11th ESBP

European Symposium on Biopolymers

BOOK OF ABSTRACTS

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Dear Participants,

Welcome to the 11th European Symposium on Biopolymers, ESBP2023!

The ESBP symposium series, inaugurated in the year 2000 in Münster, has been a beacon of knowledge and collaboration in the field of biopolymers. It's been quite a journey since the last gathering in Straubing in 2019. The global pandemic disrupted our regular biennial rhythm, but today, we stand united in the vibrant city of Brno, Czech Republic.

ESBP2023 finds its roots in the Faculty of Chemistry at Brno University of Technology and is proudly co-organised with the Brno Observatory and Planetarium. This unique collaboration provides the perfect setting for a medium-sized conference like ESBP.

At ESBP2023, we aim to facilitate connections between academics and industry experts who share a deep interest in the production, characterization, processing, and application of bio-based polymers and materials. With over 80 oral presentations and more than 40 posters, we bring you the latest breakthroughs and trends in biopolymer research. Moreover, this conference will be your canvas for sparking discussions, sharing innovative ideas, and forging new connections with experts, not just from Europe but across continents.

As you delve into the scientific program, don't forget to savour the social events we've planned. On Wednesday evening, join us for an unforgettable conference dinner at the Mendel Museum. Additionally, our terrace party on Thursday, complete with a poster session, Czech beer tasting, and a movie projection, promises a night to remember.

In another exciting development, we are thrilled to announce that New Biotechnology will be publishing a special issue dedicated to ESBP2023.

Our heartfelt gratitude goes out to our sponsors, whose unwavering support has been instrumental in ensuring the success of this conference: Wyatt Technology, Merck, Contipro, Anamet, Biotech, Delong Instruments, Hartmann, Methrom, MDPI Polymers, and Photon Systems Instruments.

In closing, we wish you an inspiring and fruitful time here in Brno. May ESBP2023 not only enrich your understanding of biopolymers but also gift you with unforgettable experiences and enduring connections.

Best wishes,

Stanislav Obruča

On behalf of the ESBP2023 Organizing Committee

PL1: **Production of Polyhydroxyalkanoates from Vegetable and Animal Oils**

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Waste oils from plants and animals are attractive feedstock to produce microbial polyhydroxyalkanoates (PHA) which is a biodegradable thermoplastic polyester [1-3]. The well-known *Cupriavidus necator* H16 can be cultivated to high cell densities on waste oils. To produce PHA copolymers such as poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (PHBHx) it is necessary to modify some genes [4]. A broader substrate specificity PHA synthase will efficiently polymerize the smaller 3HB monomer and the bulkier 3HHx monomer [5]. In addition, the supply of 3HHx monomer must be improved which can be achieved using a suitable enoyl-CoA hydratase gene (*phaJ*) [6]. Efficient *C. necator* strains have been developed that can convert waste oils into PHBHx having a wide range of 3HHx molar fractions that show various thermal and mechanical properties to suit certain applications. Gene modifications were also done to aid the downstream recovery and purification process of PHA by controlling the PHA granule size and the number in the bacterial cells. The biodegradation of the resulting PHA products in soil, rivers and marine environment were confirmed to take place from as early as 5 weeks in the case of melt-processed PHBHx sheets and within 6-8 months for injection-molded samples. The microbes involved in the biodegradation process of PHBHx were also isolated, characterized, and shown to be able to hydrolyze PHBHx microplastics completely. Since Southeast Asia has emerged as a hot spot for plastic pollution, PHA will be an attractive option to mitigate this emerging problem. To minimize production and transportation costs, it is necessary to produce PHA in the region using locally available feedstock.

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PL2: **Bioengineering of Advanced Protein-polymer Materials**

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Bacteria are prime cell factories that can produce intracellular inclusions and a variety of extracellular polymers [1]. We are rewiring bacteria to harness their capacity to synthesize and assemble functional materials to order. Synthetic biology and bioengineering approaches are applied to engineer bacterial cell factories that assemble biological building blocks into hierarchically structured materials [2]. A platform technology was developed and offers a vast design space for the scalable biotechnological production of functional nano-/micro-materials efficiently incorporating protein-based functions such as e.g. binding domains, fluorescent proteins, antigens and enzymes. We have expanded the materials design space by developing *in vitro* site-specific protein ligation methods and by developing hydrogel-based composite materials. Overall, biological synthesis and assembly pathways were engineered to design and produce innovative materials for a range of medical and industrial uses. Development and applications of tailor-made functional materials will be presented.

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PL3: **Block Polysaccharides**

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The conjugation at chain termini of two different polysaccharides provides diblock polysaccharides, a new class of precisely engineered polysaccharides. This architecture provides on one hand new solution and stimuli-responsive self-assembly properties, while retaining key properties such as biodegradability on the other.

The first part of the presentation will focus on the preparation of blocks through dioxyamine linkers [1-3]. The second part will focus on diblocks containing Ca-reactive oligoguluronates [4, 5] (derived from alginates) and their Ca-induced self-assembly studied by static and dynamic light scattering, SANS and SAXS.

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IL1: **Circularity of Polyhydroxyalkanoates**

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Polyhydroxyalkanoate (PHA) comprises an exciting class of biopolymers with large a versatility in their biosynthesis, material properties, and field of application. In the past few years, it became obvious that the natural separation into short-chain-length (C3-C5 monomers) and medium-chain- length (C6-C14) PHAs is becoming a weak separation. Especially with the introduction of chimeric and thus novel PHA polymerases, new fascinating biopolymers with novel properties could be produced, e.g., blocky PHAs [1].

The utilization of waste streams was the first approach to introduce the aspect of circularity. Recently, flue gases, such as syngas and CO_2 have gained particular interest as carbon sources for PHA production because of their abundance and their negative effect as a greenhouse gas [2, 3]. Besides novel production strains, new types of bioreactors are also needed to establish highly performing bioprocesses that allow a scale up of production and thus to achieve a good life cycle assessment.

Interestingly, the end-of-life aspects of PHAs have not that much addressed because PHAs are known to biodegrade in diverse environments.

In our study, we found that poly(3-hydroxybutyrate) is the first PHA that can fulfill the full requirements of recycling and even lead to a new, valuable organic solvent.

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IL2: **An Update on the Current Status of Industrial PHA Production**

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Despite the infinite number of different types of PHA (homo- and heteropolyesters, random and blocky structured copolyesters, short-(*scl*-), medium-(*mcl*-), or long chain length (*lcl*)-PHA), only some of them have been thoroughly studied, and only a handful reliably meet the desired property requirements of established plastics, thus allowing their industrial-scale production and use [1].

Presently, over 25 companies and start-ups and more than 30 brand owners are involved in PHA production and use for various applications. Remarkably, the PHA-product spectrum of these companies is restricted to only five types of PHA: poly(3-hydroxybutyrate) (P(3HB)), poly(4-hydroxybutyrate) (P4HB), poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (P(3HB-*co*-3HV)), poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) (P(3HB-*co*-4HB)), and poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) (P(3HB-*co*-3HHx)), plus some activities in *mcl*-PHA production at minor quantity [2]. Moreover, until recently, industrial activities were exclusively based on the use of a selected number of Gram-negative bacterial PHA production strains; only by now, the first industrial attempts towards industrial-scale production of PHA by haloarchaea have started.

However, by varying the composition of PHA biopolyesters, their properties mimic those of the seven top selling fossil plastics, produced on an annual 230 million tons scale [3,4]. Therefore, capacity expansions of 1.5 million tons over the next five years have been announced for PHA. We summarize setbacks and success stories of the past decades of the industrial production of PHA, which is just picking up speed.

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IL3: **Generalized Approach for Optimal Polyhydroxyalkanoate Extraction Conditions from Dried Biomass Using Non-chlorinated Solvents**

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Technology feasibility of pure, open, and mixed microbial culture polyhydroxyalkanoate (PHA) production methods has been demonstrated to reach a PHA-rich biomass using many kinds of substrates derived from a range of (waste) organic matter in wastewater and sludge residues. However, the downstream PHA recovery process remains a bottleneck creating risk for investments in upscaled industrial facilities. Supply chain redundancy is essential to manage risks for any industry and for obtaining scales of economy. In this context, the present work focuses on a PHA-supply chain that consists of many PHA-rich biomass production sites, supplying a strategically central purification refinery. The challenge is how a centralized recovery facility can robustly handle an incoming batchwise supply of a wide range of possible PHA-rich biomass qualities. Generalized principles for solvent extraction methods applicable to a diversity PHA-rich biomass and polymer types are required. An optimal recipe of solvent extraction, for all possible kinds of biomass, and PHA, is not possible, but an efficient generic approach to define optimal conditions for each batch is.

Optimal extraction conditions may vary for any given solvent and PHA-rich biomass combination. Suboptimal conditions may lead to reduced productivity on the one side, while detracting from the product quality control on the other side. Thermal stability of the PHA in the biomass is critical to mitigate undue molecular weight loss. Polymer recovery is a mass transport problem for which the biomass particle size distribution and morphology, as well as the PHA molecular weight also play roles. Solvent extraction furthermore requires removing co-extracted non-polymer biomass from the final product. In a production scenario it would be of benefit to be able to efficiently tune extraction conditions on a batch-to-batch basis. The question is what kinds of measurements can efficiently give information to quickly tune the extraction conditions and avoid much undue practical testing?

In the present work measurement methods with differential scanning calorimetry, thermal gravimetric analysis and solution rheology have been applied for understanding and effectively defining optimal extraction conditions of PHAs. Practical evaluations were based on extraction experiments performed at laboratory (10 mL) and pilot scales (10 L), using PHA-poor solvents like dimethyl carbonate and 2-butanol. Ideas of a generic approach were underpinned using two distinct PHA-rich biomass types. One derived from municipal activated sludge and the other was a halophilic pure culture. The purpose of this presentation will be to relate outcomes and insights on how a few simple measurements on a PHA-rich biomass and on the recovered polymer can remove much of the guess work with trial and error, and be generically applied to define optimal extraction conditions.

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IL4: **Benefits of Low Voltage Transmission Electron Microscopy in Biopolymer Research**

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Micrograph quality is assessed based on two crucial parameters: resolution and image contrast. Low voltage transmission electron microscopy distinguishes itself from conventional TEMs by offering remarkably high image contrast due to the low accelerating voltage of the primary electron beam, even with reduced staining [1].

The above-mentioned unique capability enables the visualisation of intricate structures with unprecedented clarity, as demonstrated, among others, in the case study of bacterial cells containing polyhydroxyalkanoate (PHA) granules imaging on LVEM 25E. PHA granules serve as significant intracellular storage materials in various microorganisms, playing a vital role in applications such as biodegradable plastics and biofuel production. By visualising these granules with exceptional contrast, the LVEM 25E allows a deeper understanding of their distribution, morphology, and dynamics within bacterial cells.

The LVEM 25E (Delong Instruments, Brno, Czech Republic) is an All-in-One electron microscope, providing a unique combination of multiple imaging modes and data acquisition capabilities. Its primary mode of operation is transmission electron microscopy (TEM) at an accelerating energy of 25 keV, providing high-resolution imaging. Additionally, the microscope can be equipped with scanning transmission electron microscopy (STEM) mode, advantageous for thicker samples, as well as a scanning electron microscopy (SEM) mode with backscattered electron detection, electron diffraction mode for crystallography and an energy-dispersive X-ray spectroscopy (EDS) mode for elemental analysis. Its exceptional image contrast, particularly beneficial for samples predominantly composed of light elements, such as carbon, makes it a valuable tool in biochemistry and imaging of biological sections.

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IL5: **Characterization of Molecular Structure of Biopolymers with Separation Techniques and Advanced Detectors**

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Molecular structure of polymers in general includes molar mass distribution, long-chain branching and polymer chain conformation. Detailed knowledge of the molecular structure and ability to differentiate samples prepared by different procedures and/or from different natural resources are crucial for enhanced applications of many biopolymers.

The molar mass distribution is predominantly determined by size exclusion chromatography (SEC, also known under its original name gel permeation chromatography). Although there is no principal difference between SEC of synthetic polymers and biopolymers, the analysis of biopolymers may be challenging due to often limited solubility, lack of standards of well-defined structure, and strong tendency of some biomacromolecules to interact with SEC column packing.

Multi-angle light scattering (MALS) detector not only eliminates notoriously uncertain column calibration and yields absolute molar mass distribution, but also provides additional information about biopolymers that cannot be obtained by conventional SEC with column calibration [1]. Especially the ability to characterize polymer chain branching and conformation is essential for understanding the behavior and properties of various biopolymers. However, the application of MALS detector is limited in the case of fluorescent biopolymers, namely lignin [2] and humic acids. Fluorescence, which is often more intensive than the intensity of scattered light, can be reduced by using a MALS photometer with infrared laser and filters transparent only for the incident light wavelength. The hyphenated SEC-MALS technique can be further completed with an online viscometer that extends tremendously ability of the method to characterize branching and study the polymer chain conformation – especially in the case of natural polyelectrolytes [3].

Commonly used SEC becomes limited in the analysis of biopolymers containing ultra-high molar mass fractions (e.g., some polysaccharides and natural rubber), as these fractions may be shear degraded in SEC columns. Consequently, the molar mass distribution from SEC -MALS analysis is falsely described in the region of very high molar masses. Anchoring of branched macromolecules is another limitation of SEC that leads to incorrect description of the lower molar mass region of the molar mass distribution. Asymmetric flow field flow fractionation (AF4) is an alternative analytical separation technique which separates macromolecules by flow in a channel filled in solely by mobile phase [4]. The separation is performed under markedly lower pressure compared to SEC which strongly reduces the possibility of degradation by shear forces. In addition, the lack of stationary phase completely eliminates anchoring of branched macromolecules and thus AF4-MALS represents the most suitable technique for the characterization of branched ultra-high molar mass biopolymers.

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IL6: **Designing Biopolymers with Advanced Functional Properties**

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The emergence of new technologies, such as synthetic biology, enables the creation of next-generation-advanced materials presenting smart functional properties. Among these group, biohybrid materials have recently grown in attention due to the enormous range of possibilities presented by a material that has the functionalities of living cells or simply enzymes supported on a scaffold. Bacterial biopolymers such as bacterial cellulose (BC) and polyhydroxyalkanotes (PHAs) have stood out in this field as their properties can be engineered by microbial biotechnology tools through sustainable bioprocesses combined with materials science. Moreover, the fabrication of new formulations based on BC and PHA polymers with complementary characteristics provides them with highly diverse properties, conferring them non-native features [1]. BC, an extracellular biopolymer produced by bacteria of the genus *Komagataeibacter* spp., shows excellent mechanical performance with a high capacity of retaining water in its nanofibrillar structure that can be controlled by bioprocess engineering. PHAs, intracellular biopolyesters synthetized by bacteria of many genus, highlight by their hydrophobic character which triggers this material has excellent barrier properties. Designing cell factories for the production of novel PHAs via smart metabolic engineering is key to obtain *à la carte* materials with tailored monomer composition and thus, customized mechanical and thermal properties. In this work, we used the model mcl-PHA producing bacterium, *Pseudomonas putida* KT2440 as a chassis, to assemble heterologous PHA biosynthetic modules to implement an orthogonal PHA switch in a chassis specifically constructed to override endogenous multilevel regulation of PHA synthesis in the native strain. The final goal is to design customized materials with compartments of different functionalities involving the complementary use of BC and PHA and carrying active cargos (i.e. enzymes and/or microorganisms). Several strategies will be presented and discussed such as gaining control of bacterial cellulose colonization by PHA-producing microorganisms to develop bioplasticized ultrathin films [2] and the use of biopolymers for enzybiotics delivery [3].

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IL7: **Levan-type Fructans as Functional Biopolymers in Biomedical Field**

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Levan polysaccharide has been part of our healthy diet for centuries as a microbial product of *Bacillus subtilis* in the traditional Japanese food Natto or that of lactic acid bacteria in almost all functional fermentation products. Although its presence was known as a bioactive polymer since 1881, its health promoting effects have been recognized only recently and its use in biomedical sector is still at its infancy. As a fructan homopolymer itself, levan can be considered as an understudied biopolymer when compared with the well-known inulin-type fructans. On the other hand, there is an enormous scientific and commercial interest in levan polysaccharide as its distinguishing features become uncovered by an escalating number of scientific reports on its potential applications in various fields [1].

The main bottlenecks in preventing levan to find its solid place in the Biotechnology market are its low commercial availability and high cost of production. However, the last decade witnessed a huge increase in the diversity of levan producers and that in turn also opened the ways for alternative routes for cost effective production routes. Hence levan is expected to be the polymer of choice in many high-value applications in the biomedical sector [2] and foreseeing this trend, the main goal of this study is to present a comprehensive overview on the state of the art of biomedical applications of levan polysaccharide. For this, after a brief outline of its physicochemical and biological features, various levan based systems including its films, drug delivery systems, scaffolds and hydrogels will be introduced with special emphasis on their uses in biomedical field.

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IL8: **Modification of Thermoplastic Starch to Achieve a Broad Range of Properties in Mixtures with Biopolymers**

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Thermoplastic starch (TPS) is a valuable additive to a number of polymers. It is formed by plasticization of native starches, almost entirely with glycerol, although number of other plasticizers are available. The primary aim of the addition of TPS to bioplastics is usually a decrease of the price of the final product while maintaining the biodegradability of the plastics blend.

However, modification of TPS, considering its physical and ultimate properties, is in many cases rather easy and straight forward process, and a number of different procedures are used to adjusting the final properties of the TPS.

Certainly, the final effect of the addition of TPS consists not only in the price decrease but the mixture properties and possible applications depend significantly on the ultimate properties of both the matrix biopolymers and also of the TPS.

In the lecture several options for TPS modification are briefly outlined and described, starting with the selection of native starch considering the origin of the starch. The effect of various plasticizers or even their mixtures are shown using the example of glycerol and urea. Some more advanced cases of TPS modifications will be presented, especially the effect of moisture uptake on mechanical and other physical properties of the TPS and the effect of long term storing resulting in so called retrogradation. Finally the addition of reinforcing fillers are discussed showing the effect of nanofiller precursors, namely montmorillonite, while chemical modification consisting in starch crosslinking with citric acid or dialdehydestarch is described.

Few examples are shown of mixing TPS as the most simple glycerol plasticized additive in the blends with biodegradable polybutylene adipate terphthalate (PBAT) and the comparison of the effect of the same blends using the TPS modified by various procedures demonstrates the extent to which modification of TPS may lead to interesting and in some cases advanced biomaterials with substantial amount of TPS or even based on TPS.

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IL9: **Biodegradation of (Bio)Polymers**

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All polymers that are naturally produced on our planet can be (bio)degraded and mineralized completely to carbon dioxide and water by at least one microbial species. Otherwise, non-(bio)degradable polymers would have accumulated to high polymer mountains during the history of earth. Contrary, many chemosynthetic polymers, in particular those with long chains of non-functionalized carbon atoms, can´t be (bio)degraded and mineralized by any known microbe or enzymatic action. Chemosynthetic, non-degradable polymers are in use for less than hundred years; nevertheless, they are constantly accumulating in the environment and only a partial physical/mechanical disintegration is observed leading to the formation of microplastics. Microplastic particles are constantly spread all over the world, have entered the food chain and are already regularly found in the human body. The long-term consequences of the presence of microplastic in our body are largely unknown. Therefore, the replacement of non-degradable polymers by degradable ones is necessary to prevent further negative long-time effects both on individual organisms and on the earth as a whole.

In my talk I will highlight what we can learn from natural polymers and what properties of a given biopolymer makes it biodegradable and what properties reduce or and inhibit biodegradation. Furthermore, I will summarize physical and biological factors outside the polymer molecule that have also a great impact on the fate of a given polymer in the environment.

IL10: **Engineering Strategies of Microbial Exopolysaccharide Producers towards Novel Product Properties and Increased Product Titers**

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Microbial polysaccharides have been used in industry for decades due to their thickening behavior and rheological properties. With the growing demand for biobased polymers and additives in various industrial sectors, the need for high-performance polymers also increases. For these polymers, very specific rheological properties or functionalities are of high importance. For example, polymers with a specific and adjustable linear viscoelastic range are needed for improved 3D printing applications. At the same time, these biobased polymers must be produced at high titers, high quality, and at a reasonable price to compete with their chemical counterparts. Up to now, only rare reports on genetic engineering for modified chemical structures or improved product titers exist. However, the area of synthetic biology has opened new possibilities for intensified genetic engineering, also for non-model organisms, such as most microbial exopolysaccharide producers. This talk will deal with the different possibilities for the production of modified microbial exopolysaccharides as well as their recombinant production in optimized chassis organisms. In addition, the possibilities of strain engineering towards higher product titers and product purity will be discussed. Finally, these strategies can lead to the opening of new applications of microbial exopolysaccharides in the near future.

L1: **Biotechnological Production of Polyhydroxyalkanoates from Agri-Food Residues: Sustainable Approaches**

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Polyhydroxyalkanoates (PHAs) are polyesters accumulated by specific microorganisms as cytoplasmic carbon storage granules; PHAs have plastic-like properties, are completely biodegradable and biocompatible, and thus are considered among the most promising alternatives to fossil plastics. Unfortunately, the replacement of conventional plastics with PHAs is limited by their high price, mainly due to the expensive raw materials used as a carbon source for microbial growth. To reduce costs, the production of PHAs should be based on available and cheap feedstocks such as food and crop residues. Cheese whey, slaughterhouse, and agro-food wastes are obtainable in large amounts as co-products of the dairy and meat industry and agriculture. Moreover, they can pose a threat to the environment if not disposed of correctly. Unfortunately, in nature, it is not common to find microbial strains capable of both efficiently metabolizing the complex carbon sources contained in residual biomasses and accumulating PHAs at high yields. For example, *Cupriavidus necator* DSM 545, one of the most proficient PHAs accumulators, cannot grow on such substrates. Among the possible strategies to bypass this problem, in the last 15 years, the Microbiology group of DAFNAE pursued the following approaches:

A) *C. necator* DSM 545 was engineered to acquire the ability to grow on complex substrates. With this purpose:
   i) lacZY genes from Escherichia coli or lipH and lipC genes from Pseudomonas stutzeri BT3 were cloned into C. necator DSM 545 and *Delftia acidovorans*. The resulting recombinant strains were able to grow and accumulate PHAs from wastes containing lactose or lipids.
   ii) specific amylolytic genes, such as the glucodextranase G1d from *Arthrobacter globiformis* I42 and the α-amylase amyZ from *Zunongwangia profunda* SM-A87, were co-expressed into *C. necator* DSM 545. The selected recombinant *C. necator* DSM 545 showed high hydrolytic activity on starch and, for the first time, demonstrated the one-step processing of starchy broken rice and sweet potato waste into PHAs;
B) the acidogenesis phase of the anaerobic digestion was exploited as an efficient hydrolysis step to convert starchy substrates into volatile fatty acids (VFAs), to be then used as a carbon source by *C. necator* DSM 545 to both grow and store PHAs;
C) the gaseous effluent, containing a mixture of H_2 and CO_2, and the liquid stream, rich in volatile fatty acids (VFAs), originating from an acetogenic reactor fed with agro-food wastes, were efficiently used by *C. necator* DSM 545 to accumulate PHAs under autotrophic and heterotrophic conditions, respectively.

Although future research is necessary to improve yields, the obtained outcomes pave the way to future microbiological and biotechnological solutions to process organic waste into PHAs at large scale.

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L2: **Conversion of a Xylose-rich Stream from Biomass-biorefining into Polyhydroxyalkanoate (PHA) by *Schlegelella thermodepolymerans***

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The transition to a biobased economy necessitates utilizing renewable resources as a sustainable alternative to traditional fossil fuels. Bioconversion is a way to produce many green chemicals from renewables, e.g., biopolymers like PHAs. However, fermentation and bioconversion processes mostly rely on expensive~~,~~ and highly refined pure substrates. The utilization of crude fractions from biorefineries, especially herbaceous lignocellulosic feedstocks, could significantly reduce costs. This presentation shows the microbial production of PHA from such a crude stream by a wild-type thermophilic bacterium *Schlegelella thermodepolymerans* [1]. Specifically, it uses crude xylose-rich fractions derived from a newly developed biorefinery process for grassy biomasses (the ALACEN process). This new stepwise mild flow-through biorefinery approach for grassy lignocellulosic biomass allows the production of various fractions: a fraction containing esterified aromatics, a monomeric xylose-rich stream, a glucose fraction, and a native-like lignin residue [2]. The crude xylose-rich fraction was free of fermentation-inhibiting compounds meaning that the bacterium *S.thermodepolymerans* could effectively use it for the production of one type of PHA, polyhydroxybutyrate. Almost 90% of the xylose in the refined wheat straw fraction was metabolized with simultaneous production of PHA, matching 90% of the PHA production per gram of sugars, comparable to PHA yields from commercially available xylose. In addition to xylose, *S. thermodepolymerans* converted oligosaccharides with a xylose backbone (xylans) into fermentable xylose, and subsequently utilized the xylose as a source for PHA production. Since the xylose-rich hydrolysates from the ALACEN process also contain some oligomeric xylose and minor hemicellulose-derived sugars, optimal valorization of the C5-fractions derived from the refinery process can be obtained using *S. thermodepolymerans*. This opens the way for further exploration of PHA production from C5-fractions out of a variety of herbaceous lignocellulosic biomasses using the ALACEN process combined with *S. thermodepolymerans*. Overall, the innovative utilization of renewable resources in fermentation technology, as shown herein, makes a solid contribution to the transition to a biobased economy.

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L3: **PHA Production from Lignocellulosic Enzymatic Hydrolysate for Textile Applications**

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The biodegradable and biocompatible biopolymers polyhydroxyalkanoates (PHAs) are being used to develop fibers and coatings that can be applied in different fields, namely in the textile industry. Despite the interest and potential of this biomaterial, the fibers generated still have poor thermal and mechanical properties comparing with the petro-based fibers[1, 2]. Therefore, it is important to continue investigating and developing new strategies to improve PHA properties in order to achieve new biodegradable fibers with an adequate performance.

This work aims to produce PHAs with suitable properties for their use as fibers in textile products, using sugars obtained by hydrolysis of cellulosic materials as feedstock for bacterial cultivation. The eucalyptus bark collected in the pulp mill of Cacia (Aveiro, Portugal) from The Navigator Company was used as residual feedstock, following a circular bioeconomy approach. The process began with a pretreatment stage, where the eucalyptus bark underwent a proprietary non-catalyzed steam explosion technology. This pretreatment utilized high-pressure steam without the addition of acids. The solid fraction recovered from the pretreatment stage was then subjected to enzymatic hydrolysis. Eucalyptus bark hydrolysate was obtained after 48 h enzymatic saccharification at an initial 175 g/L solids concentration (oven-dried basis), by applying Cellic® CTec3 at a dosage of 3% ($m/m_{oven\text{-}dried\ solids}$). This resulted in a sugar solution containing fermentable monosaccharides, such as glucose and xylose, derived from the main polysaccharide components (cellulose and xylan) of eucalyptus bark.

Different bacteria were able to grow and produce PHA with different composition using the enzymatic hydrolysate as sole carbon source. *P. citronellolis* NRRL B-2504. *P resinovorans* NRRL B-2649 and *P. oleovorans* NRRL B-14683 produced medium chain length PHA (mcl-PHA) with different monomeric composition. On the other hand, *B. thailandensis* E264 and *P. oleovorans* NRRL B-14682 produced the short chain length (scl-PHA) homopolymer polyhydroxybutyrate (PHB). These different types of biopolymers possess distinct physical and chemical properties, which were evaluated with the intention of their application as additives, fibers, or coatings in the textile industry.

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L4: **Sustainability Evaluation of Poly(3-hydroxybutyrate) Bioprocess Development Based on Brewer's Spent Grain Utilization**

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The increasing use and cost of petroleum-derived plastics have provided the impetus for the research of new alternatives to replace them with bio-based polymers. Poly(3-hydroxybutyrate) (PHB) has the potential to replace conventional plastics. One of the reasons that impedes the industrial production of PHB is the high production cost and the conventional solvents used for PHB purification [1]. This study presents the sustainability of PHB production derived in a brewer's spent grain (BSG) employing green solvents for PHB downstream separation purification (DSP). PHB production conducted by *Paraburkholderia sacchari* in fermentable sugars derived from BSG hydrolysate as fermentation feedstock and PHB samples treated via enzymatic bacterial cell disruption followed by PHB purification with green solvents. PHB purification led to varying recovery efficiency from the harvest cells and purity. The efficiency of PHB extraction was initially investigated using three solvents (e.g. 1,3-dioxolane, dimethyl carbonate, anisole) in enzymatically treated and untreated biomass (on either dry or wet basis) for 2 h and 6 h at 60°C and 80°C. The PHB was efficiently extracted in both cases (treated and untreated biomass), where the recovery yield was increased with increasing reaction duration. Although all solvents can effectively extract PHB from biomass, 1,3-dioxolane showed the best performance. The highest recovery yield (92.3%) was obtained with 1,3-dioxolane treatment at 80°C for 6 h, while the purity of the polymer was 97%. It was also shown that when enzymatic cell lysis was employed prior to solvent extraction, the recovery yield and purity of PHB was increased as compared to untreated biomass.

The sustainably of the process was evaluated in terms of economic and environmental pillars by carrying out techno-economic (TEA) and life cycle assessments (LCA). The whole process was designed by using the appropriate design software of UniSim (Honeywell). LCA is performed using the GaBi software and the LCA methodology CML 2001 (Jan. 2016). The system boundaries for the analysis characterized as "cradle to gate" and the functional unit is 1 kg of produced PHB. The optimal plant capacity for the PHB with the proposed bioprocess was estimated at 50 kt/year, while the fixed capital investment for this capacity was estimated as $87 million. Different cases have been considered for estimating the cost of manufacture (COM) and minimum selling price (MSP) of PHB production using different green solvents for DSP. The COM per kg PHB varied in the range of $2.6-3.8/kg and the MSP was $3.3-4.9/kg. The environmental metrics of Global Warming Potential and Abiotic Depletion Potential of PHB production from BSG by the proposed bioprocess were estimated at 1.47 kg CO2-eq per kg PHB and 45 MJ per kg PHB, respectively.

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L5: **From Non-food Agricultural by By-products to Sustainable Feedstocks for Biopolymers Production**

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The search for sustainable feedstocks for the future of chemicals and plastics production is a top priority for the industry. Most major industries, consumer brands and European regulatory agencies have set significant targets and pursuing novel approaches to reduce carbon footprint and sustainability across the value chain. Therefore, there is an urgency to transition to renewable and build up supplies of sustainable feedstocks. However, the main challenge is locating and securing alternative feedstocks. Fibers365 circular concept involves breakthrough technologies which transform agricultural by-products into products such as biogas, fibres, fertilizers, and valuable feedstocks for biomolecules and biopolymers biosynthesis. Its patented steam explosion process can process a wide variety of non-food annual plants, without any chemical addition ensuring not only the sustainability of the process but also closing the CO2 generation and soil fertility loop within 365 days. Until now the liquid stream produced during steam explosion of agricultural fibers is being used to produce Biogas, but the optimal potential of this side stream has not been yet fully explored. By submitting this stream to some downstream processes, Fibers365 has recently developed a second generation(2G) biofeedstock, Liquid365, containing up to 35% weight of total free sugars with less than 2% of solids content that allowed the successful production of lipids by yeast fermentation. Therefore, we set out to investigate whether (Liquid365) could allow the production of Bioplastics such as polyhydroxyalcanoates (PHAs). Liquid365 presented a carbon to nitrogen ratio around of 40 being glucose, xylose, acetic and formic acid the main carbon sources detected. Above all, small levels of bacterial growth inhibitors were found in Liquid365 when the growth of two different bacterial species known to produce polyhydroxybutyric acid (PHB) were tested in the presence of Liquid365. We have determined that both C6 and C5 carbohydrates as well as the organic acids present in Liquid365 were used as carbon sources. Moreover, depending on the agricultural by product source from where the feedstock was derived, these 2G biofeedstocks allowed cell proliferation and PHB accumulation, in up to 50% cdw. In conclusion, we have demonstrated that Liquid365 is a suitable feedstock for the bacterial production of PHB and could represent a way of further valorising side streams obtained after steam explosion pretreatment of agricultural wastes-derived renewable carbon sources.

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L6: **Polyhydroxyalkanoates Production from Fruit Waste at Pilot Scale: Impact of Sludge Retention Time and Organic Loading Rate on the Dynamics of Culture Growth and Polymer Storage**

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Polyhydroxyalkanoates (PHAs) production using non-aseptic mixed microbial cultures (MMCs) enables the use of waste/surplus feedstocks, contributing simultaneously to the implementation of a circular economy approach and to reduce the process operational costs associated to the traditional pure cultures' PHA production. The MMC process usually comprises three steps: the acidogenic fermentation, the enrichment of a MMC in PHA-storers and finally the PHA production [1].

The selection of an efficient MMC that displays a significant PHA accumulation potential, and a high growth rate is considered a key factor for the MMC PHA production feasibility [1], [2]. This study was focused on clarifying the impact of different sludge retention times (SRTs) (2 and 4 d) and organic loading rates (OLRs) (from 2.6 to 14.5 $gCOD.L^{-1}.d^{-1}$) on the dynamics of growth versus PHA storage of a PHA-producing culture selected under uncoupled carbon and nitrogen availabilities. These two parameters are known to have a great impact on the process productivity and were poorly studied for processes where the uncoupled feeding strategy was applied. Fruit waste was used as substrate since it is globally generated in large volumes, causing several environmental and economic problems. This study was conducted at pilot scale to ensure the results have a direct applicability in an industrial setting.

Results showed that, regardless of the OLR imposed, cultures selected at lower SRT grew faster and more efficiently using stored PHA. However, they had inferior specific PHA storage rates and total PHA accumulation capacity, resulting in lower global PHA productivity. Additionally, the polymer storage yield was independent of the SRT, and was directly linked with the abundance of putative PHA-storers in the MMC. The high PHA productivity (4.6 ± 0.3 $g.L^{-1}.d^{-1}$) obtained for the culture selected at 4 d of SRT was 80% above of that obtained for the one selected at 2 d SRT, underlining the importance of achieving a good balance between culture growth and accumulation capacity to increase the viability of the PHA-producing process from wastes.

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L7: **Polyhydroxyalkanoates Production from Tomato Waste**

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Nowadays, there has been a rising awareness for the use of environmental friendly products, such as polyhydroxyalkanoates (PHA). The production of PHA using the Mixed Microbial Cultures (MMC) presents low production costs, since it allows to use open systems and cheap feedstocks (industrial and agricultural wastes) [1]. Tomato is one of the most popular vegetables and the waste generated during its processing comprises a significant portion of pomace, including seeds, peels and fibrous components [2]. The chemical composition indicates its potential suitability for acidogenic fermentation and subsequent production of PHA. In this study the feasibility of use the tomato waste as a feedstock for PHA production was assessed using a three-stage installation composed by a 5L stirring tank reactor (STR) (acidogenic fermentation), a 2L sequencing batch reactor (SBR) (culture selection) and 1L fed-batch reactor (PHA production).

Throughout the STR operation the fermented stream produced was rich in volatile fatty acids (VFA), with a content higher than 82%. The increase of organic loading rate (OLR) from 2 to 7.5 gTS/(L.d) lead to an increase of VFA concentration but a decrease on VFA yield. The decrease of OLR to 5 gTS/(L.d) improved the reactor performance observing an increase of VFA concentration, yield and productivity. The different OLR applied to the STR resulted in different compositions of the fermented and productivities, and consequently affected the hydroxybutyrate:hydroxyvalerate:hydroxyhexanoate (HB:HV:HHx) precursors ratio. Throughout the operation, acetic and butyric acids were the main compounds presented in the fermented, accounting an average of 62% of the total VFA, while the hexanoic acid range between 5 to 27 %Cmol, depending on the OLR applied. During the SBR operation, a feast and famine (F/f) ratio of 0.05 ± 0.01 h/h was achieved, showing a good culture selection. The selected MMC demonstrated a good PHA accumulation capacity, with an average PHA yield of 0.73 ± 0.11 Cmol/Cmol. The PHA production was carried out using two different fermented stream (HB:HV:HHx of 59:9:32 and 77:7:16, molar basis), however the PHA composition was similar (HB:HV:HHx of 82:11:7, molar basis) regardless of the content of HHx precursors in the fermented stream, being the hexanoic acid driven towards HB rather than HHx. A PHA content higher than 60 %wt and an average yield of 0.75 ± 0.10 Cmol/Cmol were achieved, showing a good capacity of PHA storage by the selected culture.

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L8: **A Scale-up Story: PHBV from Lab-scale to Full-scale and from the Upstream Down to Product Development**

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After many years of much academic research, the technology to produce mixed microbial culture (MMC) poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) is primed to be scaled-up and commercialized. Paques Biomaterials, carved-out from the company Paques in 2021, has been dedicated to this mission. The scale-up story goes from grams PHBV at lab scale to kilograms PHBV at the demonstration scale plant currently in operation. It comprises upstream PHBV production, PHBV recovery, and product development, and outlines the evolving scenario towards industrial full-scale production with a maturing technology.

PHBV technology was taken out of a laboratory environment around 2012. Multiple successful pilot projects were executed with a variety of wastewaters at various sites. However, the output (0.1-0.5 kg/day) was too small to develop polymer end-use applications together with potential bioplastics stakeholders. For this reason, a demonstration plant to produce PHBV-rich biomass (25 kg/day) was realized and put into operation in 2022. This effort was accomplished by the formation of a public-private PHA2USE development consortium that consists of five Dutch water authorities as well as companies including Paques Biomaterials. The demonstration plant has been running for a year, and the presentation will discuss the achievements of PHA-rich biomass production.

The upstream PHA-rich biomass production is only a part of the story. The next critical part naturally revolves around polymer recovery and product development. PHBV recovery processes have more recently received wider spread attention in the research literature. Scale-up efforts have been limited and this limitation is a bottleneck for the commercialization of MMC PHBV. However, since 2022, the PHA2USE consortium has been undertaking scaled up solvent extraction and purification from the PHA-rich biomass produced. Successful results have made a milestone in the first kilograms of waste-derived PHBV being trialed by compounders and converters interested in market applications. The presentation will tell the story of examples of product application routes that are being investigated. In this area, there is a strong focus on products where biodegradability makes sense and where suitable polymer property specifications are readily achieved.

Finally, the presentation will explain and emphasize how the journey in scale-up efforts is essential to the development of product applications routes, and therefore towards bringing MMC PHBV to the market.

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L9: **Hot or Cold? Light or Dark? Expremophiles – New Superheroes of Microbial Biotechnology**

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Polyhydroxyalkanoates (PHA) are polyesters accumulated by numerous prokaryotes. These materials are generally considered to be biodegradable, biocompatible and bio-based alternatives to synthetic petrochemical polymers. Nevertheless, due to the high production cost of PHA, these environmentally friendly polymers can hardly compete with synthetic polymers. Extremophiles as hosts for PHA biosynthesis provide numerous benefits, most of all, processes based on extremophiles are naturally robust against contamination by ubiquitous microflora; therefore, they can be operated in semi-sterile or non-sterile conditions even under continuous or semi-continuous modus operandi which substantially reduces the cost of the process. Therefore; we focused on screening and isolation of extremophilic bacteria capable of PHA biosynthesis. At first, we identified that numerous halophiles are very potent PHA producers from various low-cost substrates such as lignocellulose hydrolysates or waste frying oils. Nevertheless; apart from many benefits, the utilization of halophiles brings also many drawbacks related to the high concentration of salt in cultivation media. Therefore; we focused on searching and screening thermophiles capable of PHA biosynthesis. It should be stated that, as compared to halophiles, PHA biosynthesis is far more unexplored in thermophiles since PHA production capacity was described only for a few thermophiles. Therefore, we systematically screened selected thermophilic strains available in public collections of microorganisms to identify novel potent PHA producers. Some of them such as *Caldimonas thermodepolymerans*, *Tepidimonas taiwanensis* or *Rubrobacter* sp. seem to be strong candidates for PHA production from various waste resources. Moreover; we also developed a unique isolation protocol enabling us to isolate PHA accumulating thermophiles from various microbial consortia such as compost or activated sludge. Therefore; we identified several very promising thermophiles which can be considered auspicious candidates for industrial production of PHA. For instance, our isolates belonging to the genus *Aneurinibacillus* reveal a unique capability to incorporate various monomer units into the PHA chain. Furthermore, since PHA are crucial with respect to the stress robustness of bacterial cells, we have been exploring the importance of PHA for phototrophic microorganisms such as cyanobacteria or purple bacteria which are potent candidates for the conversion of CO_2 into PHA and or other high-value materials.

L10: **For a Sustainable and European Value Chain of PHA-based Materials for High Volume Consumer Products (NENU2PHAR project)**

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Bio-based and biodegradable plastics clearly offer a valuable alternative to non-biodegradable, petroleum-based plastics for selected applications. Nevertheless, high costs and low yields associated with their production, together with their dependence on sugar or oilseed feedstock, are significant obstacles which remains to be tackled [1]. Polyhydroxyalkanoates (PHA) are bacterial polyesters, which constitute a very promising family of polymers both from the point of view of their properties of use and that of their environmental impact [2]. The PHA biosynthesis is particularly relevant since it avoids the traditional chemical processes, by using some of the most energy-efficient industrial biotechnology processes. The NENU2PHAR project will develop an original route of PHA production from sustainable and renewable resources using eco-friendly and ethical processes: micro-algae biomass using CO2 as carbon source and selection of specific bacteria strains able to produce PHA [3]. In this context, 8 PHA-based products with their respective end-of-life scenarios will be developed and benchmarked to their fossil-based counterparts.

The goal of NENU2PHAR is to set up a new European value chain of PHA-based bio-plastic products from a sustainable non-food biosource with an adapted end of life regarding to the use. To reach such ambitious target the NENU2PHAR project will have 6 main objectives (see the figure below). The presentation will summarize how NENU2PHAR consortium develop a PHA stream integrated in a circular economy concept, from the production to the biodegradability or recyclability of plastic products to new compounds. Special focus on micro-algae pilot production used as substrate for PHA fermentation, eco-friendly PHA extraction routes, examples of biosynthesis from this new feedstock and innovative PHA based formulations will be given. The PHA so-obtained may also be valorised for other uses, in particular for uses in cosmetic formulation as dispersible or soluble form. Previous works carried out at IRDL has demonstrated their interest as exfoliating microbeads with rapid biodegradability in the marine environment [2].

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L11: **Transition to Anaerobiosis Enhances Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) Synthesis in Purple Bacteria**

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Bioplastics produced by microorganisms are environmentally friendly materials in terms of end-of-life management. Among these innovative materials, polyhydroxyalkanoates (PHAs) are a prominent example. These polyesters primarily serve as storage for carbon and energy, while also enhance stress resistance. Additionally, their synthesis can function as an electron sink for regenerating oxidized cofactors. One particularly interesting PHA copolymer is poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), which possesses advantageous biotechnological properties due to its lower stiffness and fragility compared to the homopolymer poly(3-hydroxybutyrate) (P3HB). In this study, we investigated the potential of *R. rubrum* as a producer of this copolymer by exploiting its metabolic versatility under different aeration conditions.

We conducted shaken flask experiments with different aeration regimes, using fructose as the carbon source. Under these conditions, PHBV production was initiated, resulting in an accumulation of polymer reaching 29 ± 2% of the cell dry weight (CDW), with 75 ± 1% mol of 3-hydroxyvalerate (3HV). Propionate and acetate were also secreted. The synthesis of PHBV was exclusively catalyzed by the PHA synthase PhaC2. Interestingly, the transcription of *cbbM*, which codes for RuBisCO, the key enzyme of the Calvin-Benson-Bassham cycle, showed similar levels in both aerobic and microaerobic/anaerobic cultures. The highest PHBV yield (81% CDW with 86% mol 3HV) was achieved when transitioning the cells from aerobic to anaerobic conditions and controlling the CO_2 concentration by introducing bicarbonate to the culture. Under these conditions, the cells exhibited characteristics of resting cells, as polymer accumulation took precedence over residual biomass formation. However, in the absence of bicarbonate, the cells were unable to adapt to the anaerobic environment within the studied timeframe.

We observed that a two-phase growth approach (aerobic-anaerobic) significantly improved previous reports on PHBV production in purple non-sulfur bacteria, maximizing polymer accumulation while minimizing the production of other biomass components. The presence of CO_2 played a crucial role in this process, indicating the involvement of the Calvin-Benson-Bassham cycle in the adaptation to changes in oxygen availability. These findings highlight *R. rubrum* as a promising producer of high-3HV-content PHBV copolymer from fructose.

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L12: **Prospecting Natural Habitats for High-yielding Polyhydroxyalkanoates Producing Microbial Consortia**

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A microbiome consists of a natural mixed microbial community adapted to a natural or artificial environmental niche, in which microorganisms cooperate with each other by intraspecies and interspecies interactions. Microbiomes comprise different microorganisms, a characteristic that confers them robustness, metabolic diversity and the ability to perform tasks not possible to a single organism.

In this work, the evolution of a natural microbiome comprising sediments collected from a wetland ecosystem (Corroios, Portugal) towards a mixed microbial consortium (MMC) enriched in polyhydroxyalkanoates (PHAs) storing microorganisms was studied. The microbiome was subjected to feast and famine (F/f) cycles, as selective pressure, using acetate (5 g/L) as model substrate, in a 2 L bioreactor operated with a hydraulic retention time (HRT) of 30 days, during 90 days (corresponding to a total of 3 HRTs or 30 F/f cycles). The initial total suspended solids (TSS) were 127.900±1.03 g/L, decreasing over time to approximately 55.000 g/L after 2 HRTs (60 days) of operation, remaining stable thereafter. On the other hand, the volatile suspended solids (VSS) remained stable throughout the assay (13.000-16.000 g/L). In each 3-days cycle, acetate was depleted within the initial 12 hours (determining the duration of the feast phase), thus corresponding to an F/f ratio of 0.200. During the feast phase, cell growth and PHA accumulation occurred, followed by PHA consumption during the famine phase. Fluorescent in Situ Hybridization (FISH) showed that initially the microbiome displayed a high diversity of microorganism evolving during the assay towards enrichment in Betaproteobacteria, Gammaproteobacteria and Alphaproteobacteria, which became dominant, and are known to include PHA-storing organisms.

After completing 1, 2 and 3 HRTs (at 30, 60 and 90 days of cultivation, respectively), PHA accumulation assays were performed by inoculating a 1 L bioreactor (800 mL working volume) with 80 mL of broth collected from the selection bioreactor at the end of the famine phase, and supplying consecutive pulses of acetate. There was a clear evolution of the microbiome's PHA accumulation capacity, with the biomass collected from the selection bioreactor at days 60 and 90 (2 HRTs) reaching a PHA content of 50wt%, on a VSS basis, compared to 25±0.14 wt% for the biomass collected at day 30 (1 HRT). The microbiome's polymer yield also increased from 0.012 to 0.021 $g_{PHA}/g_{acetate}$ for the same samples.

These results show that, within less than 2 HRTs, the wetland sediments, which initially had a high microbial diversity, efficiently evolved into a specialized microbiome enriched in PHA-storing organisms. The selected microbiome demonstrated a good capacity for PHA accumulation and was maintained stable for up to 90 days of operation.

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L13: **Scalable PHB Production Using Waste Streams and Gas Fermentation**

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Cities today are not self-sufficient. The year 2007 represented a landmark in history of humankind, witnessing for the very first time the urban population outmatching the rural population. Cities have a large environmental impact—they consume 60–80% of natural resources globally, produce 50% of global waste, and 75% of greenhouse gas emissions, yet they occupy only 1% of the land surface. In this work, a technoeconomic analysis is carried out to study the conversion of urban waste into bioplastics (polyhydroxybutyrate, PHB, and its copolymers) for use in that city, avoiding outbound and inbound transportation and allowing local value creation in a circular scheme. By gas fermentation using biogas, PHB can be made without the need of agricultural primary raw materials and hence without land utilization. This work presents preliminary lab experiments, and scenarios for mass balances and use cases for urban-made and urban-used PHB, comparing different end-of-life scenarios.

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L14: **Separation Solutions for Scaled-Up PHA Bioplastics Production Needs**

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Due to the recent market growth of bioplastics such as PHA, Alfa Laval developed tailor-made separators specific for this application. A typical PHA production process requires two separation stages: cell harvesting, where bacteria are removed from the fermentation broth, and lysate extraction, where the biopolymer is concentrated after extraction.

The cell harvesting position is particularly interesting for our separators with the Bactofuge™ configuration. This mechanism was developed to discharge bacteria while preserving them. Due to the internal bowl configuration, bacteria are continuously discharged from the heavy phase outlet, avoiding cell ruptures. This is a significant improvement compared to traditional discharges, where solids are intermittently ejected at high speed. This results in a higher PHA yield, as no biopolymer is lost in the harvesting stage.

The efficiency of the PHA process can be further improved by having separators with hermetic inlet and outlet. This air-free configuration reduces power consumption by 40%, as most of the centrifugal energy is recovered as potential energy. This feature is particularly important in cleantech applications, to both cut costs and minimize the carbon footprint.

Concluding, we believe that centrifugal separation plays a major role in the PHA production process. Alfa Laval has developed tailor-made solution ensuring high yield and power savings while keeping a smooth operation.

L15: **Miniscale Extruder Prototypes with Sampling Ports and Rheo-optical Dies for the In-line Monitoring of Biopolymers Melt Processing**

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The environmental benefits brought by the substitution of oil-based plastic packages by biobased and biodegradable ones is often offset by the poor melt processability of biopolymers. For instance, in spite of the recent progress reported [1], food grade thin films made of polyhydroxyalkanoates (PHA) did not reach the market yet. The conversion of biopolymers into e.g. packages using industrial methods such as melt extrusion requires a better understanding of the relationships between process parameters and biopolymer properties and the development of new formulations, possibly using small quantities of experimental biopolymers.

Contributions to such understanding encompass the development of miniature extrusion lines equipped with tools enabling the in-situ measurements of biopolymer properties. In-line characterization is imperative as these materials are thermo-mechanically sensitive and thus degrade along the extruder or during the sample preparation for post-extrusion characterization. A set of miniature extruder prototypes equipped with sampling ports and rheo-optical dies will be presented. Outputs range from grams per hour to hundreds of gram per hours, whereas the development of the morphology and the viscoelasticity of biopolymer composites is assessed during the extrusion. Application examples with poly(lactic acid)/clay nanocomposites or PHA will be presented [2,3] whereas a new prototype dedicated to filaments extrusion for 3D printing application will be introduced.

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L16: **Techno-economic Analysis of 3D Printing Filament for Thermo-responsive Materials: A Numerical Analysis**

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The ability to create biodegradable smart materials with self-adapting shapes and qualities over time in response to external stimuli using three-dimensional (3D) printing filament suggests possible uses in a wide range of industries, including aerospace, healthcare, and automotive. In order to enhance the technological preparedness of 3D printing, it requires evaluating the techno-economic viability and studies have been done to understand the mechanism of 3D printing and the cost that goes along with it, but they do not apply to the stimuli-response mechanisms along the added time dimension or the cost of 3D printing due to the greatly increased complexity of the intercorrelated relationships between material compositions and process parameters across multiple stages. The cost of 3D printing filament with thermo-responsive polymers is quantified in this study using a techno-economic model that includes explicit relationships between cost and the material solidification chemistry and shape memory properties. The formulation of a nonlinear optimization problem yields a set of process parameters that, without compromising the desired biodegradable shape memory performance, can lower overall cost per part by 30%. So, to evaluate market-dependent and operator-oriented 3D printing parameters, a sensitivity analysis is done. The unit price of the raw material and the operator's hourly rate are the two main cost factors that have been discovered. The goal of the techno-economic evaluation is to choose the most cost-effective solution for a specific situation and set of performance requirements. It integrates findings from both investment and performance analysis.

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L17: **Fluorescence Correlation Spectroscopy in Biopolymer Research**

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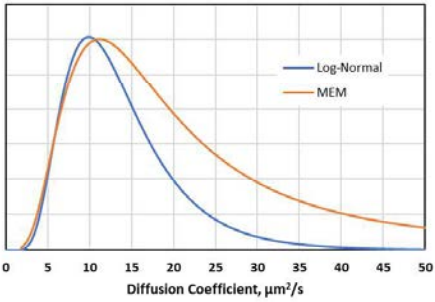

This contribution focuses on the use of advanced fluorescence microscopy, Fluorescence Correlation Spectroscopy, to study the diffusion properties of aqueous solutions of polysaccharides at very low concentrations. This type of study allows the monitoring of biopolymer interactions with, for example, supramolecular systems that may serve as suitable complexes for potential carrier systems.

The evaluation of naturally polydisperse samples of fluorescently looped hyaluronan in aqueous solutions, at low concentrations and laboratory temperature, is also described. The possibilities of interpreting the obtained results in terms of available models that take into account the polydispersity of the system and the Maximum Entropy Method [1-2] based approach (MEMFCS) are discussed.

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L18: **Characterization of Bacterial Cellulose Produced by *Komagataeibacter xylinus* Strains Grown in Styrene/glucose Mixtures**

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Styrene is a mono-aromatic compound used in the synthesis of petrochemical plastics that although being versatile and low-cost materials, are non-biodegradable and contribute to the accumulation of plastics in our ecosystems. To overcome the burden of styrene-derived plastics in the environment, we can employ microorganisms as biocatalysts for their bioconversion into less harmful compounds [1]. In this work, three bacterial strains, namely *Komagataeibacter xylinus* DSM 2325, DSM 2004, and DSM 46604 were cultivated using styrene as a co-substrate in styrene/glucose mixtures to assess their ability to biosynthesize bacterial cellulose (BC) and the impact of such cultivation conditions on its physical-chemical properties. Styrene was assimilated by all strains, although with differing efficiency for BC biosynthesis. The best-performing strain was *K. xylinus* DSM 2325 with a BC production of 2.70 ± 0.4 g/L within 18 days. Furthermore, *K. xylinus* DSM 2004 produced BC from styrene as the sole carbon source, yielding 0.32 ± 0.02 g/L BC. The presence of styrene in the cultivation media had a minor influence on BC chemical structure, thermal degradation temperature (318-337 °C), and morphology (fiber diameter ranging from 31-47 nm). The crystallinity degree of the BC pellicles, on the other hand, varied (19-46%) according to the substrate used during cultivation and the producing strain. The membranes synthesized in the presence of styrene were thinner (3-22 μm) than those produced from glucose (12-44 μm). *K. xylinus* DSM 2325 and DSM 2004 membranes had also low permeability for O_2 (1.1-2.5 barrer) and CO_2 (2.5-5.8 barrer), while those produced by *K. xylinus* DSM 46604 had a higher permeability to CO_2 (42.3 barrer) together with low permeability to O_2 (2.5 barrer). These results demonstrate the feasibility to valorize styrene as a co-substrate into a value-added polymer with tailorable properties by selecting different strains and culture mediums.

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Institute of Nanostructures, Nanomodelling and Nanofabrication – i3N, and by the European Union's Horizon 2020 research and innovation programme through Project Bio Innovation of a Circular Economy for Plastics (BioICEP), under grant agreement No 870292. Asiyah Esmail and Paloma Ortiz-Albo acknowledge FCT I.P. for PhD Grants 2021.05014.BD and SFRH/BD/139389/2018, respectively.

L19: **PHA Extraction Monitoring by Low-field NMR**

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Real-time monitoring is fundamental to enhance (biochemical)process control instead of relying on laborious *offline* analytics. Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for this purpose providing quantitative chemical information about the mixture components in solution. Compared to other commonly applied, mainly optical process analytical technology (PAT) such as Raman or NIR spectroscopy, NMR spectroscopy does not require time and cost intensive specific calibration.

While high field NMR instruments (HF-NMR) with proton frequencies between 300 and 1,200 MHz require large spaces with appropriate specific lab environment, benchtop NMR devices (ranging from 43 up to 100 MHz) represent the possibility for an easy set-up under the fume hood or at industrial site as they are compact and easy to integrate. This opens the opportunity for process coupling and *online* measurements in a bypass to obtain real time data of concentration changes within the studied process stream.

We present an approach to monitor the extraction of polyhydroxyalkanoate (PHA) bioplastics from bacterial cells. We show the applicability of a low-field NMR instrument operating at 43 MHz (LF-NMR) on the extraction process with different solvents at cell densities from 20 – 120 g L^{-1} and different temperatures, validated by *offline* high-field NMR spectroscopy and gas chromatography.

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L20: **Cyanobacterial Cells Imaged Using Uranyl-less Low Voltage Electron Microscopy**

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The studies of intracellular structures of various microorganisms rely heavily on electron microscopy, especially transmission electron microscopy (TEM). Generally, the techniques of electron miscopy require extensive sample preparation starting with fixation of ultrastructure followed by dehydration and in the case of TEM also resin embedding, sectioning and contrasting with salts of heavy metals. To date, uranyl acetate (UA) is one of the most used contrasting agents. Since the 1960s it was used for negative staining, on-section staining as well as en bloc staining [1]. Unfortunately, because of its highly toxic properties, UA is becoming a subject of rising legal restrictions for its storage, use as well as disposal. Several studies have already focused on various lanthanoid compounds as possible UA replacements, but more exotic substances such as oolong tea extracts were also tested [2,3].

Another approach, how to avoid the use of UA, is to change the imaging technique itself. If the accelerating voltage of the electron beam in TEM is lowered than the conventional 60-300kV, the contrast of the studied carbon-based specimen increases and it is possible to observe the sample without any post-staining using heavy metals [4]. It was also proven, that low-voltage TEM was able to distinguish even areas of different compositions of the polymer-blend samples [5].

In our study, we have focused on the study of cyanobacterial cells. Strain *Synochocystis* sp. PCC 6803 is a unicellular cyanobacterium significant for both industry and science because of its capability to produce various biotechnologically valuable substances such as polyhydroxyalkanoates. The samples were observed by conventional high-voltage TEM as well as by low-voltage (scanning) transmission electron microscopy to prove that electron beam voltages of 15-25kV are capable to image biological specimens without heavy metal staining and obtain results comparable to conventional staining and imaging methods [6].

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L21: **Elucidating Gene Regulation of Polyhydroxyalkanoate Production in *Ralstonia eutropha*: Identification of Transcriptional Regulators from Phasin and Depolymerase Genes**

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The stress-related storage of carbon and energy in the form of intracellular polyhydroxyalkanoate (PHA) granules is widely spread across prokaryotic species. PHA reservoirs also serve bacteria as electron sinks when facing oxygen limitation [1] and provide a shielding effect against various stresses such as UV radiation, high/low temperatures, oxidative or osmotic stress [2]. PHA metabolism involves synthesis, storage, and degradation of the polymer. In the best studied PHA producer, *Ralstonia eutropha*, in addition to two PHA synthases, eight phasins (PhaP1-PhaP8) and seven depolymerases (PhaZ1-PhaZ7) are available. Despite the great interest in engineering PHA metabolism, to date only one regulator of gene expression, PhaR, is known that controls expression of two of the phasin genes, *phaP1* and *phaP3*. Nevertheless, given its importance for central carbon metabolism, the regulation of PHA metabolism is probably controlled by a more complex network of transcriptional regulators.

Understanding the underlying regulatory network is key for developing improved PHA production strains.

We searched for additional transcriptional regulators involved in the expressional control of genes involved in PHA metabolism, specially phasin and depolymerase genes. We performed pull-down assays with putative promoter regions of *phaP1*-phasin and *phaZ3*-depolymerase genes to identify potential transcriptional regulators of these genes. Here, crude protein extracts of the P(HB-*co*-HHx) producing *R. eutropha* Re2058/pCB113 strain [3], cultivated under PHA-inhibiting or PHA-accumulating conditions, were incubated with biotin-tagged putative promoter-covering oligonucleotides bound to streptavidin-coupled magnetic beads. An extensive dataset of affinity-purified proteins was identified via Mass Spectrometry (MS) and subsequently processed to point out promising transcriptional regulator candidates. The impact on promoter activity was assayed *in vivo* using ß-galactosidase assays. Furthermore, the most promising candidates were heterologously produced in *E. coli* and their interaction with the promoters investigated *in vitro* by Electrophoretic Mobility Shift Assays (EMSAs).

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L22: **A Systems Biology Approach for Enhancing the Synthesis of Functionalyzed Polymers in *Pseudomonas putida***

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The environmental challenge associated with conventional plastics has created a need for alternative solutions, placing bio-based polymers like polyhydroxyalkanoates (PHAs) in a promising position. These polyesters are produced under nutritional stress conditions by several microorganisms including the model bacterium *Pseudomonas putida*. Carbon sources for PHA synthesis can be classified into two categories: i) PHA related substrates, primarily fatty acids, which provide high production yields, and ii) non-PHA related substrates, such as sugars, whose conversion into PHA precursors through *de novo* fatty acid synthesis route has been associated with low production yields [1]. Significant efforts have been devoted for the use of carbohydrates as carbon sources within a circular economy framework. To address this challenge, systems biology approaches, like genome-scale metabolic models, offer valuable insights into the metabolic features of PHA cycle in *P. putida* KT2440 [2]. These approaches enable the prediction of the key reaction to be deleted in order to redirect the metabolic routes of interest, and represent powerful tools for optimizing and enhancing the utilization of non-PHA related carbon sources in a sustainable manner.

In order to construct an optimized PHA producer *P. putida* KT2440 strain when using sugars as feedstock, we employed the *i*JN1411 genome-scale metabolic model to predict those genes to be deleted for eliminating potential competing pathways and redirecting carbon flux from acetyl-CoA towards PHA biosynthesis [3], and those genes to be added for using sucrose as carbon source. We obtained the engineered MT9 strain which was tested to produce PHA from sucrose. Additionally, the synthesis of the antimicrobial biopolymer PHACOS was explored by co-feeding the chemical precursor 6-acetylthiohexanoic acid (6-ATH) and glucose or sucrose [4].

The results revealed that MT9 cultures grown with sucrose as carbon sources achieved a PHA accumulation of 35% per cell dry weight (CDW) compared to 22% for the WT strain under the same conditions. Moreover, the addition of 6-ATH significantly increased the PHA content, both with sucrose and glucose, to approximately 70% of CDW, revealing the presence of 90% of functionalized 4- and 6- carbons monomers, and providing further evidence for the production of PHACOS.

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L23: **Transcriptomic Analysis Sheds Light on Enhanced PHBV Production in a *Rhodospirillum rubrum* Mutant with Unpaired Pigmentation Synthesis**

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Photosynthesis (PS) in most Purple Non-Sulphur Bacteria (PNSB) is tightly regulated to minimize unnecessary energy consumption and prevent oxidative stress when oxygen is present. PpsR, a transcriptional repressor encoded in the Photosynthetic Cluster (PSC), plays a key role in the regulation of PS. However, in some cases, the sensing function is not directly performed by PpsR itself but by other partner proteins. Rru_A0625 is situated adjacent to the *ppsR* gene, and its deletion abolishes pigment synthesis, as is the case of other PpsR anti-repressors. Due to the previously demonstrated capacity of *R. rubrum* for PHBV production, we hypothesize that mutating Rru_A0625 could harness the carbon and energy that are not being used for pigment production and redirect them towards polymer synthesis. Thus, we investigated the effects of the product of the Rru_A0625 locus (referred as HP1) on PHBV production, a promising bioplastic with similar properties to oil-derived plastics.

Both mutant and wild type strains were grown in mineral medium (RRNCO) with fructose as carbon source. The biomass, PHBV production and fructose consumption were monitored along the experiment. The main differences in growth were observed when oxygen became limiting, and the wild type strain entered in a brief lag probably due to the demanding adaptation required for PS apparatus synthesis. On the contrary, the mutant strain grew steadily resulting in a faster fructose depletion from the medium. Biomass and PHBV maximum were also reached before in the mutant strain.

Given the substantial impact of a mutation in Rru_A0625 on *R. rubrum* metabolism, we delved into the transcriptomic consequences of disrupting this gene. This approach revealed that HP1 not only affects bacteriochlorophyll and carotenoid biosynthesis, as expected, but also influences various other biological processes within the cell. Particularly notable is its impact on the transcription of genes related to nitrogen fixation (such as nifHDK). This biological process was proved to be crucial as an electron sink in anaerobic growth in some strains. Additionally, the impact of HP1 mutation extends to genes involved in stress response (including catalase, glutathione peroxidase, etc), amino acid metabolism, ion balance, polysaccharide and sugar metabolism, and other processes.

This research highlights the far-reaching effects of mutating a regulatory protein on the overall metabolic dynamics of the cell. Furthermore, it demonstrates the potentiality of deleting Rru_A0625 to redirect carbon and electron flow towards PHBV production.

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L24: **Developing a Genetic Manipulation Toolkit for *Caldimonas thermodepolymerans***

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Caldimonas thermodepolymerans DSM 15344, a moderately thermophilic Gram-negative bacterium, is an attractive microbial candidate for the Next-Generation Industrial Biotechnology due to its ability to utilize sugars from plant biomass and convert them efficiently to poly(3-hydroxybutyrate). Unlike the majority of polyhydroxyalkanoate producers, *C. thermodepolymerans* prefers xylose over glucose [1]. Production potential of this promising thermophilic bacterium could be further enhanced by genetic and metabolic engineering but suitable engineering tools are not yet available.

In this study, we describe the development of genetic engineering tools for *C. thermodepolymerans*. The work includes the optimization of transformation protocol, selection of suitable antibiotics and antibiotic resistance genes, development and adoption of plasmid-based tools for genome editing (gene insertion and deletion), and search for reliable regulatory sequences. Within the project, we confirmed the considerable impact of restriction-modification system on transformation efficiency and found origin of replication from a related bacterium, which functions well at elevated temperatures and should be compatible with *C. thermodepolymerans*. Our work contributes to the biotechnological domestication of *Caldimonas thermodepolymerans*, whose bioproduction potential can be now further enhanced by metabolic engineering.

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L25: **Transcriptome Analysis of *Rhodospirillum rubrum* – Wild Type and Mutant Strains**

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Rhodospirillum rubrum is a photosynthetic bacterium known for its unique metabolic capabilities and versatile utilization of carbon sources. It can also produce and accumulate polyhydroxyalkanoates (PHA), biodegradable polymers that serve as carbon and energy storage compounds in many microorganisms [1]. In *R. rubrum*, the production of PHA plays a crucial role in carbon and energy balance, allowing the bacterium to adapt to changing environmental conditions [2]. However, the genome-wide regulation and transcription in its genome have been studied only to a limited extent.

Therefore, a genome-scale model using Pathway Tools was created to understand better the metabolic networks presented in the strain *R. rubrum* DSM 467. Based on the model and the pathways within it, genes of interest, including PHA synthase and PHA depolymerase, were chosen for further potential study. In addition, a mutant strain (MS) with deleted two genes encoding PHA polymerases was studied to compare the genomic and phenotypic characteristics of MS and wild type (WT).

For transcriptome comparison, RNA-Seq was employed. For this purpose, RNA samples were collected from defined time points during WT and MS cultivation on SYN medium with acetate and fructose. Each time-point was covered at least by 3 replicates, and altogether 40 samples were then sequenced by Illumina NextSeq500, and as a result, 75 bp long single-end reads were obtained. Next, we performed adapter and quality trimming by Trimmomatic, removal of reads corresponding to 16S and 23S rRNA sequences by SortMeRNA together with SILVA database, and finally, reads were deduplicated using UMI-tools. In the next step, reads were mapped to the reference genome of *R. rubrum* using STAR, mapped reads were counted using featureCounts function from Rsubread package (R/Bioconductor), and finally, DESeq2 package (R/Bioconductor) was used for differential expression analysis to identify regulated genes in both strains. The overall analysis of WT and MS behaviour, especially between regulated genes, was evaluated by the gene ontology (GO) enrichment analysis using topGO package (R/Bioconductor). Finally, the expressions of selected genes of the given signalling pathways were shown using heatmaps.

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L26: **Stream-lining the Development of a Production-optimized *Xanthomonas campestris* Chassis-organism**

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Xanthomonas campestris (pv. *campestris*), especially NRRL B-1459 have been used as an industrial scale producer of the exopolysaccharide xanthan since the early 1960s. For optimized EPS production, a wide variety of scattered studies have been carried out in process optimization and investigations and modifications of metabolic pathways. In Industry, however, strain optimization is exclusively carried out by adaptive laboratory evolution because of the limitations of using GMO-free processes [1,2].

Both the use of non-defined optimized organisms and the mostly single gene focused studies on defined gene deletions towards a higher xanthan production lack a comprehensive approach for creating a defined production organism for xanthan, or even artificial hetero- or homopolysaccharides.

For this, we developed a comprehensive approach towards a production-optimized *Xcc.* Strain based on the in-house sequenced *Xcc* LMG 8031. Based on the pk19*mobsacB* suicide plasmid and Cre/*loxP* system we developed individual deletion mutants, which are deficient in flagellin, pigment, and lipopolysaccharides, and the exopolysaccharide xanthan. Our approach includes first proof-of-principle single gene deletions of single gene genes leading to the deletion of the respective pathways, including full characterization of growth behavior, substrate consumption, productivity, LCMS-based analysis of intracellular nucleotide sugars required for exopolysaccharide production. Additional to our strain characterization, the produced exopolysaccharide is characterized with respect to monomer composition, molecular weight distribution, and rheological properties.

In further steps we combine all single-gene deletions, towards a flagellin, pigment, and lipopolysaccharide deficient triple mutant, and after our strain characterization and analysis, the single-gene deletions are expanded towards full-cluster deletions using the Cre/*loxP*-system.

This unique combination strain, process and product analysis provides an extensive understanding on the combinatorial approach of previous strain- and process-based optimization approaches, while the established streamlined workflow facilitates further development steps, e.g. towards substrate uptake and metabolism.

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L27: **PUSH-based Strategy to Increase Glycolytic Flux in *Pseudomonas putida* from co-Utilized Cellulosic Sugars to Biopolymer Percursors**

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Pseudomonas putida KT2440 is an attractive host for the production of biochemicals including bacterial polyesters polyhydroxyalkanoates (PHA) from renewable lignocellulosic feedstocks [1]. It can synthesize PHA from lignin-born aromatics or cellulosic glucose. Recently, we have equipped *P. putida* with beta-glucosidase from *Thermobifida fusca* [2]. The recombinant strain showed growth and PHA formation on cellobiose, the main by-product of cellulose hydrolysis, but it preferred glucose in the mixture of the two sugars [3]. Sequential utilization of lignocellulosic substrates hinders their biotechnological valorization.

In this study, we investigate the possibilities of glucose-cellobiose co-utilization in tailored *P. putida* for the biosynthesis of valuable biopolymer precursors. A range of experimental and computational engineering techniques has been employed including cell cultures, genome editing, enzymatic assays, and *in silico* analyses using a metabolic model of *P. putida* constrained by proteomic and kinetic data.

We achieved co-utilization of glucose and cellobiose after closing the periplasmic glucose oxidation pathway [4]. Reduced growth rate and biomass yield of the mutant was compensated by the implantation of heterologous high-capacity glucose transporter. Remarkably, this PUSH-based strategy resulted in the accumulation of pyruvate in aerobic cultures of engineered *P. putida*. The overproduction of this key metabolic intermediate was explained by an improved metabolic model that suggested high glycolytic flux and enzyme saturation due to unregulated substrate uptake. We then demonstrated that the excess of pyruvate can be redirected to L-lactate and we are currently investigating the possibility of exploiting the enhanced glycolytic flux for the efficient biosynthesis of PHA from co-utilized cellulosic sugars.

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L28: **Enhanced Polyhydroxyalkanoate (PHA) Production by Enrichment of PHA-Storing Microorganisms During the Accumulation Process**

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Municipal wastewater treatment plants (WWTPs) offer a ubiquitous source of biomass for PHA production. Large amounts of surplus (waste) activated sludge (WAS) are produced daily. PHA may be accumulated directly using WAS if volatile fatty acid (VFA) rich streams can be made sufficiently available as feedstock [1]. However, the degree of enrichment (DE) of PHA storing microorganisms in WAS can vary between different activated sludge sources and biomass PHA content can be limited [2]. Low production yields due to the presence and growth of flanking populations can also limit performance.

In the present work, it is proposed to facilitate selective growth of PHA storing microorganisms in WAS directly in the accumulation process. Two parallel 1 L fed-batch reactors were used in controlled direct accumulation experiments using municipal WAS from selected Dutch WWTPs. Acetate and butyrate were employed as reference carbon sources to evaluate the hypothesis of an influence of oxygen yield on substrate (Y_{os}) for selective growth of PHA storing biomass in the process [3]. Y_{os} for PHA production was lower for butyrate than for acetate as was expected: 0.19 vs. 0.25 gO_2/gCOD, respectively. Correspondingly, given nutrient availability, after 48 hours, a higher biomass yield of 0.15 $gCOD_{Xa}$/gCOD was obtained for the butyrate fed reactor compared to acetate with 0.02 $gCOD_{Xa}$/gCOD. Biomass active growth in the butyrate reactor could be interpreted to be selective towards the PHA storing biomass fraction.

Due to the inherent increase of DE, both PHA content in the product biomass and PHA yields on substrate were significantly improved. A PHA content of 0.58 gPHA/gVSS and a PHA yield of 0.53 $gCOD_{PHA}$/gCOD were obtained after 48-hours with butyrate as the sole carbon source, compared to 0.36 gPHA/gVSS and 0.31 $gCOD_{PHA}$/gCOD with acetate. Four times more PHA was produced using butyrate. Experiments showed that a maximum PHA content of up to 0.68 gPHA/gVSS can be obtained from direct accumulation on WAS within 48-hours by promoting selectivity of growth during the accumulation process. This value is the highest known in the literature for direct PHA accumulation with municipal activated sludge. These findings lift the opportunity for municipal waste activated sludge to be a generic resource in a supply chain for industrial scale PHA production.

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L29: **Tracking Polyhydroxyalkanoate Synthesis in Thermophiles**

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Polyhydroxyalkanoates (PHAs) are considered as a promising alternative to petroleum-based plastics as they are biobased and biodegradable. However, the bottleneck in their large-scale commercialization is their high production costs compared to conventional plastics. Thermophiles, which are microorganisms that grow optimally at high temperatures, could offer a solution to reduce the pricey costs of PHA production by several ways such as minimizing energy required for cooling [1].

In this study, we navigate through the different classes of microbial thermophiles (thermotolerants, moderate thermophiles, extreme thermophiles, and hyperthermophiles) to discover their PHA production capabilities. Hence, we combined both bioinformatics (presence of the key PHA enzymes) and experimental approaches. Different bacterial strains were also introduced as examples to the different thermophilic categories to study their PHA production abilities in more detail: *Paracoccus kondratievae* (42°C), *Caldimonas thermodepolymerans* (55°C) and *Thermus thermophilus* (75°C).

P. kondratievae is a thermotolerant bacteria that we discovered its ability to accumulate PHB with a yield of 34% in cell dry weight (CDW), upon growth on glycerol or glucose as sole carbon sources. *C. thermodepolymerans* was previously described as a promising moderate thermophilic PHA producer [2]. In the current research, we extended the work on this thermophile to have a more detailed overview about its metabolism related to PHA accumulation by performing transcriptomic studies. When coming to extreme thermophiles (>65°C), interestingly a temperature border line could be deduced, beyond which the ability of microbes to accumulate PHA becomes very scarce to almost non-existent. Surprisingly, *T. thermophilus* was not found able to accumulate PHA which is contradictory to what was previously mentioned in literature [3]. An important question can be raised from this work: What is the reason for diminishing the ability of PHA accumulation in extreme and hyperthermophiles, although the wide spread of this trait in other thermophilic classes?

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L30: **Waste Animal Streams as Complex Sole Carbon and Nitrogen Sources for Polyhydroxyalkanoate Production: Influence of Extraction Temperatures**

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Fossil-based plastics and their environmental impact pose one of the biggest challenges of today's society. Substituting them with bio-based and biodegradable plastics, such as polyhydroxyalkanoates (PHAs), can positively influence our environment. Still, production capacity and their market share are low, mostly caused by high production costs.

Due to their abundance and cheap nature, waste animal materials are a promising substrate for PHA production [1]. They can be used as the carbon source for cultivation of *R. eutropha* Re2058/pCB113 to produce the copolymer P(HB-*co*-HHx) [2]. The preparation of the waste animal material includes pre-treatment, hydrolysis and phase-separation to achieve a fat, fat-protein and protein phase [2]. In this work, we evaluated how different hydrolysis temperatures and waste animal body parts influence the material characteristics and afterwards its effects on PHA production. As waste animal fats (WAF) are difficult to handle, a tailored feeding set-up [3] was used to evaluate PHA production with WAF extracted at temperatures ranging from 130 – 160°C under nitrogen limitation. Cell dry weights of > 50 g L^{-1} with a PHA content of 80 wt% and a HHx content between 16 – 19 mol% could be achieved by feeding WAF from pork bone or pork belly fat independently on the extraction temperature.

For the first time, the waste animal protein (WAP) phase was used as a complex nitrogen source during nitrogen limiting PHA production. The treatment of WAP was evaluated in terms of molecular weight distribution, sterilization, cultivation course and its effect on PHA production. Different extraction temperatures show an influence on the cultivation process and PHA yield. The protein phase was evaluated as sole nitrogen source during PHA production at laboratory bioreactor scale. The usage of the complex nitrogen source WAP has the potential to boost the growth phase of *R. eutropha* before the PHA production phase and to reduce cultivation costs by substitution of fine chemicals.

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L31: **Exploring the Potential of Cyanobacterial Microbiomes for Sustainable Bioproducts**

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Cyanobacteria have emerged as a promising source for the generation of bioproducts due to their photosynthetic abilities and capacity for the production of high-value compounds [1,2]. However, the full-scale implementation of cyanobacterial biotechnologies faces significant challenges, primarily due to significant research gaps in understanding cyanobacterial cultures and optimal growing conditions for the bioproducts of interest. The instability of cultures and limited knowledge of biosynthetic pathways leading to the generation of bioproducts hinder the development of cyanobacterial biotechnologies. Thus, it is imperative to bridge these research gaps to unlock the full potential of these microorganisms.

In this study, we investigated the production of polyhydroxybutyrate (PHB) using photosynthetic microbiomes enriched in cyanobacteria. These cultures offer a promising approach for PHB production by utilizing CO_2 and sunlight for biomass growth and biopolymer synthesis. Furthermore, the use of microbiomes instead of pure cultures requiring sterile conditions has the potential of lowering operational costs [3].

We tested the well-established feast and famine (FF) strategy, successfully applied in heterotrophic cultures [4], for the growth of PHB-producing biomass in photosynthetic microbiomes, especially rich in unicellular cyanobacteria *Synechocystis* sp. and *Synechococcus* sp. Three-liter glass photobioreactors (PBRs) were operated for over 100 days with constant repetition of FF phases

Results displayed a stable and long-term PHB production, with up to 28 % dry cell weight PHB, which represents one of the highest reported contents in wild-type cyanobacteria. In addition, positive Nile Blue A staining and Transmission Electron Microscopy images confirmed the intracellular biopolymer content in cyanobacteria, which proved to be PHB by proton Nuclear Magnetic Resonance analysis. Additionally, we observed the overexpression of gene *phaC* throughout the accumulation phase, which correlated directly with the increased PHB production. Moreover, the overexpression of genes involved in glycogen metabolism suggested the interrelation of both storage polymers as carbon sources.

Outcomes reveal that presence of cyanobacteria in the microbiome is essential for a stable PHB production over a long-term period. This research provides valuable insights into the use of phototrophic microbiomes for sustainable PHB production, which could have potential applications in various markets, including textiles, food, cosmetics, pharmaceuticals, and medicine.

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L32: **Thermophilic PHA Producer *sui generis – Caldimonas thermodepolymerans***

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Extremophiles represent a very diverse group of microorganisms with enormous potential. The application if these microorganisms is in line with the concept of 'Next generation industrial biotechnology' [1]. Thermophilic bacteria are an interesting subcategory. The use of higher temperatures brings many advantages to biotechnological processes – lower requirements for environmental sterility, higher substrate solubility, lower amounts of cooling water etc. One of the characteristics of some thermophiles is the production of biodegradable and biocompatible biopolymers – polyhydroxyalkanoates (PHA). Bacteria produce this polyester in the form of intracellular inclusions and use it as a carbon and energy store.

A very promising thermophilic producer appears to be the genus *Caldimonas,* specifically *Caldimonas thermodepolymerans* (basionym *Schlegelella thermodepolymerans*). This species of bacteria has the unique ability to utilize xylose [2]. It prefers this carbon source over other substrates including glucose. The use of xylose as a carbon source offers a wide range of possibilities, as it is the second most abundant carbohydrate on earth. It is also contained in lignocellulosic materials, which produce a large amount of waste. This contribution focuses on sugar metabolism and its interconnection with PHA synthesis in *C. thermodepolymerans* as promising candidate for PHA production within the framework of the Next Generation Industrial Biotechnology concept.

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L33: **Investigating the Relationship Between Respiratory Chain Activity and the Electron Sink Mechanism of Poly(3-hydroxybutyrate) (PHB) Accumulation in *Rhodospirillum rubrum***

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High productivity in poly(3-hydroxybutyrate) (PHB) bioprocesses requires sufficient biomass coupled with an efficient intracellular PHB accumulation. Under growth conditions, cells generate enough energy to support growth, usually via aerobic respiration, and direct the carbon flux toward biomass production instead of PHB synthesis. However, under certain conditions, PHB accumulation is proposed to serve as an electron sink, supporting growth, as seen in purple non-sulphur bacteria (PNSB). [1]

The biosynthesis of PHB involves the reduction of acetoacetyl-CoA in 3-hydroxybutyryl-CoA by acetoacetyl-CoA reductase, which requires NADPH. This electron sink step in PHB accumulation becomes particularly important for cells when they have to deal with a high intracellular ratio of reduced/oxidized cofactors, a condition resulting in reductive stress. Circumstances causing such stress are common in natural habitats but can be simulated to some extent in a bioreactor, e.g., in microaerobic and anaerobic environments.

Rhodospirillum rubrum is a purple non-sulphur bacterium (PNSB) equipped with a versatile metabolism which owns several electron sink mechanisms. [2] In particular, it has been shown that CO_2 fixation is a core electron sink for PNSB and is essential for growth under reducing conditions (e.g., low oxygen levels). [3,4] Interestingly, Narancic et al. (2016) suggested that in presence of acetate in aerobic dark conditions, PHB synthesis replaces the CO_2 fixation, suggesting that PHB biosynthesis might be as important as CO_2 fixation for handling reductive power. [5]

With this understanding, we sought to investigate the relationship between the activity of the respiratory chain and PHB accumulation in *R. rubrum* for acetate metabolism. Preliminary experiments conducted in darkness under chemoheterotrophic conditions assessed the potential of fructose and acetate as carbon sources to support growth. Conditions were tested aerobically, microaerobically for acetate, and anaerobically in both serum vials and bench-top bioreactors. While fructose metabolism was efficiently functioning across the different growth modes, acetate, as expected, could not support growth without oxygen. This observation led us to suspect that cells cannot generate energy anaerobically with acetate only in the dark. Consequently, our ongoing experiments focus on investigating microaerobic cultures under varied dissolved oxygen levels in bench-top bioreactors, enabling cells to generate ATP via the respiratory chain while managing excess reductive power through PHB production.

This study opens new directions for optimizing PHB production by leveraging its role as an electron sink in low-oxygen environments. On top of that fundamental research can also benefit from new insights into the metabolism of *Rhodospirillum rubrum* as model organism.

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L34: **Pilot Scale Volatile Fatty Acids and Polyhydroxyalkanoate Production from Cheese Whey: Process Characteristics and Microbial Community Analysis**

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Cheese whey (CW) is a subproduct in the dairy industries produced in high quatities, and due to its high organic load is one of the most polluting wastes associated with dairy industries. 90% of its content is lactose, making it easily fermentable through anaerobic digestion [1] The main products of anaerobic digestion are volatile fatty acids (VFAs), which are of great interest in industry. However, these VFA can also be converted into a type of bioplastic, polyhydroxyalkanoates (PHA), through mixed microbial cultures (MMC) [2]. PHAs are biologically synthesised polyesters that biodegrade in the environment. Their properties are similar to conventional plastics, so they can be used in many applications, such as packaging, building materials, cosmetics, pharmaceuticals, etc.

The production of PHA from wastes such as CW was studied at laboratory scale but it is also interesting to do experiments at pilot scale which allow to know the behaviour of the microorganisms in the different processes, the performance of the process, the properties of the biopolymer obtained, etc.

In this study, the production of PHA from cheese whey was studied in a pilot scale system, comprising three process step: (1) anaerobic fermentation for production of VFA, (2) enrichment of PHA-producing biomass, and (3) accumulation of PHA in the biomass. Earlier laboratory scale studies have already investigated cheese whey as feedstock for VFA and PHA production [1]. The main objective of this work was to study possible differences between the experiments at laboratory scale and pilot scale, and study the microbial community involved in both VFA and PHA production processes. In the first anaerobic reactor, VFA were produced from CW, with an acidification percentage between 70 and 80 %. In the microbial community analysis of the acidogenic reactor a novel phylum appeared, *Candidatus Saccharibacteria*, which was dominant under all the operating conditions. Other genera previously reported in other studies for the production of VFA, such as *Prevotella*, *Megasphaera* and *Bifidobactirium ,* also appeared [1]. In the second enrichment aerobic reactor, using the VFA as feed, a yield of 0.28 - 0.39 $mmolC_{PHA}/mmolC_{VFA}$ was reached. The dominant bacteria in this reactor belonged to the *Rhodocyclaceae* family, reported in other studies for their ability to accumulate PHA, although the microbial community also contained in other microorganisms at a lower percentage, such as the genus *Thauera* or the species *Plasticicumulands acidivarans* [3]. Third reactor produced PHA, with an accumulation of 0.25 - 0.45 $g_{PHAs}/g_{biomass}$. The process showed that it is possible to use a waste with a high organic matter content for biopolymer production on a pilot scale.

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L35: **Enzymatic Catalysis: A Powerful Tool for the Synthesis of Functional Oligomers and Biobased Additives**

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With the soaring of the needs of bioeconomy-based solutions, there is a great interest in agricultural waste streams as a biomass source to be used to extract high-value bio-components. This follows the concept of "second generation biorefineries", which should rely on integrated chemical and biotechnological innovations for converting food and feed residues into valuable products and returning non-toxic byproducts in the form of nutrients to the soil. This work describes the implementation of enzymatic catalysis as an environmentally friendly tool for the synthesis of biomass-derived polymers and additives [1]. In particular, the utilization of a cardanol diol deriving from cashew nut shells as a co-monomer (in combination with other biobased aliphatic diesters) for the synthesis of short oligomers to be used as poly(lactic acid) (PLA) plasticizers will be presented [2]. A synthetic route involving the combination of itaconic acid and aliphatic diamines to produce a series of bis(pyrrolidone) monomers that were subsequently polymerized using an immobilized lipase catalyst will then be described, highlighting how these materials can affect the properties of polymers such as PLA and poly(hydroxy alkanoates) [3]. Finally, a third case study on the development of glycerol-based aliphatic polyesters will be also introduced showing the extraordinary selectivity that is possible to achieve using enzymes as catalysts. These materials were then processed, through green chemical technologies consisting of non-toxic catalysts and biomass-derived solvents and utilized as hydrophilic cores to produce amphiphilic molecules having emulsifying properties and as linkers for the synthesis of peptide-fatty acids conjugates having potential biomedical applications [4].

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L36: **Simulation of GAG-analogue Biomimetics for Intervertebral Disc Repair**

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Aggrecan, one of the main components of the intervertebral disc (IVD), belongs to the family of proteoglycans (PGs) that are composed of glycosaminoglycan (GAG) chains covalently attached to a core protein. Its primary function is to maintain tissue hydration and hence disc height under the high loads imposed by muscle activity and body weight. Significant PG loss is one of the first indications of disc degeneration. A possible solution to recover disc functions is by injecting a synthetic hydrogel, into the joint cavity, hence mimicking the role of PGs. One of the hydrogels proposed is GAG-analogues, based on sulfate-containing polymers, which are responsible for hydration in disc tissue [1].

In the present work [2] we used molecular dynamics (MD) to study the effect of the hydrogel crosslinking (type and degree) on the swelling behavior of the suggested GAG-analogue biomimetics, by calculation of cohesive energy density (CED), solubility parameter, enthalpy of mixing (ΔEmix) and the interactions between the molecules at pure form and as mixture with water.

The simulation results showed that hydrophobicity plays an important role in the swelling of the hydrogel, as indicated by the linear correlation observed between solubility parameter values of the copolymers and crosslinker weight ratio (w/w) ; this correlation was found useful in predicting the amount of PEGDA needed for the desirable hydration behavior of (CS)4-peptide. Enthalpy of mixing calculations showed that all the GAG analogues, (CS)4 and (CS)4-peptide are water-soluble; radial distribution function analysis revealed that they form interactions with water molecules, which is important for the hydration process.

To conclude, our simulation results, beyond supporting the experimental data, can be used as a useful predictive tool in future development of biomaterials, such as disc replacement

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L37: **Porous Scaffold of PHBHV, Nanohydroxyapatite and Fullerene: Easy FDM Processing and Potential Application in Bone Tissue Engineering**

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3D printing is a promising technique to produce scaffolds for bone tissue regeneration, especially with porous structure. However, biomaterials for this application must not only mimic the bone structure, but also present a similar composition to the bone – which is a natural composite based on collagen and nanohydroxyapatite [1,2]. Researches are focused on developing a reproducible methodology to fabricate porous scaffolds based on polymer composites. Polyhydroxyalkanoates (PHA's) are promising candidates due to their good biocompatibility, biodegradability, and mild piezoelectricity [3]. In the present work, a hybrid nanocomposite of poly-3(hydroxybutyrate-co6%hydroxyvalerate) (PHBHV), 5wt% of nanohydroxyapatite, 1.5wt% of fullerene and 1.5wt% of vancomycin were produced by extrusion mixing (HAAKE MiniLab II) at 160ºC and 25 rpm. Filaments (Ø 1.5 mm) were obtained and used to feed a commercial fused deposition modeling (FDM) 3D printer (Prusa MK3S+). Scaffolds of 50% of porosity, with pores size of 500 µm and orthogonal orientation were designed using a 3D Autodesk Inventor software, and printed at 180ºC (without bed heating), and 20 mm/s. The printing set up resulted in a good printability and reproducibility of the scaffolds, which showed beam and pore sizes of 430 ± 56 and 631 ± 57 µm, respectively. The pores size was compatible to trabecular bone (500-600 µm) [4]. Porous structure is important to cell migration, nutrients transportation and formation of blood vessels. Small pores (30 ± 10 µm) in the surface of the beams were also observed by SEM, which can result in a favorable topography to cell adhesion. The flexural elastic modulus of the porous scaffolds at 37ºC was 0.23 ± 0.04 GPa. The dynamic compressive modulus was 0.13 GPa in the frequency range of 0.5 to 10 Hz, which is comparable to different bone regions [5]. The piezoelectric coefficient (d33) of the nanocomposite was 2.2 ± 0.5 pC/N compared to 0.22 pC/Nof the human bone [6], indicating this material can improve the cell attachment under stress. The porous scaffolds presented a vancomycin release for 2 days in enzymatic solution (proteinase K and lysozyme). This antibiotic release is an advantageous characteristic of the produced scaffold, since it is expected to prevent bacterial contamination and infection in the surgery region. The scaffolds lost 3.1 ± 0.6 % of weight during 30 days in enzymatic solution, indicating the nanocomposite in the studied geometry is stable in simulated body conditions. Therefore, these porous scaffolds are promising for bone filling applications, since they exhibit comparable mechanical properties to different bone regions, pores structure susceptible to cell migration and nutrient diffusion, piezoelectric behavior, and good stability in body conditions, with potential to match the bone regeneration period.

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L38: **Development of Porous Scaffolds Based on Polyhydroxyalkanoates (PHA) and FucoPol**

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The aim of this study was to fabricate PHA-based scaffolds for cell adhesion and proliferation, envisaging their use in tissue engineering or wound management applications. To achieve this, different PHA polymers were produced, namely, polyhydroxybutyrate, P(3HB), and polyhydroxybutyrate-co-hydroxyvalerate-co-hydroxyhexanoate, P(3HB-co-3HV-co-3HHx), which is composed of 55 wt% 3HB, 21 wt% 3HV and 24 wt% 3HHx. These biopolymers have a molar mass of 9.15×105 kg/mol and 0.94×105 kg/mol, respectively, together with crystallinity index values of 85.5% and 26.2% respectively, and glass transition temperatures of 1.65 °C and -3.5 °C, respectively. P(3HB) exhibited a melting point of 160 °C and P(3HB-co-3HV-co-3HHx) showed two melting temperatures at 144 °C and 159 °C. The biopolymers were used to develop porous structures by two distinct procedures: the emulsion templating technique and the salt leaching after hot-pressing. Both strategies produced porous white structures that supported cell (Human Caucasian Foetal Foreskin Fibroblasts, HFFF2 cell line) adhesion and proliferation. Aiming to improve the structures' biological performance, FucoPol, a bacterial polysaccharide composed of 34.3%mol fucose, 33.1%mol glucose, 26.2%mol galactose, and 9.9%mol glucuronic acid, was incorporated into the PHA scaffolds. This high molar mass (1.40×106 kg/mol) biopolymer is known for its inherent biological activity that includes wound healing ability [1] and photoprotective capacity [2]. FucoPol was incorporated into the scaffolds by impregnating the hot-pressed PHA scaffolds with a FucoPol solution followed by immersion in an iron chloride solution for FucoPol gelation, while in the emulsion templated technique FucoPol was added in the aqueous phase and acted also as an emulsion stabilizer [3]. The resulting PHA:FucoPol blends displayed improved HFFF2 cells' proliferation after 4 and 7 days, compared to the original PHA scaffolds, thus demonstrating the added advantage of incorporating FucoPol into the scaffolds. These results show the promising properties of the PHA-based scaffolds developed to harbour cells and their potential to be used in tissue engineering or in active wound management applications.

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L39: **Novel Hydrogel Membranes Based on the Bacterial Polysaccharide FucoPol: Design, Characterization, and Biological Properties**

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Hydrogel membranes (HMs) are polymeric structures that combine the dynamic mechanical properties and the water absorption characteristics of the hydrogels with the porous morphology and permeability properties of the thin membranes. These unique properties make them suitable for being used in several biomedical applications such as tissue engineering, drug delivery and wound care management [1]. FucoPol, a fucose-rich polyanionic polysaccharide, was used for the first time for the preparation of HMs, through ionic gelation, using Fe^{3+} as a crosslinking agent. This study evaluated the impact of Fe^{3+} and FucoPol concentrations on the HMs' strength. Results showed that above 1.5 g/L, Fe^{3+} concentration had limited influence on the HMs strength while varying FucoPol concentration had a more significant effect. Three different FucoPol concentrations (1.0, 1.75, and 2.5wt.%) were combined with Fe^{3+} (1.5 g/L), resulting in HMs with water content above 97 wt% and Fe^{3+} content up to 0.16wt.%, whose presence was confirmed by FTIR and XRD analysis. HMs with lower FucoPol content exhibited a porous microstructure that became denser as the polymer concentration increased. Moreover, the low polymer content HM presented the highest swelling ratio (22.3 ± 1.8 g/g) and the lower hardness value (32.4 ± 5.8 kPa). However, improved mechanical properties (221.9 ± 10.2 kPa) along with a decrease in the swelling ratio (11.9 ± 1.6 g/g) were obtained for HMs with higher polymer content. Furthermore, all HMs were non-cytotoxic and revealed anti-inflammatory activity. The incorporation of FucoPol as a structuring agent and bioactive ingredient in the development of HMs opens up new possibilities for its use in tissue engineering, drug delivery, and wound care management.

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L40: **Cryoprotective FucoPol: Studies on Crystal Size Reduction, Shape Modulation, Enhanced Ice Nucleation Control & Antifreeze Protein-like Behavior**

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The organ transplant crisis kills around 17 people per day, with a patient being added to the waiting list every 10 minutes, and while demand far outweighs supply by 10x, every 2 in 3 donor hearts are discarded during to an average shelf life of just 4 hours. While histocompatibility and organ transportation are key factors in the transplant chain, the major setback is often associated with the lack of efficient cryopreservation procedures to increase organ shelf-life. Although glycerol and DMSO are still considered gold-standard cryoprotectants, the high concentrations required for optimal action are often cytotoxic to biologicals, urging for new cryoprotectant alternatives. We have thoroughly explored the cryoprotective potential of bio-based polysaccharides for their inherent biocompatibility and vast multifunctionality. Particularly, the bacterial fucose-rich polysaccharide FucoPol has been a prime candidate for cryopreservation formula implementation and a model carbohydrate polymer in structure-function relationship studies. FucoPol has a fucose, galactose, glucose, and glucuronic acid hexamer motif (2.0:1.9:0.9:0.5 M ratio) that finely balances neutral monomers with a terminal branched chain of anionic monomers, which resembles the Thr-$(Ala)_7$ peptide sequence in the P. americanus type I antifreeze protein. Its pseudohelical structure also mimics the protein flexibility highly responsible for interacting with a growing ice front, exerting growth inhibition by a Langmuir adsorption mechanism. The polyanionic character and high viscosity of FucoPol solutions contribute to strong polysaccharide-ice interfacial interactions and hindered molecular diffusion at sub-zero temperatures, which are key kinetic drivers of ice nucleation and growth. The increased post-thaw viability of several cryopreserved cell lines in the presence of FucoPol[1] rivals the performance of the optimized cryogenic commercial formula CryoStor CS5™, and has been associated with a strong non-colligative thermal hysteresis, ice growth disruption and shape modulatory effect, reducing the size of ice crystals to innocuous sizes by acting as a crystallization initiator[2]; a strong control of the stochastic nature of nucleation, narrowing the temperature range at which crystal formation may occur and significantly increasing its induction time[3]; a very low osmolality accompanied by cell membrane osmoregulation[1]; and a consortium of high-molecular weight, high viscosity, shear-thinning and antioxidant effects[4] that further potentiate its function during several stages of mechanical and metabolic stress. The cryoprotective action of FucoPol has consolidated its use in biological deep freezing and supercooling procedures, as it provides beneficial effects in both regimes, and remains the most efficient molecule out of a study subset of 26 polysaccharides. Hence, it should be regarded

as a key chemical player in the design of safer, bio-based, low-cost biopreservation protocols with increased success rates, establishing the bridging of biotechnology with cryobiology as a feasible venture for scientific development and funding.

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L41: **Self-entrapment of Plant Growth Promoting Rhizobacteria by Gelation of Their Exopolysaccharides – Towards the Next-Generation Bioinoculants**

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Encapsulation of Plant Growth Promoting Rhizobacteria (PGPR) in hydrogel carriers represents the state-of-the-art in production of agricultural bioinoculants that are used to restore soil fertility and to enhance the yield of crops. The hydrogel matrix enhances bioinoculant performance by protecting the cells against various environmental stress factors that accompany its application, however, the encapsulation step provided by addition of an external gelation component reduces the economic feasibility of the bioinoculant production. We have proposed a novel concept of biofertilizers based on the entrapment of PGPR (from the genus *Azotobacter*) in the gel formed from alginate produced directly by the employed bacteria. We have been focusing on all the essential steps of the technology of the bioinoculant production. A complex systematic study on the processes of PGPR cultivation, gel formation, and its transformation into the form of final bioinoculant have been conducted in order to provide a necessary knowledge base for the development of the next-generation bioinoculants. Furthermore, a pilot screening of the bioactivity of the products have been performed to confirm the effect of the bioinoculants' application. Beyond providing the basic proof-of-concept on this original strategy, we have also been aiming at gaining the essential fundamental knowledge on the causal relationship between preparation procedure, structure and crucial properties of the developed bioinoculants.

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L42: **Sustainable Carbon Fiber from Lignin Precursor: Formulation, Processing, and Potential Applications**

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Carbon fibre possesses a unique combination of properties that sets it apart from most other fibres, such as a high specific strength and modulus, low density, creep resistance, high thermal and electrical conductivity, excellent chemical resistance and high temperature resistance in inert atmospheres. Translating these properties into a carbon fibre composite therefore has enabled the development of light-weight high strength and stiffness structural materials and components that have revolutionised a wide range of industries such as transportation, sporting goods, defence, wind energy, pressure vessels, and construction. At the same time, lignin, being a highly branched and amorphous macromolecule, is promising to be turned into carbon fibre; however, lignin varies greatly in its composition depending on the plant species, maturity stage, and environmental conditions.[1] Our study demonstrated that lignin, as an aromatic polymer in nature, has several advantages of high carbon content, low cost and bio-renewability which makes it attractive as precursor. The major challenge of the complex structure of lignin and its diversity that significantly restrict the spinnability of lignin was tackled by blending lignin with cellulose. We have successfully established the dope preparation, formulation, and the wet-spinning processing method of lignin carbon fibre (LCF) from oil palm waste, providing understanding on its production conditions. Specifically, a pathway was established to produce good quality LCF, of tensile strength and modulus of 364 Mpa and 30 GPa respectively, in lab and pilot scale. The findings provide perspectives on the new directions for future development of low-cost, green technology for advanced material as part of PETRONAS' sustainability effort. When compared with conventional carbon fibre from fossil fuel derived precursors, i.e. polyacrylonitrile (PAN), $15-20/lb of PAN-based carbon fibre can be reduced to $10/lb by replacing PAN with lignin as the precursor, whilst reducing the emission of equivalent CO2/kg carbon fibre up to 90%.[2]

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L43: **Cyanoflan: A Marine Cyanobacterial Polymer as a Natural Skin Care Ingredient**

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With a more environmentally conscious society, the demand for natural alternatives to replace harmful petrochemical synthetic-based compounds is rapidly increasing worldwide. In particular, the cosmeceutical and biopharmaceutical industry has been actively seeking for natural ingredients that can contribute to the quality, performance, value, and lifespan of formulations, while promoting a sustainable and eco-friendly economy. In this context, cyanobacteria are prolific sources of added-value biocompounds, being powerful phototrophic cell-factories capable of sequestering CO_2. However, despite offering an opportunity to address key challenges in distinct industries, cyanobacteria-derived products are still an unexploited resource.

Cyanoflan is a unique extracellular polysaccharidic polymer naturally secreted by a marine unicellular cyanobacterium, requiring minimal isolation steps [1]. Therefore, the biomass surplus can be used to generate value envisaging a biorefinery approach, having the potential to be commercialized as food or feed supplement, for example, due to its high content of proteins, vitamins, and minerals [2].

Cyanoflan is a complex and versatile macromolecule that can be applied in cosmetic and pharmaceutical formulations as a rheology modifier, showing a viscosity about 1.5x higher than xanthan gum [3]. Furthermore, *in vitro* and *in vivo* results demonstrated Cyanoflan biocompatibility with human cells and bioactivity, since it has antioxidant and anti-inflammatory properties, which can provide protection to the skin and promoting its regeneration [4]. Other functional and bioactive properties of Cyanoflan are being evaluated to promote the incorporation of such a natural, renewable and sustainable raw material into commodities and premium products from the cosmetics and personal care industry.

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L44: **From Lignins to Renewable Aromatic Vitrimers**

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Lignins are the most abundant sources of renewable aromatic structures on Earth. Considered as polyphenols, they exhibit a great variability of structures according to the botanical resource and the fractionation process, making them suitable for a wide range of chemical modifications [1]. Advanced properties of lignin-based materials are enhanced by its aromaticity, which improve thermal stability, mechanical strength, and chemical resistance [2]. Organosolv lignins (OSL) are obtained from solvent pulping, in sulfur-free processes. Their structures are relatively rich in β-ether bonds, the most abundant in native lignin. In this presentation we report the valorization of OSL, extracted from industrial size beech wood using aqueous acetone Fabiola™ process, with the synthesis of stimuli-responsive materials: aromatic vitrimers [3].

Vitrimers have proven in the last decade to be an important new class of polymer materials combining the main advantages of thermosets (mechanical performance and thermal resistance) and thermoplastics (recyclability and re-processability) [4]. For instance, by incorporating dynamic covalent bonds within a cross-linked network materials can undergo reprocessing cycles without compromising the properties at usage temperature [5,6].

Different chemical modifications were performed on OSL to graft reactive functional groups. Chemical and physico-chemical characterization were carried out on the resulting macromonomers. From the different macromolecular architectures, dynamic cross-linked networks were successfully obtained. The influence of lignin content and chain length of cross-linker was particularly analyzed. Chemical, thermal, and mechanical characterizations as well as rheological behavior through stress-relaxation experiment were assessed. Lastly, recyclability and self-healing ability of the different aromatic materials were studied and shown. After several recycling cycles, no major property loss was observed. The developed strategy enabled the fabrication of biobased aromatic vitrimers with tunable structural design and properties.

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L45: **Revealing the Relationship between Structural, Mechanical, and Transport Properties of Biopolymer Gels as a Route towards Novel Engineered Materials**

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Hydrogels are a group of polymeric materials with 3D hydrophilic physical, chemical and/or a combination of both, cross-linked networks formed by biopolymers, synthetic or semi-synthetic materials. From an application point of view, the structural, mechanical and transport properties of these unique materials are the most important ones. In addition, the causality between these properties needs to be in-depth clarified. The clarification of this relationship can open the new horizons in the preparation and characterization of hydrogels with tunable properties towards novel engineered materials.

This contribution presents an original concept in the design of hydrogel materials for the controlled release of charged organic compounds based on semi-interpenetrating (sIPN) and/or interpenetrating (IPN) polymer networks composed of a gel-forming polymer and linear polyelectrolyte with specific binding affinity. The original methodology, which provides a complex view of the composition-structure-performance relationship, is presented [1].

Furthermore, the unique approach to the preparation of hydrogels with gradient structures and the characterization of these materials using the most advantageous analytical techniques will be also presented, as a comprehensive description of the physicochemical techniques that demonstrate the existence of a gradient structure is still lacking. Gradient hydrogels are promising future materials that could be used in tissue engineering (e.g. scaffolds), pharmaceuticals (e.g. drug delivery systems) and many other disciplines [2,3].

The presented systematic study of the preparation-structure-performance relationship may lead to overcoming the major weaknesses of conventional hydrogels (i.e. lack of mechanical strength in the swollen state; structural disintegration over time; rapid compounds release due to disintegration), and may open new horizons in the engineering of biomaterials tailor-made for specific applications in controlled release and/or tissue engineering.

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L46: **Hybrid Gellan Hydrogel Networks – Smart Materials with Tunable Viscoelastic and Transport Properties**

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Gellan is a natural polysaccharide with broad potential applications in pharmaceuticals, cosmetics and food processing, where it can serve as suspending, stabilizing or thickening agent [1,2]. Gellan can be crosslinked into the 3D network, which can be based either on physical (non-covalent interactions), chemical (crosslinking of gellan using multivalent ions, organic cations or surfactants) but also on combined dual nature (physically crosslinked carrier network with interpenetrated additives forming additional chemical interactions with functional groups present in gellan structure) [3,4]. Dual or hybrid hydrogels combining different types of internal crosslinking (even of different natures) represent suitable candidates for use in the pharmaceutical industry and medical applications as carrier systems of various solutes. The viscoelastic and transport properties of these smart hydrogels can be manipulated via the internal structure of the hydrogel network.

The main aim of the present work was the study of viscoelastic and transport properties of selected hybrid gellan hydrogels based on carrier physically crosslinked gellan network (formed during a dissolution of gellan polymer in aqueous solution at elevated temperatures of 80°C followed by a gradual decrease of temperature to laboratory temperature) and additional modifiers (in our work we used Ca^{2+} ions, glycerol and tween 80). These prepared materials were characterized by our previously optimized combination of techniques [5], which were able to access the structural (SEM imaging, BET porosimetry), viscoelastic (rheology) and transport (macro-diffusion experiments combined with UV-VIS quantification of diffusion process; methylene blue selected as a diffusion probe) properties of studied hybrid hydrogel networks. The results of our work were straightforward. The viscoelastic properties were primarily defined by the carrier gellan network and its density (driven by a concentration of gellan in hydrogel). Moreover, the transport properties were dependent either on internal hydrogel architecture (driven by a physically bound carrier gellan network) but also on the presence of the used hydrogel modifier, which introduced into the gel matrix the functional groups affecting the transport of the used diffusion probe (methylene blue).

The results of our research have proven that changing the concentration of the gellan in a hydrogel and a suitable selection of the dispersion medium and used modifier can significantly influence the transport and viscoelastic properties of hybrid gellan hydrogels. These characteristics of hybrid gellan networks can be, in consequence, modified according to the need of a specific application.

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L47: **Poly(3-hydroxybutyrate) Production from Fruit Wastes and Property Evaluation**

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Petrochemically-derived plastic waste has gained global attention due to its environmental impact. Over 10 million t of plastic end up in the oceans from landfills each year. Poly(3-hydroxybutyrate) (PHB) is the most well-known member of the polyhydroxyalkanoate family. However, high production costs have hindered its industrial production, leading to the exploration of using crude renewable resources [1]. In this study, PHB production was developed in fed-batch fermentations with the bacterial strain P*araburkholderia sacchari* DSM 17165 using free sugars from fruit wastes as fermentation feedstock. The effect of different initial sugar concentrations was investigated followed by the evaluation of carbon to inorganic phosphorus (C/IP) ratio, phosphorous addition during feeding and volumetric oxygen transfer coefficient (k_La). PHB properties (thermal, mechanical and molecular weight) were evaluated at different fermentation times. The investigation of the effect of C/IP ratio resulted in the highest PHB accumulation (70% w/w) at C/IP 26.5 with a total dry weight of 90 g/L. At the optimal C/IP ratio (26.5), phosphorous supplementation in the feeding solution was implemented, resulting in increased total dry weight (140 g/L) with PHB accumulation of 71% (w/w). Among the k_La values used, the highest yield (0.33 g/g) and productivity (3.28 g/L/h) were observed at 206.1 h^{-1}, where the total cell dry weight was 162.6 g/L with a PHB concentration of 108.3 g/L. The effect of different cell harvesting times on the properties of PHB was subsequently investigated. The weight average molecular weight and thermal properties remained unaffected by the harvesting time. However, there was an impact on the tensile strength, which decreased from 28.7 MPa at 22 h to 13.3 MPa at 36 h. The elongation at break ranged from 3.6% to 14.8%, while Young's modulus ranged from 830 MPa to 2000 MPa.

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L48: **Novel Method for the Extraction of Polyhydroxyalkanoates from a Mixed Microbial Culture Fed with Paper-mill Wastewater**

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The high versatility and low-cost of petroleum-based plastics have significantly improved society's daily life. Nevertheless, their use has arisen environmental concerns regarding, namely, greenhouse gases and microplastics accumulation in the oceans. Polyhydroxyalkanoates (PHA) are biobased and biodegradable polymers that have emerged as a natural alternative to conventional plastics, presenting comparable thermo-physical properties but none of their environmental hazards [1]. Despite this, PHA production costs are significantly higher than those for petroleum-based plastics (EUR 1.18-6.12/ kg vs. < EUR 1/ kg) [2]. The utilization of organic waste streams as carbon source and mixed microbial cultures (MMC), in lieu of pure cultures and the required sterile conditions, has contributed to the reduction of PHA production costs. Nowadays, PHA downstream processing is one of the major economic impacting factors on the PHA production chain [3]. PHA extraction should be both economically and environmentally sustainable and adjusted to the properties of each biomass to maximize PHA recovery and purity.

The present work consists on the extraction of PHA from MMC produced at pilot-scale with paper-mill wastewater. Various methods were tested at lab-scale, to assess the most adequate to this type of biomass and its potential impurities, such as calcium carbonate and lignin-related compounds. Good results were observed when biomass was first incubated with a combination of acetic acid (CH_3COOH) and hydrogen peroxide (H_2O_2), and then treated with sodium hypochlorite (NaClO). After optimization at lab-scale, PHA extraction was applied at pilot-scale. This process recovered ca. 49% of PHA with a purity of 87% from biomass containing ca. 31% of PHA. The polymer presented molecular weight and thermal properties similar to those of the polymer extracted from the same biomass using the benchmark protocol with chloroform. Incubation with CH_3COOH and H_2O_2 shows thus potential to be an efficient and environmentally sustainable pre-treatment for PHA extraction.

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L49: **PHB Production Through a Two-stage Cultivation Fed with Syngas**

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Flue gases are crucial elements in fostering the global warming and therefore should be a key target to reduce their formation and in addition to use these gases efficiently for new products. In our strategic approach we focused on valorizing syngas, a mixture of N_2, H_2, CO and CO_2. produced mainly in steel milling industry as well as in the petroleum refining industry. Unfortunately, the key component CO is also toxic to most bacterial production strains. The aim of the project is to convert CO into another compound that is less toxic for another strain that is producing a valuable product, e.g. the bioplastic polyhydroxyalkanoate (PHA). Hydrogenophaga pseudoflava, a heterotrophic and facultative autotrophic strain, consumes CO and produces CO_2 under aerobic conditions [1]. The second strain, Ideonella sp. O-1, an autotrophic strain and CO-tolerant strain [2], consumes CO_2 and produces polyhydroxybutyrate (PHB).

In order to improve the growth of the PHB-producing strain and CO_2 production *H. pseudoflava* had to be optimized, medium and gas composition were improved using DOE models and fractional factorial designs. The maximum specific growth rate was improved by 75% to 0.07 h^{-1}, the maximum biomass concentration from 2.6 g/L to 10 g/L and the maximum CO_2 production was tripled. In a second step, *Ideonella*'s specific growth rate and PHB accumulation was also optimized by analyzing the effect of each gas component and then improving gas consumption. With these results, specific growth rate was increased from 0.085 h^{-1} to 0.31 h^{-1} and PHB accumulation from 15% to 49 %.

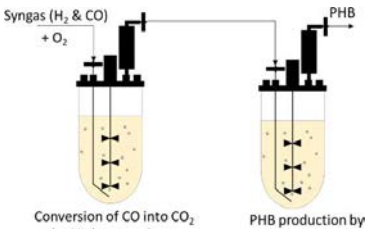

Figure 1 Description of the two-stage process based on CO consumption to PHA accumulation with two strains: H. pseudoflava and Ideonella sp. O-1.

Finally, the proof of concept for this biorefinery approach was done with a two-stage cultivation (Figure 1). In the first stage, a turbidostat, *H. pseudoflava was continuously grown and* converted CO from syngas to CO_2. The gas outlet was fed to the second bioreactor, where *Ideonella* was grown during a fed-batch cultivation. The specific growth rate was 0.27 h^{-1} and PHB was accumulated up to 45 %.

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L50: **Valorization of Salted Cheese Whey into Polyhydroxyalkanoates by Halotolerant Mixed Microbial Cultures**

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Saline side streams from agri-food industries are characterized by a high cost in their treatment for safe disposal, as the conventional biological processes are often inhibited by the high saline concentration. Among others, fishery and dairy industry are a relevant source of saline wastewaters [1,2]. The development of biological processes able to produce value-added products from saline wastewaters as feedstock is favourable to promote both environmental and economic sustainability for the agri-food industry.

In the present study, the valorisation of salted fermented cheese whey, a by-product from the dairy industry, into polyhydroxyalkanoates (PHA) by mixed microbial cultures (MMC) was investigated. The main challenges were to enrich a MMC with PHA-accumulating organisms and attain good PHA productivities under high salinity (30 gNaCl/L). Furthermore, the study investigated PHA production using the salted fermented stream supplemented with valeric acid (HVa) as feedstock, aiming to enhance the 3HV monomer content within the synthesized PHA and thereby expand the range of potential applications for this polymer.

A sequencing batch reactor (SBR) with a 2 L working volume was operated under Feast/famine regime strategy as selective pressure for the enrichment of the culture with PHA-accumulating organisms. The SBR was operated with 3 cycles/day of 8 hours each, at room temperature (20 ± 1.5 °C) with a hydraulic retention time (HRT) and a solids retention time (SRT) of 14.5 h and 3 or 4.5 d, respectively. The organic loading rate was set at 60 Cmmol/(L · d), and the C/N/P molar ratio selected was 100/5/2. The salted conditions (30 gNaCl/L) were artificially imposed through salt addition. Accumulation assays were performed with two feedstocks: the same fermented cheese whey used in the SBR, and the fermented cheese whey supplemented with HVa (relative content 30% CmmolHVa)

The two SRT tested showed a relevant impact on the performances of the selected culture in the SBR as reported in the table. The culture selected with the lower SRT presented a more efficient selection. In general, a great preference for the consumption of butyric acid was observed and the co-polymer accumulated was P(3-hydroxybutyrate-co-3-hydroxyhexanotate) with 6% of 3HHx content.

Parameter @SBR	SRT = 4.5 d	SRT = 3 d
Feast/famine [h/h]	0.09 ± 0.01	0.06 ± 0.01
PHA_{max} [%wt., VSS basis]	18.0% ± 1.8%	35.5 % ±
VFAs specific consumption rate [$Cmmol_{VFA}/(Cmol_X \cdot h)$]	0.35 ± 0.00	0.65 ± 0.09
PHAs specific production rate [$Cmmol_{PHA}/(Cmol_X \cdot h)$]	0.22 ± 0.01	0.52 ± 0.10
Specific storage yield [$Cmmol_{PHA}$ / $Cmmol_{VFA}$]	0.64 ± 0.03	0.80 ± 0.07

In PHA accumulation, the culture selected at an SRT of 3 days exhibited strong storage capacity, achieving a maximum PHA content of 73.0% wt. and 76.5% wt. (VSS basis) without and with HVa addition, respectively. Interestingly, despite being selected without HVa, the culture displayed even higher PHA content in the accumulation batch with this VFA. This resulted in a terpolymer, P(3HB-co-3HV-co-HHx), with 66% wt. of 3HB, 30% wt. of 3HV, and 4% wt. of 3HHx. In conclusion, results suggest

the viability of the valorisation of saline wastewaters into valuable biopolymers such as PHAs using MMC. This is of great interest for cost and environmental impact reductions.

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L51: **Novel Continuous-feeding Process for Polyhydroxyalkanoates Production with Mixed Microbial Cultures**

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Polyhydroxyalkanoates (PHAs) are a family of biologically synthesized polyesters which are considered among the most promising substitutes of conventional synthetic plastics being both biobased and fully biodegradable in the environment [1]. Current industrial processes for PHA production are based on the use of pure or recombinant microbial cultures grown on unbalanced growth media under sterile conditions, which contributes to the high cost of PHA manufacturing [1]. The possibility to produce PHAs from mixed microbial cultures (MMCs) on several waste feedstocks (e.g., food waste, agro-industrial wastewaters) to reduce the production costs has been widely investigated. When dealing with MMC it is essential to apply a multi-stage process which involves the microbial selection into PHA-storing microorganisms (starting from an activate sludge), typically consisting in the alternance of excess (feast phase) and lack (famine phase) of external carbon source. This is generally performed in a Sequencing Batch Reactor (SBR), in which oversized pumps are required to manage high volumes of liquid streams in a short period of time, contributing to the increasing of the process capital costs. Along this line, in this research an innovative continuous process for the microbial selection has been developed, consisting in two reactors functioning as the feast reactor (tubular configuration, 1L working volume) and the famine reactor (continuously stirred tank reactor, 5 L). The feast reactor was continuously fed with a synthetic mixture of acetic and propionic acids (65% and 35 %, respectively, in terms of Chemical Oxygen Demand, COD) at a flow rate of 6 L/d (Q_E). The two reactors were connected through a recirculation flow rate (Q_R) and the effect of the recirculation factor ($R_C = Q_R/Q_E$), in the range from 1 to 8, as well as of the organic load rate (OLR, in the range from 2.12 to 8.50 gCOD/Ld) on the process performance has been investigated. The highest average intracellular PHA content in the feast reactor (34±2%, wt/wt) was obtained at an R_C of 4 and an OLR of 2.12 gCOD/Ld. However, during the batch accumulation step the best working condition was at $R_C = 8$ with an average PHA content of 58±5 % (wt/wt) [2]. Based on these results, the condition at $R_C = 8$ has been also investigated at a high OLR (4.25 gCOD/L). Preliminary data indicated an average value of the intracellular PHA content of 9% (wt/wt) during the selection and 70% (wt/wt) at the end of the accumulation batch tests. Overall, the obtained results are promising for the development of this novel technology for MMC-PHA production and point out that the recirculation factor and the organic load rate are key parameters for the process performance.

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author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them.

L52: **Extraction of a Medium-chain Length Polyhydroxyalkanoate Using Green Solvents**

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Growing backlash against oil-based plastics has led to an ever-increasing interest in bio-sourced materials. Biopolymers such as polyhydroxyalkanoates (PHA) could be an alternative to produce more environmentally friendly materials. Not only are medium chain length PHA (mcl-PHA) biosourced, biodegradable and biocompatible, they also present elastomeric properties which make them more attractive than regular short chain PHA (scl-PHA like PHB), especially for medical applications [1,2].

This work aims at extracting mcl-PHA from bacteria using eco-friendlier alternatives to conventional solvents, already used in previous work or at an industrial scale [3]. Hence, five green solvents, namely 1,3-dioxolane, 2-Methyltetrahydrofurane, 2-butanone, Cyclopentyl methyl ether and ethyl acetate have been selected and compared with conventional solvents, acetone and dichloromethane. Those green solvents have been selected using a method based on Hansen Solubility Parameters (HSP) with Hoftyzer/Van Krevellen and Hoy approaches [4]. Soxhlet apparatus has been used to test the extraction performance for the selected solvents. Results showed higher performance for the green solvents allowing a yield of 100% within 30 min of extraction, compared to conventional solvents which reached a yield of 80 to 100% within 4 hours, with a purity always superior to 80%. The extraction yield determination is based on a PHA quantitative analysis using GC-FID and GC-MS chromatography. In addition, transmission electron microscopy has been used to compare the effect of each category of solvents on the bacteria cells, showing a faster cell membrane alteration with the green solvents used.

Furthermore, the energy consumption has been considered to compare tested solvents. The energy consumption was higher for the conventional solvents than for the green ones among which 2-Butanone was the less consuming one.

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L53: **Delving into the Genomic Basis of *Pseudomonas* sp. GK13, a Prototype Strain for Extracellular PHA Degradation**

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Polyhydroxyalkanoates (PHAs) are a well-known family of biodegradable and biocompatible polymers that are synthesized by various microorganisms as carbon and energy reservoirs. Bacterial PHAs can basically be found in two different states: i) as amorphous granules inside the cell, coated with proteins and phospholipids, or ii) denatured in the extracellular milieu, after cell lysis and release to the environment. Extracellular PHA depolymerases (ePhaZ) are carboxylesterases belonging to the α/β-hydrolase fold family, produced and secreted by a limited range of bacteria. They possess the ability to hydrolyze denatured PHAs, in contrast to intracellular PhaZ, specific for native PHA granules. *Pseudomonas* sp. GK13 is a gram-negative bacterium isolated from activated sludge, which is characterized for the production of an ePhaZ that specifically degrades medium chain length PHA [1]. Although the biological relevance of this enzyme-producing bacterium and its potential biotechnological applications, its genome was not sequenced. In this work, using Illumina and Nanopore technologies, and after a meticulous bioinformatic data analysis, we obtained its full genomic sequence in a single contig. By means of a comparative analysis with other related species, we found that *phaZ* is located in a putative cluster of four conserved genes including a TetR family transcriptional regulator, previously associated to PHA metabolism, which could be involved in ePhaZ regulation. Elsewhere in the genome, distributed in different spots, four type 2 secretion system (T2SS) were identified. T2SS is the key system for secreting ePhaZ as it is determined by its peptide sequence. Its multiple versions manifest the importance of protein secretion in this strain. Additionally, a gene cluster containing a type 4 pilus (commonly related to T2SS), a T1SS and a T6SS, commonly associated with pathogeny, were found. Interestingly, other functions related to PHA metabolism were inferred by the genomic data and experimentally validated. Finally, based on the analysis of the 16S rDNA and rpoD sequences, as well as ANI and dDDH analysis, *Pseudomonas* sp. GK13 appears to be closely related to other pseudomonads strains, such as the rhizospheric Sm006 strain and *Pseudomonas* sp. TUM22785. However, all the genetic evidence indicates that this strain sets apart from the most similar type species (*P. tohonis*). Taken together, the characterization of this biocatalyst plays a significant biotechnological role in achieving a sustainable bioplastic upcycling.

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L54: **Microbial Recycling of Biodegradable Plastics into Carboxylic Acid Platform Chemicals**

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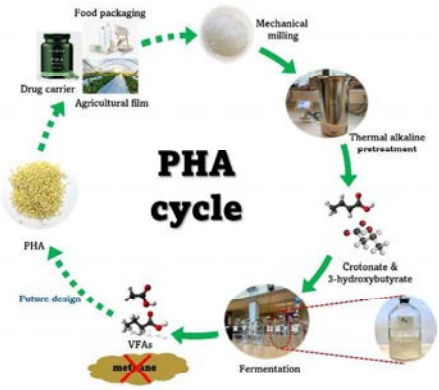
There seems to be an increasing number of plastic-based products touted as 'biodegradable'. It is clear that several biodegradable plastic products, such as certain packaging materials for vegetables, tea bags and coffee cups, are designed to be to some extent biodegradable and carry certificates such as 'Industrially compostable'. Once such materials do indeed enter composting facilities, 'organic recycling' takes place, releasing CO_2 and allowing reabsorbing by plants to close the loop. In the context of the circular economy, one can also think about potentially more effective recycling routes in which the bioplastic-based product is not mineralized into CO_2, but is microbially converted into chemical building blocks to create new biodegradable plastics, for example (see illustration PHA cycle [1]).



Our recent research envisions that the so-called carboxylate platform can be used to unlock different types of biodegradable plastics as raw materials, in addition to typical biomass waste streams [1,2]. Central to the carboxylate platform are the microbial processes that convert the waste or residual material into the desired chemicals [3]. Several industries are operating or starting commercial scale plants to produce a spectrum of carboxylic acids such as acetic acid, butyric acid, caproic acid and caprylic acid.

Here we demonstrate the successful feasibility of using 'new' PHA (polyhydroxyalkanoates) and used PLA (polylactic acid) food packaging waste to be microbially recyclable into various carboxylic acids. To allow micro-organisms to quickly absorb the bioplastics, materials were first hydrolyzed under relatively mild conditions. After this, anaerobic fermentation conditions were created to allow microbial conversions.

PHA was first found to be hydrolyzed under alkaline conditions to crotonate and/or hydroxybutyrate; after this, a mixed culture of microorganisms was able to convert apparently all solubles to acetate and n-butyrate. PLA was first hydrolyzed to lactic acids; subsequently, the mixed culture was able to convert the lactate into a spectrum of C2-C6 carbon length carboxylates. Various aspects to improve the process (such as integration of hydrolysis and fermentation) and considerations on microbial recycling of end-of-life biodegradable products will also be addressed.

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L55: **Microbial Conversion of PET Waste into Polyhydroxyalkanoates (PHA)**

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A large portion of the plastic market is held by polyethylene terephthalate (PET), a petrochemical-based thermoplastic composed of repeating units of terephthalic acid (TPA) and ethylene glycol (EG) [1], with applications in electronics, packaging, household items, textile, medicine and many other [1]. It has become a persistent pollutant worldwide due to its large-scale production and recalcitrancy to biological degradation [2]. Approximately 72% of plastic waste is not recovered [3], as current recycling rates for PET waste, especially when mixed with other plastics (i.e., multilayer packaging or fibres), are low, encouraging research into finding new sustainable technologies for PET recycling.

This work reports a new biotechnological process for converting unrecyclable post-use PET into polyhydroxyalkanoates (PHA), biodegradable, biocompatible, thermoplastic polymers [4]. In this work, Delftia spp., isolated from an aerobic activated sludge plant, treating the percolate water of a landfill, was grown on TPA from chemically degraded mixed PET plastic waste, as sole feedstock. In 2L bioreactor cultivation runs, the culture reached a biomass concentration of 1.7 g/L within 48h of cultivation, with PHA concentration of 23.8%. Overall, 14.892 g/L of TPA were consumed at that point, corresponding to growth and product yields of 0.162 gX/gTPA and 0.033 gPHA/gTPA respectively. The PHA was a 3-hydroxybutyrate (3HB) homopolymer, whose properties match those of commercial PHB and that produced from other feedstocks.

This study demonstrates the suitability of the newly isolated *Delftia* spp. strain for the valorisation of low-grade PET waste monomers into a valued-added biopolymer, thus contributing to tackle the accumulation of synthetic plastics waste in the environment.

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L56: **High Value Compounds by a *Rhodococcus* sp. Strain Using Synthetic Plastic Wastes as Feedstock**

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Synthetic plastics have become fundamental to our lives, which led to the generation of enormous plastic waste streams. Recently, innovative strategies for the upcycling of plastic wastes have been gaining attention regarding the use of synthetic plastics as carbon sources for microbial cultivation coupled with the synthesis of valuable products. The versatile metabolism of members of the *Rhodococcus* genus renders them potential towards environmental bioremediation of recalcitrant contaminants, such as synthetic plastics' depolymerization products. Furthermore, they possess the ability of storing diverse interesting compounds, including triacylglycerols (TAG) and polyhydroxyalkanoates (PHA) that are useful for various fields of applications (e.g., biodiesel production, food and cosmetic products).

This work demonstrated the ability of a *Rhodococcus* strain isolated from a plastic-contaminated site, to use chemically depolymerized polyethylene terephthalate (PET) waste containing terephthalic acid (TPA), as a carbon source. Bioreactor cultivation runs were performed, with *Rhodococcus s*p. displaying a maximum cell dry weight (CDW) of 2.7 ± 0.06 g/L while using 11.0 ± 0.32 g/L of TPA and 0.3 ± 0.00 g/L ammonium to depletion, in a 20 h batch cultivation at 30 °C, with no pH control and a dissolved oxygen concentration of 20%. The biomass had a PHA content of 5± 0.27 wt.% and a TAG content of 13± 0.43 wt.%, mainly composed by 32wt. % of hexadecanoic acid ($C_{16:0}$) and 49wt.% octadecenoic acid ($C_{18:1}$ cis-9). The biopolymer accumulated under such conditions was a co-polyester, poly(3-hydroxybutyrate-co-3-hydroxyvalerate), with a 3HV content of 60%. Fed-batch cultivations were also performed by pulse feeding of a TPA solution (7 ± 0.8 g/L), following an initial 24 h batch phase. A maximum CDW of 3.2 ± 0.03 g/L with an intracellular TAG and PHA contents of 16± 0.12 wt.% and 3 ± 0.05 wt.%, respectively, were obtained, while using 13.2 ± 0.02 g/L of TPA and 0.3 ± 0.00 g/L ammonium, at the end of the cultivation (48 h). The biopolymer produced was also a P(3HB-co-3HV), with an increased 3HV content (88wt.%). Hexadecanoic acid ($C_{16:0}$) and octadecenoic acid ($C_{18:1}$ cis-9) remained the predominant fatty acids of the synthesized TAG. The fed-batch cultivation resulted in an improvement of TAG and PHA production, with 0.48 ± 0.04 g/L and 0.09 ± 0.00 g/L, respectively, when compared to batch strategy that displayed 0.16 ± 0.05 g/L and 0.01± 0.02 g/L, respectively.

The bio upcycling for PET degradation products, namely TPA, as a carbon source, was demonstrated with the new isolate *Rhodococcus* strain being able to completely assimilate the TPA available in the medium, while presenting a good TAG production. The developed bioprocess represents a promising approach that contributes to reduce plastic waste environmental impact through a circular and sustainable approach.

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L57: **Sharing with Your Partners: The Bacterial Symbionts of the Gutless Worm *Olavius algarvensis* May Need Their Host to Degrade PHA**

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Many bacteria and archaea synthesize the bioplastic polyhydroxyalkanoate (PHA), which acts as a battery bank by storing excess carbon and energy[1-3]. For example, when bacteria and archaea have access to carbon but lack sufficient nitrogen resources to produce biomass, they store carbon in the form of PHA granules [1, 3-5]. Once limiting conditions have ended, the microorganisms can tap into their battery bank to jumpstart their metabolism and begin growing again [1, 3, 6, 7]. To degrade the stored PHA, microorganisms use an alpha/beta hydrolase called PHA depolymerase (PHAD). The primary symbiont of the gutless oligochaete *Olavius algarvensis*, *Candidatus* Thiosymbion sp., synthesizes PHA [8]. *Ca*. Thiosymbion is a sulfur oxidizing gammaproteobacteria that chemoautotrophically fixes CO2. These symbionts, along with several secondary symbionts, provide *O. algarvensis* so sufficiently with nutrition that the animal has lost its digestive system [9, 10]. Notably, symbiont produced PHA makes up over 40% of the symbiont's internal carbon storage [8]. As in all other bacteria that synthesize PHA, *Ca*. Thiosymbion sp. expresses a functional PHAD. However, other genes involved in PHA degradation and energy generation, such as a beta-hydroxybutyrate dehydrogenase, were not expressed by *Ca*. Thiosymbion. We thus asked if *Ca*. Thiosymbion sp. can use the PHA it produces to generate energy. Our metagenomic and metatranscriptomic analyses revealed that the worm host may be able to use the PHA degradation products of its symbiont and then return carbon to the symbiont for PHA synthesis. We thus hypothesize that the symbiont needs its host to use PHA for energy generation.

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L58: **Polydopamine as Conductive Coating for Enhanced Electron Transfer of *Rhodobacter sphaeroides***

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Polydopamine (PDA), a bio-inspired polymer produced by the self-polymerization of dopamine in mild and biocompatible conditions, has widespread applications as soft-material due to its ability to act as functional material [1]. PDA has a quinone-based redox matrix that can be exploited to anchor bacteria onto electrodes and for improving electron transport at the interfaces between biological and inorganic components [2]. However, efficient electron transfer requires high surface area with a precise topography to unleash the whole electrogenic power of electroactive bacteria.

Here, we show that the individual encapsulation of single bacterial cells with polydopamine ensures a better direct contact with the abiotic component and, consequently, a more efficient electron transfer. The optimization of the interface between photosynthetic bacteria and electronic components was achieved through in situ formation of polydopamine coating around single *Rhodobacter sphaeroides* cells, by exploiting the biological intrinsic machinery of living bacteria, avoiding the use of oxygen or other external oxidants to promote polymerization [3].

In bio-electrochemical systems, living and metabolically active photosynthetic microorganisms can be used for the sustainable production of energy.

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L59: **Synthesis of Phenolic Resins from Willow Biomass Extractives**

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Willow is a tree with more than 400 species and is currently grown for biomass on a 3-year coppicing cycle. Willow and its phenolic compounds have been used by humans throughout history with many applications. These include the use of its natural products as phytomedicines, use of willow foliage for veterinarian applications, pulping paper production and use as a biofuel for energy production. Additionally, willow has environmental applications acting as a biofilter for wastewater [1].

Although chemistry varies between species, stem tissue from willow biomass is predominantly composed of lignocellulosic compounds, sugars, and phenolic glucosides such as triadrin, salidroside, salicortin, salicin and its derivatives [2]. Phenolic glycosides can be easily obtained using simple extraction methods and hydrolysed for further use [3]. Resultant phenolics can be subsequently used for different applications including phenolic resin synthesis.

Phenol–formaldehyde (PF) resins are currently synthesized using petroleum derivates as the raw materials. Due to regulations and environmental concerns, the industry is finding substitutes for these raw materials. The substitutes for phenol include hydrolysed lignin (organosolv or derivate from kraft process), tannins, cardanol. While substitutes for formaldehyde include hydroxymethylfurfural, and glyoxal [4].

Our present work approaches the synthesis, properties, and potential applications of phenolic resins from willow extracts. We will discuss the use of new methods for their extraction and compare the phenolic resins synthesized partially or fully replacing the use of both phenol and formaldehyde, with the conventional phenolic resins. The chemical nature of willow extractives in addition to its biodegradability and sustainability, will potentially make it a good candidate for a greener option in polymer synthesis.

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L60: **Enzyme Based Hydrolysis for Specific Recovery of Novel Poly(lacticacid)-poly(1,5-pentanediol 2,5-furanoate) Blends Building Blocks**

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Polymer blending established as a strategy of choice in plastic industry for half of the global produced plastics amount in 2010 [1], thanks to its cost-effectiveness and versatility. As a consequence, recent efforts have been focused on new formulations, that could stand up to new biopolymer standards, such as biobased production and biodegradability.

Among the novel competitive alternatives to terephthalic acid-based plastics, 2,5 furandicarboxylic acid (FDCA) is a promising candidate, fully obtained from renewable resources. Nowadays, it is applied in innovative formulations, especially for packaging: poly(ethylene 2,5-furandicarboxylate) (PEF), poly(butylene 2,5-furandicarboxylate) (PBF), or poly(pentamethylene 2,5-furandicarboxylate) (PPeF) [2].

In order to obtained improved products for packaging, PPeF was not only characterized in its homopolymeric version, but also blended with the fully biobased Poly(lactic acid) (PLA). The resulting polymers overcome PLA brittleness and poor biodegradability issues.

Nevertheless, polymers blends imply some challenges as regards recycling, specifically in separating the single components to recover monomers.

Enzymatic hydrolysis is a valuable approach to achieve specific depolymerization. This work presents an application of *Thermobifida cellulositilytica* (Thc_Cut1) cutinase [3] for the hydrolysis of the mentioned blend. The substrate preferentiality of Thc_Cut1 proved to be focused on PPeF from PLA/PPeF blends, leaving PLA matrix intact. Moreover, it was possible to successfully recover FDCA monomer from the hydrolysate. Its high purity, assessed with ^{1}H-NMR and thermogravimetric assay, confirmed the possibility of its application in resynthesis. These results would demonstrate the full circularity of new formulations based on FDCA, from their production to post-use processing.

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L61: **Green Synthesis of Flexible Ethyl Cellulose Films Using Sunflower Oil as a Natural Plasticizer**

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Biomaterials and green synthesis are consistently successful research areas because of their sustainability and environmental observance. Plasticizers that are frequently used in the plastics industry to deform polymers are phthalate derivatives like DBP, DEP, and DBS, originating from non-renewable sources [1,2]. Despite their notable performance, they did not gain favourable attention because of their detrimental effects on both the environment and human health [3]. Research investigations are becoming increasingly interested in using organically produced substances instead of conventional plasticizers [4,5]. Vegetable oil is one such substance that has drawn a lot of interest. This study examined the use of modified sunflower oil as a plasticizer for ethyl cellulose, a popular hydrophobic biopolymer with outstanding film-forming capabilities.

The direct addition of vegetable oil to ethyl cellulose poses a concern due to its low migration, where it tends to leach out of the EC films. To address this issue and improve the plasticization efficiency without encountering oil phase separation, we have implemented a widely recognized organic reaction known as transesterification. By subjecting sunflower oil to this reaction in the presence of a base catalyst, NaOH, we have achieved remarkable results. Sunflower oil is transesterified to produce small fatty acid ester molecules, as well as intermediates like di and monoglycerides, which provide a better affinity for ethyl cellulose than sunflower oil itself. The establishment of a potent affinity between ethyl cellulose and sunflower oil is significantly influenced by the presence of NaOH. Consequently, we were able to get over the restrictions of low plasticizer migration and low deformation of EC films.

A comprehensive set of physical and chemical characterizations was conducted on the synthesized ethyl cellulose films plasticized with sunflower oil (ECSF films). The mechanical characterization of the ECSF films shows an elongation at break of about~94%, demonstrating their increased flexibility. The rheological study verified the enhanced stretchability of the ECSF films because of the shortened elastic chains. In line with the decline in tensile strength, XRD analysis confirmed the decreased crystallinity index of EC. A smooth surface devoid of porosity was detected in the SEM images of ECSF films. The FTIR spectra proved the successful transesterification of sunflower oil. Moreover, the incorporation of sunflower oil into the EC polymer matrix helped to improve its hydrophobic behavior by~21%.

To the best of our knowledge, our proposed technique for plasticizing ethyl cellulose with modified sunflower oil has yielded exceptional results, exhibiting the highest stretchability reported in the literature. The drop cast-solvent evaporation method used for the film preparation is simple, easy as well as cost-effective. This technique can be used to produce more ECSF films with varying EC: Oil ratios to have desirable applications like transdermal drug delivery systems, coating, and stretchable electronics.

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L62: **Selecting Relevant Biopolymers for Modeling of the Future Polymer Production in Germany, Belgium and Netherlands**

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To achieve a defossilisation in the chemical industry, there is a need to significantly increase the utilization of recycling and renewable feedstocks beyond current levels. However, the recycling of polymers is a complex task that requires specific processes tailored to different types of polymers, each with individual technical requirements like polymer purities and yields. Even if this recycling will be applied to a much larger extend in the future, a significant amount of the required carbon for the polymer industry has to be provided by other means [1]. According to the first assessments in the ongoing GreenFeed project, this will be at least 3 million tonnes of renewable carbon for Germany only [2]. Therefore, different types of biomass will presumably play an essential role in the defossilisation of the polymer industry.

How much biomass will be available in the future and how this biomass can be used as a feedstock for polymer production in Germany, Netherlands, and Belgium are key questions that the DBFZ is addressing in the GreenFeed project. To answer the latter question, the most important value chains from biomass to bio-polymer have been identified in terms of

- technology readiness level (TRL)
- the potential to substitute polymers that are currently produced [3]
- expected development of the actual production capacity until 2027 [4]

By attributing these value chains with suitable feedstocks and specific process step yields a connection between the projected biomass availability and polymer demand becomes possible. A feasible contribution of these pathways toward the polymer demand can thus be estimated and modelled. However, the selection and description of the value chains towards bio-based polymers are of great relevance in this project. Therefore, a presentation of the method to select the pathways and the results shall be presented and discussed with the experts at the conference.

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L63: **Greener Enzymatic Synthesis of Bio-Based Polyesters in Xylose-derived Solvents**

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With the shift towards green and sustainable chemistry, there has been an increased focus by researchers on 'greening' widely used materials such as polyesters. Namely that the feedstocks should be from recycled or bio-based sources, the method of syntheses should also be green, and the final product should be easily recyclable. Enzymatic catalysis is a sustainable alternative to traditional chemical syntheses of these materials, but in most cases, these syntheses are still performed in organic solvents with serious environmental issues such as diphenyl ether (DPE) and toluene [1,2]. DPE and other such solvents are highly hazardous for the environment and petroleum based, therefore, new solvents need to be investigated to replace them in industrial-relevant processes. One promising candidate is diformylxylose (DFX), a polar aprotic solvent derived from xylose that has successfully been used in hydrogenation, cross coupling and alkylation reactions [3].

In this work, DFX and several analogues were tested in the polycondensation of both aromatic and aliphatic monomers, in the presence of the immobilized biocatalyst Candida antarctica lipase B (CaLB). Pyridine-based monomers were selected due to their interesting thermal properties when polymerized [4] and their bio-based origin. Polymers with a Mn of up to 20 kDa were synthesised, and the polyester with the highest molecular weight was able to be cast as a film. All polymers produced had a very narrow dispersity of Mw/Mn<1.5. Lastly, a reaction solvent recycling procedure was also implemented allowing the recovery to up to 100% of the organic media. The recovered solvents were then re-used in the same polycondensation reaction leading to polymers showing the same structural features of the ones synthesized in the freshly synthesized media.

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L64: **Tunable Biobased Polyol Esters for Sustainable Future**

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The increasing awareness in sustainable, eco-friendly products and the depletion of petroleum resources have driven the market uptake of bio-based polyol esters in various applications including personal care, lubricants, and many others. Polyol esters can be directly synthesised through esterification of acid and alcohol in the presence of catalyst. The present study aims to produce a unique class of polyol esters with adjustable viscosities ranging between 25 cP to 100 cP at 25^{o}C, depending on the desired applications. This is achieved by varying the ratio between monocarboxylic and dicarboxylic acids, and from this structural uniqueness, the polyol esters are able to meet the increasing demand for biodegradable products while meeting the stringent environmental standards. The synthesis of these polyol esters uses carboxylic acids derived from palm oil and generating water as the only by-product, hence avoiding the use of petrochemical substances that can pose threats to the environment and human safety and promoting sustainability through complete biodegradability with lower environmental pollution. The final product contains less than 0.05 ppm of metal content with acid value of lower than 1 mgKOH/g.

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L65: **Exploring the Potential of Acetan-like EPS derived from *Kozakia baliensis*: Rheological Characterization in Personal Care Surfactant Systems and Galactomannan Blends**

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Rheological modifiers are the key compound in personal care products. Due to increasing customer awareness of environmental impacts and sustainability, manufacturers are forced to replace established synthetic chemicals with biodegradable compounds while retaining full product performance. The family of *Acetobacteraceae* has demonstrated their ability to produce a wide range of different exopolysaccharides (EPS) with interesting physiochemical properties [1]. One of its representatives, *Kozakia baliensis*, produces an acetan-like heteropolysaccharide. This polysaccharide exhibits a high structural resemblance to xanthan gum but lacks any additional substitutes [2].

Here, we focus on a comprehensive investigation of the physiochemical properties of EPS derived from *K. baliensis* SR-745 and *K. baliensis* LMG 27018 in selected surfactants in the field of personal care, namely lauryl glucoside (Plantacare 1200 UP), cocamidopropyl betaine (TEGO Betain F 50), sodium laureth sulfate (TEXAPON NSO) and cetrimonium chloride (Varisoft 300). The performance of both polymers was compared to native xanthan gum and a xanthan gum deletion variant lacking any acetyl and pyruvyl residues [3]. We demonstrated that minor changes in the side chain composition result in an altered performance of these polymer-surfactant compositions. Similar to native xanthan gum, blends of EPS derived from *K. baliensis* and several galactomannans displayed highly increased viscosity, thereby significantly reducing the total amount of polymer required for the desired viscosifying properties. Overall, the results of this study show the high potential of the acetan-like heteropolysaccharides derived from EPS of *K. baliensis*, which allows for overcoming downsides of native xanthan gum in industrial applications and extend the portfolio of bio-based polysaccharides in future fine-tuned personal care products.

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L66: **Biopolymer Analyses by Vibrational Spectroscopies with Applications in Microbial Biotechnology**

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Microorganisms, such as yeasts, filamentous fungi, microalgae and bacteria, are powerful cell factories for substance synthesis. Since they can produce various metabolites, including biopolymers, lipids, structural and enzymatic proteins, pigments, alcohols, and antibiotics, they have found use in the fields of industrial, pharmaceutical and agro-environmental biotechnology. Microbial bioprocess development and optimization requires technologies for process monitoring and high throughput screening, and in this respect vibrational (infrared and Raman) spectroscopy techniques have seen rapid advance and application in the recent years. Infrared and Raman spectroscopies are considered as fast, inexpensive, and highly sensitive methods for analysis of biological samples, and thus have emerged as the dominant biospectroscopy techniques. Both techniques are excellent for obtaining comprehensive and detailed information in biotechnology since they can simultaneously measure broad chemical profiles of the chemical constituents present in the bioprocess via detection of numerous functional groups.

Here, spectroscopy-based research conducted at the Norwegian University of Life Sciences will be presented [1-4]. The research group has extensive collaboration with food, feed and biotechnology industry in valorization of waste and rest materials, such as animal slaughter side-streams from meat industry, rest biomass from brewery, woody lignocellulose side-streams from forestry, and side-streams from fisheries and aquaculture industry. These waste and rest materials can be utilized and converted by microorganisms into biopolymers, biofuels, food, feed and nutraceuticals. Examples include co-production of polyphosphates, polysaccharides, such as glucans and glucosamines (chitins and chitosans), lipids and carotenoid pigments by Mucoromycota fungi [1]. Development and optimization of bioprocesses for high-yield production of target biopolymers is based on microtitre cultivation and high-throughput screening (HTS) by Fourier transform (FT) IR and FT-Raman spectroscopies [1-3]. Since wet-chemistry methods for chemical characterization of complex biopolymers are extremely time consuming, rapid vibrational spectroscopy methods are in high demand. In this respect, we have recently developed fully automated microplate-based high-throughput vibrational spectroscopy methods with multivariate models for determination of degree of acetylation (DA) for chitin and chitosan [4].

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L67: **Diving Deep into Norwegian Kelp: Sequential Extraction and Characterization of Versatile Polysaccharides**

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Norway has a well-established seaweed industry based on extraction of the high value phycocolloid alginate from wild harvested brown algae, *Laminaria hyperborea (LH).* However, alginates only account for around 15 - 40 % of the dry weight of brown algae [1] and no substantial valorization of the remaining biomass is currently performed, leaving multiple side streams containing potential valuable polysaccharides and other components unexploited.

To increase the valorization of the biomass, an integrated process for extraction of all four main polysaccharides in brown algae, namely alginate, fucoidan, laminarin and cellulose has been developed in 2023 [2]. The process was established for cultivated *Saccharina latissima* (SL) and *Alaria esculenta* (AE) but can be applied for all kelps and presents a method that reduces the generation of waste, thereby improving the environmental and economic sustainability of future seaweed biorefineries. Focus was to develop a process only applying mild chemical methods that do not compromise the molecular weights (MW) of the polysaccharides, particularly with respect to alginate - being the product with currently highest commercial value. Response surface methodology (RSM) was applied to optimize the yield of fucoidan and laminarin and assess the effect of the applied extraction parameters: pH, temperature, and time, on the MW of subsequently extracted alginates. RSM confirmed that high yields of fucoidan and laminarin can be achieved while retrieving high MW alginates. The optimized conditions were applied in a pilot scale process, where all four polysaccharides were extracted. Purity and composition of the components were evaluated combining several analytical techniques: HPAEC-PAD, NMR, SEC-MALS, CHNS Elemental Analyzer, and ash analysis. The process can be adapted to simultaneously collect mannitol, which accounted for ≤ 15 % of the dry weight. The quality of the alginates was further examined by producing hydrogels, showing that the gel properties are comparable to alginates from *LH* fronds. The study demonstrated that mild chemical extraction techniques can be combined to extract all four polysaccharides from *SL* and *AE,* providing a foundation for a multicomponent biorefinery using brown algae.

Currently we have continued to examine how introduction of targeted commercial enzymes will affect extraction yields and quality of the phycocolloids when applied within a sequential extraction process.

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L68: **The Induced Production of Extracellular Polysaccharides by *Rhodotorula* Yeast Using Sustainable Feedstock**

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Nowadays, rapid climate change has led to an increased demand for natural products that can replace chemical and synthetic compounds. This shift towards eco-friendly solutions has paved the way for the exploration of Rhodotorula yeasts, which exhibit tremendous potential as organisms capable of producing high-value polymers from food and agriculture supply chain by-products. Among all the polymers, extracellular ones have gained attention due to their unique properties and relatively simple extraction methods. Extensive research has been conducted on extracellular polysaccharides, exploring their biocompatibility, antimicrobial and antitumor properties, as well as their potential as thickeners. Despite their advantageous properties, only a few extracellular polysaccharides, primarily of bacterial origin, have been commercially produced so far.

In this study, the fermentation process for valorizing renewable substrates was developed. Five Rhodotorula yeasts were selected for high-throughput screening in Duetz-MTPS and investigated for extracellular biopolymers production. The lignocellulose hydrolysate induced production of exopolysaccharides, up to 8 $g \cdot L^{-1}$. Oppositely, synthetic media resulted in biosynthesis of only low amount of exopolysaccharides. Batch fermentation resulted in production of 13.14 $g \cdot L^{-1}$ of exopolysaccharides, 19.05 $g \cdot L^{-1}$ of biomass and 39.8 % lipids per DCW, respectively. Monosaccharide composition showed the presence of galactose, glucuronic acid, and mannose. Metal ions present in lignocellulose hydrolysate have a significant influence on exopolysaccharides biosynthesis.

Acknowledgement
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L69: **Biorefinery Development of Lignocellulosic Biomass for Bacterial Cellulose Production and Biobased Packaging Formulation**

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The aim of this work is the holistic approach of the biorefinery of protein-rich lignocellulosic biomass in order to valorise protein as matrix in biodegradable packaging films. Lignin can be used as an additive with antioxidant and antimicrobial properties to the final packaging [1]. The substrate was pretreated using a dielectric barrier discharge (DBD) non-thermal plasma reactor, and the remaining solids were enzymatically hydrolysed to produce a sugar-rich hydrolysate. Bacterial cellulose (BC) was produced by microbial fermentation using the carbon-rich hydrolysate in fed-batch fermentation and processed into bacterial nanocellulose (BNC) by acid hydrolysis with sulfuric acid (50% w/w H_2SO_4, 55°C, 48 h). Proteins were isolated from the residual solids by alkaline processing, followed by a delignification step for lignin recovery. Protein extract was composed of 53% protein and 37% lignin, due to the partially separation of these two components [2]. For the films preparation, 3.5% (w/v) of protein, 30% (w/w) glycerol, 1% (w/v) tween 20% and 10% (w/w) BNC were mixed by Ultra-Turrax (12,000 rpm, 5 min) and heated at 80° C for 30 min. Three concentrations of lignin (5, 10, 15% w/w) were incorporated in the protein-based films using two different solubilization methods, lignin solubilization at pH 12 (L) and lignin particles formed by the antisolvent precipitation method (LP). The protein-based films were characterized, evaluated on fresh salmon packaging (11 days, 4°C) and compared with conventional PVC membrane (microbial growth, sensory analysis). Mechanical test showed that the incorporation of lignin led to lower tensile strength and elongation at break. Brown Intext ranged between 3.9 and 8.2%, whereas the transparency (%$Transmittance_{600nm}$) was less than 2%. DPPH inhibition of the films was increased by the addition of LP from 39.8% to 47.2%. Shelf-life analysis showed that the protein-based films may be an alternative solution for salmon preservation at 4 °C considering microbial growth in compared with commercial PVC membrane. To concluede, lignin addition affected the final properties of the films and it is a promising antioxidant agent for packaging applications.

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P1: **Valorization of Wine Lees to Polyhydroxyalkanoates**

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In the last years, research focused on the conversion of waste biomasses, and several other residues into value-added products such as new materials and chemicals [1]. In particular, the valorization of winery waste has received significant attention. The International Organization of Vine and Wine's report shows that, in 2020, about 5 million of tonnes of wine was produced only in Italy.

Wine lees, one of the main by-products of the wine industry, are a polluting substrate, and massive volumes (up to 0.3 million t) are indeed yearly produced in Italy. [2].

This work aims to exploit wine lees to obtain high-added value products. Although these residues have been already proposed for the recovery of compounds such as tartaric acid, ethanol, and antioxidants to reduce disposal costs [3], this is the first time that wine lees were explored as a substrate for the proficient microbial production of polyhydroxyalkanoates (PHAs), a completely biodegradable and biocompatible bioplastic.

With this purpose, the PHAs accumulation by *Cupriavidus necator* DSM 545, *Hydrogenoflava pseudoflava* DSM 1034, and *Pseudomonas oleovorans* DSM 1045, was studied in different growth conditions on Prosecco wine lees and PHAs were determined in bacterial biomass. *C. necator* DSM 545 displayed the highest performances with a CDW (cell dry weight) and PHAs content of 4.90 ± 0.41 g/L and 60.30 ± 3.97%, respectively.

Although fine-tuning fermentation parameters is needed to improve PHAs yields, these preliminary results pave the way for the future industrial exploitation of wine lees into bioplastics.

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P2: **Testing Biomass Mastication for Assisting the Downstreaming of Polyhydroxyalkanoates Produced from Mixed Microbial Cultures**

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PHA downstreaming from its biomass represents 50% of its overall cost. Here, in an attempt to assist downstreaming, mastication of wet biomasses is tested as a new mechanical continuous biomass pretreatment with potential for industrial upscaling [1]. Downstreaming conditions where both product recovery and purity are low due to the large amount of treated wet biomass (50% water) were targeted with the following process: extraction of 20 g in 100 mL chloroform at 30 ºC for 2 h, followed by 4.8 h digestion of 20 g in 0.3 M NaOH. Under the studied conditions, a nearly 50% loss of PHA was seen during digestion after mastication. PHAs downstreamed by digestion with large amounts of impurities started to degrade at lower temperatures, but their melt elasticity was thermally stable at 170 ºC. As such, these materials are attractive as fully PHA-compatible processing aids, reinforcing fillers or viscosity modifiers. On the other hand, wet biomass mastication before solvent extraction improves PHA purity and thermal stability as well as the melt rheology, which recovers the viscoelasticity measured with a PHA extracted from a dried biomass. Yet, as chloroform-based processes show no sustainable industrial applicability, prospects with eco-friendly natural deep eutectic solvent [2] showing viscosity levels enabling extrusion-based downstreaming will be discussed.

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P3: **PHA Synthesis Genetics and Genomics in *Halomonadaceae* Family**

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Polyhydroxyalkanoates (PHA) are polyesters accumulated by numerous microorganisms under stress condition and excess of carbon source in the form of inclusion bodies, as a reservoir of carbon and energy source [1]. More than 150 different monomers can be combined to generate different types of PHA which are promising bioplastic due to its biodegradability, renewability, and sustainability [2]. Despite of having most prominent bacteria for PHA production, genetics and genomics of PHA production in *Halomonadaceae* family is not entirely understood.

The aim of the current work is to analyse the PHA production among halophilic bacteria isolated from former salt mine Solivar near Prešov, to identify isolates, and to analyse genetic background of PHA production. The molecular identification methods (MALDI-TOF MS analysis and 16S rRNA sequencing) reveal the presence of multiple gammaproteobacteria of *Halomonas, Idiomarina, Chromohalobacter*, and *Halovibrio* genera, some of them representing possibly new species. 5% NaCl and 2% glucose as sole C-source in cultivation media was used for PHA screening in all obtained isolates using Nile Blue A, Sudan Black B staining and confocal and bright field microscopy. PHA production was observed in members of *Halomonadaceae* but not in *Idiomarinaceae* family. Whole genome sequencing (NGS) of HP20-15 (*Halomonas* sp.), HP20-50 (*Idiomarina* sp.), HP20-59 (Halovibrio sp.) and HP20-39 (*Chromohalobacter* sp.) was performed to analyse genetic and genomic basis of PHA production.

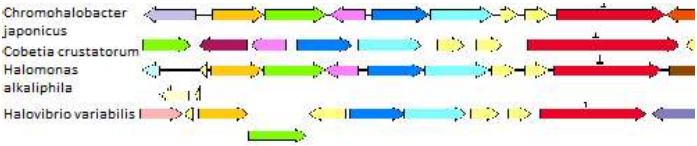

Figure 1 Genome organization comparision of PHA synthase gene in Halomonadaceae family.

In all PHA producing bacteria type I PHA synthase was detected. Comparative sequence analysis of PHA synthases in *Halomonadaceae* showed that while PHA genes are relatively well conserved at genomic level (Fig. 1) there is variability in PHA synthase gene size. Clear evidence of horizontal gene transfers among members of *Halomonadaceae* family. The outcome of this study will enlighten genetics and genomics of PHA synthesis in *Halomonadaceae* family and will contribute a tip of the iceberg in a world of bioplastic.

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P4: **Impact Of Stress and Nutritional Conditions on Growth and PHA Synthesis in the Thermophilic Bacteria *Caldimonas thermodepolymerans***

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Caldimonas thermodepolymerans, a moderate thermophile that has shown the ability to produce polyhydroxyalkanoates (PHAs) at high yields on low nitrogen xylose-rich medium [1]. PHAs in general were produced from bacteria using agro-industrial waste as feedstock, which -compared to the conventional plastics made from petroleum oil- gives this polymers family high potential [2]. In addition, *C. thermodepolymerans*' ability to grow and produce the polymer at high temperatures (45°-55°C) makes it a potential candidate for industrial appropriation. Therefore, *C. thermodepolymerans*' metabolism, physiology, and genetics are studied, with the focus on selection of significant stress factors that trigger high PHA production, scaling up to mass production, and genetic engineering to enhance productivity [3].The focus on the stress factors includes nutrients limitation, salinity, pH and oxidative stress and the identification of *C. thermodepolymerans* regulator genes related to this stress, as there is a potential in adapting the stress response for enhanced PHA production [4]. To explore the impact of stress on *C. thermodepolymerans*, RNA sequencing for different conditions was done, supported by RT-qPCR studies. In addition, the amount of polymer formed inside the cell was determined through Nile red assay and through two phases extraction. By establishing those expression profiles for the bacterium in different conditions, the stress was shown that it affects PHAs production, and it also impacts other cellular functions. For instance, PHA production is enhanced while motility ability is diminished, and the flagella related genes (*flg*, *mot* & *fli*) are down regulated under salt stress. The oxidative stress generated from hydrogen peroxide has a similar effect on PHA production.

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P5: **Production of Polyhydroxyalkanoates in Bacterial Isolates from Food**

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Polyhydroxyalkanoates (PHAs) have extensive applications in the medical field and potentially in other sectors due to their biocompatibility and biodegradability. Gaining insights into the biosynthetic pathways of PHAs could facilitate the identification of optimal conditions (substrates) for producing specific types of PHAs using particular microbial strains. This research aimed to develop a methodology for identifying potentially valuable PHA-producing bacteria. Within this study, PCR analysis revealed the presence of all four classes of PHA synthases and other genes involved in PHA formation (*fabG*, *phaA*, *phaB*, *phaG*, and *phaJ*) in 64 bacterial collection strains and food isolates. The strains encompassed various genera, including *Acinetobacter*, *Bacillus*, *Cupriavidus*, *Escherichia*, *Klebsiella*, *Lelliottia*, *Lysinibacillus*, *Mammaliicoccus*, *Oceanobacillus*, *Pantoea*, *Peribacillus*, *Priestia*, *Pseudomonas*, *Rahnella*, *Staphylococcus*, and *Stenotrophomonas*. Carbon sources such as fructose, glucose, sunflower oil, and propionic acid were utilized, and PHA production was assessed through Sudan black staining, Nile blue staining, and FTIR techniques. Class I synthase and phaA genes were most frequently observed, indicating the strains' capacity for synthesizing PHA from carbohydrates. Among the bacterial strains tested, the *Pseudomonas* genus demonstrated the ability to utilize all carbon sources examined. Notably, the *Pseudomonas extremorientalis* strain exhibited potential for biotechnological applications.

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P6: **Usage of Cyanobacteria for Carbon Capture and Polyhydroxyalkanoate Production**

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Topic that resonates strongly with society is carbon dioxide pollution. More than 35 billion tons of CO_2 were emitted to the atmosphere in 2021[1]. 100 billion tons of carbon dioxide are naturally absorbed and emitted every year through natural cycles of Earth[2]. Capture and usage of carbon dioxide together with reducing the CO_2 emission per year could slow down warming of Earth surface.

Cyanobacteria are phototrophic gram-negative bacteria belonging to the prokaryotes. They are ecologically extremely important as they are capable of oxygenic plant-like photosynthesis. This ability caused cyanobacteria played a key role not only in biological, but also in geological history of our planet[3,4].

Polyhydroxyalakanoates (PHAs) are microbial biopolymers and occur as intracellular granules. PHAs are biopolymers with similar properties as petrochemical synthetic plastics[5]. PHAs do not solve in water, they are resistant against UV light, they are biocompatible and non-toxic. They are fully biodegradable, PHAs decompose biologically to water and carbon dioxide under aerobic conditions[6,7]. Nevertheless, PHA production is not only time-consuming, but also financially demanding, for reducing the production cost the waste materials are being used as an input material while serve as an energy source for heterotrophic bacteria. As the cyanobacteria are capable of photosynthesis, they do not demand carbon source and for PHA synthesis they need only CO_2 and light source. Technology using the waste CO_2 as a carbon source for cyanobacteria producing PHAs could be greatly beneficial for environment.

In this study, two *Synechocystis* strains were tested for PHAs production, as cyanobacteria naturally produce 3-hydroxybutyrate (3HB). Both strains were also tester for copolymer production – copolymer of 3HB and 4-hydroxybuytrate (4HB) which was for the first time reported in cyanobacteria in *Synechococcus* sp. PCC7002 in 2015[8] an in PCC6803 in 2020. The composition of co-polymer differs in *Synechocystis* sp. PCC6803 and *Synechocystis salina* CCALA192 and the composition of co-polymers also varies depending on the precursor concentration. PCC6803 and CCALA192 cultivated with acetate and γ-butyrolactone accumulated co-polymer 3HB-co-4HB with different fractions of 4HB.

Role of PHAs in cyanobacterial metabolism seems to be very complex. In the beginning of our work with cyanobacteria, the production of PHB was around 2 % of PHB in cell dry weight. So deeper understanding together with well-adjusted cultivation conditions can make cyanobacteria the PHA-producers in present and in future rich in CO_2.

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P7: **Production of Polyhydroxyalkanoates from Wheat Bran Hydrolysates Using Halophilic Bacteria**

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The global issue of accumulating by-products from the food industry represents a significant challenge. Lignocellulosic materials belong to the group of these by-products. These materials have diverse applications, such as biofuel production, chemical synthesis, and the production of biodegradable polymers known as polyhydroxyalkanoates (PHAs). PHAs are biodegradable, biocompatible and can be generated from renewable resources as intracellular granules by various prokaryotic microorganisms.

Lignocellulosic materials consist of cellulose, hemicellulose, and lignin. Through chemical and biochemical pretreatment, these materials can be hydrolyzed to obtain fractions suitable for synthesizing valuable substances. Cellulose and hemicellulose can be broken down into monosaccharides by high-pressure and thermal pretreatment and enzymatic hydrolysis, which serve as a carbon source for bacteria capable of producing PHAs. Utilizing lignocellulose-based resources as a substrate for PHA production presents an opportunity to reduce production costs.

In this study, we analyzed the composition of wheat bran hydrolysates obtained by combined chemical and enzymatic hydrolysis. The hydrolysates were evaluated mainly with respect to the concentration of reducing saccharides. Subsequently, we cultivated two selected halophilic bacterial strains (*Halomonas halophila* CCM 3662 and *Halomonas organivorans* CCM 7142T) on these hydrolysates. Firstly, we cultivated these two bacterial strains in flasks and then we transferred the process to laboratory bioreactors (batch cultivations). The content of PHAs was determined using gas chromatography with a flame ionization detector. *Halomonas halophila* showed the highest PHA production in flasks (2,5 ±0,1 g/l), accounting for up to 68% of the dry cell weight. However, a higher PHA yield (3,8 ±0,1 g/l) was obtained by the cultivation in the bioreactors by *Halomonas organivorans* (≈ 37% of cell dry weight).

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P8: **Using Of "Hot" Genus *Aneurinibacillus* To Produce Unique Polyhydroxyalkanoate copolymers**

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Polyhydroxyalkanoates (PHA) are microbial polymers that serve as a source of carbon and energy for bacteria. Moreover, they are a suitable alternative to polymers produced from fossil resources. Although they have many advantages - biocompatibility, biodegradability, wide application use; their production costs are still high. Possible cheaper production is possible by the use of waste substrates as feedstock or by the recently very popular employment of extremophile producers.

Extremophilic microorganisms are those organisms that live in extreme conditions. High or low temperatures, high salt concentrations or acidic or basic pH that allows the use of semi-sterile or even non-sterile conditions leading to cheaper biotech process. PHA producers are abundant among extremophiles, and halophilic and thermophilic microorganisms seem to be of particular interest.

Over 40 thermophilic strains were obtained using an original isolation protocol that uses changes in osmotic stress as a selection. After characterization, a large proportion of these strains were assigned to the previously poorly described *Aneurinibacillus* species. However, the genus *Aneurinibacillus* appears to be a very interesting PHA producer with great potential. Firstly, it can use waste glycerol as a carbon source, but also synthesizes interesting and unusual copolymers when suitable precursors are used. Isolates of this genus are able to incorporate PHA monomers such as 4-hydroxybutyrate in high fraction content (up to 90 mol.%) into their polymers. Also, with the use of a suitable precursor, 4-hydroxyvalerate, 5-hydroxyvalerate, but also monomers of mcl- PHA namely 3-hydroxyhexanoate or 4-hydroxyhexanoate can be incorporated into the PHA structure. Moreover, their metabolism of PHA synthesis is very interesting, both due to their unique PHA synthase with very low substrate specificity. Therefore, one of the strains was also deposited as a patent culture in the Czech collection of microorganisms.

P9: *Schlegelella* or *Caldimonas,* Uncovering Genomic Features of Species *aquatica* and *thermodepolymerans*

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Genus *Caldimonas*, until recently known as *Schlegelella*, encompasses a diverse group of Gram-negative thermophilic species. The ability to utilize polyhydroxyalkanoates (PHAs) under extreme temperature conditions makes these bacteria highly attractive candidates for various biotechnological applications. To fully harness the ecological potential of *Caldimonas*, a complex understanding of their genomic features and functional capabilities is crucial. Therefore, we conducted a comprehensive genomic analysis of two *Caldimonas* species, *C. thermodepolymerans* and *C. aquatica*.

In this study, we present the crucial genomic elements involved in PHA biosynthesis and sugar utilization we identified through genome sequencing and analysis of *C. thermodepolymerans* DSM 15344^T, DSM 15264, LMG 21645 strains, and *C. aquatica* LMG 23380^T. Additionally, we conducted a phylogenomic analysis to understand the relationships of bacteria within the *Caldimonas*/*Schlegelella* genus.

Our findings reveal that the PHA biosynthetic pathway in both *C. thermodepolymerans* and *C. aquatica* is regulated by a complex network of genes, including the *phaCAB* operon. Notably, we discovered genomic elements unique for *C. thermodepolymerans* species that enable the efficient utilization of xylose as a preferred source of carbon and energy. In particular, we identified a distinct regulatory mechanism responsible for the xylose preference characterized by a unique *xyl* operon consisting of seven genes.

Furthermore, we conducted the phylogenomic classification of the *Caldimonas* genus by comparing its species to genera of the family *Sphaerotilaceae* and additional Gram-positive and Gram-negative PHA-producing bacteria. The analysis revealed a clearly distinguishable cluster of bacteria within the *Caldimonas* genus and confirmed that the genera *Schlegelella* and *Caenibacterium* present synonyms to *Caldimonas*. In addition, we proved that *C. aquatica* and *C. koreensis*, until recently classified as orphan species *Schlegelella aquatica* and *Schlegelella koreensis*, represent distinct species of the *Caldimonas* genus.

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P10: **Production and Characterization of Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) Using Methane and Valerate by Methanotrophs, *Methylocystis* sp. MJC1**

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Polyhydroxyalkanoates (PHA) are biodegradable plastics that can replace conventional petroleum-based non-degradable plastics. Methane is considered a next-generation carbon feedstock for industrial biotechnology [1]. Methanotrophs is the microbe that can utilize methane as their sole carbon and energy source [2,3]. In this study, we investigated the production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) using type II methanotroph, *Methylocystis*. Methane was continuously fed and valerate was step-wisely added. Production parameters such as pH, valerate concentration, and valerate feeding time were optimized for enhancing the yield and 3HV mole fraction of poly(3-hydroxybutyrate-co-3-hydroxyvalerate). Under optimal conditions, we obtained 20.77 g DCW/L of biomass composed of 11.2 g/L of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) with a 3HV mole fraction of 19%. The thermal and mechanical properties such as melting temperature, elongation to break, tensile strength, and Young's modulus were also analyzed.

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P11: **Valorization of Microalgae Side Streams for Sustainable Polyhydroxyalkanoates Production**

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The activity of algae biorefineries can generate a diversity of microalgae side streams that must be treated/valorized in order to assure the eco-efficiency of the algae industry. Furthermore, in the framework of a circular economy, this valorization can occur through the production of different compounds, such as the biopolymer polyhydroxyalkanoate (PHA). PHA is a biodegradable polyester with properties similar to those of petrochemical-based plastics, thus being a proper substitute to conventional plastics.

The goal of this study is to produce PHA using microalgae side streams as feedstock by employing a three-step method that uses mixed microbial cultures (MMC): i) acidogenic fermentation of the feedstock to produce an effluent rich in volatile fatty acids (VFAs); ii) enrichment of a MMC in PHA storing organisms; iii) PHA accumulation.

This project kicked-off with fermentation batch tests of different microalgae side streams (specifically *Nannochloropsis*, *Chlorella*, *Haematococcus* and a mixed phototrophic consortium) to assess the fermentation potential of each stream. Additionally, pre-treatment of the streams was conducted to evaluate the improvement of COD solubilization prior to batch fermentations.

Currently, two different reactors are being employed to carry out the first step of acidogenic fermentation of a *Nannochloropsis* side stream: a 1.75 L Up-flow Anaerobic Sludge Bed (UASB) reactor and a 1 L Continuous Stirred Tank Reactor (CSTR). This side-by-side operation will allow to compare the VFA production profile of the two systems and determine operational conditions that maximize productivity.

Subsequently, the process will be up-scaled to pilot production of around 100-200 L reactors comprising all the 3 steps of the biopolymer production.

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P12: **Unexplored Extremophile Bacterium, *Pseudohalocynthiibacter aestuariivivens*, as a Sustainable Bioplastic Producer**

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Here we demonstrate that extremophiles, a class of microorganisms that thrive in harsh environmental conditions (e.g., hyper-salinity, polluted environments, extreme temperatures) [1], possess a significant potential to be providers of novel bio-based products, including bioplastics, such as Polyhydroxyalkanoates (PHAs) [1]. PHAs are biodegradable, thermoprocessable polyesters capable of replacing petroleum-based plastics and help tackle plastic pollution. PHAs are microbially produced as an energy source to maintain growth and cell survival under stressful conditions [2]. PHAs are suited for several industrial applications, but their market success is limited due to high production and downstream processing costs, contamination prevention procedures, low productivity, and poor product quality [2].

This study reports for the first time the ability of an unexplored extremophile bacterium, named *Pseudohalocynthiibacter aestuariivivens* P96, to produce PHAs. *P. aestuariivivens* 96, which was isolated from a heavily polluted river mouth characterised by high salinity [3], showed to be able to accumulate 4.73 g/L of Polyhydroxybutyrate (P(3HB)), equal to 87% of total cell dry weight. The PHB produced was a stiff, brittle, and hydrophobic polymer in nature with properties comparable to those of synthetic plastics and good processability into porous, 3D constructs, for medical applications, as described by Esposito et al., 2023 [3].

This study represents a significant step toward the sustainable commercialization of biobased PHA's via the novel use of extremophiles.

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P13: **Exploring the Polyhydroxyalkanoates Production in Psychrophilic/Psychrotolerant Bacteria**

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Microorganisms can accumulate many specific biopolymers to store them as a carbon source in unfavourable conditions. Bacteria produce polyhydroxyalkanoates (PHA) which have great potential to replace conventional plastics due to their biodegradability. However, the main disadvantage is the high cost of production (primarily associated with the biotechnological process itself). Therefore, the research is focusing on ways to overcome this obstacle, by screening many bacterial strains that can survive extreme conditions. The main advantage that extremophiles bring from the perspective of biotechnology is a reduction in the cost of sterility of the work, which is what benefits in conjunction with PHA. Additionally, there is limited research devoted to psychrophiles and psychrotolerants, microorganisms living in cold areas.

In this work, the results of the screening involving seventy-eight cold-adapted bacterial strains isolated from East Antarctica, specifically from meltwater ponds and green snow, are presented. Both genotypic and phenotypic methods were used for the selection of potential PHA producers. The polymerase chain reaction demonstrated the presence of genes encoding PHA synthases, namely PHA synthases class I and II. From the phenotypic testing, staining with Nile Red and Sudan Black B, FTIR spectroscopy in combination with a high-throughput microculture system (Duetz-MTPS), and gas chromatography were used to explore the bacterial biomass for the presence of PHA. Cultivation was conducted on five different media, four of which were limiting nutrients.

Based on the results the highest biomass production and PHA accumulation (0.6 g/L) showed Pseudomonas sp. belonging to the green snow isolates. Interestingly, a high synthesis of PHA, specifically P(3HB), was observed on complex BHI medium with extra glucose addition, which did not provide the nitrogen limitation that is typical for the stimulation of the PHA accumulation. Although fluorescent staining did not provide indicative results for psychrophiles and/or psychrotolerants, it can be assessed that the presence of promising producers among polar bacteria was confirmed by PCR, FTIR, and GC, and the yield of PHA can be further increased by optimizing the media composition and growth conditions.

P14: **Differential Expression Analysis of RNA-Seq Data for Identification of Housekeeping Genes in *Rhodospirillum rubrum* DSM 467**

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Rhodospirillum rubrum is a bacterium that has gained attention due to its ability to produce and accumulate polyhydroxyalkanoate (PHA) in a form of intracellular granules, which serve as a carbon and energy storage mechanism. Apart from that, PHA is sustainable, biocompatible, biodegradable and also a promising replacement for petrochemical polymers. *Rhodospirillum rubrum* is currently intensively studied for its metabolic flexibility and diverse biosynthetic strategies, further expanding our understanding of PHA synthesis and its potential applications.

Differential expression analysis of transcriptomic data is one the most important tools for understanding gene regulation under specific conditions. Yet, it can also help with identification of genes that are not differentially expressed but maintain stable gene expression and preserve basal metabolism. These housekeeping genes can used as reference genes for normalization of gene expression data obtained by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR), a crucial step for accurate data interpretation. Reference genes should be chosen individually for each particular organism and that can be accomplished by analysis of available transcriptomic data [1].

In our study, we focused on identification of housekeeping genes in *Rhodospirillum rubrum* DSM 467 by conducting differential expression analysis of available RNA-Seq data using selected tools (empiReS, DESeq2, limma-voom, edgeR). We compared the results with respect to the identification of housekeeping genes. We evaluated how the selection differed among the tools and which tools are the best for identification of the housekeeping genes. Our results will be used to improve our method for identification of novel housekeeping genes based on RNA-Seq data and enable the identification of candidate reference genes for *R. rubrum*. Subsequently, these candidates will be validated through RT-qPCR experiments.

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P15: **PHB Production by Continuous Fermentation – Approach Towards Constant Product Quality?**

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Polyhydroxybutyrate (PHB) is a promising biopolymer for applications requiring fast biodegradation. However, its high production costs and the lack of matured large-scale production processes hinder its further distribution. Discontinuous batch and fed-batch processes are still the state of the art and most widely used in industrial scale PHB production [1]. Besides complete substrate consumption and other advantages, these processes do not reach maximum productivity due to repetitive set-up times.

Continuous processes for PHB production have been described in literature as an excellent means to increase overall productivity and efficiency [1] and can face the challenge to deliver constant product quality over several batches. Therefore, research at the *Technische Hochschule Nürnberg* focuses on the utilization of the developed two-stage continuous process [2] for various fields of application. Firstly, the utilization of industrial waste streams like crude glycerol. Secondly, the use of the developed continuous process in order to achieve a constant product quality over the long term and thirdly to influence the product quality by means of technically adjusted process parameters.

In a continuous process run as chemostat, parameters such as substrate, biomass and product concentration are constant during steady state. For PHB production, the process operated in two spatially separated stages exploiting the growth independent accumulation of PHB: The first stage ensures the required microbial growth, the second one delivers optimum PHB accumulation conditions. The environment for the cells such as carbon source concentration and residence time can be technically set relatively precisely and reproducibly [2,3].

So far, the hidden benefit of the link between steady state process conditions in continuous processes and stable product quality has not been addressed or proven. This is where current research focuses on. Indicators for fermentation linked product quality are the average molar mass of PHB and the width of its distribution (known as polydispersity), as the structure and chain length of the polymer molecules directly influence the material and processing properties of the later plastic [3]. The underlying working hypothesis is that the average molar masses and their distribution depend on the surrounding conditions of the cells. Further, the process parameters are optimized to influence average molar mass and the polydispersity of the produced polymers.

Due to the mentioned advantages, it is assumed that the developed process is not only well suited to study the influence of process parameters on the average molar mass, but also to increase constant product quality. Hence, the ability of predefining material characteristic during fermentation can be a viable step to facilitate the market penetration of biopolymers and other products from bioprocesses.

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P16: **Valorization of Varying Industrial Waste Streams for PHB Production by Continuous Fermentation**

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The use of industrial waste streams for the production of polyhydroxybutyrate (PHB) is of high econonomic and ethnical interest, as it holds the potential to reduce production costs and implicts no conflict with resources used for food & feed. Depending on the initially used feedstock and process parameters, waste streams show very high variablitity in compostition regarding nutrients, inhibitory substances and interfering particles between several production runs. These variabilities can challenge the use for bioprocesses conducted in batch or fed-batch operation, as product yield and quality are unpredictably affected when differing charges are used. Continuously operated processes can be an approach to overcome these side effects, as they show an intrinsic stability when run as chemostat (short for 'chemical environment is static') [1].

One focus of the research group is the application of continuous fermentation to overcome problems regarding the varying nutrient-levels in different industrial waste streams. For this work C. necator DSM545 was used, which shows no growth associated PHB synthesis: Cell growth occurs under optimal supply of nutrients to the cells (phase 1) and PHB storage occurs under nitrogen deficiency (phase 2). The PHB production was thus implemented as continuous process with two bioreactor in series. [2]

The waste streams used are crude glycerol from a plant-based biodiesel production plant and anaerobic digestate from a biogas plant processing food waste from restaurants and supermarkets. Depending on the respective feedstock and process parameters, these waste streams vary significantly in nutrient quality, composition and concentration. The glycerol concentration of crude glycerol varies from 60 to 80 % w/w. Digestate contains large amounts of ammonia, nitrate, phosphate, and various trace elements in widely varying concentrations and ratios. Both waste streams are challenging and, due to their high particle load, require pre-treatment to avoid clogging, e.g. in the tubes of the peristaltic pumps. If clogged, the feed to the reactor is hindered and continuous fermentation is terminated.

As the liquid digestate used in this work was obtained from a food disposal plant also processing packaged food and therefore contained up to 6 % foreign matter (<0.5 mm) in the form of plastic, glass and metal particles as well as organic fermentation residues, these residues must be removed from the digestate prior to use as a nutrient medium component. This is done by a combination of different separation steps such as centrifugation and filtration.

The digestate has a pH of about 8 to 9 and contains unoxidized compounds, therefor pH adjustment and aeration were identified as necessary additional steps before use in the continuous process. Preliminary experiments in shake flasks with nutrient media only consisting of crude glycerol und digestate show almost similar results in terms of growth rates and biomass gain in comparison to defined mineral media with crude glycerol as sole carbon source. Based on these promising results, the existing continuous process will be modified for the utilization of anaerobic digestate with crude glycerol for the growth phase (bioreactor 1) and PHB accumulation enhanced by feeding with crude glycerol in the second phase (bioreactor 2).

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P17: **PHA Metabolism and Stress Response in Bacterium *Rhodospirillum rubrum***

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Technological changes caused by the industrial revolution have led to environmental pollution in the recent decades. However, this phenomenon could also be reduced by using materials that are easily degradable in nature, including polyhydroxyalkanoates (PHAs), which are products of the metabolism of micro-organisms that are broken down into carbon dioxide and water. One of the promising producers of PHAs is the G^- mesophilic bacterium *Rhodospirillum rubrum*, which is interesting mainly for its versatile metabolic apparatus, which includes the synthesis of PHAs from different carbon sources.

This work was focused on polyhydroxyalkanoate (PHA) production in *Rhodospirillum rubrum*, comparing wild-type PHA-producing (*R. rubrum*) and mutant PHA-non-producing strains in terms of cell morphology. These bacterial strains were also subjected to selected stresses and the effect of PHA on the stress response was determined using advanced analytical techniques. First, both strains were cultured under aerobic conditions in the dark for 48 and 72 h. Viability of these samples after exposure to selected stresses was determined using a fluorescent probe (propidium iodide) by flow cytometry, while cell disruption was examined by electron microscopy (cryo-SEM and STEM). As an additional experiment, the production of pigments (carotenoids and bacteriochlorophyll *a*) was carried out under microaerobic conditions in the light for 120 h. UV-VIS spectroscopy was used to determine the extracted pigments. Furthermore, both strains were exposed to the same selected stresses as in previous experiments. Samples were also examined by flow cytometry and electron microscopy (cryo-SEM and STEM).

We were able to confirm the ability of wild-type stain of *R. rubrum* to produce the copolymer P(3HB-*co*-3HV). The hypothesis of the cryo-protective property of PHA granule during exposure to multiple freezing cycles was also confirmed. In the contrast, the protective function of PHA was not proven when exposed to osmotic stress. In addition, a negative effect of prolonged time of cultivation on the viability of both strains of *R. rubrum* was also proven.

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P18: **Development of *Paenibacillus polymyxa* Strains for Recombinant Dextran Production by Genetic Engineering**

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Dextrans comprise a group of polysaccharides that consist of mainly α-(1-6)-linked α-D- glucopyranosyl residues produced from sucrose-containing media by lactic acid bacteria belonging to the genus of Leuconostoc, Weissella, Lactobacillus and Streptococcus. The molecular structure strongly depends on the production strain as well as the process parameters, such as temperature and media composition. The most commonly used strain for dextran production is Leuconostoc mesenteroides NRRL B-512F which has been classified as a R2 organism recently [1]. The main fields of application of dextran produced by L. mesenteroides are in food industry as a texture enhancer and in medicine as a blood plasma substituent or cryo-preservative. Therefore, high quality standards are required for dextran and the product safety has to be ensured.

Dextran synthesis is carried out by dextransucrases, which are a class of extracellular enzymes that are induced in the presence of sucrose in Leuconostoc mesenteroids ssp. [2].

Paenibacillus polymyxa is a non-pathogenic, Gram-positive bacterium that is commonly found in the rhizosphere of plants. In recent years, P. polymyxa has gained significance in industrial and biotechnological processes as it produces a broad range of relevant biotechnological products including enzymes, 2,3-butanediol, antibiotics and exopolysaccharides such as levan and paenan. It is accessible for genome editing through highly efficient CRISPR-Cas9 tools [3]. Here we investigate the recombinant production of dextran with P. polymyxa mutants. Through chromosomal integration, different dextransucrases of Leuconostoc species. are introduced into the P. polymyxa genome. We were able to generate P. polymyxa mutants which produce dextran in titers of up to 14 g/L, and are easily accessible for further genetic engineering and at the same time have the generally recognized as safe (GRAS) status.

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P19: **Enhancing PHB production in Cyanobacteria: Modeling the Optimal Light Regime for Growth**

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The generation of bioplastics such as polyhydroxybutyrate (PHB) through cyanobacteria offers a sustainable and renewable alternative to traditional plastics [1]. In order to produce bioplastics on a competitive scale, it is necessary to obtain a culture with a sufficiently dense biomass. Mathematical modeling of the growth and formation of PHB allows to improve the concentration achieved in the laboratories. The main objective of this work is to model the growth of a cyanobacterial culture in order to optimize the production of bioplastic. After a literature review, four models were selected and compared to analyze the evolution of biomass concentration. These models were constructed through different factors [3, 5]: empirical knowledge (Gompertz), biological factors (Baranyi-Roberts), nutrient availability (Monod) and light regime (Aiba). In addition, an analysis of two PHB accumulation models [2, 4] was performed and potential improvements in the models used were explored. The objective of this study was to identify the factor that best describes the growth of cyanobacteria and the maximum amount of PHB that can be accumulated. The results obtained indicate that a light-based model provides the best description of cyanobacterial growth. Using this model, different experimental setups were simulated, and experiments were conducted to correlate photosynthetic efficiency with biomass growth. Optimization of the light regime could be useful in reducing production costs and improving the economic viability of cyanobacteria-derived bioplastics.

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P20: **Raman Spectroscopy Analysis of Polyhydroxyalkanoates Produced by Microorganisms**

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Rapid and reproducible quantitative analysis of polyhydroxyalkanoates (PHAs), biodegradable polyesters accumulated by various bacteria is a clear goal in biotechnological production when processing dedicated bacterial samples.

During the last 15 years or so, Raman spectroscopy has gained a wider acceptance as a method for the identification of biological samples (bacteria, yeast and algea). The technique of Raman spectroscopy is slowly becoming a well-recognized analytical technique because of the identification of its competitive position amongst other techniques. Raman spectroscopy has an extremely competitive position if in-situ, contactless, noninvasive, and fast analysis is required, for instance, online in biotechnological processes. The sample does not have to be prepared for analysis using solvents, the technique is label-free and with minimal interference from water. Consequently, we have demonstrated that Raman spectra generated from bacteria (*Cupriavidus necator* H16) could give sufficient information to monitor the process control of PHB production.

Here we report on quantitative Raman spectroscopy analysis of PHA accumulated by bacteria using calibration curves. Analytical results based on this calibration can be used for fast and reliable determination of intracellular PHB content during biotechnological production of PHB since the whole procedure - from bacteria sampling, centrifugation, and sample preparation to Raman analysis - can take about 10 minutes. On the contrary, gas chromatography analysis takes approximately 8 hours.

We believe that the measurement and evaluation procedure we have exploited can once become a technique of choice for real-time and in-situ monitoring of microorganisms involved in biodegradable polyesters production.

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P21: **Raman Spectroscopy and Raman Tweezers for Detection of Biopolymers and Pigments in Prokaryotic Cells**

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A Raman spectroscopy is a method that is slowly beginning to be more acknowledged when considering the analyses of microbiological samples. The simplicity of its use with the comfort of analyzing the sample as is, reasonably short detection times, and non-destructive nature of the method make it an attractive alternative to the commonly used chromatography and mass spectroscopy. For single cell analyses optical tweezers combined with Raman spectroscopy can be used. The combination of these methods is called Raman tweezers. The cells can then be sorted depending on their production of the desired compound.

Here we present the suitability of Raman spectroscopy and Raman tweezers for biotechnology applications. We analyzed a variety of bacterial and cyanobacterial species to detect biopolymers or pigments produced by the cells. Namely, we measured the presence of polyhydroxybutyrate (PHB) in bacteria *Cupriavidus necator*, *Halomonas halophila*, *Azotobacter vinelandii*, *Chelatococcus sambunathi*, and cyanobacteria *Synechocystis*. PHB belongs to the group of polyhydroxyalkanoates which can be used as biodegradable polyesters and offer a more ecological approach as opposed to basic plastics. Pigments, such as violacein or phycobiliproteins, were detected in the bacteria *Janthinobacterium lividum* and also in *Synechocystis*, respectively. Both pigments can be used for cosmetics and medicine, phycobiliproteins are also common for food applications.

Our findings show the applicability of Raman spectroscopy for the detection of polymers and pigments in microorganisms. The method has the potential to become a method of choice when online and fast detection is required or when the preservation of the living sample is mandatory.

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P22: **Protein Hydrolysates from Hempseed Waste as Actives in Hair Cosmetics**

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The quest for sustainable and environmentally friendly ingredients in hair cosmetics has led to increased interest in gaining biopolymers from the secondary products of food industry. One of the promising ingredients for such application is hemp protein hydrolysate (HPH) obtained from hempseed meal, which is a by-product of the hempseed oil extraction. Hempseed protein is predominantly composed of edestin, which account ~75 % of the total protein content [1]. Edestin contains a rich amino acid profile making it potentially beneficial for hair care applications [2].

However, the practical utilization of edestin in hair cosmetics is hindered by its low solubility, particularly at pH levels optimal for hair health [3,4]. Edestin aggregates and precipitates at pH levels below 7 [3], limiting its solubility and efficacy as a hair conditioning agent [4]. This solubility challenge necessitates innovative approaches to enhance the solubility of HPH while ensuring the formulation remains mild for hair.

To address this challenge, we employed an enzymatic hydrolysis process to produce a soluble HPH formulation that can be effectively incorporated into hair conditioners. Our approach involved experimentation with diverse hydrolysis conditions and industrial enzyme blends, aiming to achieve optimal results. To further enhance the solubility of HPH without compromising hair mildness, we incorporated various anionic surfactants into the formulation. By optimizing the hydrolysis conditions and surfactant selection, we aimed to achieve a soluble HPH formulation that retains the beneficial properties of edestin for hair care.

Our findings demonstrate the successful enhancement of HPH solubility at hair-friendly pH levels through the optimized formulation. The soluble HPH formulation offers improved solubility and compatibility with hair conditioners, enabling its efficient incorporation into hair care products.

In conclusion, this study presents an innovative approach to overcome the solubility challenges associated with hemp protein hydrolysate in hair cosmetics. By creating a soluble HPH formulation and incorporating anionic surfactants, we enable the development of sustainable hair conditioners that harness the potential benefits of edestin for hair health.

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P23: **Microbial Synthetic Consortia for PHB Production on Molasse**

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Microbial synthetic consortia, or defined mixed cultures of two or more defined microorganisms in a single bioreactor [1], are here hypothesized to be a means to achieve higher volumetric polyhydroxyalkanoate (PHA) productivities, but also to harness specific substrates using well-known and efficient PHA producing strains. Here we report two basic consortia that were studied both in shake flasks and in a benchtop 2L bioreactor.

First, a bacterial consortium with *Azohydromonas lata* and *Cupriavidus necator* was proposed to grow on molasse, an inexpensive byproduct of the beetroot-based sugar industry. The former strain produced invertase, able to hydrolyze saccharose into fructose and glucose, and therefore supporting the growth of both bacteria as well as poly(3-hydroxybutyric acid) (PHB) accumulation. The global volumetric PHB productivity obtained was 0.55 g L^{-1} h^{-1} in preliminary bioreactor experiments. This could be further optimized by tuning the nutrient limitation strategy.

Furthermore, a defined mixed culture of a yeast, *Candida utilis*, and a bacterium, *Cupriavidus necator,* was developed also to grow on molasse. The main research aspects were the design of a common growth medium, the monitoring of the development of both strains' population, as well as keeping low yeast concentrations, while enhancing PHB production. Here, a pulsed fed-batch bioreactor allowed to reach 8.5 g L^{-1} of PHA, with 45 wt% PHB content over the global biomass.

As a conclusion, synthetic consortia constitute an intriguing concept to be further developed in the field of PHA production either when specific substrates are targeted or when specific products are desired, *i.e.* a new metabolic pathway combination is required. This brings however new challenges in terms of medium design, bioprocess strategy, and monitoring of each strain growth.

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P24: **Degradation Of FDM 3D Printed Scaffolds In Simulated Body Fluid**

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A material for bone tissue engineering should meet numerous requirements for its thermo-mechanical, physical and biological properties. Resorbability in the human body environment together with the optimal rate of mechanical properties change are among them.[1] The mechanism of the degradation of aliphatic polyesters such as poly(3-hydroxybutyrate) (PHB) and polylactide (PLA) is hydrolysis, which can be catalysed by non-specific esterases present in the surroundings.[2]

In this work, the effect of hydrolysis on 3D printed samples was studied in order to gain insight into the behaviour of these structures upon implantation. Seven materials composed of various ratios of four components: PHB, PLA, plasticizer Syncroflex 3114 and bioactive hydroxyapatite/tricalcium phosphate were tested. Printing was done on a Prusa MK3s fused deposition modelling commercial printer. Tested scaffolds took the shape of normalized testing specimens for a compression test. Simultaneously, scaffolds with 50% porosity and a grid structure were created. The degradation test was performed at a temperature of 37.5 °C in a medium simulating human plasma according to [3] for five months.

The ratio of PHB/PLA was shown to have the greatest influence on the degradation of studied mixtures. The sample containing 70% PHB (the highest in the tested range) had a negligible weight change during the whole experimental period. Similarly, its mechanical properties were untouched. On the contrary, the sample with a 50/50 ratio PHB/PLA exhibited the greatest change of tested properties with a weight loss of 14% for a non-porous sample and a 6% weight loss for a 50% porous sample.

Generally, the degradation within five months was more profound for full scaffolds compared to their porous analogues. The reason is probably the autocatalysis of degradation by acid by-products, which are released to the medium, as described for PLA.[4] Overall, the samples followed the bulk degradation mechanism, which was described for 3D-printed scaffolds from PLA.[5] The only deviation from idealized behaviour is the apparent increase in weight in the fifth month of the experiment compared to the weight after four months. This is caused by the solution-precipitation of tricalcium phosphate on the surface of the scaffold, which is a desired phenomenon given the use of these blends.[6]

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P25: **Polymer Science & Innovations @ Merck**

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Merck's legendary Sigma-Aldrich Materials Science portfolio includes a range of products for polymer synthesis, biomedical applications as well as high-performance energy and electronic materials. Among these, innovations in the fields of ready-to-use bioinks for tissue/organ fabrication by 3D bioprinting and new tools for drug delivery formulation using particle synthesis with microfluidics, will be presented. These products enable researchers and professionals in various fields to create complex tissue and organ constructs, develop targeted drug delivery systems, and streamline polymer or lipid particle synthesis processes, ultimately advancing the healthcare and biotechnology industries. One part of the poster is devoted to selected greener alternative products which Merck offers to help reduce the environmental impact of research work while ensuring that results are not compromised. You will learn about new generation of greener surfactants, bio-based polypropanediol and examples of products from Merck's expanding portfolio of materials that enable alternative energy generation technologies and support research in various emerging fields with high social impact, such as biosensors or wearable electronics.

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P26: **Using Atomic Force Microscopy to Determine Morphological and Physicochemical Properties of Living Bacteria and Native Cellular Components**

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The atomic force microscopy (AFM) belongs to important instruments to determine morphological and physicochemical properties such as topography, adhesion, or elasticity of biological samples. Determination of differences in stiffness of samples could be used in various medical fields, for example in cancer and developmental biology [1, 2]. The potential of this methodology could be found also in microbiology field where the defects of surfaces of bacteria cells that were caused by peptide action were observed [3]. Therefore, AFM can play an important role in specifications of changes of biological samples in real time and in addition *in situ* mode.

In this study, we were focused on characterization of physicochemical properties of bacterial cells *in vivo* to compare to each other with respect to different intracellular content of polyhydroxyalkanoates (PHAs) in cells. As it is already known, the PHAs are not used by bacteria only as storage of carbon or energy but it is also used as protectants against a wide range of stressors (low temperature, high salinity, pH etc.) [4]. The aim of this study was to achieve topographic images of cells in high quality and measure Young's modulus to define stiffness of bacterial cells that produce PHAs and bacterial cells without this ability.

For optimization of experiments such as fixation of cells and measuring of cells in buffers by AFM, we chose bacterial strains PHA producing *Cupriavidus necator* H16 and its PHA negative mutant *Cupriavidus necator* PHB^{-4}. In next step to determine Youngs modulus to evaluate the stiffness of cells, we add also bacterial strains producing PHAs *Rhodospirillum rubrum* and its PHA negative mutant *Rhodospirillum rubrum* Δ*pha*C. We investigated innovative evaluation of stiffness of bacterial cells where the method was devised using directive lines obtained with the steep parts of the *extend* curves instead of the Hertzian data approximation model. The hypothesis of different stiffness of PHA-containing bacterial cells and non PHA-containing was confirmed where smaller deviations were observed on whole cells and not measured only on the part of cells. At the end of our study, the isolated native PHA granules were also characterized and showed the middle stiffness comparing to measured bacterial cells.

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P27: **Valorizing Food Waste Through Bacterial Cellulose Production from Waste Apple Pulp and Stale Bread**

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Bacterial cellulose (BC) is one of the most well-known microbial biopolymers due to its excelling properties, such as high crystallinity, biodegradability, biocompatibility, great water-holding capacity,and mechanical performance, which make BC suitable for application in several fields (e.g., packaging, biomedicine, etc.) [1]. Still, industrial production is considered challenging due to the high production costs associated with the cultivation media. A common strategy for cost reduction is to resort to agro-industrial waste as a carbon/nitrogen source, allowing a circular economy by turning waste into avalue-added polymer [2]. In this work, we investigated the use of waste apple pulp (supplied by Sumol+Compal S.A.) and stale bread (supplied by a local market) as potential alternative substrates forBC production against synthetic mediums, and their impact on BC's physical and chemical properties.

Both waste apple pulp extract (comprising of glucose and fructose) and stale bread hydrolysate (comprising of glucose) were used as sole substrate sources achieving a BC production of 0.94±0.0 and 0.40±0.08 g/L, respectively. However, BC production was higher in HS medium supplemented with either waste, reaching 3.38 ± 0.09 g/L for waste apple pulp and 2.07±0.22 g/L for stale bread. There was no significant impact of the substrate/medium used for cultivation on BC fiber diameter (38±7 - 55±14 nm) and chemical structure, and only a slight variation in crystallinity (55-69%). Water uptake was higher for BC produced in the non-supplemented waste apple pulp extract and stale bread hydrolysate (8552±153 and 7368±802%, respectively) than for their corresponding HS-supplemented medium (4751±219 and 5013±222%, respectively). In terms of mechanical performance, BC from the HS medium supplemented with stale bread hydrolysate showed the highest tension at break and Young's modulus (933±88 and 3200±1500 kPa, respectively), which is the closest to the requirements for wound dressing applications [3]. The membranes' gas permeability varied from 0.1±0.01 to 116±6 barrer for O_2 and from 0.1±0.01 to 2217±111 barrer for CO_2, with the lowest permeability values beingattributed to non-supplemented HS medium, and filling the barrier properties requirements forpackaging applications [4]. These results show that food waste, namely waste apple pulp and stale bread allows for higher production of BC in comparison to synthetic sugars while displaying BC with different properties that can be tuned for a specific application by the choice of the substrate and cultivation medium.

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P28: **Light Scattering for Structural Studies of Biopolymers**

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Understanding the molar mass distribution and molecular structure of biopolymers is pivotal for their effective utilization in industrial applications. To address these analytical challenges, the integration of multi-angle light scattering (MALS) detectors with size exclusion chromatography (SEC) proves to be highly efficient. The standout advantage of MALS lies in its ability to solve the calibration dilemma encountered in SEC. Unlike traditional methods dependent on retention time and molecular standards, MALS offers an indisputable advantage by providing absolute determinations of molar mass and size distributions.

The indisputable advantage of MALS is its absolute molar mass and size distributions determination independently of retention time and molecular standards, and regardless of non-ideal column interactions.

Analysis of biopolymers can be difficult due to heterogeneity, limited solubility, fluorescence (lignin and humic acids) or non-existence of well-defined standards for branching studies.

SEC may fail in case of samples containing ultra-high molar mass fractions that can undergo shearing degradation; and also, in case of branched polymers due to the anchoring of branches in the pores of SEC column packing. Asymmetric-flow field-flow fractionation (AF4) is by far the most commonly used Field flow fractionation (FFF) separation technique. AF4 is an alternative separation technique to SEC – gentle separation without shear or adverse, non-ideal column interactions, and HMW species are not filtered out or disrupted as on the column.

In this work, molar mass, branching and conformation of cellulose, vegetable oil, natural rubber, lignin and various polysaccharides were characterized using both AF4-MALS or SEC-MALS techniques.

P29: **Bioconversion of Plastics' Building Blocks into Bacterial Cellulose for Circular Economic**

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Polyethylene terephthalate (PET) is one of the most extensively utilized thermoplastics, synthesized by the chemical polymerization of terephthalic acid (TPA) and ethylene glycol (EG) monomers [1]. Poly(lactic acid) (PLA) is a bio-based and biodegradable polymer resulting from the polymerization of lactic acid (LA), whose mechanical and physical properties are suitable and in parity with conventional plastics for applications such as food and packaging [2]. PET overuse, combined with its recalcitrant nature and waste management, makes this plastic a major responsible for the large environmental burden [1]. Even though PLA is a biobased and renewable material, its inefficient disposable management approaches also contribute to the environmental impact of plastics.

In a bid to value PET and PLA waste streams, *Komagataeibacter xylinus* DSM 2004 static cultivation experiments were carried out in Hestrin-Scharmm (HS) medium supplemented with commercial LA and/or TPA. The kinetics of BC production were determined, and the most relevant BC yields were attained from HS supplemented with 10 g/L LA (1.14 ± 0.07 g/L) and with the combination of LA (10 g/L) and TPA (10 g/L) (1.09 ± 0.03 g/L). Although the BC yields obtained are higher than those observed for the standard medium used for BC production (HS supplemented with glucose, 0.83± 0.04 g/L), a preference of the bacterium for LA as a carbon source was denoted since a higher consumption of LA than TPA was observed. The physico-chemical properties of BC pellicles produced were also evaluated.

The results gathered in this study constitutes a potential green and sustainable strategy to reuse plastic monomers and to valorize them into a value-added biomaterial, thus contributing to circular bioeconomic values.

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P30: **Stress Adaptation of PHA Producing Microorganisms Studied by Microcalorimetry**

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Isothermal microcalorimetry is a promising technique for studying bacterial growth in various sample types – from soil to liquids including cell suspensions [1]. In this work, microcalorimetry was used to study stress adaptation of a wild and adapted strains of halophilic bacterium *Halomonas halophila* on microbial inhibitor levulinic acid. *H. halophila*, as a known PHA-producing bacterium, was chosen due to its wide substrate-utilization flexibility with the aim of utilizing lignocellulose-based media.

Levulinic acid together with other microbial inhibitors naturally occurs in a mixture after the hydrolysis of lignocellulose materials which can be used as a cheap substrate in PHA-producing process. Therefore, the use of lignocellulose-based media is complicated because only a very limited number of PHA-producing microorganisms can effectively utilize the mixture rich in microbial inhibitors [2].

H. halophila was cultivated in saline mineral media (66 and 35 g/l for wild and adapted strain, respectively). The concentration of levulinic acid was between 0.5 and 10 g/l (wild strain) and between 0.5 and 15 g/l (adapted strain). The samples for microcalorimetric measurements were filled into 4ml glass ampoules and inserted into a modular microcalorimeter TAM III (TA Instruments, USA). The experiments were performed at 30°C. The main conclusion from the observation of the heat flow curve is that the length of the exponential phase of the microbial growth was longer with higher levulic acid concentration.

Microcalorimetric measurements showed that the wild-type *H. halophila* strain could adapt to levulinic acid to a concentration of 5 g/l. The adapted strain, which was expected to have poorer stress management, could adapt to all observed concentrations. The microcalorimetric results were confirmed with the conventional analytical methods (i.e., gas chromatography, spectrophotometry).

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P31: **PHB in Cyanobacteria: Analyzing Production Through Images Processing and FTIR Techniques.**

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Cyanobacteria have gained significant attention in recent years due to their ability to produce a variety of valuable compounds. One such compound is polyhydroxybutyrate (PHB), a biodegradable polymer with immense potential in various industrial applications [1]. PHB is accumulated inside the cells; therefore, an extraction process is needed to quantify the biopolymer content. Nevertheless, this process is time consuming and requires environmental hazardous chemicals, such as chloroform. In the present work, we present two complementary methods developed to analyze and quantify PHB production in a cyanobacteria microbiome. The first one consists in an image processing applied on images obtained from (TEM) Transmission Electronic Microscope. It can also be applied to others type of optical and electronic microscope images. In this case, a segmentation process allows differentiating PHB grains inside cyanobacteria cells. A metric is then established by computing pixels area taken up by PHB in the whole image and in cyanobacteria cells. The second one relies on Fourier-transform infrared (FTIR) spectroscopy [2] [3], as a non-destructive and rapid method to analyze PHB. A freeze-dried sample with a 30 % dry cell weight (dcw) PHB content together with a commercial PHB pellet, as a reference sample, were used for FTIR analysis. Analyses has been conducted with a FT-IR spectrometer fitted with an ATR system and spectra covers a range from 4000 cm^{-1} to 500 cm^{-1}. Absorption peaks due to metyl, carbonyl, ester, and hidroxyl group characteristics of monomer structure in PHB are observed.

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P32: **Fucose Plays a Critical Role in Enhancing the Cryoprotective Potential of Polysaccharides**

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Advances in the cryopreservation of cells, tissues and organs have revolutionized the fields of science and medicine. Ice nucleation and growth, which are lethal physical stressors, have long been coupled to equally lethal volumetric fluctuations the cell incurs during freezing and thawing. Most chemical formulas often rely on cryoprotective agents that require high concentrations to be used, rendering them cytotoxic, and osmotic regulators that add increased compositional and procedural complexity to an inherently sensitive technique. Therefore, natural polymers, particularly polysaccharides, have been studied as bio-based, biocompatible alternatives. In this work, we studied the post-thaw metabolic viability (PTV) and histology of cryopreserved Vero cells exposed to freezing media containing 10% DMSO and one of 26 different polysaccharides, and cross-correlated PTV with their compositional fingerprints. The dataset shows that the best performing cryoprotective polysaccharides (G, P1, EPS-7, FucoPol, EPS-6) are those labelled fucose-rich, containing an average 35.6±6.9 wt.% fucose; and resulted in an average PTV of 2.8-fold (up to 3.1-fold) compared to 0.8-fold and 2.2-fold for non-cryoprotective and cryoprotective polysaccharides, respectively. Moreover, only fucose-rich polysaccharides could rival the performance of the optimized commercial cryopreservation formula CryoStor™ CS5 (2.56-fold). The best performing polysaccharides are characterized by a uronic acid (UA) content between 13.5–24 wt.%, a high fucose content of 18–35.7 wt.% and a molecular weight higher than 1 MDa (PTV=2.1-fold vs. 1.1-fold). Also, a stoichiometric balance between both features rather than a dominance of one seems more beneficial towards PTV, but a minimal threshold of both is required. To deconvolute the effects of each variable towards PTV, a principal component analysis (PCA) coupled to K-means clustering was performed. For this dataset, two major mechanisms of action explained 70% of the observed variability in a three-cluster system containing six predictor variables. While PC2 (30.25%) was composed by uronic acids and neutral monomers of opposed influence, PC1 (36.89%) was exclusively explained by a fucose content that scales with molecular weight, suggesting that optimal cryopreservation is achieved by using polyanionic, membrane-interacting polymers capable of disrupting ice growth and minimizing lethal volumetric fluctuations during freezing. Ultimately, our research highlights the critical role polymeric fucose plays in enhancing cellular cryopreservation outcomes, disputing previous assumptions that polyanionicity is the sole governing predictor of cryoprotection and that neutral monomers are chemically inert linker residues. The inherent biocompatibility of fucose-rich polysaccharides highlights exciting alternatives to the use of cytotoxic glycerol and DMSO in low-cost, bio-based formulations.

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P33: **Preparation and Characterization of a PHBV Nanoparticles Obtained from Whey for Drug Release**

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The polyhydroxyalkanoates (PHA) are a biopolymer produced by many microorganisms. The production process is based on the use of a mixed microbial culture that uses residues or industrial by-products as a substrate, such as whey [1]. In this way, a high-quality material is obtained from highly polluting waste at a low cost.

At present, research has focused on obtaining these biopolymers for use in drug delivery. This is due to its exceptional characteristics: it is a biodegradable, biocompatible material and it does not cause toxicity. For this reason, there are several studies in which they use this biopolymer to encapsulate drugs (i.e. epirubicin, paclitaxel) [2, 3]. Some proteins, such as the TGF-ß3 factor, are essential in cell differentiation processes, but they have a short life, so they must be added from time to time [4]. Therefore, the nanoencapsulation of this protein is of great interest since its degradation is avoided.

This study presents the PHA production process, the purification of PHA and the preparation of nanoparticles encapsulating TGF-ß3 factor. The PHA obtention process was carried out using a mixed microbial culture and whey as a substrate. Then, due to the presence of endotoxins, a purification of the material obtained prior to the formation of nanoparticles has been carried out, and, subsequently, the presence of endotoxins was determined. Finally, the formation of poly (3-hydroxybutyrate-co-3-hydroxyvalerate acid) (PHBV) nanoparticles with a 12% HV content was carried out for use as a protein releaser, specifically the TGF-ß3 factor.

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P34: **Implantation of Retinal Pigment Epithelium Cells via Ultrathin Nanofibrous Carrier**

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Degenerative retinal diseases such as age-related macular degeneration (AMD) impair the function of the retinal pigment epithelium (RPE), which results in failure of photoreceptors and loss of vision. Replacement of the RPE by a transplantation of retinal cells via nanofibrous membrane is therefore considered as a therapeutic option for patients with these eye conditions.

In this work, we report on the results of the subretinal implantation of human RPE (hRPE) cells on PDLLA-based nanofibrous carrier in minipig. Nanofibrous membranes were prepared by electrospinning that easily allowed an embedding of a supporting frame. Such a frame enables not only handling without irreversible folding of carrier and keeping a side-orientation of the sample while seeded with cells, but also to regain membrane's flat shape when inserted into the subretinal space during surgery. The hRPEs were collected from human cadaver donor eyes and cultivated on nanofibrous carriers. The observation period after implantation lasted 1, 2, 6 and 8 weeks, and included *in vivo* optical coherence tomography of the retina as well as post mortem immunohistochemistry using following antibodies: HNAA and STEM121 (human cell markers); Bestrophin and CRALBP (hRPE cell markers); peanut agglutining (PNA) (cone photoreceptor marker); PKCα (rod bipolar marker); Vimentin, GFAP (macroglial markers); and Iba1 (microglial marker).

The hRPEs showed cobblestone morphology, persistent pigmentation and measurable trans-epithelial electrical resistance on the nanofibrous PDLLA carrier. The implanted hRPEs were positive for HNAA and STEM121 and were located between the minipig's neuroretina and RPE layers at week 2 post-implantation, which was gradually attenuated until week 8. The implanted cells expressed the typical RPE marker bestrophin throughout the whole observation period, and a gradual decreasing of the CRALBP expression in the area of implantation at week 8 post-implantation was observed. The transplanted hRPEs appeared not to form a confluent layer and were less capable of keeping the inner and outer retinal segments intact. The cone photoreceptors adjacent to the implant carrier were unchanged initially, but underwent a gradual change in structure after hRPE implantation; the retina above and below the implant appeared relatively healthy.

The presented ultrathin, highly porous, and surgically convenient cell carriers have key characteristics in order to improve the integration and the functionality of implanted hRPE cells. The nanofibrous carriers with cultivated hRPE showed good incorporation into the host retina over an 8 weeks observation period, with some indication of a gliotic scar formation.

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P35: **PHA-Based Particles Prepared by Nanoprecipitation as Drug Carriers**

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Biodegradable polymeric particles represent an interesting approach for *in vivo* delivery of drugs, vaccine antigens, or hormones [1]. When administered, they distribute the drug more uniformly than single unit administration, which reduces local irritation as well. In polymeric particles, active substances can be entrapped, dissolved, or adhere to the surface [2]. Such systems generally use three different described mechanisms to release the encapsulated substances, including monophasic release with diffusion; biphasic release with a high initial burst followed by saturation; triphasic profile starting an initial burst, then polymer degradation and ending with a slow diffusion release [3]. The efficiency of the performance of particles in the body also depends on their size, as smaller sizes increase their intracellular uptake and mobility to the target sites [2]. Various top-down and bottom-up methods are reported for the preparation of polymeric particles [4,5], and nanoprecipitation is one of them [6]. This method allows changing the size of the obtained polymer particles very easily by changing processing parameters, such as temperature, stirring rate, or polymer concentration.

Polyhydroxyalkanoates (PHAs) have great potential in this area, especially due to their biocompatibility, biodegradability, and their wide range of mechanical properties [7]. Besides, their hydrophobicity is an advantage, as the intestinal uptake of hydrophobic polymers, when used as drug delivery carriers, was higher than the hydrophilic ones [8,9].

Herein, we have designed drug delivery carriers based on different types of PHAs. The effect of varying the parameters of nanoprecipitation as a preparation method on the resulting particle sizes has been described. This work shows the release kinetics of the encapsulated substances studied in different environments. The tests induced different pH values, which also vary naturally in the human body depending on the site. The effect of acidic and neutral environments on release was compared.

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P36: **Functionalized Combined Nanostructures as Potential Wound Dressings**

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Wound healing requires a suitable environment to promote the healing process. Therefore, treatment and management of wounds largely depend on the dressing material, which provides surface protection, drug delivery and allows tissue regeneration. Previous studies showed that the unique properties of nanofibers such as nanoporosity and large surface area make them an efficient tool to promote wound healing [1].

The aim of the presented work was to develop a drug delivery system based on PHB-liposome-enriched PHB nanofiber mats. Materials were functionalized by active compounds in the form of antimicrobial substances.

Scanning electron microscopy and fluorescence microscopy were used to characterize the morphology of the prepared combined nanostructure. The size and colloid stability of particles were defined by dynamic light scattering and zeta-potential. Materials were further tested for the encapsulation efficiency and *in vitro* release of the incorporated drug into the model environment by chromatographic method. Antibacterial activity against strains of gram-positive and gram-negative bacteria was also evaluated.

To assess whether prepared nanostructured materials are suitable for further local skin applications cytotoxicity assay on HaCaT cell line was carried out. Combined nanostructures showed promising results for local treatment of skin as prospective advanced wound dressings.

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P37: **Monitoring of the Biological Effects of Prepared Biopolymer-based Wound Dressings**

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Currently, appropriate wound care is given a lot of attention. According to this notion, there is a rising demand for the availability of high-quality and effective wound dressings. Due to these reasons, this work was focused on optimising the preparation of nanofibrous and non-fibrous wound dressings and testing interaction of prepared materials with human cells.

Alginate and chitosan were used to create non-fibrous dressings, while poly(3-hydroxybutyrate) was used as a basis for nanofibrous dressings. Two methods, electrospinning and forcespinning, were used to create PHB nanofibers. Selected active compounds functionalized both types of dressings, and their gradually release was established. For potential application in medicine prepared dressings were tested for safety on immortalized human keratinocytes (HaCaT). Determination of cytotoxicity was performed by MTT assay and LDH assay. A scratch test, which measures how quickly a wound is healing, was also carried out.

The findings revealed no cytotoxicity effect, indicating that the produced materials are suitable for use in medicine. For example, as a skin wound dressing for opened and closed wounds. Moreover, cosmetics could be another area of potential use.

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P38: 3D Printed Scaffolds Based on Poly(3-hydroxybutyrate) and Its Blends for Use in Medical Applications and Tissue Engineering

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Currently, several bioplastics and biodegradable polymers, such as poly(3-hydroxybutyrate), polylactic acid, polycaprolactone, and their blends and copolymers, find applications in the field of medicine. These materials are utilized to enhance regeneration, facilitate tissue restoration, and expedite the healing process. [1]

Tissue engineering stands as one of the forefront methodologies in modern regenerative medicine. A pivotal component of this approach is the porous structure, or scaffold, which provides essential support for cells and becomes seamlessly integrated into the human body. The scaffold's critical feature is its appropriate porosity and three-dimensional architecture, facilitating cellular growth throughout its entirety and enabling the formation of new tissue. [2]

Recently, tissue engineering has started using 3D printing to produce scaffolds from polylactic acid, poly(3-hydroxybutyrate), polycaprolactone, and others. [3] Furthermore, tricalcium phosphate as well as hydroxyapatite (HAp) can be used as a bioactive filler to promote *in vivo* osteogenic differentiation of mesenchymal stem cells (MSCs). [4]

In this work, poly(3-hydroxybutyrate) based composite blends for bone medical applications and tissue engineering were prepared and characterized. The PHB used for the study was sourced from both commercial and chloroform-free extraction methods. The PHB was blended with poly(lactic acid) (PLA) or polycaprolactone (PCL) and plasticized using oligomeric adipate ester (Syncroflex, SN). Tricalcium phosphate (TCP) particles were incorporated as a bioactive filler.

The thermal properties were evaluated through differential scanning calorimetry, and the mechanical properties, including tensile strength, three-point flexural strength, and compression strength, were investigated to assess the material's performance. Surface properties of the materials were studied in two biocompatible blends based on plasticized poly(3-hydroxybutyrate)/poly(lactic acid) with and without tricalcium phosphate addition. Confocal microscopy was used to observe the morphology, while contact angles with different liquids were measured to calculate the surface energy. 3D printed scaffolds with various grid sizes were employed for further testing.

The results demonstrated that the mechanical properties of the PHB-based composite blends were comparable to human trabecular bone, with strengths around 40 MPa and moduli around 2.5 GPa. The calculated surface energies of all blends were approximately 40 mN/m. Furthermore, the study showed that scaffolds with a grid size of 50 µm were a suitable compromise for osteoblasts. Confocal microscopy revealed that parallel roughness was around 2 µm for the material without tricalcium phosphate, while the composite showed roughness between 4 to 6 µm. Both blends of PHB/PCL were non-cytotoxic, however biological tests *in vitro* didn´t show significant differences among the various scaffold structures.

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P39: **Hyaluronic Acid: Nature's Versatile Biopolymer for Regenerative Medicine and Skin Care**

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Hyaluronan (HA) is a natural biopolymer, which commonly occurs in the human body (e.g., in the extracellular matrix, synovial fluids, or cartilage). The main properties of HA are biocompatibility, high solubility, and ability to bind water. It is advantageous for a wide range of medical and cosmetic applications. For some specific applications, where the properties of native hyaluronan are not sufficient, the chemical modification of the HA backbone can be utilized. Many approaches and strategies in modification of the molecular structure of HA were developed, including crosslinking, functionalization, and grafting of various moieties. [1,2] These modifications can be finely tuned to alter HA's mechanical strength, degradation rate, and bioactivity. HA is commonly produced in the form of a white powder, yet its conversion into various final forms is achieved through developed processing technologies, thus broadening its application potential. Within the scope of the research of HA forms in Contipro a.s., the attention is focused primarily on the fabrication of nanofibers, microfibers, thin films, hydrogels, and amphiphilic carriers.

Among the various forms that can be prepared from HA and its derivatives, nanofibers have attracted considerable interest due to their exceptional mechanical properties and high surface area-to-volume ratio in a thin layer. These nanofibers find applications in cosmetics and skin regeneration, wound healing, tissue engineering scaffolds, and drug delivery systems. [3,4] Microfibers, on the other hand, offer unique opportunities for cell culture and drug encapsulation, enabling precise control over drug release kinetics. These fibers can also be knitted into more advanced structures to improve their mechanical and absorbing properties. [5,6] Thin films based on HA have demonstrated promising potential as wound dressings and antiadhesive barriers, providing a biocompatible, non-adhesive interface between damaged tissues. [7,8] Hydrogels, as three-dimensional networks capable of absorbing large amounts of water, are another versatile form of HA. These hydrogels have been extensively studied for tissue engineering, drug delivery, and regenerative medicine applications, due to their ability to mimic the natural extracellular matrix environment. [9,10]

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P40: **Aminopolysaccharide-based Personalized Bone Implants Prepared by Direct Low-temperature 3D Printing**

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The objective of the *ProfiBONE* project is to establish close multidisciplinary cooperation between Czech and Icelandic partners with a focus on the research and development of biofunctionalized polymer-ceramic ink for low-temperature 3D printing of patient-specific bone implants. The premise is to improve the mechanical, degradation, osteoinductive, and antibacterial properties of bone implants by modifying the ceramic cement with a binder based on biodegradable synthetic polymers (developed at BUT), bioactive aminopolysaccharides (provided by Genís h.f.), and proteins. The advantage of this approach is avoiding the denaturation conditions such as radiation (laser or UV) or high temperatures. The whole process takes place at laboratory temperature (printing) or at 37 °C (curing of the final product). Therefore, it is possible to add bioactive additives directly to the paste during printing without losing their biological activity. This procedure also guarantees an even distribution of bioactive substances in the implant, which positively affects their release and the complex function of the implant. The parameters of both the printer and the composite paste were optimized for direct 3D printing of samples with different extrusion coefficients, compositions, degradation times, and mechanical properties. The results showed that the use of different internal structures greatly affects not only the resulting mechanical properties but also the suitability for cell seeding, where the original regular structure was inappropriate. The modified structure prevented cells from falling through the scaffolds and ensured the uniform inoculation of the materials with SAOS-2 cells for all types of samples with different internal porosity. The *in vitro* biocompatibility tests on differentiated bone cells and mesenchymal stem cells are being performed gradually. The osseointegration properties of the printed implants are monitored using an established rat femoral bone defect model and are tested on a new rat parietal bone defect model. The recent histological evaluation confirmed osseointegration of new bone into the printed implant without significant inflammation of surrounding tissue. In vivo resorbability of 3D printed implants in skull defects are being currently studied.

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P41: **Fructan Biopolymers in Functional Biotics Production**

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It has been well-known for a long time now that there is a direct relationship between human health and the consumption of pro-, pre- or synbiotics and that they significantly affect the gut flora and hence the emergence of various diseases ranging from cancer to psychiatric disorders. Today, newly emerging biotics like paraprobiotics, postbiotics, psychobiotics, nutribiotics, and gerobiotics gained huge popularity due to their added multifaceted beneficial effects as well as the feasibility of their industrial production processes [1]. Fructans are homopolymers of fructose with a wide range of applications in the food, medical, pharmaceutical, cosmeceutical and chemical industries. Depending on the glycosidic linkages, they are classified as levan-type (β-2,6 linkages) and inulin-type (β-2,1 linkages) fructans [2]. Thanks to their well-established prebiotic effects, inulin and inulin-type oligosaccharides have the largest market share in the functional food market worldwide. Also, escalating number of reports point to levan as an emerging prebiotic polymer. With many probiotic members, most lactic acid bacteria have the capacity to synthesize inulin- and/or levan-type fructans. For this reason, fructanogenic (fructan-producing or metabolizing organisms) lactic acid bacteria are invaluable assets for obtaining different biotic products.

The main purpose of this study is to obtain starter culture to produce biotic products from fructanogenic lactic acid bacteria and to use these products in functional foods in the future. For this firstly, various starter cultures from Nuvita Inc. (İstanbul, Turkey) were assessed for their fructanogenic characteristics and selected strains were cultivated under optimized growth conditions in bioreactors and their biotic products were characterized in terms of their antibacterial, antioxidant, anticarcinogenic characteristics as well as their antibiotic resistances. At the last step, industrial production in pilot scale will be conducted, resulting in a lyophilized final product.

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P42: **Synthetic Biology and Physics-based Modelling for Creating New PHAs**

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Polyhydroxyalkanoates (PHAs) form an interesting group of fully biobased polymers, which have the potential to replace many currently fossil-based materials. However, the huge design space of more than a hundred different monomers and their different combinations in random and block copolymer structures remains largely unexplored. We combine synthetic biology, polymer science, and physics-based modelling with the aim to improve PHA material properties and reveal the potential of yet undiscovered PHA structures.

Our synthetic biology approach has focused thus far on controlling PHA copolymer structure in recombinant yeast *Saccharomyces cerevisiae* [1]. We have used modified Tet-On system to control expression of lactate dehydrogenase gene (*ldhA*) from *Leuconostoc mesenteroides*. Binding of synthetic transcription factors upstream from *ldhA* was adjusted by varying doxycycline concentration in the cultivation media. With this method, we produced a set of poly(D-lactate-co-3-hydroxybutyrate) [P(LA-3HB)] copolymers with varying D-lactate monomer contents from 6 mol% to 93 mol%. We are currently analyzing thermal and barrier properties of these different P(LA-3HB) copolymers to create a validation database for our modelling approaches.

At the same time, we are developing both physics-based and data-driven modelling workflows to understand how material properties follow from the primary structure of the studied polymers [3]. Our first analyses target the barrier performance of PHA films, based on molecular simulation of permeant solubility and transport. Ultimately, we aim for a modelling platform for testing novel PHA designs before their experimental synthesis.

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P43: **Impact of the Absence of RfbC and FucS on the Extracellular Polysaccharides of *Synechocystis* sp. PCC 6803**

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Cyanobacteria are photosynthetic prokaryotes capable of producing extracellular polymeric substances (EPS). These substances can either remain attached to the cell (CPS) or be released into the extracellular environment (RPS). The EPS produced by cyanobacteria are mainly heteropolysaccharides, which exhibit an unusually high diversity of monosaccharides, including acetyl, methyl, and amino sugars, as well as peptide moieties and sulfate groups, making them attractive for several biotechnological and biomedical applications [1]. However, despite recent efforts [2,3], their full potential remains untapped due to limited understanding of the intricate mechanisms involved in cyanobacterial EPS production and export. Aiming at better understanding how to control the production and/or to fine-tune the characteristics of these polymers, the model cyanobacterium *Synechocystis* sp. PCC 6803 was used to engineer knockout mutants on genes encoding proteins putatively involved in the biosynthesis of the deoxyhexoses dTDP-L-rhamnose and GDP-L-fucose: *rfbC* (*slr0985*) and *fucS* (*sll1213*), respectively [4]. Both mutants exhibit a clumping phenotype at low cell densities and the *fucS* mutant demonstrated growth impairment compared to the wild type. The *rfbC* mutant produces significantly less RPS than the wild type, but its monosaccharide composition showed no major differences. Interestingly, transcript levels of another putative copy of *rfbC* (*slr1933*) seem to be higher. In contrast, the monosaccharide composition of the RPS from the *fucS* mutant showed no detectable levels of fucose and rhamnose, while the CPS exhibit diminished levels of rhamnose. Further studies, namely the generation of double/multiple mutants, are required to clarify various steps of these complex biosynthetic pathways.

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P44: **Production of Poly(3-hydroxybutyrate) with Recombinant *Corynebacterium glutamicum***

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Corynebacterium glutamicum is a well-characterized model organism and working horse for the white biotechnology industry. It is mainly used to produce amino acids like L-glutamate and L-lysine, which have already reached the million-ton scale [1]. This study aimed to genetically engineer *C. glutamicum* to produce poly(3-hydroxybutyrate) [PHB]. PHB belongs to the polyhydroxyalkanoates class, one of the most important biological degradable biopolymers, and has desirable plastic-like properties. Naturally, PHB is synthesized and accumulated intracellularly by a broad range of microorganisms for carbon and energy storage [2, 3]. As *C. glutamicum* is not a natural PHB-producer, the PHB biosynthesis genes (*phaCAB*) from *Ralstonia eutropha* H16 were plasmid-borne overexpressed, and furthermore the precursor supply of acetyl-CoA was increased by deleting the citrate synthase gene (*gltA*) in *C. glutamicum* [4]. By modifying the culture condition, a yield of about 40 % PHB CDW^{-1} was reached. Here we demonstrated that *C. glutamicum* is a suitable host for the biosynthesis of PHB, but further studies have to be done to aim for an economically feasible PHB production.

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P45: **Tailored Production of Various Biopolymers by Engineered Strains of *Yarowia lipolytica***

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Finding bio-based alternative routes for the production of polymers makes the 12th sustainable development goal of the UN. The oleaginous yeast *Yarrowia lipolytica* has recently been used for the tailored production of different polyesters with valuable properties such as medium chain length polyhydroxyalkanoates (mcl-PHAs) and polylactic acid (PLA).

The strategy for mcl-PHA production involves the engineering of the beta-oxidation pathway that generates 3-hydroxy fatty acids used as substrates by the PHA synthase. Depending on our metabolic engineering strategy, we produced an mcl-PHA homopolymer of C12-OH fatty acid, or an mcl-PHA heteropolymer for which we identified C8/C10/C12/C14-OH fatty acids. Interestingly, our strains accumulate more than 25% (g/g) of mcl-PHA polymers. A more detailed characterization highlights our polymers have distinct physicochemical properties relating to their composition [1].

The metabolic engineering strategy for PLA production involves identification and deletion of the lactate consumption pathway and the expression of two enzymes: a lactate activating enzyme and an engineered PHA synthase. We tested different subcellular localisations for these two enzymes to optimize the production of the CoA-intermediate and the production of the polymer. We depict the first production of PLA in a eukaryotic host with a 5% (g/g) accumulation of high molecular weight homopolymers [2].

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P46: **Plastics in the Soil and the Changes in the Microbial Communities**

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Polymeric materials (plastics) buried in the soil can influence the microflora in their vicinity. This could be related to many factors, among them, the chemical composition of the polymeric materials and the eventual biological availability of the carbon in them and to some extent the concentration of the plastics in the environment. Because plastics is a composed of particles that could not be often distributed homogenously in the environment, we can investigate the changes in relation to the distance from the material. Here we present a preliminary result from such a study with PLA, PBAT, PBSA, and PHB as the polymeric materials. The soil was sampled right on the material and then at some specified distances from the materials during the three-month time interval. The microbial community composition and dynamics were investigated by 16S and 18S rDNA amplicon sequencing. Specific bacterial and fungi taxa were promoted in the immediate vicinity of all materials. Nonbiodegradable and slowly biodegradable polymers influenced only the closest layer of the soil while the biodegradable polymers projected their influence at least several millimeters from the materials and even more in the case of fungi. Differences between the materials and time the evolution of the microbial communities is discussed. The results should help to understand the scale and dynamics of the changes induced by the introduction of plastics into the soil and possibly establish a safety limit for plastic contamination in the soil.

P47: **Investigation of Biodegradability of Polymers in Lab-scale Respirometers**

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Globally, the annual usage of plastics has been increasing, leading to a significant environmental pollution problem [1]. In order to prevent plastic pollution and pursue sustainable development, the international community actively promotes the production and use of biodegradable plastics, and relevant regulations are being enacted. As a result of this global trend, there is growing interest in biodegradable plastics, and numerous research institutes and companies are conducting research and development in this field.

For the sake of reproducibility and accuracy, there are ISO international standards and individual country-specific standards such as ASTM for measuring the biodegradability of polymers. Each standard analysis method is described in detail, taking into account factors such as the biodegradation environment (e.g., soil/water), biodegradation measurement methods, measurement scales, and polymer characteristics. Despite the existence of various standard analysis methods, there are challenges in accurately adhering to the standard specifications due to 1) the specific characteristics of environmental samples, 2) variations in the physical/chemical properties of biodegradable polymers, 3) the time-consuming nature of biodegradation measurements, and 4) the requirement for facility costs to ensure accurate biodegradability assessment. As a result, there are only a few cases reported where the biodegradability of polymers has been reported in strict compliance with standard analysis methods. Most studies have reported polymer biodegradation using facilities and systems developed internally, leading to a lack of reproducibility and reliability in the measured biodegradability of the polymers [2]. Consequently, the need for establishing biodegradability analysis methods and facilities that adhere to international standards, ensuring high accuracy and reproducibility, as well as the necessity for biodegradability certifications, are becoming increasingly important.

Furthermore, due to the inherent characteristics of biodegradable polymers, the biodegradability varies depending on the environment in which the biodegradation takes place, such as industrial compost, topsoil, liquid media, and natural seawater. Therefore, biodegradability certifications for biodegradable polymers require measurements of biodegradability in various environmental conditions, and it is necessary to specify the actual environments and conditions in which biodegradation occurs [3].

In this study, we will cover the definition of polymer biodegradation and discuss the measurement methods for biodegradability depending on the biodegradation environment.

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P48: **The Use of Biopolymers for Development of New Type of Bacterial Bioinoculants**

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Azotobacter vinelandii is a nitrogen-fixing rhizobacterium (PGPR), capable of synthesizing two completely different biopolymers with great potential for applications. Polyhydroxyalkanoates (PHAs) are produced and stored in the form of intracellular granules as energy storage, while alginate is produced extracellularly and used for bacterial protection. In agricultural demand, the use of PGPRs as bio-inoculants to increase the yield of plants and crops can be an alternative to conventional fertilizers. Encapsulation of bacteria into hydrogel-based carriers substantially improves the application potential of the bioinoculant, as well as the viability and robustness of the bacterial culture.

In this work, we screened several beneficial effects of different strains of *A. vinelandii* as the production of iron-chelating compounds (siderophores), phytohormones (Indole Acetic Acid) and bacterial ability to solubilize phosphates. Of all above six different strains we selected one strain with the best results (CCM 289) for IAA production (46.07μg/ml) and use it for inoculation of the soil with *Lactuca sativa* as a model plant easily affected by even small changes in the environment. The results between inoculated plant and the control were significant in the length of plants and leaves and chlorophyll content. According to our results, the bacteria trapped in a gel matrix seemed to be viable and had beneficial effects on plants, which confirms the feasibility and viability of the novel concept.

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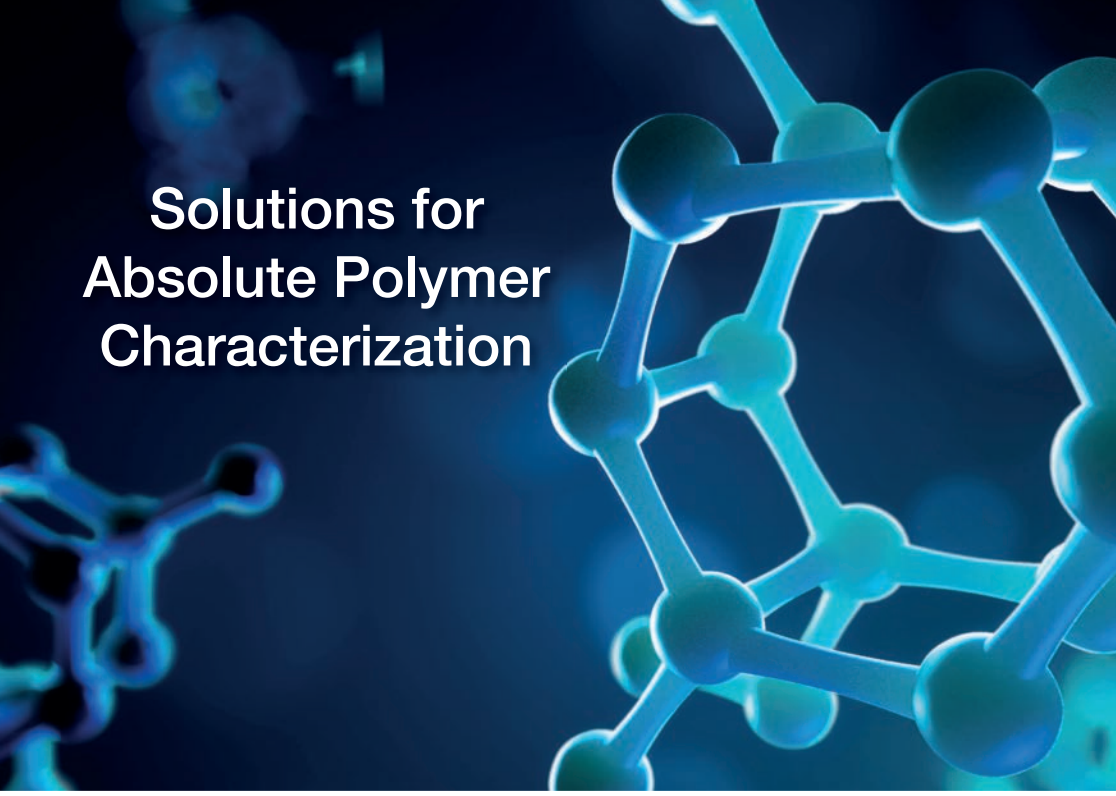

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