induce pore formation in the final material, the use of a solvent such as water is essential. Water is ideal for generating porosity, as its slow evaporation via freeze-drying preserves the integrity of the porous structure.

We applied this technique to develop porous PHBHHx networks. To create structures with tunable hydrophobic/hydrophilic balance, poly(2-oxazoline) (POx) was incorporated. The resulting porous PHA materials exhibit density and thermal conductivity comparable to expanded polystyrene, representing a significant advancement toward lightweight, biodegradable, and bio-based materials. Notably, we successfully formulated a highly porous PHA with an ultra-low density of 0.041 $g \cdot cm^{-3}$ and enhanced hydrophilicity due to the presence of POx. The swelling ratio in water reached 392% [3].

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P45. Composites of Bacterial Cellulose and Blends of Polyhydroxyalkanoates

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Scl-PHAs, such as polyhydroxybutyrate (PHB), are highly crystalline, brittle, and stiff with low elongation at break. On the other hand, mcl-PHAs are mostly amorphous, with low melting temperatures and high elongation at break. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co3-hydroxyhexanoate), (PHBHVHHx), is considered one of the most promising additions to the polyhydroxyalkanoates (PHAs) family that includes short-chain length (scl) and medium-chain length (mcl) monomers into terpolyesters with interesting novel properties that combine the tensile strength properties of the PHB homopolymer with the flexibility of mcl-PHA polymers [1]. Although PHAs are tailorable and have interesting features, they can still underperform mechanically. To solve this, PHA can be combined with other materials to form biocomposites with broader functionality. Bacterial cellulose (BC) is a natural polysaccharide synthesized by some species of bacteria and owns properties such as high crystallinity, high water-holding capacity, high tensile strength and fine web-like network structure [2]. In this study, blends of PHBHVHHx [1] with an mcl-PHA [3] were prepared by dissolution of both biopolymers in chloroform with 25 g/L of each PHA. The resulting solution was mixed with 1g of wet BC paste (5.6% BC content on a dry basis) in NaOH (3 mL), at a 1:10 (v/v) ratio, and emulsified by mixing at 9000 rpm for 30 minutes. The emulsion was cast on a glass plate and the composite membrane was obtained by slow solvent evaporation, at room temperature, in the fume hood. The composite membrane had a thickness of 400 □m, it was white and opaque, with a porous structure, as shown by SEM analysis. Its properties (mechanical and barrier properties, water absorption and swelling in water) were compared to those of a blend of PHBHVHHx:mcl-PHA (with no BC) and to membranes of each individual PHA, to highlight the advantages of incorporating BC into the PHA blend. The good mechanical resistance (Young's Modulus of 20 MPa) and the flexibility (elongation at break of 31%) showcase the valuable properties of the produced PHA:BC composite, whose applications in several areas can be explored, including technical uses like textiles and packaging or as biomedical materials.

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P.46 Polysaccharides Rich in Rare Sugars Secreted by Newly Isolated Bacterial Strains

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Polysaccharides are essential natural metabolites found in all living things, including microorganisms, animals, and plants, with diverse biochemical structures and biological functions [1]. Due to their biodegradability, biocompatibility and general non-toxicity [2], polysaccharides are widely used in high value applications such as pharmaceuticals, food and cosmetics [3]. In this study, two bacterial strains were isolated from plant roots and river sediments. The strains were identified as *Lelliota* sp. strain RD5 and *Bacillus* sp. strain SC4, and synthesized extracellular polysaccharides (EPS) rich in fucose (FPol) and glucosamine (GlcNPol), respectively. Culture experiments with each bacterium were carried out in 2-liter bioreactors using glycerol as the only source of carbon. The EPS produced in each assay were recovered from the culture medium by centrifugation and ultrafiltration of the cell-free supernatant. The resulting samples were lyophilized and characterized in terms of composition, molecular mass distribution and functional properties, including their viscoelastic properties, formation capacity and emulsion stabilization, capacity of gelification and as potential reducing agents and/or stabilizers in the synthesis of nanoparticles (NPs). Both the FPol and GlcNPol produced aqueous solutions with shear thinning behaviour, however the aqueous solutions of GlcNPol were considerably less viscous than those of FPol. Emulsion assays were performed using various oil/water ratios (3:2 and 2:3, v/v) and aqueous solutions containing 1% (w/v) of EPS. Both biopolymers formed good emulsions that remained stable in both proportions tested for several days, at room temperature. Regarding gelification, FPol formed homogeneous spontaneous gels in the presence of Fe(III), while GlcNPol gelified in the presence of Cu(II). FPol also showed good reducing and/or stabilizing capacity in the synthesis of gold, silver and selenium NPs. Given these characteristics, these polysaccharides are promising candidates for use as suspending agents or bioemulsifiers in various applications, or for the development of hydrogels. Their high content in rare sugars confer them additional value, given their known biological activity.

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P47. An Eco-Sustainable Route for mcl-PHA Extractions and Novel PHA-Based Polyurethanes

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Recent advancements in the development of novel biopolymers, has been mostly focused on sustainable, innovative materials designed to both minimize environmental impact and to decrease the depletation of fossil fuels [1]. With the rising demand for eco-friendly and biodegradable, biobased options, bacterial biopolymers polyhydroxyalkanoates (PHAs) retained attention. PHAs have a great applicative potential in various fields, but its downstream processing usually using organic solvents makes it less eco-sustainable. In the presented study, greener extraction routes employing deep eutectic solvents (DES) composed of menthol and fatty acids and commercial enzymes efficient in selective mcl-PHA rich biomass degradation are presented. The mcl-PHA extracted from bacterial biomass, through greener downstream processing, was further used as a polyol component in novel bio-polyurethanes synthesis. Polyurethanes (PUs)—commonly utilized in foams, coatings, elastomers, adhesives, and biomedical applications—represent a high percentage of global polymer market. Current perspectives in the bio-based PU synthesis have focused on substituting fossil-based diisocyanates and polyols with bio-derived alternatives, aiming to create polymers that are both high-performing and environmentally friendly [2]. Addresing these demands, this study explored the potential of mcl-PHA and castor oil used in different ratio as polyol components for PU synthesis, using hexamethylenediisocyanate (HMDI) as the crosslinking agent. The bio-PU films were obtained via solvent casting and characterized in terms of structure, thermal properties, mechanical properties, wettability and toxicity (using ATR-FTIR spectroscopy, SEM, TGA, X-ray diffraction, mechanical testing, water contact angle measurements) [2]. The findings originated from this study suggests an alternative, eco-sustainable extraction of mcl-PHAs and their further utilisation for bio-PU synthesis, opening promising route for greener PU production.

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P48. A Screening Method for Detecting Gel-Forming EPS Extracted from Biological Aggregates

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Wastewater treatment processes convert organic pollution (COD) into sludge, which is currently utilized in agriculture or as an energy source through incineration. When advanced treatment technologies using granular sludge (GS) are employed, the resulting sludge contains a significant amount (up to 25% by dry weight) of biopolymers. Among these biopolymers, structural extracellular polymeric substances (sEPS), particularly hydrocolloids, represent a valuable resource for industrial applications such as in concrete production, etc. Given the substantial amount of COD treated in wastewater treatment plants, the hydrogel production potential is high, paving the way for the development of cost-effective production pipeline. This transformation creates opportunities for innovative applications converting waster into a commercially viable product like hydrogels.

The sEPS extracted from GS are composed of polysaccharides, proteins, lipids, humic acid and DNA [1]. This work focus on evaluating their functional properties, particularly their ability to form gels. Conventional rheological methods can assess these properties, but require significant amounts of EPS, which usually unavailable from laboratory-scale pilot systems. To overcome this limitation. Bou-Sarkis et al. (2023) developed a miniaturized method for assessing the gelling capacity of sEPS [2]. This poster describes the development, optimization and evaluation of the method.

This miniaturized screening technique detect gelation via ionic crosslinking, using only 1 to 3 mg of tested sample (BSA, DNA, alginate (ALV) or EPS). The method involves calculating a reactivity percentage (%R) using UV-Visible spectra and determining the gel volume percentage (%Vg) formed after the addition of calcium. In a 96-well microplate, 100µL of the sample is mixed with 100 µL of $CaCl_2$. After incubation and centrifugation, two phases are formed: a gel and a supernatant. The volume of the supernatant (Vs) is measured and the pellet volume (Vg), representing the formed gel, is calculated as the difference between the initial total liquid volume (Vt) and the supernatant volume (Vs).

To prevent the formation of $Ca(OH)_2$ precipitate, the supernatant is diluted. Since EPS are a complex mixture of molecules, the dilution factor varies between proteins and polysaccharides. The diluted supernatants are then analysed using UV-Vis spectroscopy over a wavelength range of 220 to 500 nm. Absorbance values are adjusted by multiplying them by the ratio of Vs to Vt to account for changes in liquid volume caused by gelation after calcium addition. The %R and %Vg values are combined to calculate the gelling factor (GF). The work proposed here describes the optimization of this method and its evaluation on different standard molecules, pure or mixes as well as on extracted polymers from GS. After testing pure molecules, a classification of gelling capacities was established as follow: GF(ALV) > GF(ALV+DNA) > GF(BSA+ALV+DNA) > GF(BSA+ALV) > GF(DNA) > GF(BSA+DNA) > GF(BSA).

This method provides a rapid and low-cost approach to assess the gel-forming capacity using minimal material and time. It enables efficient screening of extracts and can guide purification processes by tracking the enrichment of gelling polymers.

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P49 (Flash). Environmental Risks of Biodegradable Polymers in Soil: Myth or Reality?

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Biodegradable polymers, especially biopolyesters like polyhydroxyalkanoates, are increasingly applied in agriculture as environmentally friendly alternatives to persistent petro-plastics, particularly in mulch films and controlled-release systems for fertilizers or pesticides. These materials are designed to undergo microbial decomposition into benign end-products, and their usage is generally considered compatible with sustainable soil management. However, a growing body of literature has begun to scrutinize potential environmental risks of biodegradable polymers in soil, particularly in their fragmented forms, the so-called “microbioplastics”. These concerns include alterations in soil´s microbial community structure, enzymatic activity, and nutrient cycling dynamics.

While such scrutiny is warranted, many recent studies raising alarms rely on experimental setups that fail to reflect realistic field conditions. One of the most pervasive issues is the use of unrealistically high polymer loading rates. These methodological flaws may lead to misleading conclusions, as effects observed at such exaggerated concentrations, e.g., destabilization of soil organic matter or suppression of plant growth, are often presented as inherent risks. Moreover, many reported negative effects appear to be transient and reversible as the material particles migrate and get diluted, degrade, and microbial communities recover.

This contribution critically examines the current discourse around biodegradable polymers as soil contaminants, highlighting the need for environmentally relevant exposure levels, time scales that reflect agricultural cycles, and the inclusion of appropriate and relevant positive controls from nature in experimental setups. It also calls attention to the importance of distinguishing between intrinsic risks and those arising from improper application or unrealistic experimental assumptions and design.

We argue that while biodegradable polymers are not without environmental consequences, but their impact must be assessed in a fair and scientifically grounded context. With proper design and usage, these materials can support circularity and sustainability in agriculture without posing significant risks to soil health.

P50. Composite Biopolymer Nanofibre Delivery System for Topical Wound Treatment

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Electrospun nanofibres offer several advantages over traditional wound dressings and have attracted increasing interest in the field of wound management. Encapsulation of bioactive compounds of natural origin has been shown to endow these materials with multifunctional therapeutic properties and the potential to combat antimicrobial-resistant infections [1,2].

In the present study, a composite material consisting of layered poly(3-hydroxybutyrate) (PHB) and gelatine electrospun nanofibres was developed. The nanofibres were functionalised with two different antimicrobial agents and the analgesic ibuprofen, to achieve a combined therapeutic effect. The selected antimicrobial compounds and the electrospun nanofibres were evaluated for their antioxidant activity, antimicrobial efficacy and potential synergistic effects against selected gram-positive and gram-negative bacterial strains.

The cumulative release profiles of the encapsulated compounds in a model environment were determined using HPLC analysis. Furthermore, the biocompatibility of the nanofibre mats was assessed using the MTT assay on human keratinocytes (HaCaT cell line).

The results showed synergistic antimicrobial effects, significant antioxidant activity and
non-cytotoxic behaviour of the composites. In conclusion, the developed biopolymer-based delivery system shows great promise for local dermal therapy of infected wounds.

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P51 (Flash). Development of Stable Oil-In-Water Emulsions Based on the Hyaluronic Acid-Like Polysaccharide Produced by the Marine Bacterium *Vibrio alginolyticus* Mo245

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Hyaluronic acid (HA) is a glycosaminoglycan (GAG) being increasingly used for medical and cosmetic applications due its hygroscopicity and biocompatibility. HA most common production lacks process efficiency and relies on the use of pathogens such has *Streptococcus zooepidemicus*, bringing safety concerns [1]. The production of GAGs by non-pathogenic marine bacteria is a promising alternative approach to tackle those issues due to their ability to secrete high molecular weight (Mw) polysaccharides with physicochemical and biological properties similar with HA. In particular, the strain *Vibrio alginolyticus* Mo245 has been reported to produce a HA-like extracellular polysaccharide (HA-like EPS) with potential to be an alternative to HA, due to its composition, gelling and viscoelastic properties [2]. In this study, *V. alginolyticus* Mo245 was cultivated using glycerol as the sole carbon source to produce the HA-like EPS, followed by the development of HA-like EPS based emulsions, which will be further designed into cosmetic formulations. V. *alginolyticus* Mo245 HA-like EPS, mainly composed of equimolar amounts of amino sugars and uronic acids, had a high Mw (2.01±0.01 x10^6 Da), similar to HA [3]. The emulsifying capacity of the HA-like EPS for the development of oil-water (O/W) emulsions using different oils was demonstrated. Castor oil was selected based on the high emulsification index (100%) and stability (70% over a period of 30 days) of its emulsions with the EPS at concentrations of 1.0 and 1.5 wt%, and a O:W ratio of 3:2. The prepared emulsions were characterized as oil-in-water (O/W), which represent two thirds of the emulsified cosmetic products in the market due to their good sensorial properties [4]. Further, rheological analyses on the HA-like EPS/Castor oil emulsions showed a shearthinning behaviour and a gel-like structure. The ingredients concentration of the HA-like EPS based emulsions with castor oil were optimized to enhance the emulsification index and rheological properties towards the development of suitable cosmetic formulations. This study demonstrated the promising features of the HA-like EPS produced by *Vibrio alginolyticus* Mo245 using glycerol that render it highly interesting for the development of cosmetic formulations.

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P52. Fabrication Of Polyhydroxyalkanoate (PHA) Structures Loaded with Biogenic Gold Nanoparticles

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Polyhydroxyalkanoates (PHAs) are accumulated inside bacterial cells as carbon and energy storage compounds and, depending on the size of their monomers, they have different properties, ranging from brittle and rigid thermoplastics (e.g. poly(3-hydroxybutyrate)), to flexible and elastomeric polymers (e.g. medium-chain-length-PHA) [1]. These hydrophobic, biocompatible and biodegradable polymers can have diverse applications, particularly in biomedical and nanotechnology fields. FucoPol, on the other hand, is a hydrophilic, high molecular weight heteropolysaccharide mainly composed of fucose, galactose, glucose, and glucuronic acid [2]. This exopolysaccharide (EPS) is known for its film and hydrogel forming capacity, flocculating, thickening, emulsifying, anti-inflammatory, antioxidant, anti-aging, UV protective, cryoprotective and, even, wound healing ability [3–5]. Also, due to its anionic character and functional groups, FucoPol can reduce and stabilize metallic nanoparticles (NP), allowing the control of particles' shape, size and dispersion [4]. One example of this, is the synthesis of gold nanoparticles (AuNP) in presence of FucoPol. These particles have emerged as promising materials for countless biomedical applications including drug delivery systems, imaging, biosensors to detect toxins or pathogens, cancer therapy and antibiotic development [6–8].
In this study, the fabrication of PHA structures loaded with biogenic AuNPs synthesized in presence of FucoPol. The resulting bionanocomposites were characterized to assess their structural, thermal, and functional properties. The integration of AuNPs into PHA materials provides these structures with bioactive properties demonstrating the potential of sustainable functional bionanocomposites to be used in different applications, including biomedical and active packaging.

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P53 (FLASH). Biosurfactant-Assisted Preparation of Polyhydroxyalkanoates Nanoparticles: a Comparative Approach

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The development of sustainable surfactants is critical to reduce reliance on synthetic compounds in nanoparticle synthesis for biomedical and environmental applications [1]. This study evaluates bacterial biosurfactants—FucoPol, a fucose-rich polysaccharide, and HA-EPS, a hyaluronic acid-like exopolysaccharide—as eco-friendly alternatives to polyvinyl alcohol (PVA) for fabricating biodegradable polyhydroxyalkanoate (PHA) nanoparticles via solvent evaporation. Four types of PHA, poly(3-hydroxybutyrate) (PHB), poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and a medium chain-length PHA (mcl-PHA), were processed using a hybrid protocol combining high-speed homogenization (12,000 rpm), ultrasonication, and filtration, with surfactant concentrations ranging from 0.1% to 10% [2]. With HA-EPS, PHB nanoparticles of 43.4 nm were achieved at a 0.5% concentration, comparable to PVA's 28.4 nm at 1%, while FucoPol yielded optimally sized PHBHHx nanoparticles (312 nm) at 0.1%. Notably, biosurfactants required 10-fold lower concentrations than PVA to achieve similar results, though FucoPol exhibited aggregation (>10,000 nm) at concentrations ≥1%. Both biosurfactants demonstrated broad applicability for mcl-PHA, producing nanoparticles (123–978 nm) within the size range of PVA-synthesized counterparts (113–1517 nm). These findings position bacterial biosurfactants as scalable, sustainable substitutes for PVA, offering nanoscale precision and rapid biodegradability. The optimized protocol bridges lab-scale innovation to industrial feasibility, with sub-50 nm PHB nanoparticles promising for targeted drug delivery and mcl-PHA systems applicable to environmental remediation (e.g., pollutant degradation). By reducing chemical inputs and enhancing process efficiency, this work advances green nanotechnology for diverse socio-environmental challenges [3].

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P54. Bacterial Cellulose-Fucopol Composite Hydrogel Dressings for Advanced Wound Treatment

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Chronic wounds present considerable challenges, burdening patients and healthcare systems with substantial costs, especially due to factors such as obesity, diabetes, and aging populations. Effective wound care depends on dressings that prevent infection and facilitate healing, driving research into advanced therapeutic dressings for the development of affordable solutions [1]. Bacterial cellulose (BC), a glucose homopolymer, is highly regarded for wound dressings due to its mechanical strength, nanoporous structure, high water-holding capacity, and biocompatibility [2]. While BC promotes debridement and maintains moisture for wound healing, it lacks essential bio-functional properties, which can be addressed through functionalization with other compounds. FucoPol (FP), a polyanionic, fucose-rich exopolysaccharide, offers wound-healing potential alongside adhesive, photoprotective, and bioactive properties [3,4].

This study combines BC and FP to develop a composite hydrogel designed for wound dressings, leveraging the unique properties of each polymer. BC was impregnated with FP through agitation and gelation using Fe^{3+} (0.26–5 g/L) as a crosslinking agent. Rheological analysis revealed that 2.5 g/L Fe^{3+} was optimal for consolidating the materials and enhancing gel strength compared to BC alone. Increasing FP concentrations (0.5–2.0 wt.%) further improved the composite's mechanical strength, but it reduced its crystallinity index (43-38%), water holding capacity (72-39 g/g) and water retention, compared to BC by itself (crystallinity index: 59%; water holding capacity: 123 g/g). Moreover, the hydrogels demonstrated non-toxicity, with cell viability exceeding 80% in HaCaT cells (human skin keratinocytes) and NCTC clone L929 (mouse fibroblast) cell lines. Additionally, they supported HaCaT and NCTC clone L929 cell adhesion and enhanced wound recovery, validating their suitability for wound dressing applications.

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P55 (Flash). Hydrogels of *Halomonas* Levan Polysaccharide

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Levan is a fructose-based homopolysaccharide renowned for its unique properties, including exceptional adhesive strength, self-assembly capability, low viscosity, and bioactivities such as prebiotic, anti-cancer, anti-inflammatory, and anti-diabetic effects. These characteristics have created increasing interest in levan-based biomaterials over the past decade, positioning levan as a highly under-explored biopolymer for a wide range of applications, from medicine to cosmetics. As a result, levan-based hydrogels have emerged as promising biomaterials in drug delivery, tissue engineering, and cosmetic formulations, owing to their extracellular matrix-mimicking structure, tunable mechanical properties, and controlled cargo release capabilities [1, 2]. Our research group is currently involved in producing both physically and chemically crosslinked hydrogels with levan produced by *Halomonas smyrnensis* halophilic microbial system, *Halomonas* levan. Some of the hydrogels are injectable while others are used for bioprinting microneedles. These hydrogels are used for controlled release of polyphenols and other bioactive compounds like resveratrol [3] and cannabidiols. With their high-water retention and proven biocompatibility, the produced levan hydrogels are targeted to be used for biomedical, food and cosmetic applications.

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P56. Bioactive Membranes of Alginate and Bacterial Cellulose Enriched with Rhamnolipids for Biofilm Inhibition

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Chronic wounds represent a major global health concern, impacting more than 200 million individuals worldwide and placing a substantial strain on healthcare systems due to persistent infections and the presence of resilient microbial biofilms [1].
Among the emerging candidate's materials for wound care applications, bacterial cellulose (BC) has garnered considerable attention owing to its outstanding biocompatibility, high water capacity, and adaptable structural properties [2]. Nevertheless, the broader application of BC is limited by factors such as low production efficiency and high production costs. Moreover, its lack of inherent antimicrobial activity poses a significant drawback for use in environments prone to microbial infection [3]. In response to these limitations, the present study explores the functionalization of BC with rhamnolipids (RL), which are naturally derived biosurfactants known for their high antimicrobial and anti-biofilm properties [4]. Another important aspect of this study is its commitment to sustainability, demonstrated by the use of commonly discarded food waste (banana peels) as feedstock for BC production. Additionally, the use of the non-pathogenic bacterium *Burkholderia plantarii* DSM 9509^T to produce RL ensures a safe and environmentally friendly production process.
The development of this membrane was achieved by combining BC with alginate (Alg), and RL were incorporated into the matrix through ionic crosslinking using calcium chloride ($CaCl_2$). This approach aimed to develop a hydrogel capable of a more sustained release of RL, thereby minimizing the risk of burst release commonly associated with simpler impregnation methods such as soaking.
Utilizing banana peel waste as a substrate for BC production resulted in a notable ~56% enhancement in yield compared to the traditional Hestrin–Schramm medium. Additionally, the BC/Alg membranes functionalized with RL exhibited remarkable antibacterial activity. This was clearly demonstrated by the formation of a well-defined inhibition zone measuring 0.15 ± 0.05 cm, confirming their effectiveness against *Staphylococcus aureus*, which is a prevalent pathogen commonly associated with wound infections. Notably, biofilm formation was almost entirely inhibited (< 92%, by colony forming units (CFU) counts), thereby offering a strategy for improving the functional performance of BC-based materials in antibacterial biomedical applications. In addition to its antibacterial benefits, the membrane exhibited no cytotoxicity and demonstrated WHC and mechanical properties consistent, and in some cases better, with those reported for commercially available wound dressings.

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P57 (Flash). Green Polysaccharide Binder Systems for Aqueous Electrode Slurry Processing

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Electrode slurries play a critical role in the performance of lithium-ion batteries. An electrode slurry consists of a suspension of active material in a solvent medium, along with conductive carbon and polymer binders. The current state-of-the-art slurry uses polyvinylidene difluoride (PVDF) as a binder and N-methyl-2-pyrrolidone (NMP) as a solvent. Focusing on eco-friendly alternative battery materials, our study aims to replace toxic and fluoride chemicals with biobased aqueous polysaccharide systems.

In our research, we investigate the effect of the polysaccharide structure on the rheological and coating characteristics of electrode slurries. For that, we compare the wild type (wt) xanthan with genetically modified structures, such as ΔgumFG and ΔgumFGL, lacking the different acetyl and pyruvyl decorations in a systematic way [1]. The absence of acetate and pyruvate influences the adhesion/cohesion properties of the slurries, leading to distinct behaviour.

The function of the binder during the processing is to thicken the slurry, suspend the components, and prevent settling, which impacts the adhesion of the electrode to the current collector. Understanding the rheological properties of slurries provides insights into the internal structure of the slurry and subsequent manufacturing steps like coating and drying.

The slurries' formulation exhibits higher elastic (G´) and viscous (G´´) moduli than the pure xanthan structures, confirming the formation of particle-polymer networks and interaction between the components. In general, the stability range is reduced, which is one more indication that the slurries are a suspension.

Due to the increase in viscosity in all the slurries compared to the pure polysaccharides, flow behaviour indicates the formation of interactions between the components. The rise was more evident in the xanthan wt and ΔgumFG slurries, confirming that the pyruvate groups are involved in the adhesion/cohesion properties. In fact, the slurry prepared with the ΔgumFGL variant displayed poor adhesion properties during the electrode preparation.

Further surface, microstructure, and electrochemical performance investigations are conducted to support the findings in the structure-property observed behaviour.

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P58 (FLASH). Alginate – Pullulan Hydrogels for Wound Healing

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Significant advancements are being made every day in the biomedical and wound care field, especially in bleeding control, and thus traditional care methods are being replaced by organic or inorganic hemostatic materials, based on polymers or composites, due to their low cost, efficiency, low toxicity and low environmental impact [1-2].

The current study emphasizes the development of alginate – pullulan hydrogels that could stop the bleeding and regenerate wounds. In order to successfully obtain hydrogel samples, pullulan was oxidized with sodium periodate [3] and then crosslinked with alginate in a calcium chloride solution. The developed samples were characterized by FT-IR and Raman spectroscopy, X-ray diffraction and XPS. Then they were evaluated in terms of porosity, toxicity, swelling and charge on the surface, by using SEM, cell viability assays, water up-take, and mechanical tests. The oxidation of pullulan improved greatly the liquid absorption capacity of the materials, the samples had different pore sizes because of the freeze-drying processes applied, and the crosslinking between the biopolymers succeeded. The bioactivity of the scaffolds was demonstrated by SEM analysis of the samples immersed in simulated biological fluid, and the cell viability of keratinocytes indicated no cytotoxic effect; the cells not only survived but proliferated. These results indicate their potential to stop bleeding, and therefore further *in vivo* tests will be carried out. Moreover, some other active ingredients such as bioactive glass or cerium nanoparticles could be added to the sample composition to improve their properties.

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P59. Development of a Multifunctional Hydrogel Based on Natural Polysaccharides for Diabetic Wound Healing

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Diabetic wounds (DW) are a major health concern affecting more than 60 million people worldwide [1]. As conventional treatment approaches are not efficient, DW severely impact patients' quality of life, often leading to recurrent hospitalizations and limb amputations [1]. DW are clinically challenging due to an extensive inflammatory phase caused by the high oxidative stress characteristic of these wounds, and the potential presence of bacterial infections.

This work aimed to develop novel advanced wound dressings based on natural polysaccharides that are produced from renewable resources, are biodegradable, biocompatible, and possess biological activity. The fucose-rich polysaccharide produced by *Enterobacter* A47, FucoPol, with wound-healing, antioxidant, and anti-inflammatory properties [2,3,4], was combined with an antimicrobial polysaccharide, either chitosan (Cs), a biopolymer widely used in biomedical applications, or a copolymer of chitosan and glucan (CsGC) [5], to prepare hydrogels with unique properties suitable for DW healing. CsGC hydrogels were obtained by alkaline dissolution (2 wt% in 5 M NaOH) and polymer coagulation of the polysaccharide produced by the GRAS yeast *Komagataella pastoris*. Cs hydrogel membranes were prepared by electrospinning using acetic acid solution (1.8 wt% Cs in 90% acetic acid) as solvent, poly(ethylene oxide) (0.4 wt%) as a co-spinning agent and glutaraldehyde vapour as the crosslinker. FucoPol was incorporated in both porous freeze-dried Cs-based aerogels through the soaking method (6 mg aerogel in 5 mL of 5 g/L of FucoPol). Morphological analysis revealed that CsGC hydrogels presented a higher porosity compared with Cs structures, which led to a higher FucoPol incorporation (1.7 times higher). The presence of FucoPol in the hydrogel's structure improved its mechanical properties and promoted a higher keratinocyte's adhesion. Additionally, FucoPol loaded Cs-based hydrogels enhanced cell proliferation and migration, demonstrating their wound healing potential.

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P60. Eco-Friendly Formulation Based on Polyhydroxyalkanoates and Natural Polymeric Surfactants for Fabrics' Dyeing with the Bacterial Pigment Prodigiosin

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Bacterial prodigiosins are naturally occurring red alkaloids characterized by a linear tripyrrole chemical structure, which confers their distinctive biological activity [1]. Prodigiosin is primarily synthesized during the late stages of bacterial growth, and its production is highly dependent on environmental conditions. It has attracted significant attention for its therapeutic properties and demonstrates potential for a wide range of applications, including use as a natural colorant [1,2]. Polyhydroxyalkanoates (PHAs) are natural, non-toxic, and biodegradable thermoplastic linear biopolymers. Their barrier properties make them a promising coating agent for textiles, offering a potential approach to optimize material performance [3]. In this study, a newly isolated bacterial strain, *Serratia* sp. VJ20, was grown in Medium E* [4] supplemented with glycerol at a concentration of 40 g/L, yielding pink-pigmented biomass. The pigment was identified to belong to tri-pyrrole prodigiosin family. Multi-fiber strips and different types of fabrics (cotton, hemp and linen) were dyed using a formulation comprising PHA powder (extracted from bacterial cells by enzymatic treatment [5]) and the freeze-dried pigmented biomass, which was mixed with an aqueous solution of a surfactant/suspending agent and emulsified at 10000 rpm, for 10 min. The formulation's composition and the conditions for its preparation were optimized. Natural polymeric surfactants were tested to replace synthetic ones, thus rendering the formulation eco-friendlier. The formulation was cast on test fabrics' strips that were subjected to a fixation involving drying at temperatures of at least 100 ºC, followed by hot-pressing at 130-180 ºC and 1-9 ton. The conditions were adjusted to obtain homogeneous coating of the samples and a good pigment fixation, assessed by applying several washing cycles and evaluating the color retention. A good prodigiosin fixation on the fabrics was achieved, which renders this procedure highly promising for textile uses.

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P61. Melt Electrowriting of Polyhydroxyalkanoates for Enzymatically Degradable Scaffolds

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Melt electrowriting (MEW) enables precise scaffold fabrication for biomedical applications. With a limited number of processable materials with short and tunable degradation times, polyhydroxyalkanoates (PHAs) present an interesting option. Here, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and a blend of PHBV and poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (PHBV+P34HB) are successfully melt electrowritten into scaffolds with various architectures. PHBV+P34HB exhibits greater thermal stability, making it a superior printing material compared to PHBV in MEW.

The PHBV+P34HB scaffolds subjected to enzymatic degradation show tunable degradation times, governed by enzyme dilution, incubation time, and scaffold surface area. PHBV+P34HB scaffolds seeded with human dermal fibroblasts (HDFs), demonstrate enhanced cell adherence, proliferation, and spreading. The HDFs, when exposed to the enzyme solutions and enzymatic degradation residues, show good viability and proliferation rates. Additionally, HDFs grown on enzymatically pre-incubated scaffolds do not show any difference in behavior compared those grown on control scaffolds. It is concluded that PHAs, as biobased materials with enzymatically tunable degradability rates, are an important addition to the already limited set of materials available for MEW technology [1].

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P62. Comparative Analysis on Sustainable Biomass and Substitution Potentials

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The shift towards a sustainable transition in the chemical industry necessitates a comprehensive analysis of raw material availabilites, technological advancements, and possibilities to use existing refinery infrastructures for processing. The aim of the project Bio2x was to conduct a comparative analysis of sustainable biomass potentials in order to highlight possible substitution potentials in refineries for Germany and Europe [1,2]. The comparative analysis (meta-analysis) of sustainable biomass potentials was conducted as a systematic investigation and classification of current studies on German and EU biomass potentials with a focus on biogenic waste, residues and by-products. Overall five potential studies are compared in detail, focusing on criteria such as number and kinds of biomasses covered, potential level, mobilisation rates of residues, competing uses, system boundaries, data sources and reference year. The potential of sustainable biomass generally covers a wide range, which primarily results from the potential level under consideration, the scope of the individual biomasses under consideration, the consideration of competing uses and sustainability requirements, the geographical reference, the data basis, the time horizon and any associated assumptions (scenarios). The results of selected potential studies in the form of average scenarios and bandwidths are compared for Germany and Europe in terms of energy-specific units (in the form of calorific values). Overall, there is currently only a very limited potential of corresponding residual and waste materials available for process chains or products that necessarily have to rely on resources containing oil and fat. Direct substitution of mineral oil within the established process chains is therefore hardly possible at present since the suitable and available (oil and fat-containing) biomass potential in Germany and the EU is relatively low. On top, the already sufficiently developed biobased conversion processes are not suitable on the basis of this potential to directly substitute the current product portfolio of the mineral oil industry (in terms of breadth and quantity). The integration of biogenic resources therefore requires the targeted (further) development of suitable processes and process chains. Based on specific product and technology assumptions made, specific product potentials for biogenic substitutes for diesel, kerosene, naphtha, ethanol, methane and methanol were identified. Overall, the potential of sustainable biomass for substituting previously mineral oil-based supply chains for fuels and other products has a wide range. Depending on the study, the bioenergy potential in Germany can replace 7-28% and in the European Union 10-45% of the current refinery output in the European Union (see Figure 1). With regard to the mobilizable technical potential the result ranges from 3-9% (EU: 8%), also in terms of energy content [1,2]. When considering sufficiently mature conversion processes the subsequent substitution potential primarily results from the conversion of biogenic by-products, waste and residues into biomethane and biomethanol. Based on a significantly reduced demand for fuels and combustibles due to electrification in transport, this relative share would be significantly higher.

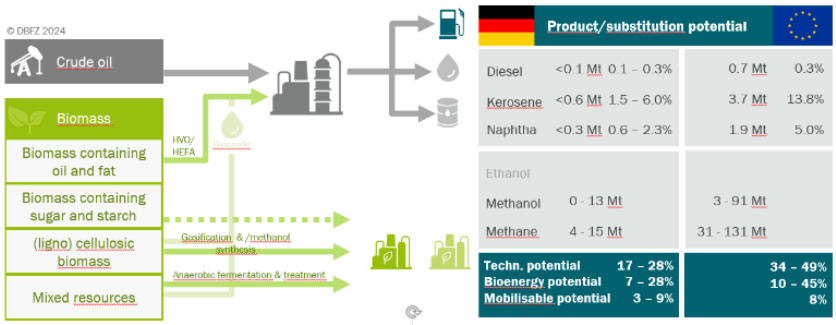

Figure 1: Bio-based substitution potential for products from mineral oil refineries for Germany and Europe [1] Notes: fuel-specific values: reference to mean values and mean scenarios of the studies (quantities in Mt oil equivalent, 1 kg oil equivalent = 42 MJ) | Total potential bandwidths also include min/max scenarios,Resources not included here: starchy crops, sugar from sugar beet (ethanol) | rape seed, sunflower, soya seed (diesel, kerosene, naphtha) | lignocellulosic crops, stemwood, forest wood (methanol)

At the conference, the results of the currently only in German published DBFZ background paper [1] will be presented in English for the first time. The results are able to give insights into methodological differences between biomass potential assessments as well as the need for strategic process development for certain biomass feedstocks.

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P63. Assessing the Ecotoxicological Impacts of Chronic Dietary Exposure to Petroleum-Based and Bio-Based Microplastics in Gilthead Seabream (*Sparus aurata*)

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Since the production of the first plastic, this industry has been growing year by year to keep up with population growth and the demand from plastics products [1, 2]. However, alongside this comes a panoply of problems. Currently, plastic waste is generated at a rate of 400 Mt year^{-1} [2], and when these enter the environment (terrestrial or aquatic) they take several years to degrade completely, depending on the type and the factors in the environment [3, 4]. Food packaging sector, is, in fact, one of the main industries responsible for most plastic pollution around the world, especially due to single-use food packaging materials [5]. To address this issue, industries have developed biodegradable and recyclable bioplastics derived from natural sources as potential alternatives to convention petroleum-based plastics [6]. However, the ecotoxicological effects of these bioplastics/biopolymers on marine life remain largely unexplored when they breakdown into microplastics fragments [7, 8]. Hence, this study aimed to evaluate the physiological responses of gilthead seabream (Sparus aurata), a species of ecological and commercial significance, to chronic dietary exposure (28 days) to microplastics particles from different polymers, i.e. conventional petroleum-based polyethylene terephthalate (PET), and the bioplastics polybutylene succinate (PBS) and poly[propylene] fumarate (PPF). Fish (~100 g) were maintained in recirculating aquaculture systems (RAS) under optimal conditions (19ºC, > 7 mg/L O2) and were fed ~1% of their body weight with a control diet (CTR) or experimental diets, containing ~60 particles PET, PBS, PPF g^{-1} of feed, during 28 days. Then, fish underwent a 7-day depuration period during which they were only fed with the non-contaminated diet (CTR). Fish (n=12) were sampled at days 28 (post-exposure) and 35 (post-depuration), and liver, brain and gut were collected to analyse key physiological biomarkers indicative of oxidative stress (catalase activity [CAT], superoxide dismutase activity [SOD], lipid peroxidation [LPO] and glutathione S-transferases [GST]), aerobic metabolism (lactate dehydrogenase [LDH] and citrate synthase [CS]) and digestive activity (α-amylase, pepsin and trypsin). Data analysis is still ongoing. However, preliminary findings suggest that PPF may be less harmful compared to PET and PBS, indicating that this bio-based polymer could be a more sustainable and environmentally friendly alternative to conventional plastics, as well as other commercially available bioplastics (e.g. PBS).

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P64. Let's Pave the Way for Industrial Applications: Thermohpilic PHA Production from Fibers365 Lignocellulosic Hydrolysates

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Polyhydroxyalkanoates (PHA) are biodegradable and biocompatible polyesters synthesized by various microorganisms as intracellular carbon and energy storage materials. Their potential as sustainable alternatives to conventional plastics arises from their renewable production sources and environmental degradability. However, large-scale PHA production faces challenges, particularly the high costs of carbon substrates. To address this limitation, alternative, cost-effective substrates are being explored.

In this work, thermophilic bacteria, specifically wild-type and adapted/genetically engineered strains of *Caldimonas thermodepolymerans*, were employed for PHA production. These thermophiles offer cultivation advantages of minimized contamination risks and reduced sterilization requirements. We collaborated with Fibers365, a company specializing in the processing of lignocellulosic waste using a unique chemical-free SteamFiber technology. This method yields valuable side products rich in free carbohydrates, which present an attractive and sustainable carbon source for microbial growth.

Cultivation experiments were conducted using lignocellulosic hydrolysates provided by Fiber365, testing the PHA production capabilities of both wild-type and genetically modified *Caldimonas thermodepolymerans* strains. The study highlights the feasibility of utilizing industrial lignocellulosic residues for biopolymer production, promoting a circular bioeconomy while reducing production costs and environmental impact.

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P65. Acidogenic Fermentation as a Valorization Strategy of Lipid-Based Waste

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Annually, several tons of food frying waste are generated from household and restaurant kitchens and industrial processes potentiating environmental burden due to incorrect management or disposal. From these, spent frying oil (SFO) is particularly problematic due to its toxic compounds that pose significant challenges for wastewater treatment plants (WWTP) and for the wastewater resulting from the cleaning of its transport tanks (TTWW). Thus, this study aims to address these challenges through the biological valorization of SFO and TTWW through acidogenic fermentation (AF) to assess the production of short-chain organic acids (SCOAs) [1]. An alkaline hydrolysate of SFO (SFOH) was also conducted to allow its use as substrate in AF experiments, with 100% free fatty acids (FFA) and a 76% yield. SFO contained 97.9% of triacylglycerides, with esterified oleic (49.1%), linoleic (26.6%), and linolenic (14.5%) acids as the main constituents, and had a chemical oxygen demand (COD) of 1402.45 g COD/L. On the other hand, TTWW was composed by 64.8% of carbohydrates, with glucose from starch as the main constituent, and revealed a COD of 90.04 g COD/L, and a total nitrogen concentration of 1.990 g/L, where 0.229 g/L corresponded to nitrate concentration, and 7.58% and 9.34% of volatile and total solids, respectively. Four inocula, two aerobic (AES and AESER) and two anaerobic (ANS and ANSER) sludges, from municipal WWTPs, were used to determine the most suitable for AF with food to microorganism (F/M) ratios of 1:1, 1:2, and 2:1 g COD/g volatile suspended solids (VSS). The two latter ratios were only studied for AES and ANS. To assess the pH effect on SCOAs yield and variability, two pH values were used, 5.5 and 10.5, with ANS as inoculum at a F/M ratio of 1:2. All assays resulted in significant SCOAs production, with the ones using TTWW resulting in the highest acidification degree (AD) and, globally, the highest diversity in produced SCOAs, particularly of acetic, butyric and propionic acids, as well as faster production, when compared to assays with SFO and SFOH as substrate. In addition, the assays performed at pH 5.5 and 10.5 allowed to produce a higher variety of SCOAs, including, iso-butyric and valeric acids and the others previously referred, although in similar yields. The mixtures of SCOAs obtained will be further used as substrate for the production of polyhydroxyalkanoates (PHAs) by mixed microbial cultures.

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P66. Post-Consumer Pet Waste Upcycling into Bioplastics: Unlocking the Power of a Natural Microbiome

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The persistent accumulation of polyethylene terephthalate (PET) waste presents a significant environmental challenge due to its resistance to degradation and contribution to plastic pollution. Tackling this issue requires not only effective strategies for reducing PET disposal but also the development of sustainable, biobased, and biodegradable alternatives. Thus, upcycling approaches that combine chemical depolymerization with microbial bioconversion are gaining attention as promising strategies for PET waste valorisation [1]. Among these, polyhydroxyalkanoates (PHAs), biodegradable and biocompatible bioplastics produced from renewable resources, offer a promising solution [2].

This study explores an innovative upcycling strategy that converts chemically depolymerized post-consumer PET into PHAs using a mixed microbial culture (MMC). A microbiome sourced from Tagus River marshland sediments was enriched in a 2 L bioreactor through a 113-day cyclic feast-and-famine (F/f) regime with 4-day cycles, a hydraulic and solids retention time (HRT/SRT) of 40 days, and terephthalic acid (TPA) derived from PET waste depolymerization [3] ,as the sole carbon source. The prolonged HRT and SRT, coupled with a strategic cyclic 4-day F/f approach, allowed the system's stability over only one retention time, resulting in a consortium with a high PHA accumulation capacity using REX-TPA substrate, maintaining stability until the end of the assay. In this period (29 h) the consortium consumed up to 6.93 ± 0.03 g/L TPA and achieved PHA contents 71.32 ± 0.34 wt%. The microbial community attained at the end of selection was mainly composed by *Gammaproteobacteria*—particularly the *Halomonadaceae* and *Zoogloeaceae* families known for PHA production. The biopolymer produced was identified as poly(3-hydroxybutyrate) (PHB) with a molecular weight of 352 kDa, melting temperature of 174 °C, and maximum degradation temperature of 253 °C. PHB films demonstrated tensile strength of 18.8 ± 1.9 MPa, Young's modulus of 422.5 ± 142.3 MPa, coupled with good gas and moisture barrier properties. This work demonstrates, for the first time, the feasibility of transforming non-biodegradable PET into biodegradable PHAs using a microbial consortium and a TPA-rich feedstock, offering a promising approach for sustainable plastic upcycling and circular bioeconomy advancement.

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P67. Valorizing Sewage Sludge and Agri-Food Waste for Sustainable Biopolymer Production

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Agri-food residues are generally rich in carbon, proteins and other compounds, making them prone to valorization through biotechnological processes. Added value compounds, such as volatile fatty acids (VFAs), can be produced by anaerobic fermentation (AF) using agri-food residues [1]. On the other hand, wastewater treatment plants produce high volumes of waste sludge (WS), and its treatment and disposal represent between 11-60% of the total operating costs [2]. Its rich content in carbohydrates and proteins also makes WS a suitable feedstock for VFA production via AF. These waste-derived VFAs can be used for polyhydroxyalkanoates (PHAs) production, therefore increasing their sustainability and economic value. Co-fermenting agri-food residues and WS might be beneficial by increasing the C/N ratio, diluting toxic compounds, and increasing the presence of macro- and micronutrients essential for microbial activity. Therefore, optimizing PHA production from agri-food residues and WS can lead to better management of both wastes and potentially increase PHA production yields [1]. To determine the feasibility of AF of the above-mentioned wastes, batch assays were performed using fruit waste (kiwi, tomato, and orange) and nutrient-exhausted WS as substrates (S), and anaerobic sludge from a wastewater treatment plant as inoculum (I). AF assays were carried out at 30ºC, pH 5, and a S/I ratio of 1 gVS/gVS for 10 days. The individual AF performance of each fruit and WS was assessed, and AF of a 50:50 fruit-mixture:WS and a 50:50 fruit-mixture-without-orange:WS was also performed. For the individual fruit assays, a maximum VFA concentration between 99-113 Cmmol/L was attained after 7 days of operation, with all fruits showing a similar VFA profile after 10 days, mostly composed of acetic acid (AA, 37-43%) and propionic acid (PA, 32-40%). Tomato presented the highest acidification degree (71.7±0.4%), followed by kiwi (60.4±0.4%) and orange (61.7±0.1%). For the two mixtures tested, higher VFA concentration and acidification degree were obtained using the fruit-mixture:WS (81.7±0.7 Cmmol/L; 42.0±0.3%, respectively). As in the case of the individual fruits, a similar VFA composition was attained after 10 days (31-42% AA and 30-41% PA). The AF of WS resulted in the lowest VFA concentration (20.3±0.3 Cmmol/L) and acidification degree (10.5±0.1 %), possibly because WS is a more complex and less readily biodegradable substrate. Based on these preliminary results, it is expected that the PHA produced from the tested wastes will be composed of HB and HV monomers. This work was developed under the scope of the project ReLeaf (HORIZON-JU-CBE-2023-IA-02-101156998), being the main goal of our task to optimize the valorization of a mixture of nutrient-exhausted WS and agri-food waste through the production of PHA that will be used in the formulation of controlled-release bio-based fertilizers.

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P68. Production of Biodegradable Polymers from Fruit Waste for Agricultural Applications

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Agricultural waste materials and by-products can be valuable resources for diverse biotechnological applications. In a circular economy approach, the return, recycling, and reuse of resources within the same sector are of paramount importance. Polyhydroxyalkanoates (PHA) are bioplastics that can be produced from waste substrates and mixed microbial cultures (MMC) in a three-step process [1]. Here, rejected blueberries with no commercial potential are fermented into short-chain organic acids (SCOA) (1st step). This fermented stream will be utilised to select microbial mixed cultures with PHA accumulation capacity (2nd step). Further on, the fermented stream and the selected biomass are combined to maximize PHA production (3rd step). The resulting bioplastic will be utilised for agricultural applications, namely in the form of a mulch film. Substituting the prevalent fossil fuel-based plastic for PHA helps reduce fossil fuel dependency and plastic waste.

The fruit waste was submitted to an acidic pre-treatment optimised using design of experiments (DoE), where acid concentration, fruit concentration, time, and temperature were considered. The resulting feed was used in a continuous fermentative acidification reactor with a hydraulic retention time of 5 days to convert the available sugars into SCOA, the preferred substrate for producing polyhydroxyalkanoates by mixed microbial cultures. The acids profile varied when using different pH values (5.5, 6, 7, and 8), achieving the production of acids with 2 to 6 carbons, with concomitant hydrogen production for lower pH values. Acetic acid was dominant in all conditions, followed by propionic at higher pH values and butyric at lower values. Valeric and hexanoic acids were produced at pH 5.5 and 6.

The fermented stream was used for selecting a mixed microbial population with the capacity for PHA production. The reactor was operated in a sequencing batch mode with 8-hour cycles under feast-famine conditions, with uncoupled carbon and nitrogen addition (dump-feed), using a retention time of 2 days (SRT=HRT). The inoculum rapidly adapted to the fermentate, maintaining a feast-to-famine ratio between 1.2 and 1.8.

The batch polymer production will be carried out using the selected culture and the fermented stream in pulse feed mode. The characterization of the polymer properties and the identification of the microbial population present in the reactors will be analysed closer to the project end.

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P69. Tomato Pomace and Brewer´S Spent Grain Oil for PHA Production

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Amid mounting environmental concerns and the urgent need to reduce dependence on finite fossil resources, the circular and bio-based economy has garnered significant attention as a promising strategy to promote environmental sustainability and enhance resource efficiency [1]. Polyhydroxyalkanoates (PHA), have emerged as a pivotal focus in biotechnology due to their broad range of potential applications across diverse sectors. Agroindustrial residues, such as tomato pomace and brewer's spent grain (BSG), offer a sustainable and cost-effective alternative as feedstocks, providing abundant nutrients for microbial growth and polymer synthesis [2].

Cupriavidus necator was grown in BSG oil extracted with ethyl acetate and reached a maximum PHA content of 87.73 ± 2.04 wt.%, while in tomato pomace oil extracted with ethyl acetate the maximum PHA accumulation was of 79.31± 0.00 wt.%.

Tomato pomace oil extracted with ethyl acetate was found to be primarily composed of linoleic acid (11.62 wt%), oleic acid (5.94 wt%), and palmitic acid (4.91 wt%). Whereas BSG oil extracted with either hexane or ethyl acetate exhibited a predominance of linoleic acid (13.47–12.67 wt%), palmitic acid (8.55–7.17 wt%), and oleic acid (5.74–6.04 wt%).The solid fraction remaining after oil extraction will be further evaluated as feedstock. This approach aims to fully valorize agroindustrial residues by enabling the simultaneous utilization of both lipid and sugar fractions, thereby enhancing the overall efficiency and circularity of the biopolymer production process.

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P70. Optimized Biotechnological Strategies for Polyhydroxybutyrate Production from Agrifood Wastes Using *Cupriavidus necator* and Extracted Coffee Oil

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The growing scarcity of natural resources and the environmental impact of plastic waste have driven the search for sustainable materials. Biopolymers, such as polyhydroxyalkanoates (PHAs), provide advantages by reducing the consumption of fossil-based conventional plastic as well as CO_2 emissions. PHAs are bio-based polymers produced through microbial fermentation and stored as carbon and energy reserves within cells [1]. In this study, three different agrifood wastes, i.e. bread waste and by-products derived from the potatoes and artichokes processing chains, were tested as substrates for biopolymer accumulation with *Cupriavidus necator* DSM 428. These different food wastes were preliminary subjected to an hydrolysis step in order to release fermentable sugars (i.e., glucose or fructose) suitable for polymer storage. The efficiencies of enzymatic hydrolysis of bread waste and acid hydrolysis of potato and artichoke wastes were assessed on glucose (Y_G) and fructose yield (Y_F). Results on hydrolysis treatments' pointed out that Y_G accounted for 65 g/100g dry-bread and 32 g/100g dry-potatoes, while Y_F accounted for 33 g/100g dry-artichoke. Saccharides recovered from bread, potato, and artichoke food-wastes, containing initial concentrations of glucose and fructose at 25 g/L and 10 g/L, respectively; were used for the cultivation of *C. necator*. Under these conditions, the polyhydroxybutyrate (PHB) intracellular content was 24 (%, wt/wt), 32 (%, wt/wt), and 16 (%, wt/wt), respectively.

Furthermore, Extracted Oil Coffee (EOC) obtained via *Soxhlet* extraction from coffee waste was supplemented at a specific concentration (i.e., 1.5 %, *v/v*) in order to boost PHB storage capacity [2]. Hydrolysates derived from agri-food by-products, with an initial sugar concentration of 5 g/L, were utilised as substrates in combination with EOC. These strategies highlighted that the PHB intracellular content reached a value of 37 (%, wt/wt) from bread waste, 47 (%, wt/wt) from potato waste, and 45 (%, wt/wt) from artichoke waste.
Notably, the presence of EOC in the mixture of fermentable sugars obtained from organic wastes did not significantly affect the molecular weight (Mw). Indeed, Mw of polymer stored at 25 g/L of sugar derived from potato waste hydrolysis accounted for 104 KDa, while when EOC was supplemented, the Mw accounted for 226 KDa.

These preliminary results demonstrated the feasibility of employing various food wastes as substrates or biopolymer production through the fermentation process of *C. necator*.

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P71. Turning Ulva Rigida Seaweed to Biodegradable Plastics

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The environmental issues caused by petrochemical plastics, along with the growing awareness of society on these matters, highlight the urgent need to develop materials with similar properties that are both environmentally and economically viable[1]. Macroalgae can be the source for these materials.

Sulfated polysaccharides, such as ulvan from *Ulva* genus (7-29 % d.w.basis)[2] [3] emerge as alternatives for film production [4]. However, to ensure their practical application, namely in food packaging solutions, it is necessary to develop formulations to improve film properties such as low mechanical resistance and water-solubility[5].

On the other end the biopolyesters- polyhydroxyalkanoates (PHA), produced by bacteria are excellent candidates, offering physical and mechanical properties comparable to conventional plastics [6]. However, they are not economically viable, as the primary cost driver is the carbon sources required for bacterial growth[7].

In this study, *Ulva rigida* carbohydrates were upgraded to ulvan films and PHAs. Ulvan was extracted from the cell walls through hot water treatment. The ulvan was recovered via precipitation using ethanol and integrated into a film matrix along with sodium carboxymethyl cellulose (CMC), pectin or sodium alginate and glycerol as plasticizer. The films composed of ulvan, CMC, and glycerol demonstrated the best mechanical properties. However, ulvan alone did not exhibit the ability to form viable films.

The remaining seaweed carbohydrates were pre-treated by autohydrolysis in a pressure reactor and the recovered solids enzymatically treated to glucose-rich hydrolysates. These were incorporated into culture media as the sole carbon source in preliminary shake flask assays using *Halomonas halophila* as PHA-producer. These assays have shown this halophile to be tolerant towards potential inhibitors produced during the hydrolysis (HMF- hydroxymethyl furfural) and to have a high capacity to produce PHAs. Fed-batch cultures in 2L bioreactors using Ulva hydrolysates as feed are currently going-on.

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P72. A Comparative Analysis of Tomato Pomace Oil Extraction Processes Based on Mineral Composition, Thermal and Kinetic Properties, and Polyhydroxyalkanoates Production

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The tomato processing industry generates significant amounts of tomato pomace (peel and seeds), which is mostly unused, leading to waste of valuable resources and an environmental burden. Nevertheless, research into using waste materials as feedstocks for biosynthesis aims to reduce the consumption of non-renewable resources. In this study, tomato pomace oil (TPO) extracted by supercritical carbon dioxide (TPO-sCO_2) and by Soxhlet (TPO-HEX) with hexane was used as substrate to produce medium-chain polyhydroxyalkanoates (mcl-PHA) by Pseudomonas putida KT2440. The physical-chemical characteristics of TPO-HEX and TPO-sCO_2 were evaluated, showing that TPO had high thermal stability and a suitable physicochemical profile for PHA production. Both oils presented similar carbon content, 75.41 and 75.97 wt.%, respectively, and Na, K, Mg, Ca, Fe, and P were the main minerals. Thermogravimetric analysis (TGA) showed peak decomposition of 411 °C for TPO-sCO_2 and 415 °C for TPO-HEX. Additionally, kinetic modelling of TPO pyrolysis was performed to evaluate the activation energy (E_a). Additionally, kinetic modelling of TPO pyrolysis was performed to evaluate the activation energy (E_a) using Flynn-Wall-Ozawa (FWO), Kissinger-Akahira-Sunose (KAS), and Friedman (FR) models. The E_a of TPO-sCO_2 were 50.80, 112.09, and 134.92 kJ/mol, respectively by FWO), KAS and FR models. Moreover, E_a of TPO-HEX were 50.17, 110.50, and 149.38 kJ/mol, respectively. TPO-HEX presented higher mineral composition and better thermal properties when compared to TPO-sCO_2. Batch shake flask experiments at 10 g/L of TPO-sCO_2 and TPO-HEX yielded maximum cell dry weight of 0.51 and 0.58 g/L, respectively, after at 72-hr cultivation, with PHAs contents of 14.5 and 16.5 wt.%. Produced mcl-PHA consisted mainly of 3-hydroxyoctanoate (40%) and 3-hydroxydexanoate (44%). The findings showed TPO-HEX was a better carbon source for mcl-PHAs production by P. putida KT2440. In addition, the obtained results have shown that tomato pomace oil was a good economical, renewable feedstock for PHA production.

Keywords: Medium chain length polyhydroxyalkanoate; Tomato pomace oil; Pseudomonas putida KT2440; Waste valorization.

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P73. Improvise. Adapt. Overcome – Baltic Sea Bacteria and Their Polymer Degrading Ability

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As plastics production and consumption today continue to contribute to petrochemical plastic pollution, greener packaging alternatives such as biopolymers and biobased compostable materials are becoming a major focus of research. However, these materials can also pose environmental problems in areas lacking the appropriate degradation conditions. Despite global recycling efforts, plastics frequently end up in landfills and in natural environments such as seas and oceans. Several studies have reported significant microplastic pollution in the Baltic Sea; therefore, the diversity of microorganisms present in these waters may indicate the existence of enzymatic systems capable of degrading polymeric packaging materials.

In this study, several tests were conducted to evaluate the degradation capabilities of bacteria isolated from the Baltic Sea on different types and forms of biobased polymers. The formation of halozones was observed and confirmed using agar plate assays with emulsified polymers – poly(3-hydroxybutyrate), poly(butylene succinate) and poly(lactic acid). Selected polymers in granule or film form (prepared via hot pressing or solvent casting methods) were exposed to the bacterial consortium, and material property changes were monitored. Major structural changes were observed using scanning electron microscopy (SEM), while physicochemical property changes were analyzed using differential scanning calorimetry (DSC) and gel permeation chromatography (GPC-SEC/MALS). Additionally, biochemical activity was assessed by measuring biological oxygen demand (BOD) and the optical density (OD) of microbial cultures. Microbial consortium was separated and analysed on MALDI-TOF and sequenced.

The results indicate that certain bacteria exhibit potential to for the biodegradation of various polymers. Further research will examine these microorganisms in more detail in future studies.

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P74. Elaboration and Characterization of Xanthan Gum/Hemicellulose-Based Biomaterial Films

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This work is part of the characterization of films based on Xanthan, hemicelluloses, and sorbitol. The main objective is to demonstrate from a characterization study the performance and viscosity of the films obtained for food and pharmaceutical application.

The variation of viscosity was studied as a function of shear rate. The viscosity, although it decreases with shear stress, increases with decreasing temperature and this for all the 5 solutions prepared. The same trends were observed for the rheological behavior of solutions at 25°C compared to solutions at 40°C.

FTIR spectra of the five samples were recorded to compare the changes in their chemical structure and the observed characteristic IR wavenumber. The reaction of Xanthan with hemicellulose took place during the polymerization process.

DSC thermograms of the five samples of films were analyzed. The heat flux curves showed endothermic peaks of 10.7, 10.8, 10.2, and 12 °C, respectively. A single endothermic peak was also found for all films, located between 10.5 and 12 °C. This indicates that the crystal structure did not change during the production of each film. The films obtained have good properties and better functional quality.

Key words: DSC, films, FTIR, hemicellulose, sorbitol, Xanthan.

P75. Recovery of PLA Building Blocks from Single-Use PLA Waste with Commercial Savinase

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Poly(lactic acid) (PLA) is the most widely produced biopolymer globally, primarily applied in single-use plastics due to increasing demand for sustainable packaging solutions. It biodegrades under industrial composting (~60 °C, high humidity), as the only currently available end-of-life option, limited by loss of biomass-derived monomers. Alternatively, enzymatic depolymerisation of PLA waste streams offers a route to PLA waste management at lowered temperatures and perpetual recovery of PLA building blocks, omitting the need for continual biomass input. Advancing closed-loop recycling technologies is critical for the circular economy of PLA. Therefore, this work investigates the repurposing of commercial alkaline protease Savinase® 12T preparation, already produced on a large scale for the detergent industry, for the degradation of consumer-grade PLA. Reaction conditions were optimized and depolymerisation of a wide scope of consumer-grade PLA items of crystallinities (*Xc*) from 10 – 42% was assessed. Savinase degraded the PLA single-use cup at 42 °C at a rate of up to 166 $mg \cdot day^{-1} \cdot mg^{-1}_{enzyme}$ [1]. Savinase was found to be highly specific towards PLA among other tested bioplastics (PCL, PHB, PHO), with Asn155 and Ser125 identified by molecular modelling as key residues involved in the specific PLA recognition. It exhibits both endo- and exo-type PLA scission with lactic acid as the main PLA degradation product throughout depolymerization.

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P76. Potential of Proteins Derived from Invasive Crayfish Shell to Be Utilized for Biopolymer Film Production

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The spiny-cheek crayfish (*Faxonius limosus*), introduced to Europe for aquaculture, has now spread to 22 countries, where it has severely impacted native crayfish due to its high reproductive capacity, disease resistance, and role as a carrier of the crayfish plague. In Serbia, first recorded in 2002 near Apatin, it has since colonized major rivers and is expected to outcompete indigenous species in the coming decades. One possible approach to this situation is targeted harvesting aimed at reducing the population of the invasive crayfish. Driving force for such harvesting could arise from the broad potential for exploitation, with crayfish meat used as food and the shell serving as a source of valuable compounds. Crayfish shells are most commonly used as a source of chitin and chitosan, with deproteination being common step in their extraction. Precisely this process of removing associated proteins from chitin enables the isolation of a high-value protein fraction, which in this study was tested for its film-forming potential [1-3].

Two protein isolates were used in this study. In the first extraction method, crayfish meat was separated without boiling, leaving a higher amount of residual muscle tissue in the shell; in the second method, the meat was separated after brief boiling, which resulted in less residual meat and some protein loss during cooking. Films were prepared by solution casting from both protein isolates at two concentrations (5% and 7.5%), with the addition of 30% glycerol as a plasticizer.

The films were analyzed for their thickness, mechanical properties (tensile strength and elongation at break), and sensitivity to moisture, which included measurements of moisture content, contact angle, swelling percentage, and solubility. Increasing the protein concentration from 5% to 7.5% resulted in thicker and mechanically stronger films; in the cooked shell series this came at the expense of elasticity, while in the uncooked shell series, 7.5% produced a well-balanced film with both good strength and flexibility.

The films of the uncooked shell series, containing a higher protein content, exhibited lower water solubility—an important feature for moisture-resistant packaging—along with greater swelling (indicating a more hydrophilic nature without disintegration), increased thickness, and generally better mechanical stability; in particular, the 7.5 % film demonstrated the best balance of strength and flexibility, making uncooked shell series -based films more suitable for food packaging applications that require both water resistance and mechanical integrity.

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P77. Different Fractions of Proteins Derived from Invasive Crayfish Shell, Composition and Antioxidant Activity

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Crayfish shells contain a variety of proteins that are crucial for their structure, function, and color. The main proteins in the shell are cuticular proteins, chitin-binding proteins, mineral-associated proteins, and crustacyanin. These proteins work together to form a chitin-protein matrix, strengthen the exoskeleton, contribute to calcification, and provide coloration and protection [1-3].

In this study, the possibility of utilizing the shell of the invasive crayfish Faxonius Lymosus, caught in the part of the Danube River flowing through Serbia, was investigated. After removing the edible part of the crayfish meat, the shell was subjected to a chemical demineralization process followed by deproteinization using hydroxide and elevated temperature. From the extract, proteins were fractionally precipitated at pH 3.5; 4.5 and 5.1. The amino acid composition of these isolates was examined and their antioxidant activity was tested.

The total amino acid content (protein indicator) was 59.90 g/100 g for the isolate at pH 5.1, then 61.02 g/100 g for the isolate at pH 4.5 and 72.23 g/100 g for the isolate at pH 3.5. High content of glutamic and aspartic acid in all isolates can be related to chitin-binding proteins - because these acids often form negatively charged domains that bind to chitin or calcium. Increased content of aromatic amino acids (Phe, Tyr): Phe (5.13%), Tyr (3.63%) might have a role in pigment-binding proteins or proteins with a protective (antioxidant) role. Crustacyanin has a pH close to 4.5, but fragments and similar proteins can precipitate at pH 3,5.

Relatively high content of basic amino acids (Lys, Arg), Lys (3.95%), Arg (4.31%), His (2.69%) might be related to antimicrobial peptides that are present in the hemolymph and may remain in the shell after deproteinization.

The antioxidant activity of the isolates in the DPPH• scavenging ability test ranged from 68.9-71.7% at an isolate concentration of 2.5 g/ml, to 77.7-89.1% at a concentration of 7.5 g/ml. Similarly, in the ABTS test, the radical inhibition values ranged from 17.9-52.9% for the lower and 85.4-94.9% for the upper concentration.

The obtained results indicate a significant antioxidant activity of the protein fractions isolated from the shell of the crayfish in a concentration dependant manner. The pH 3.5 fraction is particularly noteworthy, showing the highest activity in both tests (ABTS and DPPH), which correlates with its highest content of total amino acids (TAA), especially aromatic (tyrosine, phenylalanine) and acidic amino acids (aspartate and glutamate), which are known to contribute to the elimination of free radicals.

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P78. Lactate-Driven Selection and Enrichment of Mixed Cultures for PHB Production: Insight on Organic Loading Rate

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The study examines the possibility of producing polyhydroxybutyrate (PHB) from mixed microbial cultures (MMCs) by 1) using a lactate solution as a substrate instead of conventional volatile fatty acids (VFAs) solutions, and 2) evaluating the effect of different organic loading rates (OLRs) during the selection and enrichment phase of PHB-accumulating biomass.

The selection phase was conducted in parallel using two identical automated sequencing batch reactors, operated over a 50-day period and adopting an uncoupled C/N strategy. Each reactor was run on 12-hour cycles, with hydraulic and solids retention times (HRT and SRT) set to 1 day. Two OLRs were compared: 40 and 130 mmolC/L/day. Biomass samples collected 4.5 hours into the cycle were subjected to accumulation tests to assess PHB content after 24 hours. The accumulation test was carried out under pH-controlled conditions (pH 8) using a 0.5 M lactic acid solution.

Preliminary results indicate that the two OLRs did not have significant effects on PHB accumulation either during the selection phase (OLR40: 30 ± 7%; OLR130: 29.8 ± 0.7%) or during the accumulation phase (OLR40: 47.5%; OLR130: 53.3%). However, notable differences were observed in terms of active biomass concentration (X_a) in the selection phase. This parameter is crucial, since given the same PHB biomass storage performance, a higher value allows higher volumetric productivities (P_{PHB}). Indeed, the volumetric observed productivity in the selection phase is 23 ± 5 mgPHB/L/h in the case of OLR40 and 61 ± 21 mgPHB/L/h in the case of OLR130.

These findings support the feasibility of using lactic acid, obtainable quickly and directly via dark fermentation of lactose-containing agro-industrial residues such as cheese primary and secondary whey, as a cost-effective and sustainable substrate for PHB production. Moreover, the importance of the OLR in the selection is highlighted as a key parameter in increasing PHB productivity using MMCs, as also reported in other studies conducted using different carbon source [1-3].

Further studies are underway to determine the threshold value of OLR that allows for effective selection of PHB using lactate solutions as the carbon source, in view of the optimization of the overall process.

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P79. Development of Bio-Based Polymeric Membranes Incorporating Deep Eutectic Systems (Des) for Skin Dressings

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The skin acts as a vital physiological barrier against physical damage, but it remains highly susceptible to acute and chronic wounds. Effective and rapid healing is essential to prevent infections and complications. Traditional wound dressings, such as gauze, provide limited therapeutic support and often rely on non-biodegradable polymers and toxic solvents, raising environmental and biomedical concerns.

In recent years, the development of bio-based polymeric membranes has emerged as a sustainable and promising approach to support skin regeneration, maintain moisture balance, and improve biocompatibility. In this work, membranes based on chitosan (CS), cellulose acetate (CA), and polylactic acid (PLA) were prepared via solvent evaporation, incorporating Deep Eutectic Solvents (DES) composed of biocompatible and naturally derived components such as lactic acid, glucose, choline chloride, glycerol, and citric acid. These solvents are biodegradable, inexpensive, tunable, and environmentally friendly, making them attractive candidates for drug delivery systems.

Six DES formulations were synthesized, including lactic acid:glucose:water (5:1:3), choline chloride:lactic acid (1:2 and 1:10), choline chloride:acetic acid (1:2), choline chloride:glycerol (1:2), and citric acid:L-proline:water (1:2:6). Their properties were characterized by Polarized Optical Microscopy (POM), Karl Fischer Coulometry (water content), and Fourier Transform Infrared Spectroscopy (FTIR). After solubility tests of the biopolymers in each formulation, the most suitable combinations were selected for membrane preparation.

The resulting membranes were evaluated in terms of morphology (SEM), composition (FTIR-ATR), hydrophilicity (swelling and contact angle), mechanical performance (puncture resistance), thermal stability, drug release profile (UV–VIS, using diclofenac as the model API), and gas permeability (O_2 and CO_2).

Preliminary results show that DES incorporation modulates membrane properties. Chitosan-based membranes demonstrated high puncture resistance compared to some commercial dressings, along with enhanced hydrophilicity and drug release rates, though they exhibited limited aqueous stability and lower gas permeability. By contrast, PLA and CA membranes showed more controlled swelling, higheraqueous stability, and mechanical properties suitable for biomedical use.

Overall, these findings highlight the potential of DES-based biopolymeric membranes as sustainable wound dressing systems capable of controlled transdermal drug delivery and effective skin regeneration support.

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P80. Liquid Crystal Biopolymer Networks Inspired by Nature for Sustainable Water Harvesting

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While scientific research in this field has surged in recent years, cellulose-based materials has existed for millennia [1]. Nature has long harnessed cellulose-based structures to respond to external stimuli, particularly water. A remarkable example is the awns of the *Erodium* fruit [2], which exhibit intricate coil and uncoil motions driven by humidity changes. When detached from the fruit, these straight awns naturally from a right-handed helix due to their intrinsic curvature. Upon expose to moisture, they unwind, enabling the seed to embed itself into the soil. In this study, we identified structural features resembling liquid crystalline elastomers at the core of these dynamic movements. These motions arise from anisotropic cellulose-based layers that contract differently in response to humidity. Similarly, cellulose-based ribbons, derived from liquid crystalline solutions, can also exhibit moisture responsiveness [3].

Here, we investigate stimuli-responsive, cellulose-based liquid crystalline films produced using shear-casting techniques with a multi-layer design. Their preparation, characterization, and potential flexible water collecting systems are explored. Nature-inspired materials hold immense promise for developing novel soft, responsive systems. These advancements could pave the way for intelligent water and energy-harvesting devices.

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P81. Innovative Thermoplastic Starch Composites Reinforced with Agrifood By-Products for Advanced Barrier and Antioxidant Materials

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Fossil-based polymers remain dominant in the plastics sector, yet their environmental impact has intensified the demand for sustainable alternatives. Starch-based bioplastics offer biodegradability but are hindered by limited mechanical strength and barrier performance. The valorisation of agricultural byproducts offers a promising strategy to overcome these drawbacks while fostering a circular economy. In this work, thermoplastic starch was recovered from potato washing slurries and reinforced with powdered onion peels (OP) and garlic peels (GP) to produce thermoplastic composites with different loaded percentages of OP and GP at different loadings (5-20%) by hot-pressing. The influence of OP and GP incorporation was assessed in terms of chromatic features, internal structure, melt flow index (MFI), and thermal stability. Mechanical performance (Young's modulus, tensile strength, elongation at break), water barrier properties (water vapor transmission rate and wettability), and antioxidant capacity were also evaluated. The incorporation of OP and GP imparted orange and brownish tones, respectively, with colour intensity proportional to filler content. Increasing filler load reduced the MFI by up to 400% without compromising thermal stability. The TPS composites displayed up to 150% higher Young's modulus, accompanied by decreases in tensile strength (50%) and elongation at break (66%). Wettability increased by 30% while the water vapor transmission rate decreased by 75%. Furthermore, OP and GP conferred antioxidant activity, achieving complete $ABTS^{+\bullet}$ inhibition within 15 min. Overall, these results demonstrate the successful upcycling of potato starch-rich slurries, onion peels, and garlic peels into thermoplastic starch composites with enhanced barrier, mechanical, and antioxidant properties. The approach underscores the potential of agrifood by-products in designing functional bio-based materials tailored for sustainable applications.

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P82. Cold Sintered Bioglass/Polymer Composites for Bone Tissue Engineering

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Major health concerns in an aging population include bone diseases like osteoporosis or degenerative disc disease. In some cases, replacements of the broken- or worn-down bones may be necessary. This study investigated the cold sintering of bioglass and bioglass/polymer composites as potential bone replacement materials for non-load bearing and compressive applications.

Bioglass and bioglass/polymer pellets were fabricated using the cold sintering method at ≤100°C at ≤250MPa. These were further characterized by scanning electron microscopy (SEM). Bacterial fermentation using different feedstock options was used to produce various natural biocompatible polymers using 'shaken flask' experiments in which multiple variations were applied to obtain data for polymer synthesis and further upscaling.

Cold sintered dense pellets of bioglass and bioglass/polymer composites were fabricated and characterized using SEM. 75:25 wt% composition of Bioglass:P(3HB) showed the best surface properties with well-formed pores. Bacterial production of the polymer was performed using 'shaken flask' experiments and analysed by gas chromatography/mass spectroscopy (GS-MS). The homopolymers P(3HB), P(3HV), P(3HO) and copolymer P(3HB-co-3HV) were produced using Paracoccus dentrificans and a bacterium from genus Pseudomonas.

The successful use of cold sintering to create dense bioglass pellets at low temperature permitted the integration of bioactive polymers in the sintering process, resulting in the fabrication of dense composites. SEM images revealed a two phase (bioglass/polymer) dense microstructure. The SEM analysis also revealed highly sought after pore-like structures needed for cells in order to attach and be able to grow within the centre of the composite. Findings within this study agreed with literature that pore formation is needed for cell to growth. Also, bacterial synthesis of polyhydroxyalkanoates (PHAs) including P(3HB), P(3HV), P(3HO) and P(3HB-co-3HV) was demonstrated and was consistent with the scientific literature.

The ability to cold sinter dense bioglass and bioglass/P(3HB) composite pellets was demonstrated. High volume of Bioglass to polymer ratio (75:25) wt% was achieved using the cold sintering method, it allowed incorporation of the polymer into the Bioglass matrix without its degradation due to the relatively low temperature used to densify both materials together. Previously, the composites made were using polymers as matrix and Bioglass as filler. This study has successfully demonstrated that the reverse combination of Bioglass as matrix and polymer as filler is possible without polymer degradation. Bacterial experiments produced biocompatible

homopolymers P(3HB), P(3HV), P(3HO) and copolymers P(3HB-co-3HV), with potential for bone tissue engineering. We propose that cold- sintered bioglass/biopolymers composites may offer a novel solution for bone disease treatment.

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P83. Kinetic Model for the Production of Polyhydroxyalkanoates with Mixed Microbial Cultures in a Continuous-Flow Process

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Polyhydroxyalkanoates (PHAs) are a family of fully biodegradable and bio-based polyesters produced by microorganisms, representing with their properties a promising alternative to conventional oil-based plastics. Industrial PHA production processes involve the use of pure microbial cultures, which requires sterile conditions and high cost of maintenance. As an alternative, the use of mixed microbial cultures (MMCs) is being largely investigated, resulting in a typical multi-stage process. The main stages usually consist in the microbial selection of PHA-storing microorganisms, through the alternance of the excess and lack of external carbon sources, followed by the accumulation stage, to maximize the intracellular PHA content. This kind of process is usually performed in a Sequencing Batch Reactor (SBR) but recently, a continuous-flow process is being investigated at laboratory scale, with the main objective of decreasing the overall MMC-PHA production costs and facilitating the scale-up. [1]

This continuous-flow process entails three reactors (i.e., a continuously fed tubular feast reactor, a CSTR famine reactor, and a CSTR accumulation reactor) with two different feeding sources for the feast and the accumulation reactors and a recirculation flow stream connecting the feast and the famine reactors. Therefore, it presents a complex dependency of the design variables. In this work, a mathematical model was developed to describe the kinetics of the transformations in the reactors and assist the design and optimisation of this process. First, a kinetic model was adapted and extended from previous work [2], to focus on the description of the production of the poly-3-hydroxybutyrrate-co-3-hydroxyvalerate (PHBV) copolymer, from a mixture of acetic and propionic acid as a substrate. Then, the model parameters were calibrated based on experimental data from two batch accumulation tests characterized by spike-feeding and validated with a third independent test. Finally, the model for the whole process was built and implemented in Matlab R2024 allowing to investigate the effect of key operation parameters (e.g., Organic Load Rate and Recirculation Factor), the feed composition, and their optimal combinations for the performances of the continuous process.

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P84. Bioinformatic Identification of New Latex Clearing Proteins.

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Rubber is a major environmental concern due to its large volume and poor biodegradability. Tyres contain poly(cis-1,4-isoprene) derived from *Hevea brasiliensis*. Certain microorganisms can degrade polyisoprene using latex clearing protein (Lcp), a b-type cytochrome-dependent oxidase [1]. Various Lcps have been reported from *Gordonia polyisoprenivorans* and *Gordonia westfalica* [2], *Nocardia farcinica* [3], *Rhodococcus rhodochrous* [4], *Nocardia nova* [5], and *Streptomyces* sp. K30 [1]. Among these, LcpK30 is the best studied, and its crystal structure has been resolved [6]. LcpK30 cleaves poly(cis-1,4-isoprene) into oligomers with keto and aldehyde end groups [1], which can be detected by Schiff reagent in liquid [7] and solid media [8].

To expand the diversity of Lcps, we screened the NCBI database for candidates and clustered them. Several variants with 25–62% sequence identity to LcpK30 were selected for further study. After cloning, expressing, and purifying, we characterized the proteins spectroscopically. Four candidates showed a Soret band at 412 nm, confirming the presence of the heme cofactor [1]. Activity was evaluated on latex-agarose stained with Schiff reagent, and three proteins, from *Streptomyces* sp., *Nocardia* sp., and *Nocardioides* sp., exhibited latex-clearing activity. Finally, we corroborated by LC-MS analysis the formation of isoprene oligomers. Based on this evidence, we conclude that we identified *bona fide* Lcps, which we are further investigating for their potential application in sustainable plastic degradation.

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