6th Symposium on Biotransformations for Pharmaceutical and Cosmetic Industry

June 17-21, 2024, Kraków, Poland

Book of abstracts

CONFERENCE ORGANIZERS
Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences
Faculty of Food Technology, University of Agriculture in Kraków

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ICSC PAS, KRAKÓW 2024

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Venue and other locations

Conference Venue
Jerzy Haber Institute of Catalysis and Surface Chemistry Polish Academy of Sciences
ul. Niezapominajek 8
30-239 Kraków

Minibrewery, Centre for Innovation and Research on Prohealthy and Safe Food, University of Agriculture in Krakow
Balicka 104
30-149 Kraków

Gala Dinner at Kawaleria Restaurant
ul. Gołębia 4
31-007 Kraków

Kraków by Night
Meeting point: Planty Park between St. Florian's Gate and Barbican

Live streaming

Day 1 - Monday 17.06.2024 Day 2 - Tuesday 18.06.2024 Day 3 - Wednesday 19.06.2024

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The best poster award will be announced during the Gala Dinner on Tuesday (18.06.2024).

Program at a glance

MONDAY 17.06.24	*TUESDAY 18.06.24*	*WEDNESDAY 19.06.24*
7:30 - 9:00 Registration		
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9:15-10:15 Invited lecture IL1 Zbyněk Prokop		
10:15-10:55 Oral presentations O1-O2	*10:00-11:00* Oral presentations O10-O12	*10:40-11:10* Coffee Break
10:55-11:30 Coffee Break	*11:00-11:30* Coffee Break	*11:10-11:50* Oral presentations O23-O24
11:30-13:10 Oral presentations O3-O7	*11:30-13:10* Oral presentations O13-O17	*11:50-12:50* Closing Lecture IL5 Johann Heider
		12:50-13:10 Closing ceremony
13:10-14:30 Lunch	*13:10-14:30* Lunch *Scientific Committee*	*13:10-14:30* Lunch & Departure
14:30-15:30 Invited lecture IL2 Paweł Satora	*14:30-15:30* Invited lecture IL4 Sandra Vojnović	
15:30-16:10 Oral presentations O8-O9	*15:30-15:50* Oral presentation O18	
16:10-16:30 Flash presentations FO1-FO3, FO8	*15:50-16:15* Flash presentations FO4-FO7	
16:30-18:00 Poster Session I FO1-FO3, FO8, P1-P17	*16:15-17:40* Poster Session II FO4-FO7, P18-P34	
18:00-22:00 Social events	*19:00-22:00* Gala Dinner Best Poster Award	

General information

Symposium on Biotransformations for Pharmaceutical and Cosmetic Industry has been organized biennially since 2014 although the first meeting took place in 2008 in the Institute of Organic Chemistry Polish Academy of Sciences in Warsaw. It is a scientific event where specialists from many branches of broadly understood biotransformation can meet.

The Symposium initially spun from the EU project "Biotransformations useful in the pharmaceutical and cosmetic industries", coordinated by the Wrocław University of Technology. However, it quickly evolved into an international meeting, with the participation of recognized global authorities in biotransformation. We aim to transform the biannual Symposium on Biotransformations into a conference integrating the Central European academic community with biotechnological and chemical industry representatives.

The sixth edition of the conference is an excellent opportunity to meet representatives of academia, scientific institutions and industry and to discuss the latest trends in biotransformation processes, as well as prospects for the future in this field. The invited lectures and talks cover many aspects of biotransformations, including the pharmaceutical, cosmetic, chemical and food industries. As in the previous editions, the scope of the conference has been expanded to biotransformations in environmental protection. We have also guests from industry presenting bioprocesses that are currently being implemented in Poland as well as research equipment and methods that are invaluable in studies of biotransformations and enzymatic processes.

The major topics of the Symposium are:

- Enzymatic and chemoenzymatic reactions
- Whole-cell biotransformations
- Cascade processes
- Green Chemistry
- Sustainable industrial processes, biorefinery and bioeconomy
- Bioprocess engineering and enzyme application
- Structure-function analysis and enzyme optimization
- Computational analysis for enzyme discovery and design

Bioprocessing workshop

This year, the conference meeting is accompanied by the Bioprocessing Workshop (June 19-21, 2024). The workshop takes place at the ICSC PAS demonstration biorefinery installation. Participants will be introduced to the processes of handling high-density bacterial cultures. In two parallel 5L systems, cultures will be conducted to achieve high concentrations of Escherichia coli, enabling high-efficiency enzyme expression. Additionally, the process demonstrated at a 30 L scale will familiarize participants with the production of PHA biopolymer in high bacterial cell density. Participants will also learn about post-processing biomass treatment, specifically using an industrial centrifuge from the company Cepa.

Media patronage

Sponsors

The project is co-financed from the state budget, granted by the Minister of Science and Higher Education Republic of Poland under the Excellent Science II Program.

ORLEN Południe is one of the key companies within the ORLEN Capital Group. Our production facilities are located in Trzebinia in Małopolska, and Jedlicze in Subcarpathia. We focus our business activities on key product segments related to the production of biofuels and biocomponents, propylene glycol glycerin, paraffin production and sales, as well as the production of paraffin-based specialties, solvents, heating oils, and base oils generated during the regeneration of waste lubricating oils. In ORLEN Południe, we implement a future-oriented economic model where resources circulate in a closed loop. Their added value is maximized, and waste generation is minimized.

We consistently develop new technologies, emphasize the development of our own know-how, and incorporate environmentally conscious design throughout the value chain, along with a series of optimization activities. We rationally utilize raw material resources, forming the basis for the development of our company towards to the biorefinery. We undertake key projects related to transforming renewable resources into high-quality "fine chemicals" used in various industrial sectors.

In 2021, we launched the first plant in Poland and the largest in Europe for the production of green propylene glycol. The "ECO" designed plant significantly reduces overall greenhouse gas emissions (GHG) per unit of propylene glycol production compared to conventional technologies – reducing emissions by 60-80%. This feature is significant for most recipients, including large enterprises concerned with their eco-friendly image and implementing a sustainable development policy.

In line with the strategy aiming for emission neutrality within the ORLEN Group, we are developing a new branch of future fuels – high-purity hydrogen 5.0 for buses and passenger cars.

In the Trzebinia refinery of ORLEN Południe, an innovative technology for producing lactic acid using microorganisms is being developed. We are also implementing an ambitious program for the production of advanced fuels, including second-generation bioethanol. The plant for its production is underway at our manufacturing facility in Jedlicze.

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- Flash chromatography systems columns and accessories.
- Size exclusion chromatography (GPC/SEC) systems, detectors, software and columns for characterizing natural and synthetic polymers, copolymers, peptides, proteins and conjugates.

- Osmometers dedicated primarily to clinical, research, research and development laboratories in pharmaceutical companies.
- Gas generators: nitrogen, hydrogen, and air.
- Clinical reference materials and chromatographic assay kits for medical diagnostics in-vitro.
- Analyzer for nitric oxide in exhaled breath and liquid samples, Sievers NOA 280i.

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shim-pol

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We are the sole distributor in Poland of Shimadzu, Kratos, Biotage, Dr. Maisch, iX Cameras and many other companies' solutions. Our strategic partner is SHIMADZU from Kyoto – Japanese world pioneer of analytical technologies. Our purpose of providing laboratories with the best analytical solutions and knowledge manifests in the recent opening of our R&D center.

Every year we organize numerous educational events and trainings: Analytical Chemistry Academy, Shim-pol Days, LabSolutions software workshops, chromatography courses and equipment demonstrations.

Day 1 - Monday 17.06.2024

7:30-9:00	**Registration**
9:00-9:15	**Opening ceremony**
Chairman: Maciej Szaleniec	
9:15-10:15	**IL-1 Discovery and engineering of biocatalysts powered by microfluidics** Zbyněk Prokop, *Department of Experimental Biology and RECETOX, Masaryk University* ***Discussion***
10:15-10:35	**O-1 Synbio4Flav and Beyond - Biocatalytic Modification of Flavonoids** Jarosław Popłoński, *Department of Food Chemistry and Biocatalysis, Wrocław University of Environmental and Life Sciences*
10:35-10:55	**O-2 Hydroxylation of flavonoids in a cascade reaction using recombinant enzymes** Kinga Dulak, *Department of Food Chemistry and Biocatalysis, Wrocław University of Environmental and Life Sciences*
10:55-11:30	**Coffee Break**
Chairman: Ryszard Ostaszewski	
11:30-11:50	**O-3 Triple expression system for efficient and controlled production of the enzymatic cascade for flavonoid rhamnosylation** Agata Matera, *Department of Food Chemistry and Biocatalysis, Wrocław University of Environmental and Life Sciences*
11:50-12:10	**O-4 Fusion proteins as a versatile biocatalyst for different reactions** Anna Christina Ngo, *Microbial Biotechnology, Ruhr-Universität Bochum*
12:10-12:30	**O-5 The use of Ascomycota and Basidiomycota strains in the synthesis of natural pigments and flavors** Filip Boratyński, *Department of Food Chemistry and Biocatalysis, Wroclaw University of Environmental and Life Sciences*
12:30-12:50	**O-6 Kinetic properties of whole-cell Baeyer-Villiger oxidation depending on the oxygen transfer rate** Patrik Cabadaj, *Chemical and Biochemical Engineering, Institute of Chemical and Environmental Engineering, Faculty of Chemical and Food Technology, Slovak University of Technology*
12:50-13:10	**O-7 Biocatalysis in the synthesis of valuable substances from cyanobacterial cells**

	Sunday Ocholi Samson, *Faculty of Chemistry, Department of Biochemistry, Molecular Biology and Biotechnology, Wrocław University of Science and Technology*
13:10-14:30	**Lunch**

Chairman: Danuta Gillner

14:30-15:30	**IL-2 Biotransformation in production of fermented beverages** Paweł Satora, *Department of Fermentation Technology and Microbiology, University of Agriculture in Krakow* ***Discussion***
15:30-15:50	**O-8 Conversion of renewable raw materials – transformation to biorefinery** Aleksandra Pajor, *Technology and Development (R&D), ORLEN Południe S.A.*
15:50-16:10	**O-9 Enzymatic Production of Bioactive Pentacyclic Triterpenes: A Case Study of Upcycling** Agnieszka Wojtkiewicz, *Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences*
16:10-16:30	**FO-1 One-Pot Two-Step Sequential Photo-Biocatalytic Deracemization of sec-Alcohols Combining Photocatalytic Oxidation and Bioreduction** Aleksandra Rudzka, *Faculty of Chemistry, Warsaw University of Technology* **FO-2 Synthesis of Optically Active Alcohols Using Photocatalytic Oxidative Cleavage of Alkenes Followed by Carbonyl Stereoselective Bioreduction** Natalia Antos, *Faculty of Chemistry, Warsaw University of Technology* **FO-3 Chromatography-free Lipase-Catalyzed Kinetic Resolution of Secondary Alcohols Using Vinyl 3-(dimethylamino)propanoate as an Acyl Group Donor** Beata Zdun, *Faculty of Chemistry, Warsaw University of Technology* **FO-8 Biotransformation of renewable raw materials into lactic acid** Justyna Więcławik, *ORLEN Południe S.A.*
16:30-18:00	**Poster Session I FO1-FO3, FO8, P1-P17**
18:00-18:30	**Transfer to the Minibrewery**
18:30-21:00 / 20:00-22:00	**Visit to the Minibrewery, Centre for Innovation and Research on Prohealthy and Safe Food, University of Agriculture in Kraków / Kraków by Night**

Day 2 - Tuesday 18.06.2024

Chairman: Tomasz Borowski

9:00-10:00	**IL-3 Lytic polysaccharide monooxygenases; a history and insights into current progress**

	Morten Sørlie, *Faculty of Chemistry, Biotechnology, and Food Science, Norwegian University of Life Sciences* ***Discussion***
10:00-10:20	**O-10 Metal: mobile or stationary to enable catalysis?** Ulf Hanefeld, *Biotechnologie, Technische Universiteit Delft*
10:20-10:40	**O-11 Theoretical studies on ectoine synthase** Justyna Andrys-Olek, *Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences*
10:40-11:00	**O-12 Unraveling the catalytic properties of benzylsuccinate synthase from a novel toluene-degrading strain *Aromatoleum* sp.** Gabriela Oleksy, *Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences*
11:00-11:30	**Coffee Break**

Chairman: Ewa Żymańczyk-Duda

11:30-11:50	**O-13 Shimadzu Solutions in Biotransformations and Metabolomics** Paweł Stalica, Shim-Pol A.M. Borzymowski
11:50-12:10	**O-14 Reasons to choose a corona CAD detector for research** Dominik Duczmal, *Adam Mickiewicz University, Faculty of Chemistry, Department of Applied Chemistry; Polygen sp. z o.o.*
12:10-12:30	**O-15 Enzymatic membrane reactors as versatile tools for sample preparation in analysis of new micropollutants** Jakub Zdarta, *Faculty of Chemical Technology, Poznan University of Technology*
12:30-12:50	**O-16 Microbial biomass as biocatalysts and bioaccumulation tool in two-step cascade process** Agnieszka Raczyńska, *Department of Biochemistry, Molecular Biology and Biotechnology, Wroclaw University of Science and Technology*
12:50-13:10	**O-17 Development of Chemical and Enzymatic Pathways for Modifying the Amino Group of 6-Aminopenicillanic Acid by Introducing Bacterial-Derived 3-Hydroxyacids into the Molecule** Maciej Guzik, *Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences*
13:10-14:30	**Lunch / Scientific Committee (room 206)**

Chairman: Maciej Guzik

14:30-15:30	**IL-4 Biotransforming waste streams into biomolecules and biomaterials** Sandra Vojnović, *Eco-biotechnology and drug development, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade* ***Discussion***
15:30-15:50	**O-18 Valorization of anthropogenic waste with microbial tools**

	Małgorzata Brzezińska-Rodak, *Faculty of Chemistry, Department of Biochemistry, Molecular Biology and Biotechnology, Wroclaw University of Science and Technology*
15:50-16:10	**FO-4 Designing a functional nanoemulsion using lactose esters and modified monomers of bacterial polyhydroxyalkanoates to improve anticancer activity of SN-38** Wojciech Snoch, *Department of Molecular Biology, University of Gdańsk* **FO-5 Innovative approach to removing pharmaceutical impurities using MOF-laccase systems** Agnieszka Rybarczyk, *Institute of Chemical Technology and Engineering, Faculty of Chemical Technology, Poznan University of Technology* **FO-6 The lipase – initiated chemoenzymatic cascade reaction leading to the C=C double bond cleavage** Anna Brodzka, *Institute of Organic Chemistry, Polish Academy of Sciences* **FO-7 Green Technology for Sustainable Environment 2024** Agata Zdarta, *Institute of Chemical Technology and Engineering, Faculty of Chemical Technology, Poznan University of Technology*
16:10-17:40	**Poster Session II FO4-FO7, P18-P34**
19:00-22:00	**Gala Dinner at Old Town with Awards for the Best Poster**

Day 3 - Wednesday 19.06.2024

Chairman: Małgorzata Brzezińska-Rodak

9:20-9:40	**O-19 Active and stable biocatalyst for the continuous flow synthesis of C8-C18 carboxylic acids esters of biomass-derived furfuryl alcohol** Anna Wolny, *Department of Chemical Organic Technology and Petrochemistry, Silesian University of Technology*
9:40-10:00	**O-20 Heterogeneous enzyme processes - reactors design** Katarzyna Szymańska, *Silesian University of Technology*
10:00-10:20	**O-21 S-1-(4-hydroxyphenyl)-ethanol dehydrogenase from *A. aromaticum*: catalytic stability studies** Mateusz Tataruch, *Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences*
10:20-10:40	**O-22 Exploring the Influence of Interfacial Hydrogen Bonding on Surface Properties and Foam Stability in Saponin Mixtures** Mateusz Jamroży, *Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences*
10:40-11:10	**Coffee Break**

Chairman: Maciej Szaleniec

11:10-11:30	**O-23 *Rhodotorula mucilaginosa* as a catalyst in biotransformation of phosphonates** Ewa Żymańczyk-Duda, *Faculty of Chemistry, Department of Biochemistry, Molecular Biology and Biotechnology, Wroclaw University of Science and Technology*
11:30-11:50	**O-24 Research on fungal enzymatic system active toward organophosphorus compounds.** Magdalena Klimek-Ochab, *Faculty of Chemistry, Department of Biochemistry, Molecular Biology and Biotechnology, Wroclaw University of Science and Technology*
11:50-12:50	***Closing Lecture* IL-5 Enzymes in Biotransformation: Overview and Outlook** Johann Heider, *Laboratory for Microbial Biochemistry, Department of Biology, Philipps University Marburg* ***Discussion***
12:50-13:10	**Closing ceremony**
13:10-14:30	**Lunch & Departure**

IL-1 Zbyněk Prokop

Discovery and engineering of biocatalysts powered by microfluidics

Z. Prokop[1,2], M. Vasina[1,2], D. Kovar[1,2], M. Marek[1,2],

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Intensive growth in biocatalysis requires the application of effective methods for the search and optimization of enzyme variants that meet the requirements of industrial processes. Microfluidics is a technology that offers high analytical throughput, reduced sample and power consumption, and facile process integration and automation. While the potential of microfluidic methods is already well exploited in nucleic acid analysis and screening of large metagenomic and directed evolution libraries, there is still room for broader applications in high-throughput biochemical characterization [1]. This lecture will focus on droplet microfluidics methods and their utilization for the systematic collection of functional data [2,3] as well as recording precise transient kinetic data, providing detailed insight into the mechanisms of enzyme catalysis [4] and protein (un)folding kinetics and stability [5]. The synergy of high-throughput microfluidic data collection, modern numerical methods for global data analysis, and molecular modelling will be provided in the examples [4,5]. The perspective potential of combining automated data collection by microfluidics with machine learning methods will also be discussed [1,6].

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Biotransformation in production of fermented beverages

P. Satora

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The production of fermented alcoholic beverages involves numerous processes in which microorganisms and enzymes convert components derived from the raw material into a wide range of compounds that affect the sensory characteristics of the resulting product. It is estimated that there may be as many as 800 to 1,000 such compounds in wine and about 800 in beer. These compounds belong to different chemical groups such as esters, alcohols, carboxylic acids, carbonyl compounds, polyphenols, sugars and many others. Some of them are primary metabolites (whose formation is linked to the growth of microorganisms), others are secondary - often requiring the formation of specific precursors [1,2].

Among these, terpenes and thiols, which are formed by plants and occur in a bound form, are an interesting group. Terpenes occur as glycosides bond to a glucose or disaccharide molecule, while thiols form disulfide bonds with amino acids, peptides and proteins. In this form, due to their low volatility, they do not significantly affect the sensory characteristics of beer/wine. To change this, it is necessary to use enzymes with β-glucosidase or lyase activity, which can be introduced in the form of enzyme preparations or as microorganisms with the appropriate activity [3,4].

The aim of our research was to determine the quantitative and qualitative changes in terpenes during enzymatic maceration using different commercial enzyme preparations with beta-glucosidase activity, as well as during the subsequent winemaking stage - fermentation. Three varieties of Polish grapes - Solaris, Seyval Blanc and Johanniter - were used in the experiments. Three Polish grape varieties - Solaris, Seyval Blanc and Johanniter - were used in the experiments; prob analyses (HS-SPME-GC-MS) were carried out during maceration, after sedimentation and in finished young wines. Enzymatic maceration increased the content of most terpenes analysed, with Solaris grapes being the richest in bound forms of terpenes. The greatest increase in terpenes was found after the clarification stage, in the must to be fermented. The fermentation process had the greatest effect on the terpene content of the finished wine. Some of these terpenes were degraded by the yeast (such as α-terpineol, rose oxide, etc.), while at the same time terpenes not present in the grape (such as α-terpinene, limonene, γ-terpinene, p-cymene-8-ol, etc.) were formed as a result of yeast metabolism. To maintain a high level of terpenes in the finished wine, *S. cerevisiae* strains should be used for fermentation, preserving the varietal characteristics of the grapes.

In the second experiment, the aim was to increase the amount of terpenes in beer obtained by so-called dry-hopping. Lager beer was hopped with Marynka hops and at the same time enzyme preparations with beta-glucosidase activity and pectinolytic agents were added.

The obtained results showed that the addition of enzyme preparations significantly influenced the content of aroma compounds in the tested beer. On the basis of the obtained results, it was noted that the addition of hops to beer (dry hopping) increased the concentration of some

terpenes and terpenoids, such as β-myrcene, humulene and caryophyllene. In the samples with addition of hops and Pectopol T-400 enzyme. Higher levels of humulene and β-myrcene were detected. Beer with hops and the enzyme complex (aromazyme and rapidase) was characterised by a higher content of nerol and geraniol. On the other hand, the application of two enzymes, Aromazyme and Pectopol T-400, increased the content of linalool. The complex of all three enzymes increased the amount of caryophyllene oxide, humulene and β-myrcene. The beer with the addition of hops was characterised by the lowest amount of aromas, with mainly floral and slightly fruity notes detected. In all the samples with the addition of enzymes, more intense floral, fruity and hop-derived aromas appeared. The results obtained prove that the use of enzyme preparations during dry hopping can contribute to the creation of a unique and distinct beer aroma profile.

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Lytic polysaccharide monooxygenases; a history and insights into current progress

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Biological conversion of plant biomass depends on peroxygenases and peroxidases acting on insoluble polysaccharides and lignin. Among these are cellulose- and hemicellulose-degrading lytic polysaccharide monooxygenases (LPMOs), which have revolutionized our concept of biomass degradation. In the CAZy database, LPMOs are categorised into eight of the seventeen auxiliary activity (AA) families (AA9-11 and AA13-AA17) based on sequence similarities. Despite large differences between the sequences of LPMOs in these different families, there are several conserved features evident in the secondary structure that unify all LPMOs. Central to LPMOs is an active site comprising a universally conserved histidine brace where a copper-ion is coordinated by three nitrogen ligands. Today, the prevailing view on the reaction catalyzed by LPMOs entails that the LPMO-Cu(II) is first reduced to LPMO-Cu(I) by a priming reduction step, followed by binding of H_2O_2 and homolytic cleavage. This is believed to generate a Cu-bound hydroxide species and a hydroxyl radical where the latter abstracts a hydrogen from the Cu-bound hydroxide to generate a Cu(II)-oxyl species, the formation of which is generally accepted. The Cu(II)-oxyl species then abstracts a hydrogen from the C-H bond of either C1 or C4 of the carbohydrate substrate, followed by hydroxylation of the bond, ultimately destabilizing the bond and leading to glycosidic bond cleavage. The lecture will briefly go through the history prior to the LPMO discovery, the transition of LPMOs to be viewed as monooxygenases to be peroxygenases, and key enzymatic features allowing the powerful catalysis of hydroxylation of saturated C-H bonds.

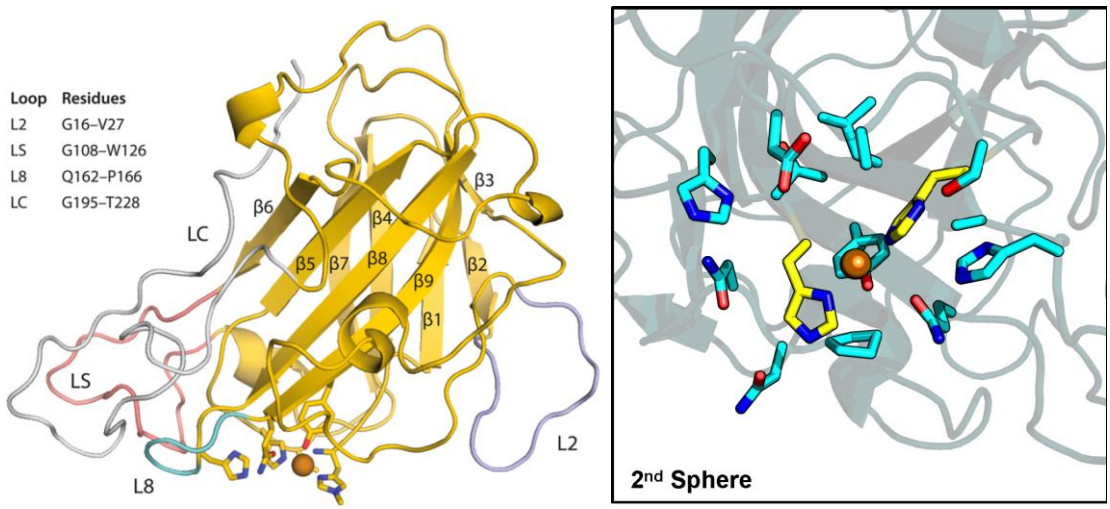

Figure 1. Crystal structure of an LPMO and possible 2nd sphere residues.

IL-4 Sandra Vojnović

Biotransforming waste streams into biomolecules and biomaterials

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Our consumption habits—from food to cosmetics, clothing, and various other products—urgently require a shift towards being more sustainable and environmentally friendly. Biotechnology emerges as a promising solution to address this pressing need. Microorganisms, though unseen to the naked eye, play a pivotal role in maintaining the health of our global ecosystem due to their abundance and diversity. They actively participate in crucial processes such as carbon and nutrient cycling, and contribute to human, animal, and plant health. Additionally, they serve as a valuable source of diverse products spanning industries like pharmaceuticals, chemicals, food, environmental management, and agriculture. Microorganisms can be turned into efficient factories for the production of various compounds and materials. Leveraging microbial capabilities, we can extract economic and environmental value through bio-upcycling, converting diverse waste streams into biomaterials (polyhydroxyalkanoates and bacterial nanocellulose), but also into next-generation, eco-friendly therapeutics. Focus is placed on bacterially derived natural products such as pyocyanin, prodigiosin, and actinomycin, which exhibit proven bioactivities like anticancer, antifungal, antibiofilm, and antiviral properties. Their greener production, processing and, formulation using innovative techniques such as fermentative bioprocess intensification, structural optimization via biocatalysis and formulations using metals, as well as biopolymeric drug carriers will be highlighted. In this way, harnessing the capabilities of microorganisms, we can effectively address both environmental and biomedical challenges, ushering in a new era of sustainability and innovation.

Enzymes in Biotransformation: Overview and Outlook

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This presentation will introduce some currently used enzyme-based biotransformation reactions, focused on oxidoreductases, which play a major role in modern production processes such as sterol transformation, generation of chiral compounds, biofuel and other value-added products. Moreover, strategies to implement new processes will be discussed, such as combining existing enzymes to larger synthetic pathways, establishing whole-cell systems by introducing recombinant proteins (Fig. 1), uses of rational mutagenesis or directed evolution and exploring the use of unusual novel enzymes from the microbial diversity for special purposes. Some examples of potentially useful enzymes will be introduced that are still underrepresented in industrial applications.

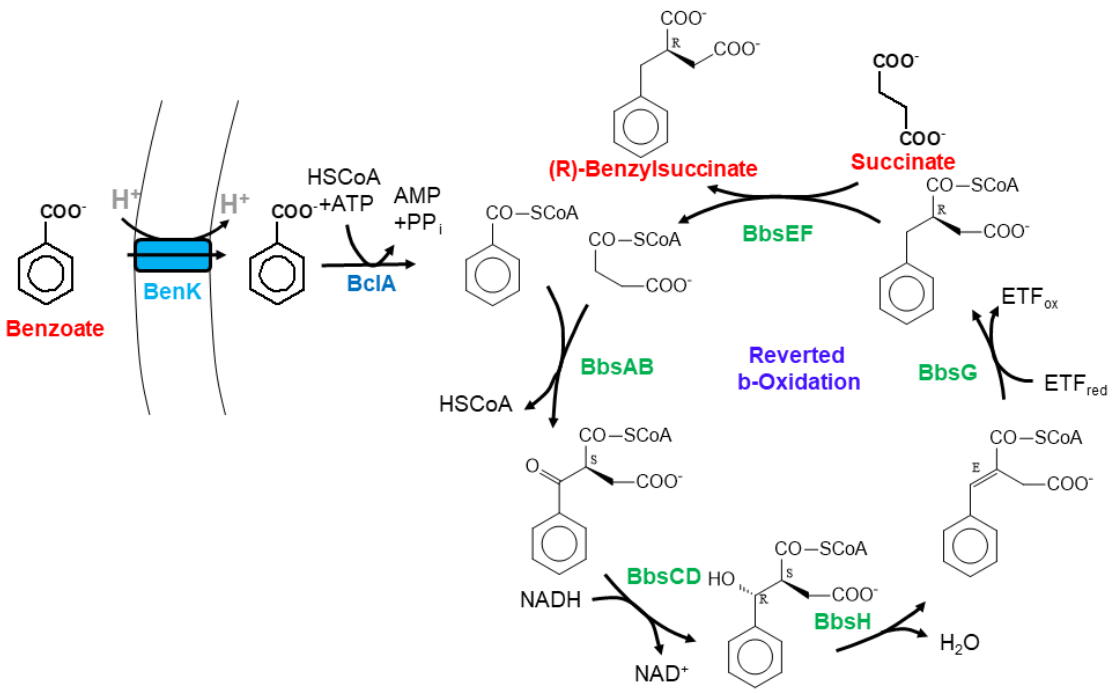

Figure 1. Whole-cell system of benzyl-succinate production. Benzoate is supplied to an anaerobic culture of recombinant *E. coli* carrying the genes for a benzoate trans-porter, a benzoate CoA ligase and the enzymes of benzylsuccinate degradation to benzoyl-CoA. Together with succinate produced from fermentation of glucose, benzoate is transformed to benzylsuccinate via the reverse-acting degradation enzymes [1].

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Synbio4Flav and Beyond - Biocatalytic Modification of Flavonoids

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The SynBio4Flav project aimed to establish a standardized pipeline for the production of numerous flavonoids using synthetic microbial consortia [1]. Flavonoids, widely used in functional food, beverages, cosmetics, and pharmaceuticals, are predominantly sourced from plants due to challenges in chemical synthesis and biotechnological approaches. SynBio4Flav address this limitation by breaking down complex metabolic pathways involved in flavonoid biosynthesis and distributing catalytic functions among different microbial species within defined consortia. The main outcome is a standardized platform comprising many optimized cell systems, enabling combinatorial *de novo* production of various flavonoids.

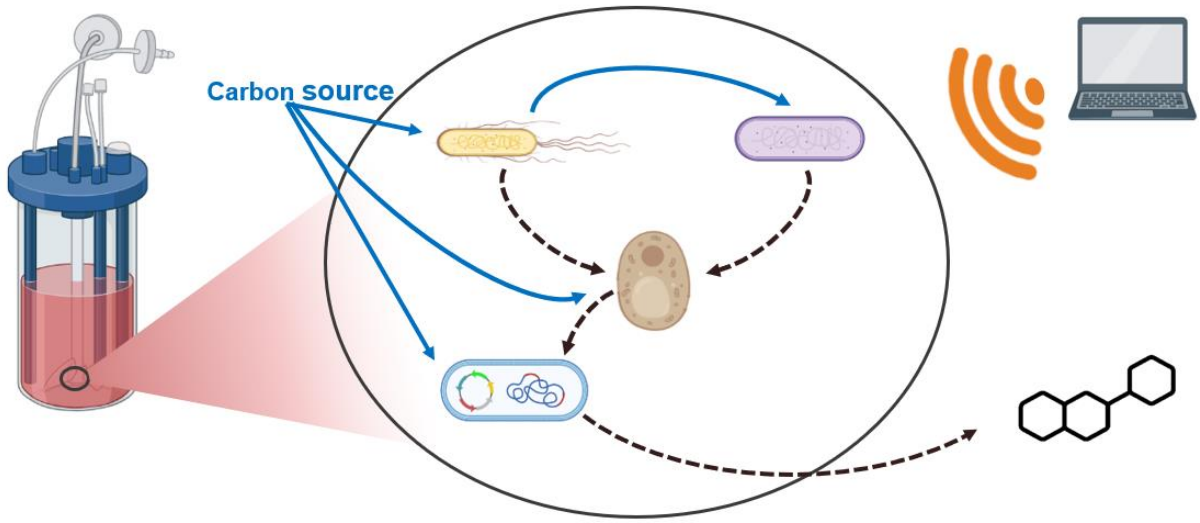

Figure 1. General scheme of SynBio4Flav synthetic microbial consortia based flavonoid production.

We would like to present our work on the preparation of functionalization modules and shed some light on the final stage of the biosynthesis of various flavonoid structures. We will also present recent advances in hydroxylation, methylation, and glycosylation, with special attention to biocatalytic cascade reactions that can also be utilized in vitro.

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[1]. www.SynBio4Flav.eu

Acknowledgments

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Hydroxylation of flavonoids in a cascade reaction using recombinant enzymes

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The search for new biocatalysts for efficient, tightly directed reactions is a leading concern in current biotechnology. This seems particularly relevant for types of reactions that are difficult to carry out using classical synthetic chemistry, such as the hydroxylation of aromatic compounds. Traditional screening method using collection of microorganisms with interesting catalytic properties are now in decline, replaced by *in silico* assays, that offers the possibility of quickly discovering new enzymes or expanding the knowledge of partially characterized biocatalysts [1]. Natural compounds remain an undiminished reservoir of new pharmaceuticals or their precursors. Furthermore, metabolic pathways for their synthesis or degradation represent an ideal source of potential enzymes with desirable catalytic capabilities [2].

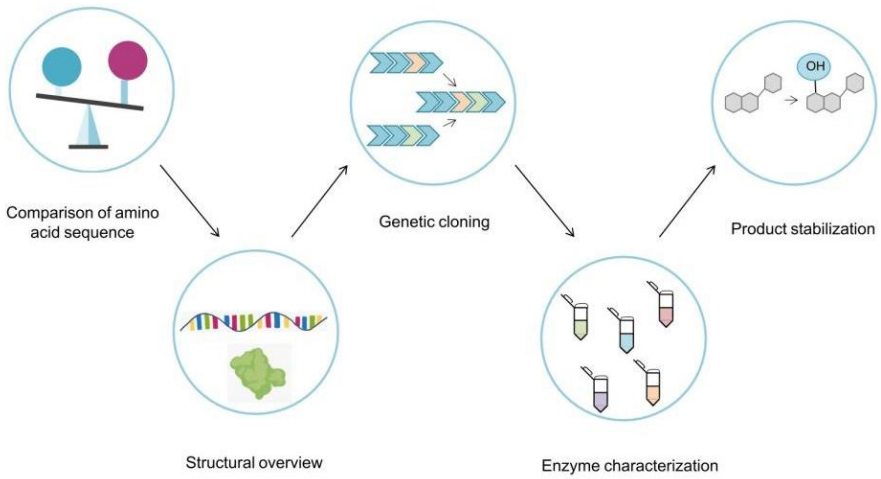

Figure 1. Flow scheme of the work.

This work demonstrate evidence for this approach. Based on preliminary bioinformatic analysis, a set of enzyme was selected and produced in *Escherichia coli* cells. This was followed by biochemical analyses and substrate specificity assays for each of the tested enzymes. The final step was reaction engineering for stabilization of easily degradable hydroxyderivatives of flavonoids. The application of the above workflow (Fig. 1) allowed us to obtain products enriched with an additional hydroxyl group at the C6, C8, and C3' positions, using biocatalysts from different hosts, belonging to different enzymatic classes and requiring different cofactors.

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This research was funded by the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreements no. 814650 (SynBio4Flav) and by the Wrocław University of Environmental and Life Sciences (Poland) as part of research project no. N070/0006/23.

Triple expression system for efficient and controlled production of the enzymatic cascade for flavonoid rhamnosylation

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The traditional concept of biotransformations, conducted by unmodified whole-cell biocatalysts, is diminishing in significance in favour of genetically engineered microorganisms, recombinant enzymes, or enzymatic cascades. Artificially designed biocatalysts are engineered to be more efficient, robust, and easy-to-implement. Thus, the biocatalyst design is gaining well-deserved attention as a vital step of process optimisation. Here, we present a characterisation of a set of bacterial plasmids tailored for recombinant expression of enzymes of interest in the broadly used *Escherichia coli* or *Pseudomonas putida.*

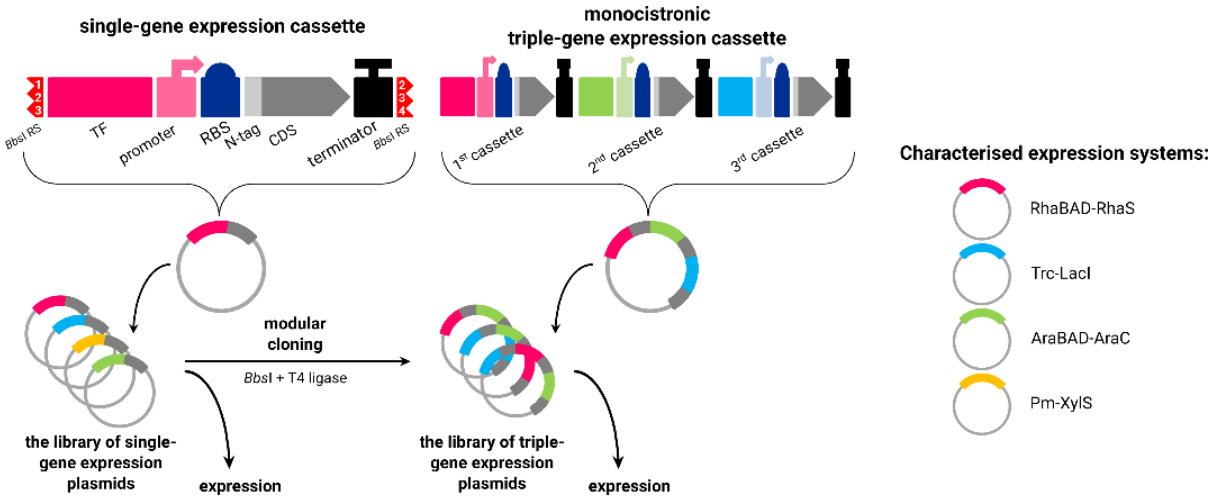

Figure 1. The framework of the expression cassettes design. Abbreviation: TF – transcriptional factor; RS – restriction site; RBS – ribosome binding site; CDS – coding sequence.

The plasmids set consists of four different bacterial expression cassettes (Fig. 1), which can be freely combined in up to three-gene expression plasmid using Golden Standard MoClo assembly [1]. Due to the independent induction of each cassette, it is possible to produce recombinant enzymes in desirable proportions, to get optimal cascade activity. The expression of triple-enzyme cascade consisting of sucrose synthase, UDP-rhamnose synthase and flavonol-7-*O*-rhamnosyltransferase was used as an example of designed plasmids utilisation.

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Acknowledgments

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Fusion proteins as a versatile biocatalyst for different reactions

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Synthetic dyes have become important to food, pharmaceutical, and textile industries. However, usage of these dyes has environmental and health implications. To circumvent these problems, biological approaches such as the use of whole-cell and enzymatic systems have been investigated. Enzymatic approaches involve the use of proteins such as azoreductases (AzoRo) to reduce synthetic dyes. However, AzoRo require the use of expensive co-substrates such as NAD(P)H and thus, makes the application quite impractical. The use of NAD(P)H recycling systems such as formate dehydrogenases (FDH) has been promising. Therefore, the combination of AzoRo and FDH makes it an efficient duo.

We previously investigated the fusion protein comprised of FDH and AzoRo with histidines as the peptide linker. As the construct improved substrate scope, we further investigated the linkers – using different linkers with varying length and properties. Based on our results, linker properties affect dye reduction with 2x helical linker being the best with 12 out of 20 dyes tested being reduced. We have also observed the production of hydrogen peroxide with and without the addition of FMN up to 2 to 3 mM – making our fusion constructs having oxidase activities. We also coupled our construct with an unspecific peroxygenase (*Cvi*UPO) to fuel hydroxylation and sulfoxidation reactions. *Cvi*UPO with 1 mM H_2O_2 alone cannot produce 1-phenylethanol at all but linker constructs that showed less and slower hydrogen peroxide production exhibited activities and improved enantioselectivity of 1-phenylethanol.

Therefore, our construct shows that we can have a plug-and-play system for different reactions. We can have a myriad of downstream applications like NADH production, dye degradation, H_2O_2 delivery, and cascade reaction with UPO all available from one biocatalyst.

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The use of Ascomycota and Basidiomycota strains in the synthesis of natural pigments and flavors

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Currently, natural pigments in comparison to the synthetic colors have taken a lead in a rapidly changing industry. Considerable attention has been paid to exploring the biotechnological applications of several *Monascus* sp. for pigment production. An endophytic Ascomycota strain isolated from leaves of *Origanum majorana* was identified as *Monascus ruber* SRZ112 produced following pigments: rubropunctamine, monascin, ankaflavin, rubropunctatin, and monascorubrin. As a first step towards developing an efficient production platform of red pigments, the suitability of seven types of agro-industrial waste was evaluated. To increase yield of red pigments, favourable culture conditions including incubation temperature, incubation period, pH of moistening agent, inoculum concentration, substrate weight and moisture level were evaluated. Additionally, yield of red pigments was intensified after the exposure of *M. ruber* SRZ112 spores to 1.00 KGy gamma rays [1]. In our further studies, this mutant was employed in the immobilization technique using various entrapment carriers. Subsequently, we optimized the culture medium for maximal red pigment production using the Response Surface Methodology. Finally, these immobilized cultures were successfully utilized for red pigment production using a semi-continuous mode of fermentation. Importantly, this study marks the successful production of *Monascus* red pigments in a semi-continuous mode using gamma rays' mutant strain [2].

The second part of presented research is placed within the context of natural flavor biogeneration. More specifically, our work has been conceived to find a biotechnological solution to the current shortage of natural fragrant benzaldehydes. Indeed, during the last twenty years, the major food brands gradually eliminate artificial flavors from their commercial products. This general trend has created a problem to the worldwide supply of natural vanillin and piperonal, which prices have increased steadily. Herein, the microbiological alkene cleavage of propenylbenzenes, including isosafrole, anethole, isoeugenol [3] as well as reduction of benzoic acid derivatives, namely *p*-anisic, vanillic, veratric, piperonylic, and eudesmic acids [4], to produce the corresponding fragrant aldehydes will be discussed. We found that different Basidiomycota strains efficiently perform this transformation, with good chemical selectivity and tolerance to the toxicity of substrates and products.

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Acknowledgments

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Kinetic properties of whole-cell Baeyer-Villiger oxidation depending on the oxygen transfer rate

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The biocatalytic Baeyer-Villiger (BV) oxidation is a green and reliable way to produce carboxylic acids esters or lactones from ketones. The biocatalytic approaches of such systems can be performed either with whole-cells, purified enzyme, or their immobilized form. In the presented work, whole-cell recombinant *Escherichia coli* BL21(DE3), harboring cyclohexanone monooxygenase (CHMO) original from *Acetobacter sp.* were used as a biocatalyst. However, the use of this type of biocatalyst in such a complex system brings various challenges, which must be coped with. An efficient BV oxidation requires availability of reduced coenzyme flavin adenine dinucleotide ($FADH_2$) and reduced cofactor nicotinamide adenine dinucleotide phosphate (NADPH). Therefore, regeneration of the oxidized components of the enzyme network is necessary through glycolysis of glucose. For this purpose, oxidative regime of the biocatalyst is desirable. Although, oxygen is consumed in the catabolism of bacteria, it is also the second substrate of the BV oxidation, so that oxygen air supply should be sufficient. Bioreactor experiments showed that similar bicyclic lactones production rate 1.68 mmol L^{-1} h^{-1} were obtained for the oxygen transfer rate (OTR) 4.83 mmol L^{-1} h^{-1} at different dry cell concentrations (0.5 – 4.2 g L^{-1}). However, when OTR was increased up to 18.29 mmol L^{-1} h^{-1} the reaction rate also increased to 7.81 mmol L^{-1} h^{-1} for the highest biocatalyst concentration. This observation means that, at low OTR and higher biomass concentrations, there was not enough oxygen for the BV oxidation and a major part of dissolved oxygen was utilized in the metabolic pathways. For better understanding of BV oxidation reaction, and its further optimization, it is essential to determine the oxygen consumption by cells and in the bicyclic ketone oxidation. The specific oxygen uptake rate by bacterial cells was determined to be 1.69 mmol g^{-1} h^{-1}. If an oxygen transfer rate higher than the uptake rate was chosen, the lactones production rate could be adjusted. An optimal ratio between biocatalyst concentration (g L^{-1}) and the oxygen transfer rate (mmol L^{-1} h^{-1}) was set to 1:5. Another factor influencing enzymatic reaction rate was the bicyclic ketone initial concentration. It was shown that the affinity of CHMO to ketone substrate is high. However, the lactones production rate kept constant in substrate concentration range 0.35 – 2.25 g L^{-1} and steep reaction rate decrease was obtained at bicyclic ketone initial concentration above 4 g L^{-1}. Furthermore, optimized reaction conditions like OTR and biocatalyst concentration ratio, and initial substrate concentration will be studied to increase the productivity of the whole-cell BV oxidation.

Acknowledgements

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Biocatalysis in the synthesis of valuable substances from cyanobacterial cells

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Microbial biotransformation can be considered as a mechanism by which a compound (substrate) is transformed (by means of microbial enzymes known as biocatalysts) into a desired substance(s) with ecofriendly, economic, and financial importance. In this research, cultures of several strains of cyanobacterial whole cells (*Synechococcus bigranulatus*, *Nostoc cf-muscorum*, *Kamptonema animale*, *Limnospira maxima*, *Limnospira indica*, and *Leptolyngbya foveolarum*) were selected as biocatalysts due to their robust catalytic mechanisms [1]. The productive potential of cyanobacterial strains to catalyze organic redox reactions and/or enzymatic hydrolysis of the target substrates in this research, phosphonates (vinyl and epoxy phosphonates) and 1-phenylethyl acetate and transforming them into valuable substances (Figure 1 b and c) has remained largely unexplored because of their unyielding and competitive inhibitors to several biocatalysts [2,3]. Biocatalysis of phosphonic compounds and 1-phenylethyl acetate has the potential for the synthesis of optically pure compounds that could present a platform for further applications in agrochemicals, flavors, cosmetic and pharmaceutical industries [4]. The current research could also be targeted at resolving the challenges of chemical synthetic approach resulting in significant emission of harmful substances and pollution of the ecosystem. Cyanobacterial biocatalytic approach on the other hand, presents a safe, limited pollution and toxicity.

Bioconversion methods employed were as follows: a) preparation of a 21-days cyanobacterial cultures (for phosphonates substrates) in a growth chamber, and 7/14-days cultures (for 1-phenylethyl acetate substrate) in phytotron growth chamber; b) Biocatalytic reactions between substrates and microbial cultures under sterile and favourable growth conditions; c) centrifugation for biomass removal; d) extraction; e) monitoring of results via Liquid Chromatography with Mass Spectrometry detector (LC/MS); Nuclear Magnetic Resonance (NMR) of isotopes ^{1}H and ^{31}P; Infrared Spectroscopy (IR); and Gas Chromatography (GC) (for 1-phenylethyl acetate bioconversion).

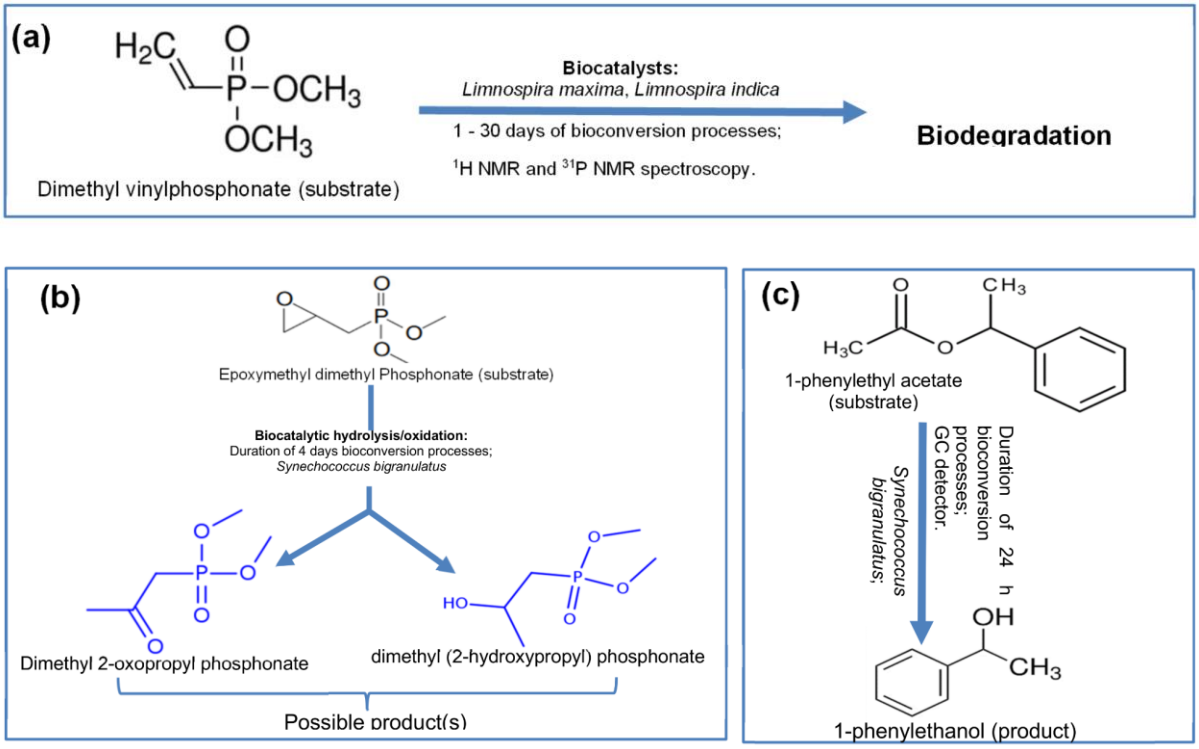

Figure 1. Possible sequences of photobiocatalytic reactions (a, b, and c).

Possible biocatalytic product, 1-phenylethanol (Figure 1c) is a secondary alcohol with increasing high demand in industries such as fine chemical, flavours, agrochemicals and pharmaceutical industries [3]. Dimethyl (2-oxopropyl)phosphonate (Figure 1b) has been explored as a reagent for the homologation of aldehydes to alkynes, which is a valuable transformation in organic synthesis [5]. Biodegradation observed in the case of vinyl phosphonate biotransformation process (Figure 1a) demonstrated the potentials of selected cells to degrade some phosphonates and using them as nutrient source. This finding could be ecologically important in limiting or eliminating pollution and toxicity of vinyl phosphonates or other similar undesirable phosphonic compounds in the environment.

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Conversion of renewable raw materials – transformation to biorefinery

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The chemical industry is one of the key branches of the processing industry. In Poland, it's one of the important elements of the economic system and one of the most important components of GDP (Gross Domestic Product). The increasing energy demands, gradual depletion of fossil fuels and rise of crude oils price are foremost motivations for exploration of renewable resources for sustainable production of energy, fuels, organic chemicals, fine chemicals and polymers [1]. All of this is also extremely important in the context of European Union regulations.

There have been many definitions and types of biorefinery. One classification covers the whole range of biomass and is based on well-known conversion technologies. Additionaly, technologies and products are expected to be expanded in near future in response to use of novel feedstock and further scientific advancements [2].

Utilization of renewable raw materials gain importance in the chemical conversion of substances in industry. Partial or even complete adjustment of economies to renewable raw materials require completely new approaches in research, development, and production. As predicted chemical and biological sciences play a leading role in the building of future industries [3]. To develop this direction, chemists should support this change and collaborate with colleagues in adjoining disciplines, for example biotechnology, agriculture, forestry.

ORLEN Południe rationally utilize raw material resources, forming the basis for the development of our company towards to the biorefinery. It is significant for most recipients, including large enterprises concerned with their eco-friendly image and implementing a sustainable development policy.

In 2021, we launched the first plant in Poland and the largest in Europe for the production of green propylene glycol. The "ECO" designed plant significantly reduces overall greenhouse gas emissions (GHG) per unit of propylene glycol production compared to conventional technologies – reducing emissions by 60-80%. This feature is significant for most recipients, including large enterprises concerned with their eco-friendly image and implementing a sustainable development policy.

In line with the strategy aiming for emission neutrality within the ORLEN Group, we are developing a new branch of future fuels – high-purity hydrogen 5.0 for buses and passenger cars. In the Trzebinia refinery of ORLEN Południe, an innovative technology for producing lactic acid using microorganisms is being developed. We are also implementing an ambitious program for the production of advanced fuels, including second-generation bioethanol. The plant for its production is underway at our manufacturing facility in Jedlicze.

During the speech, specific examples and results of completed investments, implemented technologies and ongoing research and development (R&D) projects will be presented, which are implemented by ORLEN Południe in accordance with the biotransformation strategy.

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Enzymatic Production of Bioactive Pentacyclic Triterpenes: A Case Study of Upcycling

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Recently we proved that 3-ketosteroid dehydrogenase from *Sterolibacterium denitrificans*, known as AcmB2 conducts an efficient and regioselective oxidative dehydrogenation of lupenone to glochidone [1]. The lupenone precursor – lupeol, a common natural compound derived from birch bark, is available in quantities of up to 20 g per kg of dry matter. While glochidone, also extractable from medicinal plants, occurs in significantly lower amounts, ranging from 11.4 to 83 mg/kg, representing the plant production 24 to 181 times lower. Furthermore, the extraction of glochidone from natural sources on a scale measured in milligrams does not suffice to meet the commercial demand for this biologically active compound. Addressing this challenge, enzymatic synthesis offers a solution, enabling the production of glochidone on a scale measured in grams, thereby exemplifying, in addition, an effective upcycling process.

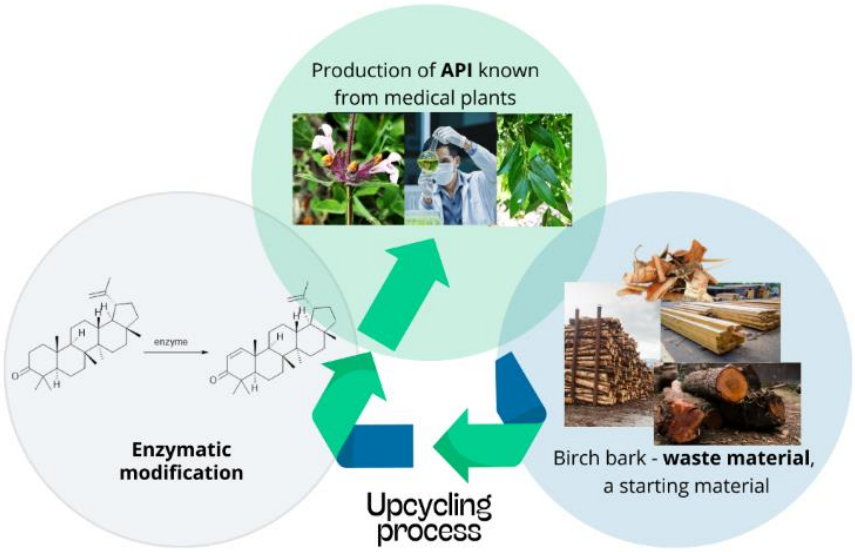

Figure 1. Synthesis of glochidone, active PCT known from medical plants by 3-ketosteroid dehydrogenase from lupenone sources from birch bark, a waste material.

Pentacyclic triterpenes (PCTs), natural compounds present in tree bark or leaves due to their diverse biological properties, are the subject of intensive research regarding their applications in pharmacy, food industry, and cosmetics. Documented properties of PCTs are anticancer, anti-inflammatory, antiviral, and antioxidant activities [2]. In some cases PCTs are even proposed as a beneficial alternative for steroidal chemotherapeutic or rheumatoid arthritis

treatment [3], [4]. Among other PCTs, glochidone, due to the presence of the C1-C2 double bond, remains a more potent active agent with therapeutic properties [5].

In our work we evaluated glochidone's cosmetic properties unveiling its significant rejuvenating potential, particularly its robust sirtuin induction activity, making it an attractive candidate for cosmetic formulations aimed at enhancing skin health [1]. Our study provides valuable insights into enzymatic synthesis and the biochemical properties of AcmB2, offering promising avenues for the development of bioactive compounds in the pharmaceutical and cosmetic industries.

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Acknowledgments

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Metal: mobile or stationary to enable catalysis?

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Enzymes are the catalysts of life and a third of them are metalloenzymes that require a metal cofactor for their catalytic activity. This metal cofactor is seen as static. It is perceived as fixed in one single position and to be motionless. We recently observed that the substrate can induce a 2.4 Å shift of the metal from a resting state to an active state. In the resting state, the metal is coordinated by three residues of the enzyme *Sw*HKA (E145, D171 and S116'), a Class II pyruvate dependent aldolase. In contrast, in the active state two residues (E145, D171) bind the metal, and a pyruvate derivative (not shown) is coordinated as substrate. Remarkably all this occurs without significant changes in the protein structure [1,2].

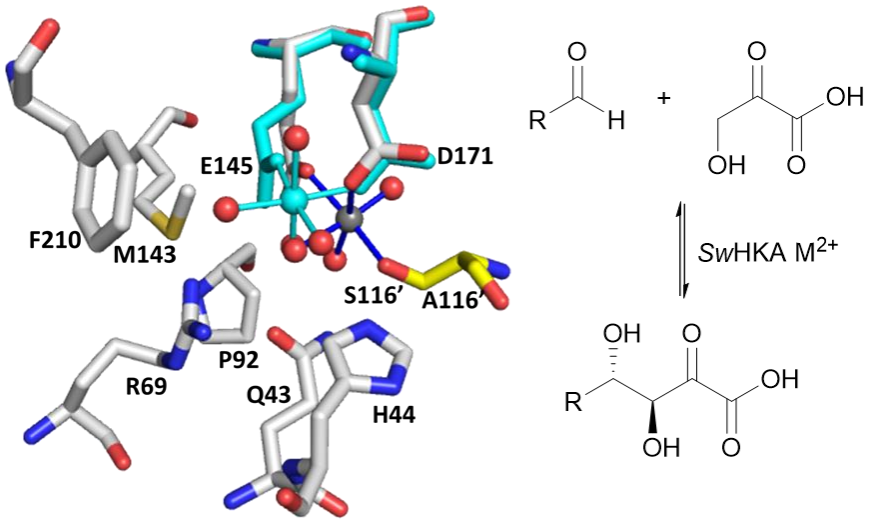

Figure 1. Metal shift in SwHKA. Mg(II) moves more than 2 Å upon docking of the substrate.

To gain insight into the generality of this metal shift for catalysis we extended our studies to other Class II pyruvate dependent aldolases [3]. Remarkably the closely related *Bp*HKA does not show this metal shift. Both enzymes require a divalent metal for their catalytic activity yet they differ in how they utilize this metal. Interestingly, they also differ in regard to phosphate. *Sw*HKA is activated by phosphate with *Bp*HKA does not display any differences in activity in the presence of phosphate.

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Theoretical studies on ectoine synthase

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Ectoine, a chemical chaperone produced by bacteria to counter osmotic stress, has gained significant interest from pharmaceutical and cosmetic industries due to its hydrating and cell-protective properties [1]. However, the reaction mechanism of its final synthesis step remains elusive. This step is catalyzed by ectoine synthase (EctC), a Fe^{2+}-dependent homodimeric cytoplasmic protein. To address this knowledge gap, we combined Mössbauer spectroscopy, molecular dynamics simulations and QM/MM calculations to investigate (1) the coordination geometry of the Fe^{2+} ion, (2) the geometry of an active site of enzyme-substrate complex (3) and finally propose a mechanism for the EctC-catalyzed reaction [2,3].

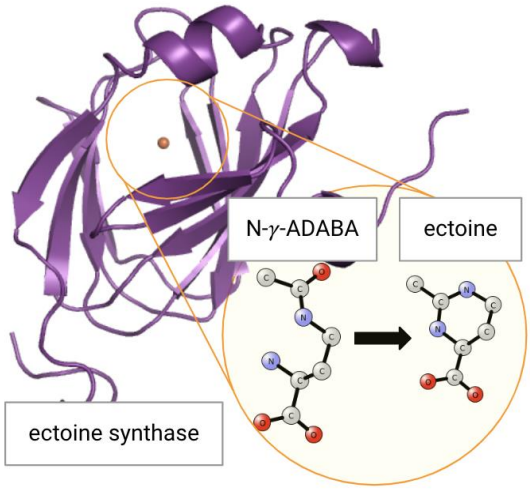

Figure 1. Model of EctC monomer with a scheme of catalyzed reaction of N-γ-ADABA cyclization [3].

Our findings indicate that the ligands needed to fill the first coordination sphere of the Fe^{2+} cofactor are the three amino acids (Glu57, Tyr84, and His92), along with one water molecule and one hydroxide ion. The latter two act as critical proton donors and acceptors during the cyclization reaction. Molecular dynamics simulations of the *Paenibacillus lautus* EctC (PlEctC) protein in dimeric form show that the presence of the substrate stabilizes the protein structure, notably affecting a short helix near the entrance to the active site [4]. Furthermore, amino acids crucial for substrate binding were identified as Trp21, Arg25, Asn38, Thr40, and Tyr52, which is consistent with previous experimental data [4,5]. The studies shine new light on the active site geometry and ligand interactions, providing insights into the dynamic nature of EctC-N-γ-ADABA complex and energetics of the ectoine biosynthesis reaction. This knowledge can help to design an efficient biocatalyst, which can be used in industrial production of ectoine.

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Acknowledgements

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Unraveling the catalytic properties of benzylsuccinate synthase from a novel toluene-degrading strain *Aromatoleum sp.*

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The petroleum industry is one of the main causes of the excessive release of hydrocarbons into the environment. Aromatic hydrocarbons contribute to the deterioration of soil quality and biodiversity depletion. Moreover, as these compounds enter the food chain and contaminate potable water, aromatic hydrocarbons are hazardous to human and animal health, increasing the risk of carcinogenesis, miscarriage and kidney and liver damage [1]. The areas affected by oil spills are often anoxic or anaerobic. Microorganisms capable of survival under such extreme conditions have developed a unique enzymatic machinery, allowing them to incorporate recalcitrant saturated and aromatic compounds into the central metabolic pathways even without oxygen [2]. One of the biodegradation strategies is fumarate addition to the methyl group of toluene and its analogs, which is catalysed by benzylsuccinate synthase (BSS).

Benzylsuccinate synthase is a representative of the glycyl-radical enzymes family. The reaction mechanism is started by a radical transfer from Gly to Cys residue, yielding thiyl radical, which enables the activation of the methyl group of toluene. Intermediate benzyl radical addition to the double bond of fumarate results in the enantioselective formation of a product-based radical, which is quenched by hydrogen abstraction from Cys, thus forming (*R*)-benzylsuccinate and regenerating the protein-bound radical (Fig. 1) [3]. Due to the high oxygen sensitivity of BSS, reports on successful activity tests are scarce.

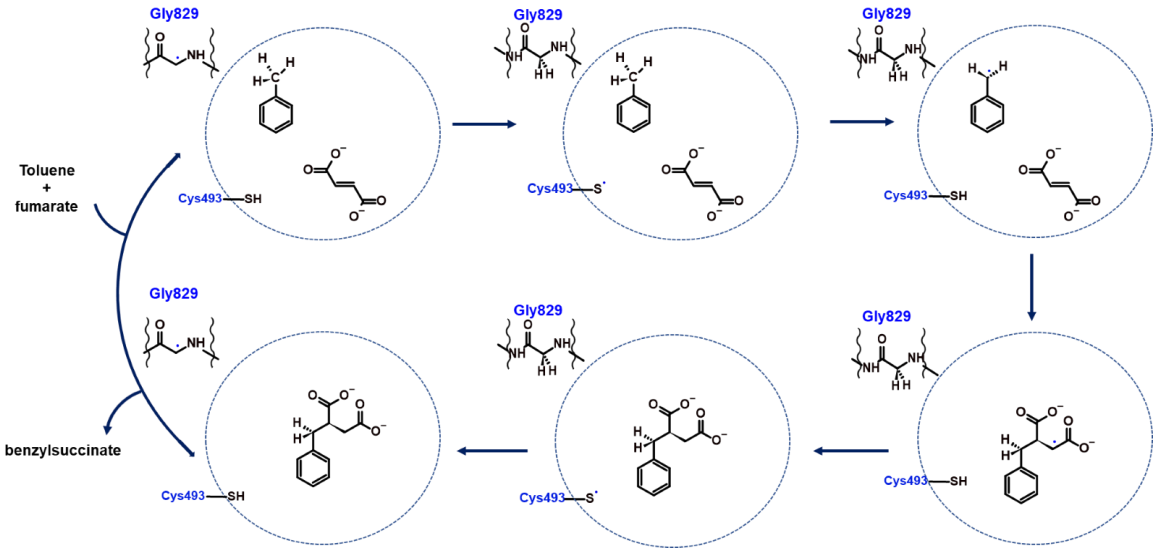

Figure 1. Proposed benzylsuccinate synthase mechanism (Heider et al., 2016, modified)

Throughout our research, a novel toluene-degrading strain of *Aromatoleum sp.* was discovered. Whole genome sequencing confirmed the presence of *bss* operon. Novel BSS isozyme shares the highest resemblance to the BSS isozyme from *Aromatoleum petrolei* ToN1. We have examined the catalytic properties of novel BSS, including the kinetic isotopic effect in a direct and competitive assay and H/D exchange on benzylsuccinate in the presence of 40% D_2O. Additionally, we have tested BSS activity with some of the toluene analogues, including cresols and xylenes isomers. All the activity tests were conducted under a strictly anaerobic atmosphere, using the cell-free extract, acquired from toluene-grown *Aromatoleum sp.* Samples collected from the reactors were subjected to LC-DAD and LC-MS/MS analysis, allowing the identification and quantification of benzylsuccinate and its derivatives.

According to our measurements, BSS isozyme produced by a novel strain *Aromatoleum sp.* exhibits a strong KIE, equal to 2.1 ±0.1 and 3.7 ±0.3 for a direct and competitive assay, respectively. These values are in agreement with the results obtained for BSS produced by *T. aromatica* T1 [4]. H/D exchange in the reaction product was proven by LC-MS/MS measurements in single ion monitoring mode, which provides experimental support for H/D exchange at the catalytic Cys493 residue [5]. At last, we have confirmed that BSS from *Aromatoleum sp.* can convert all three xylene isomers, suggesting that the strain may exhibit wider substrate preference which is an important factor for the removal of aromatic hydrocarbons from the contaminated sites.

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Acknowledgments

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Shimadzu Solutions in Biotransformations and Metabolomics

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Shimadzu supports the development and proliferation of biotransformations technologies by providing solutions combining mass spectrometers, imaging mass microscopes, databases, and software, which cover quantitative metabolomics, non-target analysis, imaging and multi-omics. With the capability to acquire up to 200 high resolution spectra per second we can create MS and MS/MS methods for targeted and untargeted metabolomics using a single DIA method. This workflow creates cycle times of <1 second for a mass range of m/z 50-1000 with high resolution accurate mass MS and MS/MS data.

Core ion beam technologies first developed and fully optimized on the Shimadzu triple quadrupole LC-MS/MS systems delivering class leading sensitivity at very fast acquisition rates. The same ion beam focusing and ion transfer innovations are part of the LCMS-9050 creating a high sensitivity QTOF.

The combination of highly sensitive and stable Q-TOF LC/MS and multivariate analysis software provides an effective workflow for non-target/target metabolomic analysis. The combination of triple-quadrupole MS and database with a wide range of registered primary metabolite analysis conditions makes it possible to obtain large amounts of information efficiently, including simultaneous quantitative analysis of 475 compounds. Currently available are such method packages as:

- Primary metabolites
- Metabolic enzymes
- D/L amino acids
- Cell culture profiling
- Lipid mediators
- Phospholipid profiling
- Short chain fatty acids
- Bile acids etc.

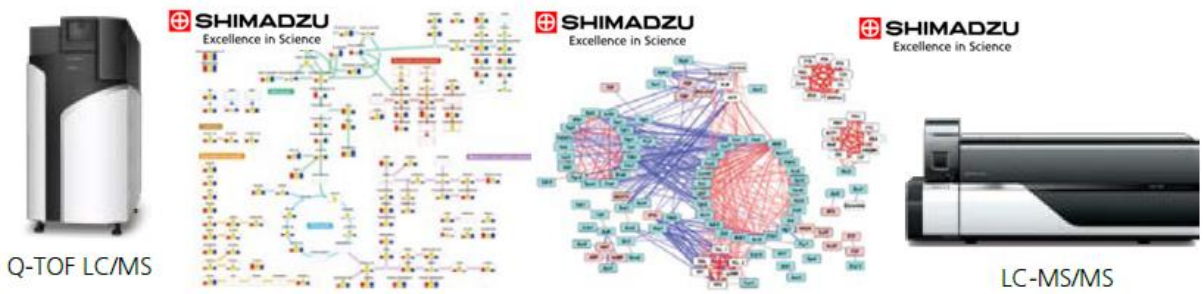

Figure 1. Shimadzu Solutions in Biotransformations and Metabolomics.

Reasons to choose a corona CAD detector for research

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In high-performance liquid chromatography (HPLC), the charged aerosol detector (CAD) offers an effective method for detecting non-volatile and semi-volatile compounds. The detection process involves nebulising the effluent from the HPLC column, charging the droplets through corona discharge, evaporating the solvent, and measuring the charged particles. This charge is correlated with the analyte mass for quantitative analysis. The CAD's capacity to identify a diverse range of compounds, including those with poor UV absorbance, such as sugars and lipids, in addition to its sensitivity and adaptability to gradient HPLC, renders it invaluable for complex matrix analysis. It is widely employed in the pharmaceutical industry for the detection of drugs and impurities, and in the food and beverage industry to measure sugars and lipids. CAD's versatility also extends to industrial applications, analyzing polymers and detergents, thereby broadening the applicability of HPLC beyond UV-detectable substances.

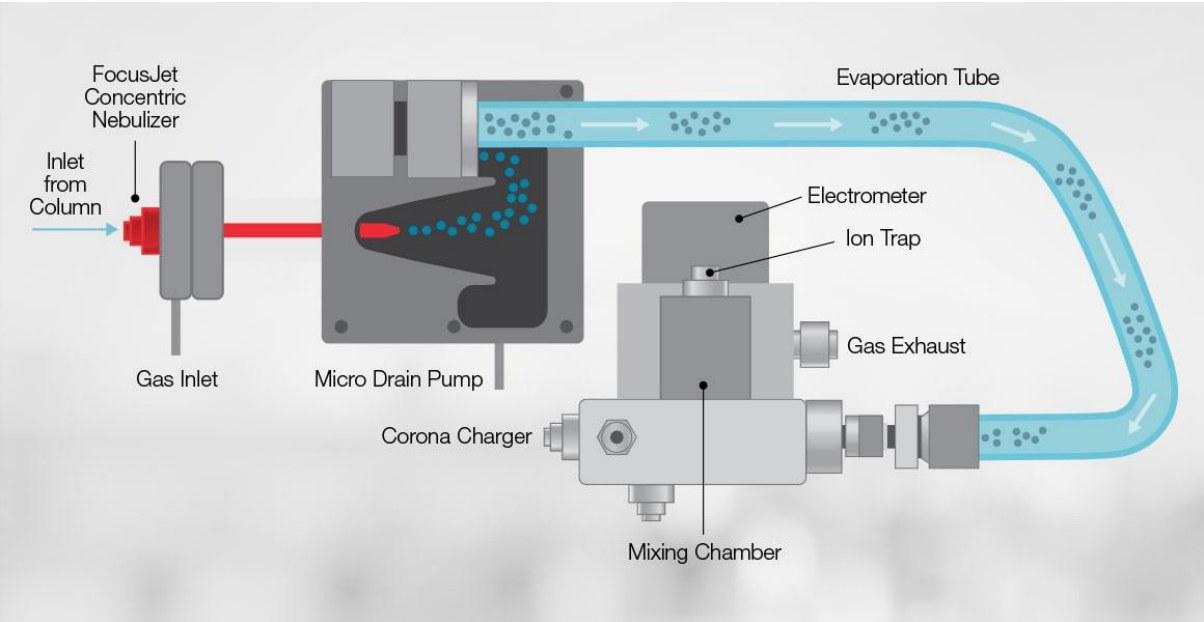

Figure 1. Schematic diagram of the Charged Aerosol Detector components

Building on the findings of research presented in article [1] on synthetic cannabinoids, the objective of our study was to investigate the quantitative detection of other cannabinoids in

hemp-derived products using a corona detector, with the analysis based on a single standard, such as cannabidiol (CBD).

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Enzymatic membrane reactors as versatile tools for sample preparation in analysis of new micropollutants

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Extensively growing market of goods and services as well as dynamic development of industry results in constantly raising amount of various pollutants in soil and water bodies. Beside well-known pollutants, such us heavy metal ions, oils or micropollutants including pharmaceuticals, dyes, personal care products or pesticides, nowadays new micropollutants, including microplastic, arouse particular interest [1]. Microplastics are synthetic, high-molecular weight compounds that due to the action of various factors have been micronized into particles smaller than 5 mm. Microplastic particles are known by their low biodegradation rate and mostly remain in the environment and adversely affect the entire ecosystems and human body [2]. Although the removal of microplastic is a final global goal, also developing of standardized methods for monitoring the occurrence, distribution, and movement of microplastics in the environment is a serious challenge [3]. For this reason a series of research has been initiated to determine the effect of various substances in the sample on microplastic analysis and to develop universal protocols for removal of such an impurities and inhibitors for effective microplastic examination [4]. Nevertheless, the challenge is to propose one-pot approach for sample purification and its further analysis with limited negative effect on microplastic structure.

Hence, in the main goal of the presented study is to develop multienzymatic biomembrane composed of enzymes from various catalytic groups, for use in enzymatic membrane reactors for sample purification prior to microplastic analysis. As a main component of the system, aluminium- and gold-coated membranes were applied on the surface of which enzymes such as cellulase, lipase, protease and laccase were deposited. These enzymes are capable of removal of the most common impurities including cellulose, lipids and oils, peptides and biomass residues, respectively. The purpose of this research was to determine the most suitable conditions for membrane preparation and to examine enzymes order for retention of high catalytic properties and for efficient sample purification. After obtaining, membranes were thoroughly characterized to evaluate their physicochemical and morphological properties and were finally tested in removal of model impurities affecting microplastic analysis.

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Microbial biomass as biocatalysts and bioaccumulation tool in two-step cascade process

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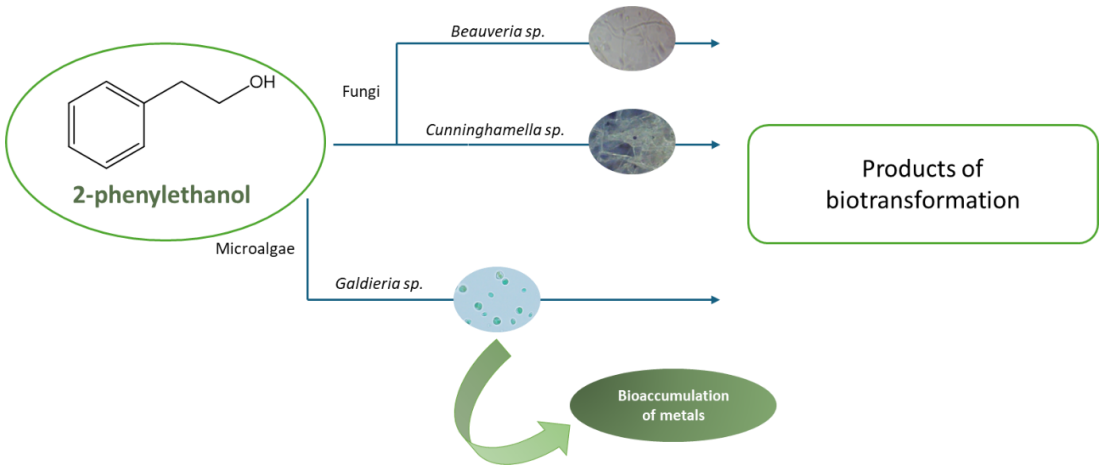

Figure 1. Graphical abstract: Microbial biomass as biocatalysts and bioaccumulation tool in two-step cascade process.

Whole-cell biotransformations are constantly explored field of possibilities of excluding chemical processes burdening the environment. They are low-cost methods of obtaining desired derivatives, especially important in case of chiral fine chemicals synthesis. As an inexpensive and widely available chemical compound 2-phenylethanol can be a valuable substrate for the preparation of many different products of antioxidant, anticancer or anti-inflammatory activity. These include among others 1-phenylethane-1,2-diol, 4-hydroxyphenylacetic acid and hydroxytyrosol [1].

Experiment started from the applying of the heterotrophic, eukaryotic biocatalysts. Fungi of the genus *Beauveria* and *Cunninghamella* are active towards 2-phenylethanol and were used as biocatalysts. To increase their specificity and protect against the possible substrate interactions, the mycelia of *B. bassiana* and *B. brongniartii* were subjected to different immobilization methods (e.g. calcium alginate, agar-agar and polyurethane foams). This was not possible for the *Cunninghamella* genus, because of its culturing features.

In the next approach photobiocatalysts were employed. The red alga *Galdieria sulphuraria* is known for its bioaccumulative abilities [2]. This gives the possibility to design the cascade two-step process applying this microalgae as biotransformation tool and then as biomass able to bioaccumulate the rare earth elements.

Cultivation and biotransformation conditions (e.g. temperature, rotation, medium, time) were individually adjusted to each microorganism.

Analysis of the reaction products was carried out using high-performance liquid chromatography (HPLC). Algal biomass tested towards rare earth elements accumulation was sent to analysis by inductively coupled plasma-mass spectrometry (ICP-MS).

All tested microorganisms (*B. bassiana, B. brongniartii, C. elegans, C. blakesleeana, C. echinulata, G. sulphuraria*) were positively verified as active towards 2-phenylethanol. However, depending on the strain selected, there were differences in the reaction efficiency and the resulting products. *G. sulphuraria* showed bioaccumulation potential towards rare earth elements but it was lower than in other tests [2], which means that previous biotransformation can influence the course of bioaccumulation.

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Development of Chemical and Enzymatic Pathways for Modifying the Amino Group of 6-Aminopenicillanic Acid by Introducing Bacterial-Derived 3-Hydroxyacids into the Molecule

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Semi-synthetic penicillins represent a crucial class of antibiotics globally, often preferred for treating infections due to their efficacy against a broad spectrum of Gram-positive and Gram-negative bacteria. They function by disrupting bacterial cell wall synthesis, specifically targeting peptidoglycan, which is absent in eukaryotic cells, thus ensuring their safety for humans and animals. However, the increasing prevalence of antibiotic-resistant bacterial strains has reduced the efficacy of many β-lactam antibiotics, highlighting the urgent need to develop novel antibiotics and β-lactamase inhibitors or find new modification methods.

This research project explores novel pathways for penicillin synthesis by modifying the side chain at the C6 position. The starting molecule is 6-aminopenicillanic acid (6-APA), which is modified using derivatives of polyhydroxyalkanoates (PHA), a diverse group of chiral (*R*)-3-hydroxy acids. Poly-3-hydroxynonanoate (P(3HN)) and poly-3-hydroxy-5-phenylvalerate (P(3H5PV)) were synthesized *via* bacterial fermentation and then linked to 6-APA benzyl ester through both chemical synthesis with T3P condensing reagent and enzymatic biosynthesis using *Thermomyces lanuginosus* TL-IM lipase. Six 6-APA derivatives featuring side chain modifications were obtained. These PHA-modified penicillins exhibited potent antibacterial activity against Gram-positive and Gram-negative bacteria, particularly of the *Proteus* genus, with a similar spectrum to penicillin G. Furthermore, the new derivatives displayed lower genotoxicity compared to penicillin G and ampicillin.

The pathways developed here utilizing structurally diverse PHA monomers could provide a framework for further modifications of the 6-APA core, leading to the discovery of new, more effective antimicrobial compounds.

Acknowledgments

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Valorization of anthropogenic waste with microbial tools

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Nowadays, post-consumer textile wastes are a big problem that has not yet found a satisfactory solution. A small part is degraded and most of the used textiles of various origins are burned or landfilled mainly in poorer countries such as Chile and Kenya. Only 12% is recycled and less than 1% is used to re-manufacture clothes [1]. Textiles can contain natural fibers of plant (cellulose) or animal origin (fibroin) and different amount of synthetic fiber addition (such as polyester). This results in the fact that the organic part of the resulting waste (cellulose fraction) can be managed as feedstock for production of biofuels (e.g. biomethane, bioethanol), while the synthetic fibers created after degradation can be reused in accordance with the circular economy. Although recycling can be beneficial, unfortunately the limiting factor is the wide variety of fibers and dyes used, which means that such waste must be properly sorted and prepared (e.g. removal of dyes, shredding, etc.) before further processing. Current methods mostly involve the use of chemical hydrolysis [1] and the use of enzymatic catalysis is mainly limited to auxiliary processes (e.g., increasing the solubility of cellulose) [2] or requires prior separation of chemical and organic fibers [3]. Our research focuses on finding a suitable biocatalyst (fungi or bacteria) capable of decomposing cellulose fibers directly in the fabric sample, without first separating the organic part. The aim of the research is to degrade the cellulose and use the released sugars for cellular purposes if a whole-cell biocatalyst is used, or to enzymatically hydrolyze the biopolymer and increase the solubility of sugar part (post-culture extracellular enzyme cocktail). In both cases, the resulting synthetic fibers will be directed for reuse (Figure 1). Due to limited literature reports, process optimization requires both the determination of optimal conditions for the cultivation of microorganisms and cellulase production, as well as the fabric transformation process itself. Preliminary research allowed to establish favorable conditions for the production of cellulases by the fungus *Trichoderma viride* and bacteria *Ideonella sakainensis* and to confirm the method of detecting the progress of the reaction (scanning microscopy). Further research will focus on quantifying the level of activity of the produced enzymes, selection of the appropriate method of preparing textile waste samples and determining the impact of external factors (temperature, time) on the efficiency of hydrolysis of cellulose fibers in the tested samples.

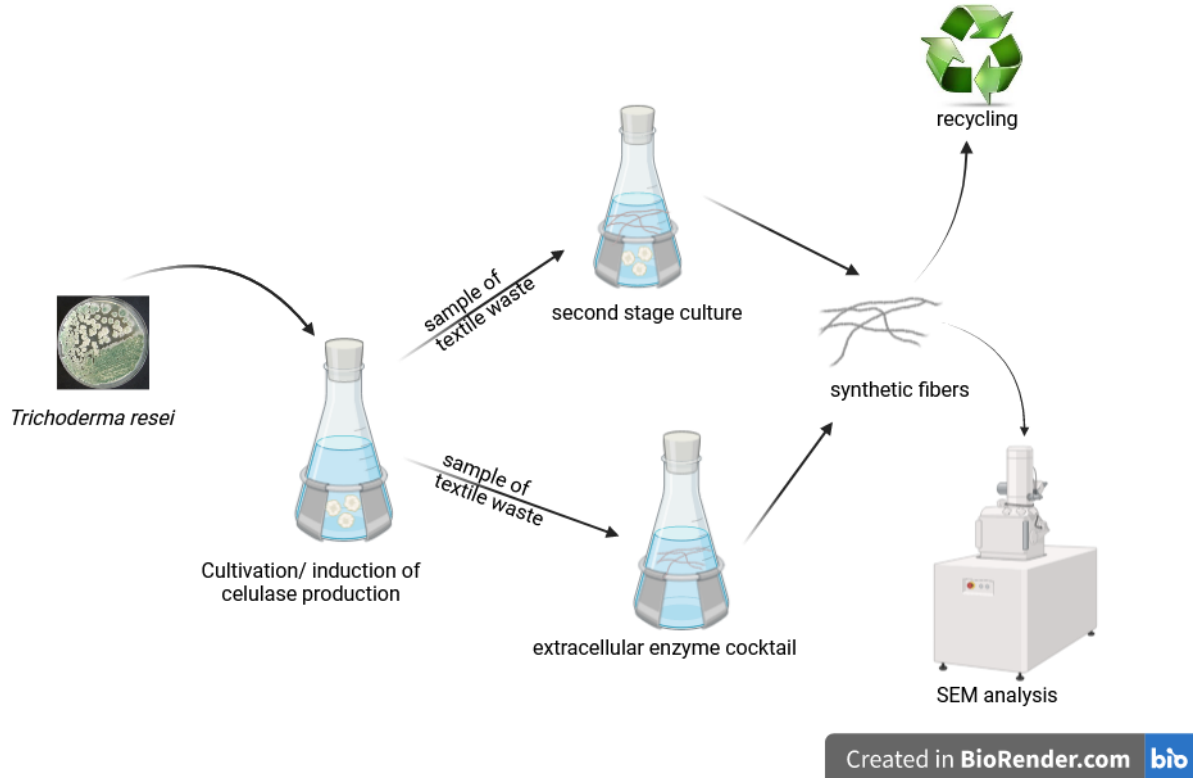

Figure 1. Simplified scheme for the valorisation of textile wastes.

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Active and stable biocatalyst for the continuous flow synthesis of C8-C18 carboxylic acids esters of biomass-derived furfuryl alcohol

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The availability of lignocellulosic biomass offers a promising avenue for sustainable chemical manufacturing, aligning with the principles of circular economy and carbon neutrality. As the demand for selective catalysts and environmentally friendly production methods continues to grow, biocatalysis emerges as a crucial solution. The biodegradability and high activity of enzymes enable their action under mild conditions, ensuring safety and effectiveness for the environment. However, challenges persist in improving the recycling and stabilization of enzymes in chemical processes [1, 2].

To respond these challenges, we explored immobilization methods employing various types of silica-based supports. Previous literature indicates that hydrophobic surfaces increased lipase activity, and introducing surface modifiers, such as alkyl groups on silica, enhances catalytic performance [3, 4]. Therefore, we modified $MgO{\cdot}SiO_2$ matrix with triethoxy(octyl)silane (C_8) to maintain the hydrophobic properties of the material. The modified matrix served as both a support and stabilizer for adsorbed lipase from *Aspergillus oryzae* (LAO). By combining expertise in biocatalyst design and support surface chemistry, we demonstrated the first application of a continuous flow system for furfuryl ester synthesis using the $MgO{\cdot}SiO_2$-C_8-LAO biocatalyst.

Initial studies conducted in a batch system showed excellent catalytic activity of $MgO{\cdot}SiO_2$-C_8-LAO in the esterification of furfuryl alcohol and caprylic acid, with 90% conversion observed after 45 min. The biocatalyst exhibited versatility towards other acids as well, achieving high conversions within short reaction times. Encouraged by these promising results, we transitioned to continuous flow synthesis, which proved to be an effective strategy for enhancing biocatalytic ester production. The transformation from batch to flow synthesis enabled us to achieve 97% conversion of furfuryl alcohol with optimized flow parameters. The space-yield-time for the active $MgO{\cdot}SiO_2$-C_8-LAO in the continuous flow system reached 458.4 ($gh^{-1}mL^{-1}mg^{-1}$). Visual representation of the designed continuous flow synthesis of furfuryl caprylate in the presence of the $MgO{\cdot}SiO_2$-C_8-LAO biocatalyst is presented in Figure 1.

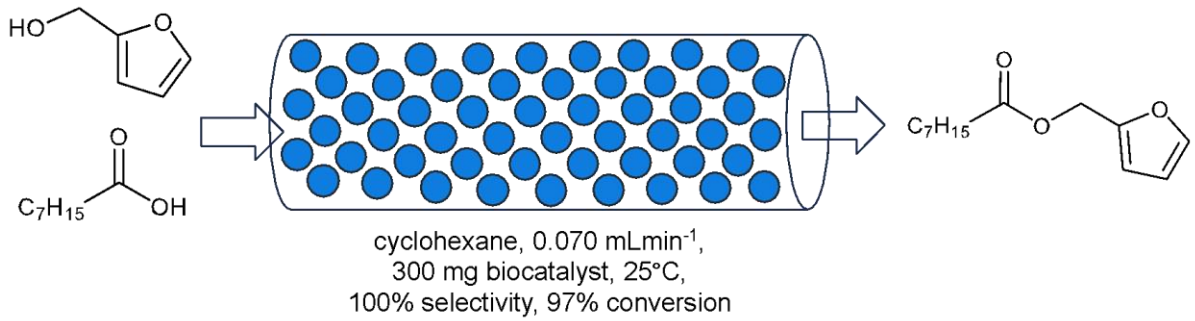

Figure 1. Continuous flow synthesis of furfuryl caprylate ester in the presence of $MgO{\cdot}SiO_2$-C_8-LAO.

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Heterogeneous enzyme processes - reactors design

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Since enzymes are by nature very active catalysts, their use in processes requires effective mass and heat transfer. This requires the design of suitable reactors. A basic distinction is made between batch reactors and continuous flow reactors. Nevertheless each of them should ensure protection of the immobilised biocatalyst from mechanical damage, in addition to effective mass and heat transfer [1, 2].

Here we present the possibility of using silica monoliths with a hierarchical pore structure as enzyme supports and their application in flow systems (Fig. 1A) and in a reactor with a stationary catalyst bed, StatBioChem (Fig.1B). In the flow system the hierarchical pore structure allows efficient mixing of the reactants. Due to the pore size of the silica material (μm), it is also possible to use it effectively in basket reactors. The efficiency of the reactors will be presented using selected biotransformations as examples.

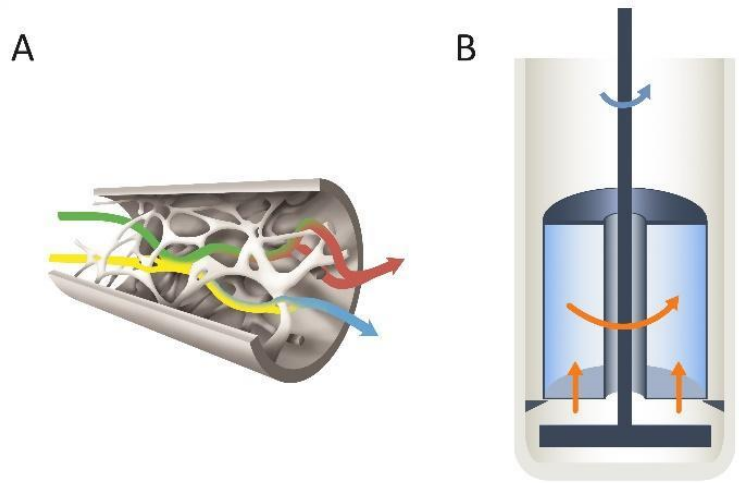

Figure 1. Scheme of continuous flow monolithic reactor (A) and StatBioChem reactor (B).

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S-1-(4-hydroxyphenyl)-ethanol dehydrogenase from A. aromaticum: catalytic stability studies

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Biocatalysts play a pivotal role in various industrial processes due to their selectivity and efficiency. However, their structural complexity often makes them susceptible to degradation, resulting in a progressive loss of activity. Therefore, alongside selectivity, the robustness of biocatalysts against inactivating conditions during reaction and storage is one of their most desired properties. Specifically, resistance to thermal inactivation and inactivation induced by extreme pH is crucial.

Derived from the denitrifying bacterium *Aromatoleum aromaticum* EbN1 (Azoarcus sp.), the enzyme *S*-1-(4-hydroxyphenyl)-ethanol dehydrogenase (S-HPED) belongs to the short-chain dehydrogenase/reductase family (SDR) and represents promising biotool for the stereoselective synthesis of chiral aromatic alcohols [1-3].

The presented study focuses on evaluating the activity and stability of S-HPED both under storage and process conditions within the pH range of 5.5 to 9.0. The relationship between the dynamics of aggregation and activity loss under various pH levels, and in the presence of glucose as a stabilizer, was analyzed. Based on inactivation experiments, the mechanism of thermal inactivation under storage conditions at pH 9.0 was modelled. The irreversible first-order mechanism of S-HPED inactivation was verified through isothermal and multi-temperature evaluations.

Our findings indicate that *S-HPED*, alongside *R*-1-(4-hydroxyphenyl)-ethanol dehydrogenase, is the second enzyme belonging to the SDR family for which a one-step thermal inactivation mechanism was confirmed under similar pH conditions [4]. These results provide the prerequisite for drawing initial conclusions on the inactivation mechanism of the entire SDR family. Confirming the first-order mechanism across a broader spectrum of enzymes from this group would provide stronger evidence to support such a general hypothesis.

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Acknowledgments

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MT acknowledges Erasmus+ programme (project number: 2021-1-PL01-KA131-HED-000005650) and Polish Academy of Science Study Visit Abroad programme for providing financial support for the research visits to Slovak University of Technology in Bratislava, where part of presented data was obtained.

Exploring the Influence of Interfacial Hydrogen Bonding on Surface Properties and Foam Stability in Saponin Mixtures

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Due to the constantly increasing pollution of the aquatic environment with detergents, it is crucial to develop modern, effective alternatives to commonly used synthetic surfactants. The ideal detergent must have a high surface activity to obtain a dispersed system with minimal biosurfactant content [1]. It should also be a biocompatible and hypoallergenic compound so that it can be used in industrial processes and in cosmetic and medical applications. A key feature of modern biosurfactants should also be the ease of removal of the compound from the aqueous environment and fast controlled biodegradation as soon as further activity of the compound is unnecessary.

Saponin is a plant-derived biosurfactant extracted from various plants, making it biodegradable and safe for humans and the environment. Saponin is used in cosmetics and medicine as a safe, bioactive and effective emulsifying agent. In vitro and in vivo studies exhibited their anti-inflammatory, antimutagenic, antiviral, antibacterial, antifungal and antitumor activities. Moreover, by reducing surface tension, saponins increase the solubility and absorption of medicinal substances sparingly soluble in water. The use of saponins makes it easier for drugs to penetrate cell barriers.

In recent years, various biotransformation methods of the original saponin extract, e.g. by fermentation, have become increasingly important [2]. The main way of transformation is through hydrolysis of saponin glycosyl groups to transform natural saponins into rare saponins containing low sugar chains. The generated conversion products have better bioavailability or more potent biological activity than the original saponins.

In our research, we want to develop safe methods for increasing the surface activity and foam-forming ability of saponins and their derivatives. Saponin is a compound capable of forming complexes and other advanced spatial structures with other biopolymers and chemical compounds through electrostatic interactions or via hydrogen bonds. Saponin can be both a hydrogen bond donor and an acceptor [3-5]. We aim to demonstrate that it is possible to use hydrogen bonds and/or electrostatic interactions to form complexes between saponin and other chemical compounds in order to 'modulate' surfactant surface and foaming properties.

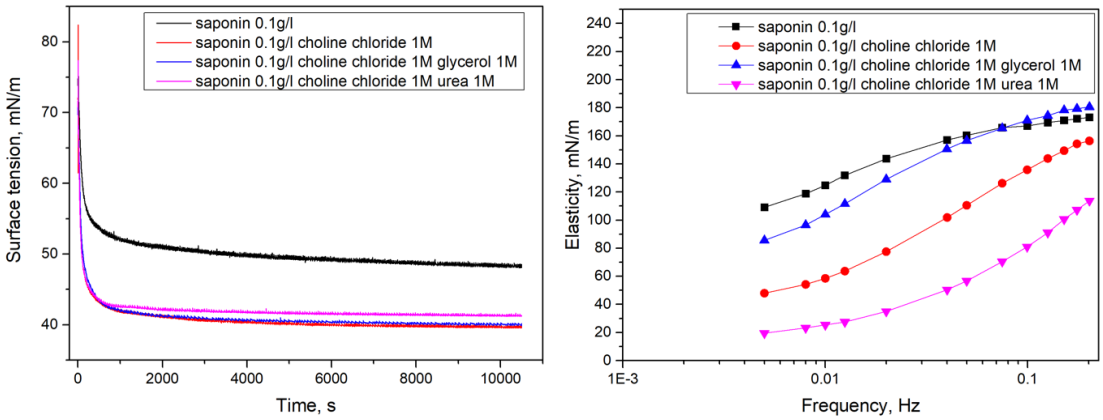

Figure 1. Surface activity and dilational elasticity in saponin - choline chloride - glycerol - urea mixtures.

The present study analyses the effect of adding glycerol, choline chloride and urea on saponin surface activity. As seen in Figure 1, adding these chemical compounds leads to significant changes in the adsorption layer. For example, choline chloride significantly increases the mixture's surface activity relative to the saponin solution's surface activity. Choline chloride also reduces the surface dilatational elasticity of the tested mixture.

We also compared changes in surface properties with changes in foam-forming properties and the stability of the foams obtained in the foam-forming column and in the Multiscan apparatus.

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Rhodotorula mucilaginosa as a catalyst in biotransformation of phosphonates

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As asymmetric organic synthesis has its challenges, which is the use of expensive asymmetric catalyst or harmful reagents, the application of biocatalysis is an attractive alternative. Enantiomers usually have different biological activity and it is imperative to synthesise an optically pure compound. Phosphonates have their applications as herbicides (i.e. Roundup), antivirals, antibiotics (i.e. fosfomycin) or chelating agents. *Rhodotorula mucilaginosa* is a known biocatalyst and was chosen for this experiment as it is able to grow in in the presence of phosphonate, as well as keeps its reductive properties in anhydrous conditions – here in hexane.

Immobilised on Celite R 630 and lyophilised cells of *Rhodotorula mucilaginosa* were the catalyst in biotransformation of 1,1-difluoro-2-oxo-phenylethylphosphonate diethyl into 1,1-difluoro-2-hydroxy-phenylethylphosphonate diethyl. Substrate, immobilised cells, and the addition of ethanol or isopropanol or a mixture of them both, in anhydrous hexane were shaken on a rotary shaker 130rpm for 3, 6 and 9 days. After biotransformation the solvent was evaporated on rotary evaporator. Samples for ^{31}P NMR were made with deuterated chloroform. The addition of quinine (a chiral solvating agent) in the samples made it possible to distinguish an optically pure product on ^{31}P NMR, which was the objective of the biotransformation.

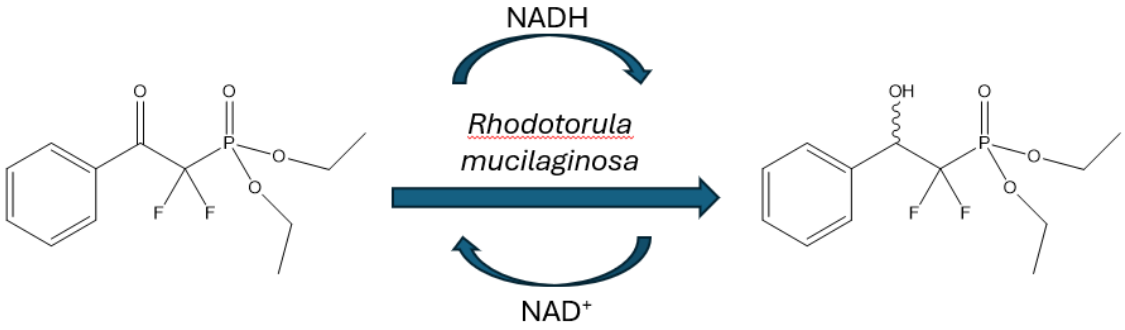

Figure 1. The process of biotransformation of the substrate into the product by *Rhodotorula mucilaginosa*

Research on fungal enzymatic system active toward organophosphorus compounds

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Organophosphonates are a group of compounds, both synthetic and biogenic, characterized by the presence of a stable carbon to phosphorus bond (C-P bond), which makes them very resistant to degradation [1]. Such compounds are believed to play a particularly important role in the biogeochemical circulation of phosphorus. Phosphonates enrich the global pool of phosphorus compounds, as synthetic compounds are widely used in industry and enter the environment in large quantities as xenobiotics [1, 2], but on the other hand, they are ubiquitous in biological systems

Phosphorus is a limiting nutrient in many environments and hence organisms have evolved metabolic pathways that can release phosphate from phosphonates of various origin. Several strategies have been discovered, including hydrolytic, oxidative, and reductive processes [3, 4] but the hydrolases activities towards phosphonate molecules are thought to contribute significantly to global P-cycling [2]. The most predominant biogenic P-C compound is ciliatine (2-AEP), which is very often found as a membrane lipid head-group analogues to phosphatidylethanolamine [4]. The amount of 2-AEP in phosphonic resources is dominant [5], so the degradation of this molecule containing P-C bond by microorganisms appears to be critical for the phosphorus cycle in various types of ecosystems. It is worth to stress, that since the phosphonates metabolism is, in most described cases, dependent on the presence of inorganic phosphate in the environment, data on the capability of microorganisms to mineralize P-C compound regardless of the phosphate status of the cell are relevant, but still incomplete, particularly for eukaryotic microorganisms.

The research presented here concerns the characterization of the two-step process of 2-AEP biodegradation by *Penicillium commune* strain. It was confirmed that the fungal decomposition process is independent of the phosphate status of the cell. For the first time, an eukaryotic phosphonatase catalyzing the enzymatic cleavage of the stable C-P bond in the phosphonoacetaldehyde moiety was isolated and partially purified. The enzyme was characterized and compared with analogous bacterial enzymes described in the literature. The partially purified fungal phosphonatase probably belongs to the enzymes of the HAD superfamily and, like its bacterial counterparts, requires the presence of magnesium ions (Mg^{2+}) for its activity.

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One-Pot Two-Step Sequential Photo-Biocatalytic Deracemization of *sec*-Alcohols Combining Photocatalytic Oxidation and Bioreduction

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Asymmetric synthesis of enantiomerically pure compounds constitutes one of the major pillars of the modern organic chemistry. In this context, stereocontrolled synthesis of non-racemic alcohols is critical for the production of varied high-value-added products such as pharmaceuticals, agrochemicals, flavours and fragrances [1].

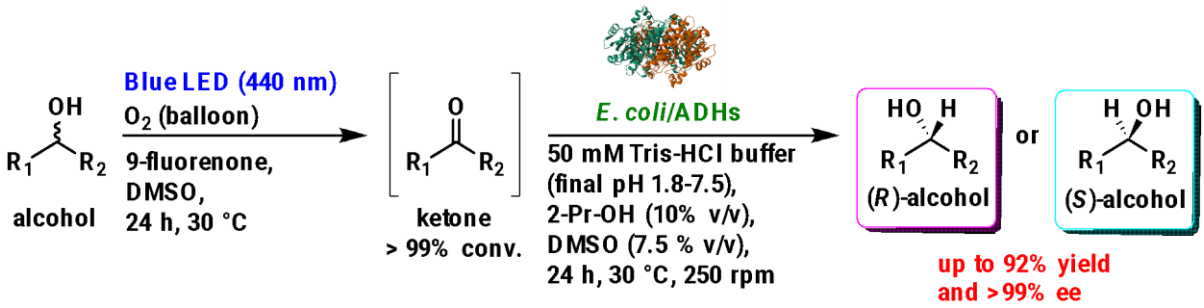

Figure 1. Photo-biocatalytic deracemization of racemic alcohols using 9-fluorenone-O_2-blue LED-*E.coli*/ADH system.

Herein, we report on the development of a one-pot, two-step sequential photo-biocatalytic cascade for the synthesis of enantioenriched alcohols (**Figure 1**) [2]. In order to quantitively oxidize a broad range of racemic (hetero)benzylic alcohols into prochiral ketones we employed a slightly modified protocol reported by Das et al. [3], which consisted of 9-fluorenone as a metal-free photocatalyst, molecular oxygen as the terminal oxidant, and DMSO as the hydrogen peroxide-neutralizing agent. The *in situ*-formed carbonyl intermediates were subsequently converted into optically pure alcohols using stereocomplementary recombinant alcohol dehydrogenases (ADHs) as biocatalysts. In conclusion, the elaborated linear telescopic cascade strategy allowed us to prepare pharmaceutically valuable chiral alcohols with excellent conversions (up to >99%) and enantiomeric excesses (up to >99%) in a stereocomplementary fashion.

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Acknowledgments

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Synthesis of Optically Active Alcohols Using Photocatalytic Oxidative Cleavage of Alkenes Followed by Carbonyl Stereoselective Bioreduction

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Oxidative cleavage of alkenes to obtain carbonyl compounds has traditionally been carried out by ozonolysis, which relies on the *in situ* generation of ozone as a final oxidant. However, the risks related to the disposal of ozonides and peroxides, large amounts of hazardous waste, demand for using specialized equipment, and lack of selectivity of this reaction enforced chemists to discover safer and more sustainable synthetic protocols [1].

In the last decade, photobiocatalysis has gained considerable attention as an efficient synthetic tool in asymmetric organic synthesis. This stems from the fact that merging both of the aforementioned catalytic strategies brings many benefits for particular processes in terms of desired reactivity, selectivity, and ecological feasibility. So far, photobiocatalytic methods for obtaining optically active alcohols consist of deracemization of racemic alcohols combining photocatalytic oxidation and enzyme-catalyzed bioreduction [2,3], photocatalytic asymmetric C–H bond oxyfunctionalization of activated alkanes (i.e., ethylbenzene) followed by carbonyl stereoselective bioreduction [4] or selective activation of C−H bonds in a photo-biocatalytic cascade process [5].

In this study, we report on a one-pot, two-step sequential photo-biocatalytic synthetic procedure for the preparation of optically active alcohols from terminal alkenes.

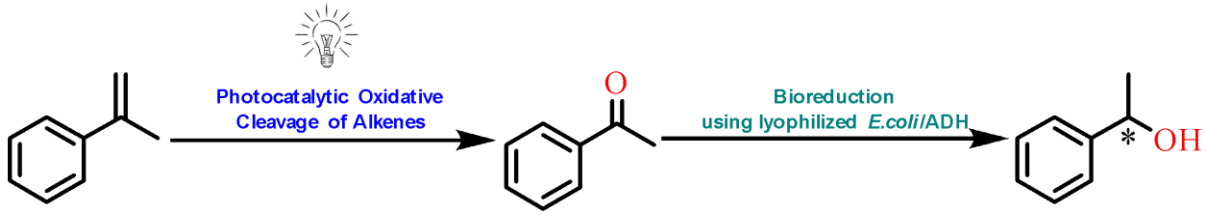

Figure 1 Synthesis of optically active alcohols using photo-biocatalytic cascade reaction.

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This research was funded by the National Science Center (NCN) of Poland grant "OPUS 24" (Grant No. 2022/47/B/ST4/00139). Statutory support by the Faculty of Chemistry at Warsaw University of Technology (WUT) is also acknowledged. N.A. acknowledges financial support from the IDUB project ("Scholarship Plus" program for Ph.D. students). The University of Graz and the Field of Excellence BioHealth are recognized for financial support.

Chromatography-free Lipase-Catalyzed Kinetic Resolution of Secondary Alcohols Using Vinyl 3-(dimethylamino)propanoate as an Acyl Group Donor

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Enzymatic kinetic resolution is one of the most popular tools for the synthesis of optically active compounds, which mostly stems from the enormous biocatalytic potential of hydrolases, especially lipases [1]. Unfortunately, the use of EKR is associated with unpleasant technological consequences, including low process efficiency and the requirement to purify the products using preparative column chromatography. The second-mentioned obstacles related to the purification step generate the high costs of the particular process and also significant amounts of environmentally harmful waste, including volatile and toxic organic solvents as well as cancerogenic silica gel. In recent years, much effort has been made to circumvent this limitation and obtain high-yield optically pure compounds without chromatographic purification [2].

Herein, we report the development of a convenient and practical method for the chromatography-free enzymatic kinetic resolution of secondary alcohols (**Figure 1)**. The employed vinyl 3-(dimethylamino)propanoate as an acyl group donor enabled highly enantioselective (E>200) lipase-catalyzed resolution of racemic alcohols with up to 98% ee. Moreover, the KR products are easily separated via a liquid-liquid extraction work-up using a 1M HCl aqueous solution.

The developed method can be applied to the industrial synthesis of chiral secondary alcohols where the other techniques fail in the separation work-up.

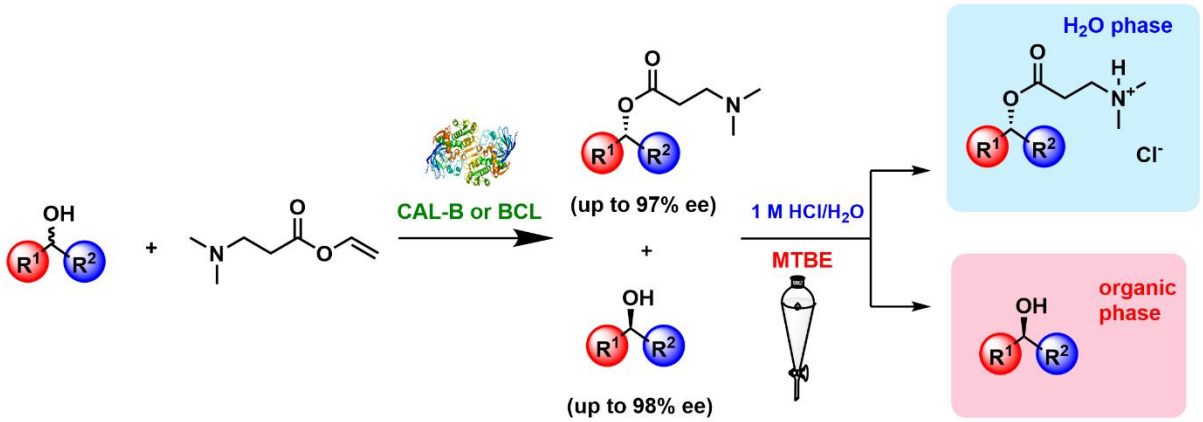

Figure 1. Chromatography-free lipase-catalyzed kinetic resolution of racemic alcohols.

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Designing a functional nanoemulsion using lactose esters and modified monomers of bacterial polyhydroxyalkanoates to improve the anticancer activity of SN-38

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Colon cancer is one of the most frequently diagnosed and deadliest cancers worldwide hence the number of drug discoveries for the disease is still growing [1]. To improve the solubility and *in vivo* stability of promising chemotherapeutics numerous delivery systems are being designed and tested. Our study involved chemo-enzymatic methods to perform a novel emulsion to provide sufficient solubility and delivery for one of the most promising anticancer agents: SN-38 [2][3]. The oil phase consisted of selectively fluorinated monomers from bacterial poly-(*R)*-3-hydroxynononanoate-co-heptanoate (PHN) which are fully described in our previous studies (involving ^{1}H NMR, ^{19}F NMR, IR, UHPLC-MS/MS analysis). A mixture of PHN-monomer-based lactose esters (also previously described) and DMSO served as an emulsion stabiliser and water was the hydrophilic solvent. Dynamic Light Scattering analysis indicated the average size of micelles was 500–1000 nm and their stability was maintained for seven days. MTT assay showed that the novel emulsion significantly improved the activity of the investigated chemotherapeutic by decreasing the viability (IC_{50} and IC_{80}) of the selected colon cancer cells (and concentrations were: 0.125 and 0.25 mg ml^{-1}). An experiment on zebrafish embryos (*Danio rerio*) showed relatively low toxicity of the carrier alone (embryos were treated with >0.4 mg ml^{-1} for 5 days). Experiments on mice with colon cancer explants (HCT116) indicated a drop in tumour growth inhibition factor (TGI) while treating them with SN-38 in the novel emulsion system compared to those treated only with SN-38 in DMSO. The results allowed for patent claim nr P.447923.

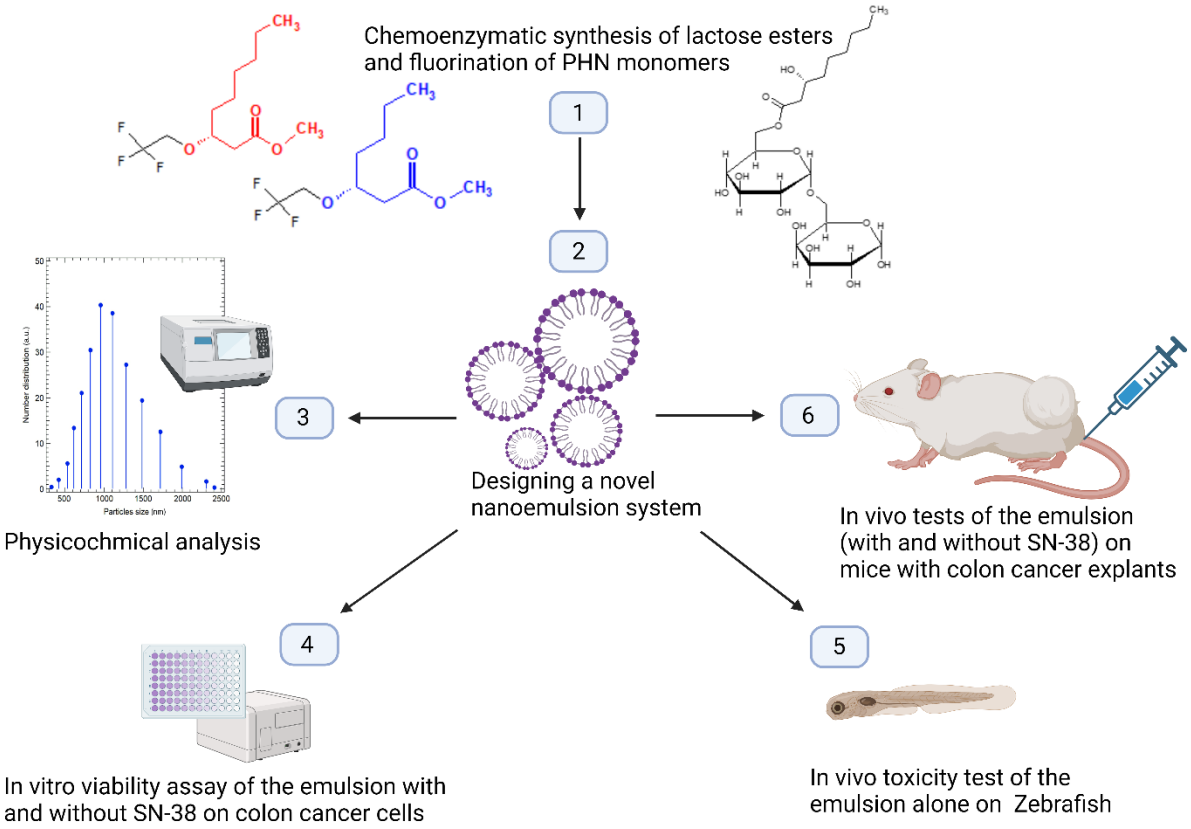

Figure 1. Scheme of research: Designing and testing a novel emulsion system for SN-38 delivery. Created with BioRender.com

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Innovative approach to removing pharmaceutical impurities using MOF-laccase systems

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Growing concerns about contamination of surface waters with pharmaceuticals have spurred significant research and development efforts to find effective solutions. One promising approach involves the use of enzymes immobilized on solid supports to facilitate the removal of these contaminants [1]. Recently, metal-organic frameworks (MOFs) have attracted attention due to their unique structural properties, such as complex architecture and high porosity, making them ideal candidates for such applications. Incorporating enzymes into MOFs brings several benefits, including enhancing the thermal and chemical stability of the enzymes, thereby prolonging their activity and effectiveness under harsh environmental conditions [2]. In addition, such integration improves catalytic performance, allowing faster and more accurate degradation of pharmaceutical impurities. Moreover, the porous nature of MOF increases accessibility to the active sites on the enzymes, maximizing their effectiveness in degrading contaminants [3]. Laccase, meanwhile, known for its ability to degrade impurities, was chosen for immobilization on CuBDC MOF [4]. Optimal immobilization parameters were determined through thorough analysis, followed by a series of studies to assess enzyme loading, immobilization efficiency, retained activity, and kinetic properties. The biocatalytic system's reusability was examined, alongside investigations into the effects of temperature and pH on the immobilized laccase's catalytic behavior. Subsequently, the efficacy of the developed catalytic system was put to the test in the degradation of 17β-estradiol, a pivotal facet of the ongoing research endeavor. This comprehensive approach not only addresses the urgent need for efficient pharmaceutical pollutant removal but also underscores the promising potential of MOF-based enzyme technologies in tackling contemporary environmental challenges.

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The lipase – initiated chemoenzymatic cascade reaction leading to the C=C double bond cleavage

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Biocatalysis plays an important role in modern organic synthesis. Among others, lipases are the most widely used enzymes as they catalyze “natural” reactions such as hydrolysis, esterification, and transesterification, often in a stereoselective or chemoselective way [1]. However, lipases also catalyze non-natural reactions, what makes these enzymes even more attractive. This ability of enzymes is known as a promiscuity [2].

Recently, we have focused our attention on enzymatic Michael addition reaction. However, when we studied the reaction of cinnamic acid esters with dimedone in the presence of lipase we did not obtain Michael adduct, but discovered a new activity of these enzymes as xanthendione derivatives products were obtained (Figure 1).

The unpreceded lipase activity towards the Michael addition leading to the C=C double bond cleavage was discovered. Various esters of cinnamic acid derivatives were converted to valuable xanthendiones in the reaction with dimedone. It is worth to note, that under special conditions, two various tetraketones were obtained from one ester molecule.

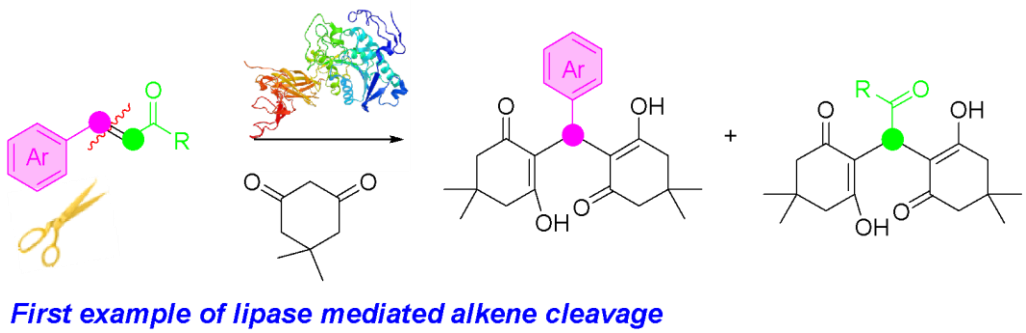

Figure 1. Chemoenzymatic cascade reaction leading to alkene cleavage.

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Green Technology for Sustainable Environment 2024

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GTSE24 is the fourth edition of the conference series Green Technologies for Sustainable Environment and also its first European edition. The conference topics include environmental protection, development of green technologies, sustainable development, and circular economy.

GTSE 2024 aims to promote research in the field of green technologies and sustainable environment and facilitates the exchange of new ideas in these fields among academicians, engineers, scientists, and practitioners. The GTSE24 will be held on-site from September 22nd to September 26th, 2024 at the Poznan University of Technology Campus.

Scope of the conference:

- Advanced waste/wastewater treatment and reuse
- Air pollution control and monitoring
- Redefining of environmental pollutants
- Disruptive environmental technologies
- Solid waste and water resource management
- Applied technology/nanotechnology for environmental applications
- Advanced analytical techniques for environmental monitoring
- Energy and resource recovery from waste and wastewater
- New micropollutants in the environment – current achievements, challenges. and predictions

The high scientific level of the organized event was appreciated by the Ministry of Education and Science, getting funding from the state budget, granted by the Minister of Education and Science under the “Excellent Science II” Program.

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P-1 Weronika Badzińska

Novel biocatalytic systems Al_2O_3-Fe_3O_4/polyelectrolyte/laccase and Al_2O_3-Fe_3O_4/laccase/polyelectrolyte for estrogen bioconversion

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Economic development and climate changes have led to an increase in the amount of generated wastes, as well as the amount of chemical compounds produced and released into the environment. A wide array of organic compounds have been recognized as pollutants of high concern due to their controlled or uncontrolled presence in environmental matrices. Diverse organic pollutants, including pharmaceutical compounds, phenolic compounds, synthetic dyes, and other hazardous substances are becoming more prevalent in the water sources of the globe, which has detrimental repercussions for both human health and the ecosystems [1]. Among these potentially dangerous organic compounds estrogens should be mentioned. There is no doubt that estrogens are necessary for the proper functioning of the human body, however their accumulation and consumption above a safe limit can cause negative health effects. First of all, the risk of breast cancer incidence in women [2] and prostate cancer in men [3] or cardiovascular disease increased [4]. Due to the fact that estrogens are resistant to removal applying classical methods of remediation, the advanced water remediation processes are still needed to effectively remove these compounds from waters [5]. One of the promising methods of estrogens removal is enzymatic conversion. Unfortunately, enzymatic techniques are associated with difficulties such as problems with separation from the solution and related possible reuse. However, enzymes immobilization on supports not only minimizes these issues but also improves their stability over a wide range of process conditions. This work presents the effectiveness and stability of innovative systems Al_2O_3-Fe_3O_4/laccase/polyelectrolytes and Al_2O_3-Fe_3O_4/polyelectrolytes/laccase type.

The produced biosystems were firstly characterized physicochemically to confirm the immobilization of the enzyme – laccase onto the support and its modification with three polyelectrolytes - polydopamine (PDA), polyethylenimine (PEI) and poly-L-lysine (PLL). In the next step, stability, reusability in the next ten cycles, as well as the efficiency of estrogens (estradiol and 17α-ethynylestradiol) removal were evaluated. The obtained results clearly indicate positive immobilization of the enzyme and modification of the support. The produced exihibited magnetic properties related to the use of an oxide system containing magnetite. As a result of the conducted research, it was shown that the systems produced are effective in the bioconversion of estrogens. Moreover, they exhibit higher ability to adsorb laccase and remained stable over a wider range of temperatures and pH as compared to free laccase. Enzyme immobilization onto the support also enabled produced systems to maintain their efficiency even above 60% after eight catalytic cycles.

In conclusion, the obtained Al_2O_3-Fe_3O_4 oxide system has features that predispose it to be used as a support for enzymes immobilization process. The experimental data obtained clearly indicate the enormous application potential of the produced biocatalytic systems in the removal of estrogens from aqueous solutions. Moreover, the magnetic properties of the produced systems favor its quick and effective separation from the reaction environment, which increases the purity of the post-reaction mixture and avoids complicated methods of isolating biocatalysts from the solution.

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P-2 Wioletta Barszcz

Possibility of using apple pulp after supercritical extraction in CO_2 as an antibacterial agent

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The cosmetics market is still a promising market, which is due to, among others, growing consumer awareness and growing disposable income. An increasing percentage of consumers pay attention to the quality and effectiveness of products and issues related to ecology and the origin of individual ingredients. The rising production of natural cosmetics generates increasingly larger waste streams. One of the substrates used in cosmetic production is apples and apple pulp, from which bioactive ingredients are recovered in the process of supercritical extraction in CO_2.

In the presented research results, about the "cradle to cradle" concept of sustainable development of cosmetic products, the resulting apple waste after supercritical extraction in CO_2 was thermochemically transformed into biochar. The produced biochar was additionally activated with steam to increase its surface area. The pyrolysis process was carried out under cascade heating conditions until temperatures of 600, 700, and 800ºC were reached. It was assumed that thanks to the implementation of zinc ions into the structures of the obtained biochar, it would acquire antimicrobial properties, which would allow for the continuation of research on its use as an active ingredient in cosmetic products for the care of human skin. The modern cosmetics market offers several cosmetics containing activated carbon, made from selected types of wood. Carbon's high ability to absorb pollutants makes it fit into the new trend of using cosmetics of natural, non-animal origin. Cosmetic products containing activated carbon are effective in removing contaminants, i.e. dirt, dust, and toxins (i.e. dioxins, benzopyrene, sulfur dioxide). The biochar produced as part of the task will not only contribute to the protection of natural resources but will also have antimicrobial properties.

Thanks to the use of physical activation with steam, the produced biochars were characterized by an extensive specific surface: from 408 m^2/g (600°C) to 1119 m^2/g (800°C), with a predominant amount of micropores (less than 2 nm). Sorption of Zn^{2+} ions from an aqueous solution with a concentration of 50 mg/dm^3 was carried out for 60 min using the dynamic contact method at a constant temperature. Despite the lack of functional groups on the produced biochar, the sorption efficiency of Zn^{2+} ions was high - 96%. Biochars with zinc ions in equilibrium were selected for microbiological tests and their activity against the following bacteria: *Escherichia coli* and *Staphylococcus aureus* was determined. These tests were carried out using two different methodologies: qualitative (diffusion method) and quantitative (testing the kinetics of growth in various breeding environments) and were assessed for compliance with the PN-EN ISO 17516:2014-11 standard *Cosmetics - Microbiology* -

Microbiological limits. It has been shown that the tested biochars have a bacteriostatic effect on selected strains, and quantitative tests have shown that with the increase in the presence of nutrients, the effect of the modified biochar is lower and, consequently, the risk of multiplication of these microorganisms increases.

Enzymatic Glycosylation of 4′-Hydroxychalcones: Unveiling Nature's Catalytic Potential

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Chalcones, including 4′-hydroxychalcones, have garnered significant attention in the area of drug discovery due to their diverse pharmacological properties, such as anti-inflammatory [1], anti-oxidative [2] and anti-cancer [3] effects. However, their low water solubility and bioavailability, limit their efficacy *in vivo* [4–6]. Glycosylation presents a promising approach to enhance the water solubility, stability, and metabolic properties of chalcones [7-9]. Enzymatic biotransformations offer higher selectivity compared to whole-cell biocatalysis, making them attractive for glycosylation reactions [10,11]. Studies show that in case of whole-cells biotransformations, either bacteria or fungi and yeasts reduce double C-C bond of chalcones [12–14] and is not suitable for obtaining chalcones glycosides.

This study aimed to investigate the enzymatic glycosylation of eight 4′-hydroxychalcones obtained by chemical synthesis using eight different glycosyltransferases (from bacteria, fungi and plants). Among five tested enzymes, exhibited remarkable versatility for glycoside production, and for large-scale biotransformation, flavonoid 7-O-glucosyltransferase Sbaic7OGT from *Scutellaria baicalensis*, was selected as the most effective. As a result of the experiments conducted, eight *trans*-chalcone glycosides were obtained (Figure 1). During the purification of the reaction products, we also observed the isomerization of products, which resulting in eight additional *cis*-chalcone glycosides.

Our findings underscore the potential of enzymatic biotransformation as a selective and efficient method for glycosylation of 4′-hydroxychalcones, offering new avenues for the development of bioactive glycoconjugates with improved pharmaceutical properties.

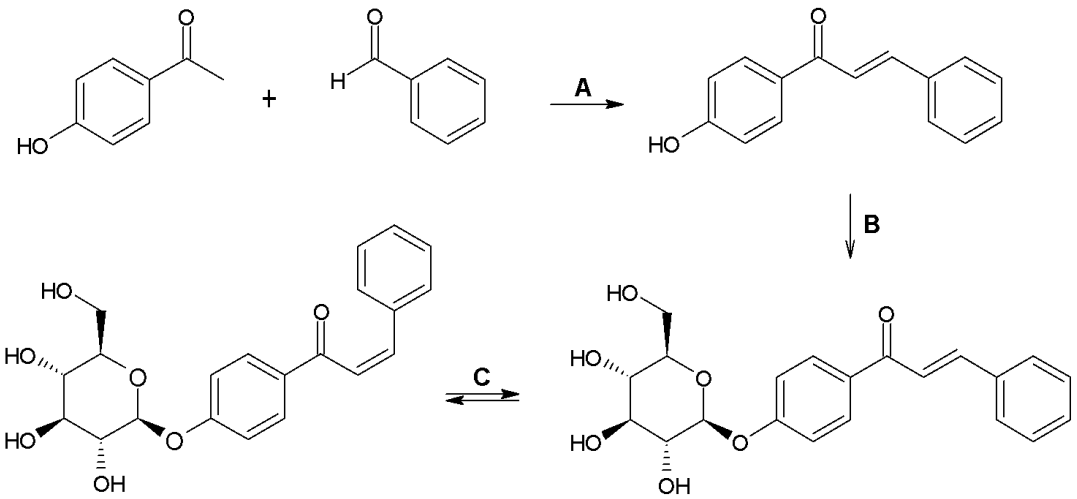

Figure 1. Process of glycoside formation: **A**) chemical synthesis of *trans*-chalkones; **B**) enzymatic glycosylation of 4′-hydroxychalcones; **C**) isomerization process induced by UV radiation.

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Biotransformation of hops-derived compounds in beer

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In recent years, there has been a growing interest in hop-heavy beers. Although hops have traditionally been used to bestow a beer its characteristic bitterness, the modern beers are not always very bitter. Often, a greater emphasis is put on the aroma which can be obtained when a skilled brewer uses adequate hop varieties. In this way, beers with the herbal, spice, floral, citrus, fruity, pine and grassy aromas can be produced. Those fragrances are largely obtained by the addition of hops at various stages of the beer production process. Due to hops varietal differences, and resulting differences in the composition of terpene alcohols, esters and polyfunctional thiols among others, beers with these aromas can be produced without the use of special raw materials, or artificial flavors [1]. However, the aroma of a given hop variety is usually far different from the aroma of beer made with its use. The final aroma of a beverage is a result of a complex combination of substances derived from malt, hops, other additives, and yeast metabolism. This intricate matrix of compounds undergoes dynamic changes during the beverage production process, due to physical, chemical as well as biochemical processes.

A great interest has been directed towards the “hidden” aromatic potential of hops. Beside these readily flavor-active compounds, the hops contain precursors of flavour compounds, which can be released if correct conditions are applied. This hidden potential is seen by many brewers as a way to obtain more aroma-rich beverages. As a result, the biotransformation became an important field in the brewing research. The research has mainly focused on the two classes of compounds: terpene alcohol glycosides, and bound polyfunctional thiols. These compounds can be transformed into flavour-active counterparts by the yeast enzymatic activities. Other aromatic compounds, such as terpene alcohols, or esters can be transformed into alternative ones.

The terpene alcohols can undergo many transformations, which are presented in the figure 1 [2]. They are usually associated with floral and fruity aromas. Many yeast species, as for example *S. cerevisiae* might synthesize trace amounts of terpenols *de novo* [3]. According to the literature the glycosylated forms of terpene alcohols are mainly found in a form of pentose-hexose monoterpenols [4]. These compounds can be released by the action of yeasts exo-1,3-β-glucanase and β-glucosidase [5]. Precursors of polyfunctional thiols are found as cysteinated and glutathionylated conjugates [6]. These compounds are of great interest, as they are sensory active in ng/L. They bestow tropical aromas. As it is understood, these compounds can be released by the action of yeast β-lyases [7]. The degree of these yeast activities is highly strain dependent, and currently, not yet fully understood.

The aim of this report was to present the knowledge regarding the biotransformation of hop derived terpene alcohols, and precursors of terpene alcohols and polyfunctional thiols.

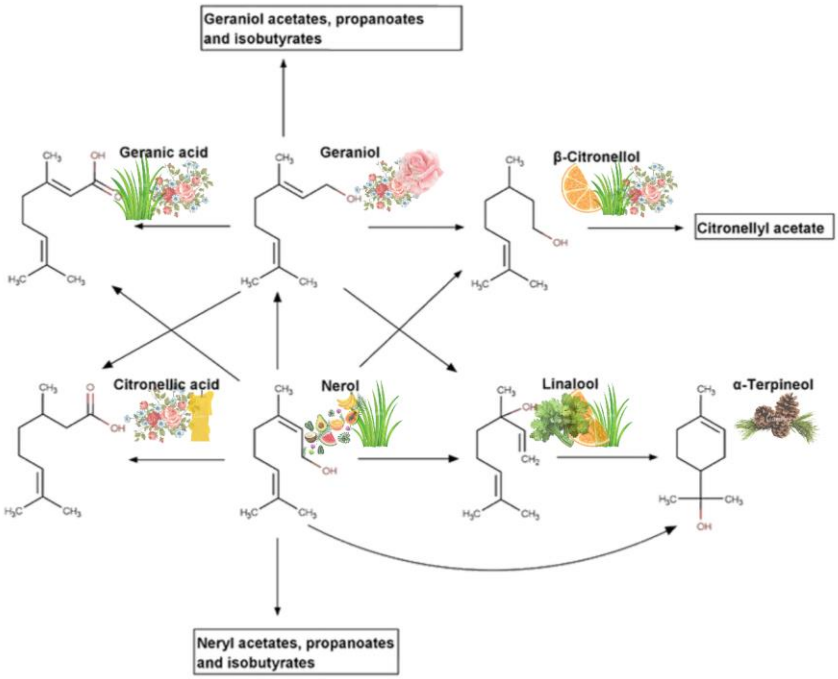

Figure 1. Known biotransformation reactions catalyzed by *S. cerevisiae*. Mentioned esters could undergo further hydrolysis [2].

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P-5 Oliwia Degórska

Unconventional API synthesis - Biocatalytic processes in flow reactors

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Biocatalysis in the industry is becoming more and more widely used in i.e. the removal of contaminants from aqueous solutions, biodiesel production as well as in the pharmaceutical industry due to emerging difficulties with enantioselectivity and purity of synthesis processes [1]. The important factors to consider are the extra measures taken to remove unwanted substances from the final product or the sale of drugs in racemic form. In the latter case, patients are required to take a higher dose than necessary due to the presence of the inactive or less biologically active second enantiomer [2]. Enantioselective synthesis might provide a solution to that issue. An innovative approach to biocatalysis opens the way to newer and more catalytically active systems characterized by high mechanical stability and selectivity.

Electrospun materials can be tailored to specific process needs by selecting appropriate polymers. It is possible to match the properties of the electrospun material to the properties of the enzymes used due to the variety of their properties, which range from hydrophilic to hydrophobic materials [3]. Electrospun mats are used as a support in immobilization processes of proteins such as enzymes, however immobilization processes, for example by adsorption, may result in the potential washing out of the enzyme from the surface of the material. Core-shell systems consisting of an outer (shell) and inner (core) layer of the fiber enable the encapsulation of the catalyst inside the fiber, ensuring its very limited leaching and high stability and activity. A properly designed material allows free diffusion of substrates and products through the shell pores, ensuring efficient biocatalysis [4].

In the presented research, a core-shell (PVP-PCL) nanofiber material was produced in order to check the catalytic properties and further application in biocatalytic reactions. During the research, attention was focused on embedding lipase in the core with the addition of ionic liquid (BMIM TFSI) to stabilize the structure and increase the activity of the enzyme. The outer layer (shell) was electrospun with the addition of a pore former to enable the diffusion of substrates into and products out the enzyme surface. Various pore former contents were tested to investigate the activity of the system. The core was crosslinked with a salt solution to ensure entrapment of the enzyme in the hydrogel and prevent diffusion of the enzyme through the pores out of the nanofibers. The photograph displayed below (Fig. 1) depicts the anatomy of the material, which confirms the presence of a core-shell structure. The formulation and process parameters were optimized to ensure a stable process yielding a nanofiber mat without visible defects and consisting of smooth nanofibers with a core-shell structure.

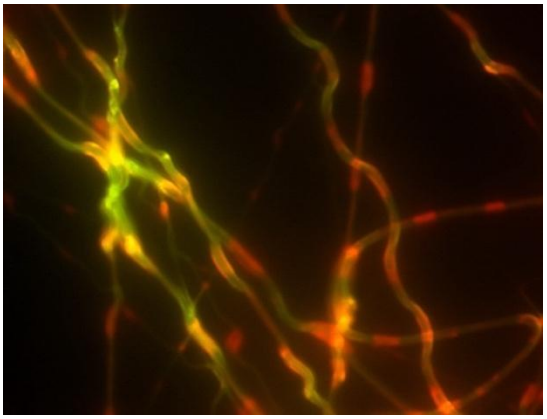

Figure 1. Fluorescent microscope image of core-shell system PVP/PCL with encapsulated enzyme in the core, supported by ionic liquid. Nanofiber layers were stained with the coumarin (core) and Rose Bengal (shell) dyes.

The produced systems will be used as biocatalysts, among others, in the separation of enantiomers of the psychotropic substance. The (S)-enantiomer is characterized by much higher biological activity, hence an enantiomerically pure synthesis would enable shortening the synthesis steps.

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Upgrading bioplastic via a fermentation process

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The research focused on preparing substrates for fermentation from commercial polymers, such as PLA, PCL, and PHA, for the production of biopolymers through bacterial fermentation processes. Substrate preparation involved chemo-mechanical degradation methods, including acid methanolysis, thermal treatment, and alkaline hydrolysis combined with digestion. Analytical tests of the prepared substrates were conducted using techniques such as GC, HPLC, IR, and NMR to assess their quality and purity. Additionally, a screening study was conducted to find bacterial strains producing PHB using organic acids, which are monomers of known polymers [1]. The results indicated the potential of specific bacterial strains, such as *C.necator* B4383 and *Z. denitrificans*, for producing biopolymers from specific organic acids derived from PHA degradation. Fermentation experiments using organic acids as carbon sources showed promising results, with the *C. necator* 4383 strain demonstrating growth on different substrates and high PHB content in dry mass [Fig1][2]. The last step of the research involved using depolymerized polymers (PLA, PCL, and PHB) as carbon sources in liquid cultures of the *C.necator* 4383 strain, which resulted in promising growth and PHB accumulation.

Figure 1. Determination of dry biomass and P(3HB) content in bacterial biomass

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P-7 Yvonne Gemmecker

Tungsten enzyme catalyzed reductions

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Molybdenum and tungsten are transition metals that are both present in biological systems. These metals occur in the active site of Mo- and W-dependent enzymes where they are coordinated to some form of metallopterin cofactor and are usually involved in the catalysis of various redox reactions. Although molybdenum and tungsten share similar chemical properties, they differ in the redox potentials of their biologically relevant oxyanions. Therefore, some reactions of this enzyme family have been found to be exclusively dependent on tungstate. This includes the direct reduction of carbonic acids to the corresponding aldehydes without any need of prior activation. One example for these W-dependent enzymes is the aldehyde oxidoreductase from *A. aromaticum* EbN1. This member of the bacterial subfamily of AORs has been shown to catalyze the oxidation of various aldehydes as well as the reverse reaction. The reverse reaction provides a versatile tool in the reduction of various substrates of interest especially in combination with other enzymes, like alcohol or aldehyde dehydrogenases for example. Here we show the potential mechanisms that ensure the highly selective incorporation of tungsten during the cofactor maturation of AOR as well as possible enzymatic cascade reactions.

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P-8 Tatjana Ilic-Tomic

Actinomycins from *Streptomyces anulatus* BV365 efficiently functionalize silk for biomedical applications

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A newly isolated strain *Streptomyces sp.* BV365 producing high amounts of orange extracellular pigments on mannitol-soy flour agar was identified as *Streptomyces anulatus* BV365. The producing strain *Streptomyces anulatus* BV365 was isolated from the ectomycorrhizosphere soil of the black truffle *Tuber melanosporum*. Crude cell extract of this strain was fractionated and orange pigment fraction showed very strong antimicrobial and cytotoxic activities. On further analysis, the strain was found to produce metabolites actinomycin D, C2 and C3 and nonactin. The application of purified actinomycins in the dyeing of multifiber fabric was assessed. Actinomycins exhibited a high affinity towards protein fibers (silk and wool), but washing durability was maintained only with silk. The morphologies and chemical components of the treated silk fabrics were analyzed using scanning electron microscopy and Fourier transform infrared spectroscopy. In addition, a skin irritation test in 3D-reconstructed human epidermis model was conducted to evaluate the biocompatibility of the tested fabrics. The results showed that the dyed silk had a safe biological properties.

Acknowledgments

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P-9 Mateusz Jamroży

Interactions of Saponin with Hydrogen Bond Donor or Acceptor Compounds at Water-Air and Water-Oil Interfaces: Influence on Emulsion Stability

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Our research focuses on comparing how selected chemical compounds of simple structure, acting as hydrogen bond donors or acceptors, affect the interfacial properties of the tested saponin-based emulsions. The chemical compounds mentioned above are: urea, thiourea, glycerol, choline chloride, betaine and nicotinic acid.

Saponin is a natural, biodegradable compound that exhibits relatively high surface activity. Thanks to its properties, this biosurfactant can be used in the cosmetic and food industries, as well as in medicine. Due to the complex chemical structure that saponin mixtures form (the occurrence of hydrogen bonds and electrostatic interactions), the precise elucidation of surface-active properties is a challenge in the context of experimental research.

A study was conducted on the effect of the above-mentioned chemical compounds on the adsorption of saponin at the water-air and water-oil interface. The stability of the produced emulsions was analyzed from the moment of formation, as well as with the passage of time. In our research, we use techniques that allow us to quantitatively and objectively characterize the stability of dispersion system samples without destruction, avoid the interference of subjective factors and show the cause of instability (aggregation or migration). For this purpose, we use the MultiScan 2.0 device, which scans the sample using near-infrared light and develops transmission and backscattering spectra as a function of the sample height and destabilization time. Moreover, we examine changes in the morphology of emulsion samples using optical microscopy. Based on studies [1-3], the relationship between the properties of adsorption layers and those of the corresponding emulsions was investigated. In particular, the focus was on how the additives used affect the destabilization mechanisms of the emulsions formed.

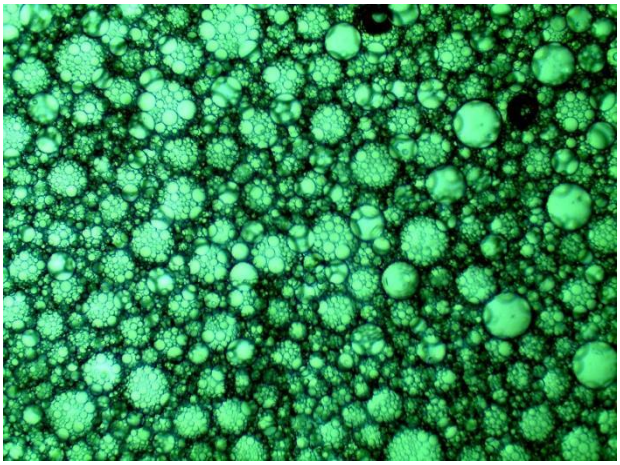

Figure 1. Microscopic photo of saponin based emulsion with urea addition.

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Enzymatic Alcoholysis of Dialkyl Phosphites

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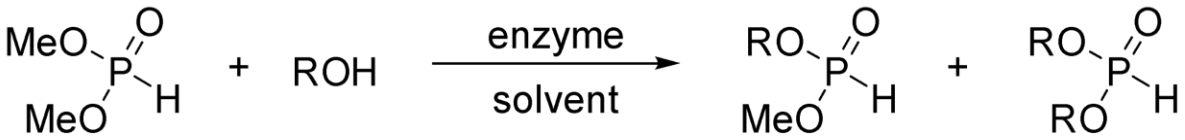
The carbon-heteroatom bond formation is the fundamental reaction in organic chemistry and materials science. Among these reactions the phospha-Michael addition is the intensively developed field and attracts much attention of the synthetic chemists in recent years [1]. *H*-phosphonates bearing different alkoxy groups on the phosphorus atom are valuable intermediates for the synthesis of P-chiral organophosphorus derivatives. The synthesis of dialkyl phosphites with two different alkyl groups is, however, not easy and requires special methods. Due to exceptional low environmental and physiological impact as well as high selectivity and mild reaction conditions enzymes were found to be also attractive for industrial-scale synthesis.



Scheme 1. Enzymatic alcoholysis of dimethyl phosphite.

The results of our studies on enzyme type impact on the reaction course, leading to the target dialkyl phosphites will be presented (Scheme 1). The influence of the reaction conditions, and the reaction media on the reaction course will be discussed.

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P-11 Mateusz Kutyła

Modelling of green biocatalytic (*R*)-(+)-limonene oxidation using the mycelium of psychrophilic *Cladosporium cladosporioides* 01

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Limonene is an olefinic hydrocarbon found in over 300 essential oils of plants, mainly orange, lemon and fir. Nowadays, limonene is mainly produced as a by-product of the citrus juice industry. Its annual production is 70 000 tonnes. The structure of limonene allows easy chemical modification due to the presence of two double carbon bonds, a chiral centre, and a six-membered hydrocarbon ring. Limonene epoxidation yields limonene monoepoxide or diepoxide, which can then be polymerized or used as bioactive compounds [1,2]. Development of an environmentally friendly and cheap process of chemoenzymatic epoxidation of limonene is important due to the need to replace environmentally unfavorable chemical methods.

The lyophilized mycelium of psychrophilic *Cladosporium cladosporioides* 01 has been found to be an efficient biocatalyst for green chemoenzymatic epoxidation of (*R*)-(+)-limonene in ethyl acetate. The research included mathematical optimization of the main process variables using RSM methods. Modelling of the reaction parameters (temperature, H_2O_2, biocatalyst, substrate concentration and stirring speed) contributed to an approximately 5-fold increase in the efficiency of epoxidation, compared to that of the non-optimized process. After 4 hours at 55 °C without stirring, 99.8% oxidation of limonene to limonene 1,2-epoxide (56.3%), limonene 8,9-epoxide (0.3%) and diepoxide (43.2%) was achieved using 232.7 µl of H_2O_2, 88 µl of acetic acid, 284 mg of a biocatalyst and 57.6 µl of limonene. Modelling of epoxidation depending on the limonene : biocatalyst ratio was also performed. The time of achievement of the maximum yield of 1,2-epoxide and limonene diepoxide was determined, which makes it possible to design the process rationally to obtain the desired product (Figure 1).

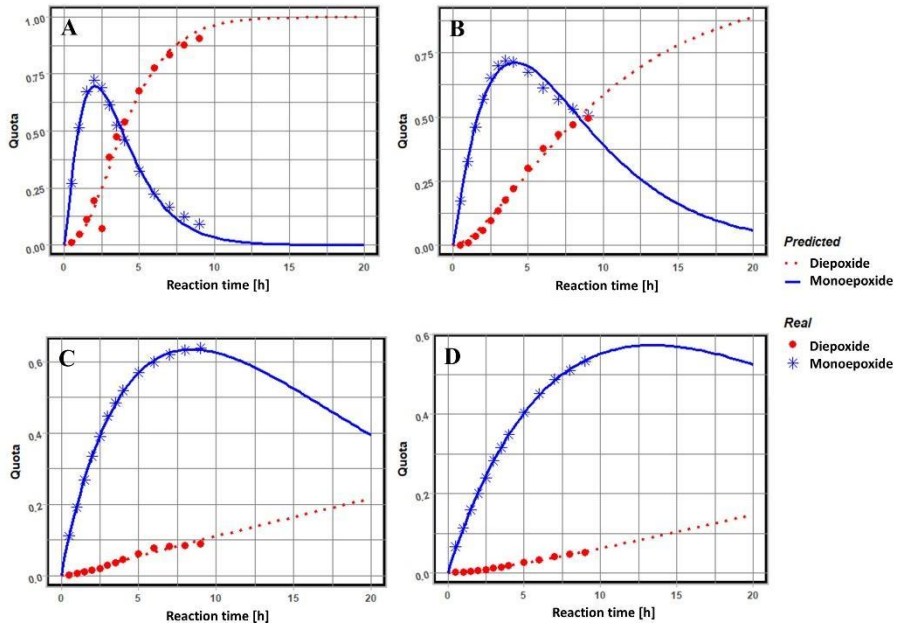

Figure 1. Time dependence of the amount of limonene monoepoxide and diepoxide at the limonene : biocatalyst ratio: k = 0.2 (A), k = 0.5 (B), k = 1.0 (C), and k = 2.0 (D)

For the first time, the profitability of the green biotechnological method of obtaining limonene epoxides with the use of a new biocatalyst was calculated. Using 2.45 grams of (*R*)-(+)-limonene, the predicted gain for 20 hours of epoxidation is about 223.5 USD, making this process very advantageous for use on an industrial scale (Figure 2).

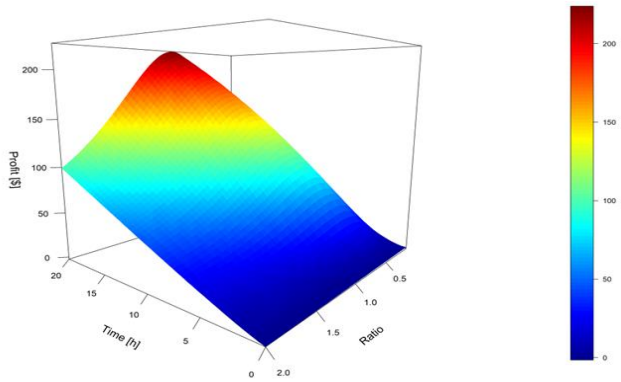

Figure 2. The gain of the reaction depending on the duration of the epoxidation and the quantitative ratio of limonene to the biocatalyst

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P-12 Mateusz Kutyła

Chemoenzymatic epoxidation of terpenes by lyophilized mycelium of psychrophilic *Cladosporium cladosporioides* 01

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Terpenes are a readily available group of compounds with many applications in both industry and everyday life. Large quantities of hydrocarbon monoterpenes are obtained from waste biomass from the forestry (a source of α- and β-pinene) and agricultural (a source of limonene) industries [1]. These readily available and inexpensive terpenes are important resources with a variety of uses in pharmacy, perfumes, and flavourings [2]. Bearing in mind the availability, terpenes could be regarded as good substrates for the preparation of value-added chemicals, of which epoxides are particularly important [3].

The aim of the research was to determine the influence of both the amount of hydrogen peroxide and the type of solvent on the chemoenzymatic epoxidation activity of freeze-dried mycelium of *Cladosporium cladosporioides* 01. This study also aimed to determine the operational stability of the fungal biocatalyst and its ability to mediate the epoxidation of different terpene substrates.

Efficient epoxidation of limonene occurs in a system with a 4-fold excess of 30% H_2O_2 in relation to the substrate. With this amount of hydrogen peroxide, complete oxidation of the substrate occurred after 4 hours of reaction. The results show that ethyl acetate is the most efficient solvent in chemoenzymatic epoxidation using *C. cladosporioides* 01 mycelium. Apart from ethyl acetate, benzene and toluene appeared as good solvents for the epoxidation reaction (Figure 1).

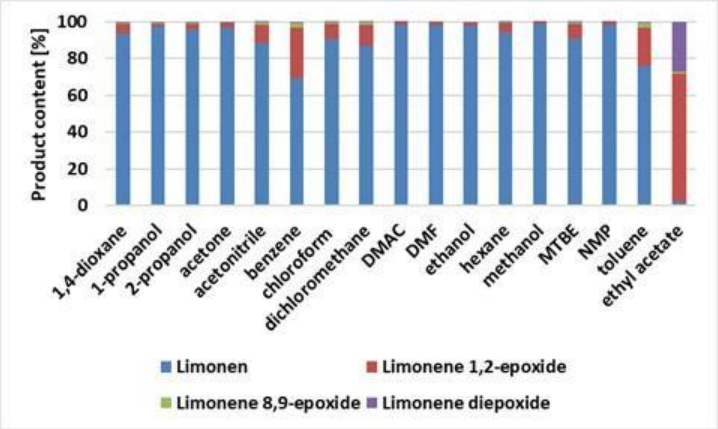

Figure 1. Effect of solvent type on the efficiency of the chemoenzymatic epoxidation of limonene

After the first cycle the biocatalyst was activated which resulted in an acceleration of the reaction in the second catalytic cycle affording quantitative conversion of limonene to 1,2-epoxide (57%) and diepoxide (43%). The increased biocatalytic activity of the *C. cladosporioides* 01 mycelium was maintained until the 8th catalytic cycle (Figure 2).

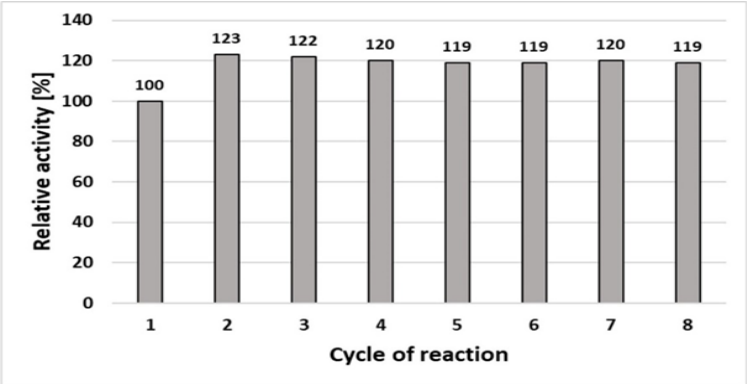

Figure 2. Reusability of C. cladosporioides 01 in a chemoenzymatic reaction cycle for the epoxidation of limonene

The biocatalyst can be successfully used in the epoxidation of other terpene substrates such as: linalool (92.8% oxidation), citronellol (90.1%), citronellen (89.3%), myrcene (89.4%), α-pinene (78%), β-pinene (45.5%), myrtenol (91%), citronellal (50%) and verbenol (75%).

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P-13 Aleksandra Lambarska

Biocatalysis in the continuous synthesis of chiral cyanohydrins

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Chiral cyanohydrins are used in the horticultural, cosmetic and pharmaceutical industries, but mainly in the chemical industry for the production of *fine* chemicals. They are a group of compounds that are precursors for the production of α-hydroxy acids, α-hydroxy aldehydes or ketones, primary and secondary and β-hydroxy amines [1-2]. Due to the complexity of the steps involved in the synthesis of chiral cyanohydrins, it has been proposed to produce them using biocatalysis rather than conventional chemical catalysis. However, in order to reduce the costs associated with the use of a biocatalyst, its immobilization is necessary. This procedure allows multiple use of the heterogeneous enzyme, as well as extending the range of their optimum temperature and pH [3].

Achieving a high enantiomeric excess in a batch reaction is extremely difficult. Therefore, it is proposed to run the reaction in a continuous system. Continuous removal of product from the reaction allows the reaction equilibrium to be shifted towards product formation and a high enantiomeric excess to be achieved. The combination of heterogeneous biocatalysis with synthesis in a continuous system influences the simplification of the downstream process, as well as the reduction of reaction volume and energy consumption, thus reducing costs [4]. All these aspects are part of the principles of *green chemistry*.

Here, we investigated the immobilization of hydroxynitrile lyase from *Granulicella tundricola* with triple mutation of the active site amino acids (*Gt*HNL-3V) on organically modified, silica monoliths (MHs). The influence of flow rate and stability of the immobilized biocatalyst was checked in a continuous flow *R*-mandelonitrile synthesis.

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P-14 Anna Christina Ngo

Degrading synthetic dyes using a synthetic microbial consortium

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Dyes are quite important to various industries but the release of these xenobiotics to the environment has become an issue because of health and environmental implications [1]. Although physical and chemical treatments are available, these options also have risks and hazards as either they are not cost-efficient or also harmful to the environment. Therefore, the use of biological agents for water treatments has become a promising alternative [1].

Here, we present a synthetic microbial consortium comprised of *Rhodococcus* and *Gordonia* isolates. With selective pressure approach, we have improved the substrate scope of the consortium from 1 to 10 substrates and improved the rate for even up to 10-fold for brilliant black bn. We have also improved biomass output by using phenols as a pre-treatment – ensuring that the consortium uses them as a carbon source and triggering the production of relevant enzymes for further reactions. Moreover, we have elucidated the degradation pathway for brilliant black bn using LC-MS – allowing us to understand how the consortium degraded this diazo dye.

As a proof of concept for downstream applications, we transferred our setup to small bioreactors and fed the consortium with only dyes as a carbon source. From our results, we could demonstrate the decrease in organic carbon and total nitrogen but an increase in inorganic carbon by almost 7-fold. This result show how the consortium consumes the dye and might also go through mineralization process. Our study offers insights on how we can trigger and improve dye degradation process using small aromatic compounds like phenols as a selective pressure and how we can use it for further applications such as in wastewater processes.

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Biotransformation of renewable raw materials into lactic acid

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Lactic acid in the form of pure L(+) enantiomer can be gained by fermentation using appropriately selected microorganisms. Biotechnological conversion of organic raw materials into lactic acid using microorganisms, typically lactic acid bacteria, is usually based on the biotransformation of renewable resources especially agro-industrial residues or waste from the food industry, including agricultural byproducts, waste from the dairy industry and other sugar waste, to higher value product with a wide range of applications [13].

Conducted research demonstrating a well-defined optimization process of developed biorefinery pilot plant performance with many aspects influencing on fermentation process like substrate type and source, fermentation medium composition, stirring, significant limits of particular chemical individual concentrations involving substrate and product as well as biomass content. The presented results concern the developed fermentation technology carried out on a pilot scale, which is an essential step enabling an in-depth investigating of the crucial aspects of the process as the scale of production increases. Data allowed to perform the necessary process optimizations providing a developed solution to be scaled-up on an industrial scale.

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P-16 Brana Pantelic

Plastic waste up-cycling potential of *Streptomyces* spp.: a genomic examination

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The accumulation of plastic waste has become an ever-growing global problem, with world production of plastic materials reaching >380 million tons annually and only predicted to increase in the coming years. An efficient means of disposing and recycling plastic waste is urgently needed. Due to environmental risk factors and high energy consumption of mechanical and chemical recycling methods research focus has shifted towards biological means of recycling. Biocatalysis offers an environmentally friendly and potentially very efficient strategy for plastic waste degradation and valorization by utilizing the reaction products in downstream biosynthetic reactions (up-cycling) (1).

Streptomyces spp. are highly regarded as bioactive secondary metabolite producers, however, the genus proved a promising source of industrially relevant enzymes as well (2). Leveraging this unique combination of biosynthetic and biocatalytic capabilities a collection of Streptomyces strains was screened for their plastic-degrading potential using different polyester-based polymers. Strains that could degrade and utilize plastic polymers and monomers as the sole carbon source were sequenced and the genomes searched for homologs of known plastic-degrading enzymes and biosynthetic clusters for bioactive compounds. Enzymes capable of degrading both conventional petrochemical and bioplastics were detected in the genomes of all tested strains. Interestingly, enzymes closely related to highly active poly(ethylene terephthalate) degrading enzymes were found in most strains. As expected, analysis of the biosynthetic potential yielded numerous gene clusters associated with polyketide, non-ribosomal peptide and lassopeptide synthesis. Finally, the ability to convert plastics to biologically active metabolites was confirmed using *Streptomyces* sp. PM1. When grown on polyurethanes as the sole carbon source this strain showed antimicrobial activity against *Staphylococcus aureus*.

In conclusion, this work highlights the potential of *Streptomyces* strains to biotransform and up-cycle a variety of plastics into bioactive molecules while underlying mechanisms can be elucidated by genome mining.

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P-17 Aneta Pater

Biotransformation of compounds derived from hops with the participation of yeast used in the production of non-alcoholic beers *Saccharomyces cerevisiae* var. *chevalieri*

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Biotransformation in brewing is defined as the interaction of compounds from hops with yeast (*Saccharomyces spp.*). This leads to the formation of a new aromatic compound as a result of an enzymatic reaction (hydrolysis) [1]. This process plays a key role in the beer production process, influencing not only the taste, aroma and quality of the final product, but also its durability and nutritional value [2]. The key compounds involved in biotransformation with microorganisms are terpenoids, which are introduced into beer through hopping [3]. The aim of the research was to analyse the biotransformation process occurring during beer fermentation, using yeast for the production of non-alcoholic beers (*Saccharomyces cerevisiae* var. *chevalieri*). The control sample were beers fermented with traditional brewing yeast *(Saccharomyces cerevisiae* US-05). The chemical composition and aroma components of the resulting beers were analysed using different chromatographic techniques (GC-FID, GC-MS and GC-O). Samples fermented with yeast for the production of non-alcoholic beers were characterised by a very rich aromatic profile, which included higher alcohols, esters and terpenes. In the case of this strain (*Saccharomyces cerevisiae* var. *chevalieri),* during fermentation there was a transformation of geraniol into b-citronellol with a rose aroma and a significant increase in the content of linalool, which gave the beer a flower and lavender aroma.

Obtained beers were characterised by rich sensory profile with high consumer acceptability. In addition, the production of non-alcoholic beers using the *Saccharomyces cerevisiae* var. *chevalieri* contributed to the production of beers with the desired sensory profile.

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Evaluating the Efficacy of a Sucrose Synthase-Based In Vitro Cascade for the Biosynthesis of Flavonoid Galactosides

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Sucrose synthase (SuSy) is a glycosyltransferase found mainly in plants but also in bacteria. The enzyme catalyses the reversible transfer of a glucosyl moiety between fructose and a nucleoside diphosphate. The main biocatalytic application of this enzyme lies in easy and fast production of NDP-glucose from sucrose, what is commonly used in cascade reactions with other glucosyltransferases [1]. There are many described applications of this cascade [2], however, the introduction of enzymes capable of modification of NDP-glucose within the cascade enables broader utility of this enzyme.

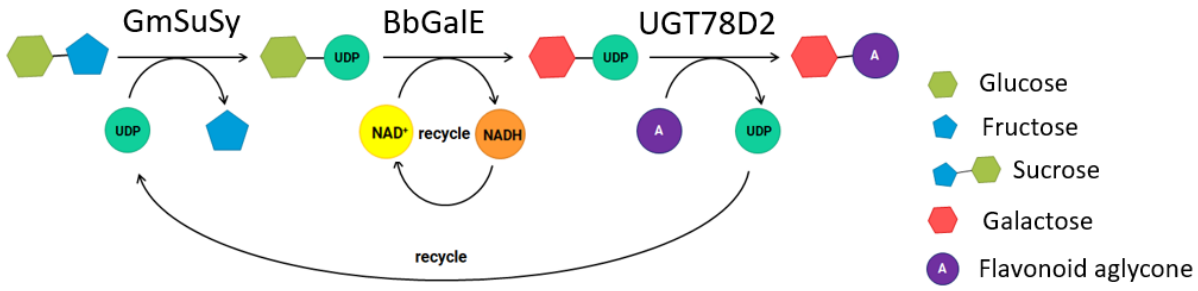

Figure 1. General reaction scheme of enzymatic cascade leading to flavonoid galactosides.

Herein we would like to present our recent progress in the incorporation of UDP-glucose 4-epimerase from *Bifidobacterium bifidum* (BbGalE) and selective galactosyltransferase from *Morella rubra* (UGT78D2) within the cascade with sucrose synthase from *Glycine max* (GmSuSy) in the production of flavonoid galactosides [3-4].

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P-19 Oliwia Rożnowska

Sustainable management of waste biomass through biotransformation into polyhydroxyalkanoates

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The waste biomass presents a significant challenge to the current economy, but it can be transformed into high-value products through biotransformation in biotechnological processes. Biopolymers produced in this way, such as polyhydroxyalkanoates (PHA), provide an alternative to commonly used plastic polymers and can also be applicable as drug carriers or raw materials to bio-based cosmetics components. Sustainable waste management aims to prolong the life cycle of these materials and minimize the generation of new waste, maximizing their potential.

Polyhydroxyalkanoates (PHAs) are large-molecule compounds classified as bacterial biopolymers. They are synthesized by various strains of microorganisms as well as genetically modified plants. During synthesis, they accumulate intracellularly within bacterial cytoplasm, forming characteristic granules that serve as a reserve material. Microorganisms utilize these granules in situations where there is a shortage of essential nutrients such as nitrogen, phosphorus, or sulfur, but an excess of carbon is present. PHA presents an attractive alternative to synthetic polymers due to their similar mechanical properties, biocompatibility, and ability to biodegradation. Despite their advantages, the production costs of biodegradable polymers remain high. However, by integrating waste resources into their synthesis, we can significantly reduce their production costs [1].

In industry, PHA commonly exists in several forms: medium-chain length (mcl-PHA), poly[(R)-3-hydroxybutyrate] (PHB), as well as copolymers such as poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx). These compounds are synthesized by various bacterial strains including *Pseudomonas putida, Ralstonia eutropha, Alcaligenes latus, Escherichia coli*, and *Aeromonas hydrophila* [2]. *Pseudomonas putida* is a Gram-negative bacterial strain capable of producing mcl-PHA from carbon sources such as volatile fatty acids resulting from methane fermentation, fatty acids from plant extracts, as well as glucose, fructose, or glycerol [4-6]. Waste in the form of coffee grounds and post-extraction rapeseed meal poses a significant challenge in managing waste from the food industry sector due to their substantial quantity. The potential transformation of these wastes through biotransformation into useful high-quality products represents a promising pathway to increase their added value, in line with the principles of sustainable economy.

This study focuses on valorizing spent coffee grounds and post-extraction rapeseed byproduct, as a carbon sources for PHA synthesis using *Pseudomonas putida.* The research began with the utility of coffee oil as a carbon source for microorganisms to conduct the PHA biosynthesis process. To achieve this, the spent coffee grounds were spread on a tray and dried. Subsequently, they were subjected to extraction using a Soxhlet extractor with hexane as the solvent. The extractions were carried out for 2 hours. The obtained oil dissolved in

hexane was purified by evaporation using a rotary evaporator (temperature 60°C, 50 rpm) for approximately 15 minutes. The next stage of the research involved chromatographic analysis using LC-MS of the obtained oil to exclude potential bacterial growth inhibitors [6]. In another part of the experiment, acid hydrolysis (1% H_2SO_4, temperature 121^{o}C) was performed for post-extraction rapeseed meal and coffee grounds, and the composition of the resulting hydrolysate was examined [4]. The research results represent the initial stages of developing technology for the utilization of bio-waste. The extraction efficiency from coffee grounds is promising. It is worth noting that the extraction process also generates post-extraction waste. The remaining material can be utilized as a potential carbon source, thus aiming for maximum waste utilization.

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P-20 Oliwia Rożnowska

Surface characterization of an inorganic membrane-enzyme system for environmental applications

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Currently, microplastic (MP) detection methods are getting more developed, although the sample preparation step becomes a bottleneck of the studies. This multi-step, time-consuming and expensive environmental sample preparation procedure for microplastic analysis requires simplification and standardization. For complex matrices like wastewater, a single sample preparation procedure takes approx. 17 days and require multiple sample treatments with different chemicals/reagents and several filtration steps. One of the most time-consuming steps is enzymatic treatments of the sample, to remove proteins and cellulose from the analysed material. Besides their length, taking at least 7 days, they also require additional sample filtrations, which carry the risk of microparticle loss during multiple repetitions of this procedure. Moreover, due to catalyst usage, this procedure is also expensive.

According to the literature, the use of membranes is the most efficient method of physical microparticle separation [1] whereas the enzymatic treatment of the sample allows impurities removal in mild conditions, which is especially important for further investigation of unaltered MPs. Based on this knowledge, new solutions in sample preparation procedures are still emerging to achieve better results in terms of the separation of a wide range of micro-sized polymers with appropriate shapes and sizes found in the environment. For this purpose, inorganic membranes were used to serve as matrixes for enzymes from various catalytic groups to form biologically active systems. Within the performed research advanced physicochemical as well as dispersion-morphological characterization and comparison of the membranes before and after immobilization were analyzed to understand their mechanism of action and properly design further steps of the sample preparation process.

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P-21 Aleksandra Rybak

Microbial enzymatic adaptations for biotransformations of selected API

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Aquatic organisms are constantly exposed to emerging contaminants from wastewater treatment plants (WWTPs). Chemotherapy medications: Methotrexate (MX), Vinorelbine (VINO), 5-Fluorouracil (5-FU), and Mycophenolic acid (MPA) – an immunosuppressant medication are concerning contaminants as their increasing use with the growing number of cancer patients and solid organ transplant recipients worldwide increases the potential risk to aquatic organisms exposed to wastewater discharges. Therefore, the main task of the presented study was a thorough analysis of the impact of the test compounds (MTX, 5-FU, VINO, MPA) at different concentrations on the growth of the microorganisms and their enzymatic activity.

Analyses were conducted for strains isolated from the artificial Lake Malta in Poznan. Individual strains were isolated from water samples and identified with the use of the matrix-assisted laser desorption/ionization (MALDI) technique. Further, the selected strains and concentrations of the test compounds were then used to establish cultures for enzymatic activity analyses. Samples were taken from the cultures at regular intervals, and proteins were isolated. Their concentrations were determined using the Bradford method and changes in the activity of selected enzymes (catalase, superoxide dismutase, S-glutathione transferase) were analyzed.

Agents such as Methotrexate, and 5-Fluorouracil belonging to the group of antimetabolites share structural similarities with compounds formed during normal cellular metabolism. Most antimetabolites have high cell cycle specificity and can target and arrest DNA replication in neoplastic cells[1]. Methotrexate is used in chemotherapy and as an immunosuppressant in auto-immune diseases. MTX may be dangerous if administered improperly. The most serious possible adverse effect is severe myelosuppression. This is responsible for the majority of the relatively rare deaths caused by MTX. Other side effects include bone marrow suppression, liver fibrosis, pneumonia, and baldness [2]. 5-Fluorouracil is still a common cancer drug. It has played an important role in the treatment of colon cancer since 1957 and is also used in patients with breast and other cancers, such as head and neck cancer [3]. Vinorelbine is a semi-synthetic vinca-alkaloid with a broad spectrum of anti-tumor activity. The mechanism of action is to interfere with the polymerization of tubulin. Tubulin is a protein responsible for building the microtubule system that forms during cell division. Among the alkaloid anticancer drugs, VINO was frequently detected in aquatic environments. These compounds have a high potential for persistence and bioaccumulation in sediments and organisms [4]. Mycophenolic acid is commonly used to prevent graft rejection in solid organ transplant recipients. The main concern with long-term use of immunosuppressive drugs is the risk of developing cancer [5, 6].

The effect of the tested compounds was different for each strain. Moreover, variations in bacterial enzymatic activity suggest various adaptation mechanisms for the metabolism of the tested compounds.

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P-22 Aleksandra Rybak

Influence of seasons on fresh-water bacterial community changes – a biochemical approach

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Community analysis is a valuable tool in environmental research, giving an opportunity to follow the effects of species cooperation and in the common effort of stress mitigation [1]. The seasons of the year are one of the factors occurring naturally, but due to the current climate changes, the extremes of each season are changing rapidly. This research shows the effect of seasons on the river water bacterial community in the neighborhood of the wastewater treatment plant discharge station.

To analyze microbial community changes, samples were taken every two months, starting from October 2023 to April 2024. The sampling points were chosen based on the location of the main Poznan WWTP discharge point: 1 km upstream (W-1) of the WWTP, directly at the WWTP discharge point (W0), and 1 km downstream (W+1) of the WWTP. During the sampling period, an abnormally high level of water was observed in February 2024 (above the warning level).

For the community analysis, the EcoPlates were used, to evaluate the functional diversity of bacterial communities by measuring their ability to oxidize carbon substrates. The EcoPlate is a 96-well microplate containing 31 common carbon sources from a total of five compound groups - carbohydrates, carboxylic and ketonic acids, amines and amides, amino acids, and polymers - plus a blank well and a control, all replicated three times to control for variation in inoculum density [2,3]. Based on the results, the Shannon diversity index was calculated and the studied consortia were compared in terms of their ability to metabolize selected compounds. The results of the analysis show bacterial shifts in time, depending on the sampling point, making visible also the impact of additional microorganisms from the WWTP discharge.

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Exploring Biotransformation of Phosphonates Using Cyanobacteria

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Biotransformation is a green, sustainable, and cost-effective process in which bacteria, yeast, fungi, plant tissues, cell extracts, and isolated enzymes can be used as biocatalysts [1]. The selection of suitable biocatalysts for specific reactions is a key stage of the process. In this study, we highlight the potential of cyanobacteria as an efficacious biotransformation platform for the synthesis of phosphonates. Cyanobacteria, photosynthetic prokaryotes, hold significant advantages, including minimal nutritional requirements; physiological resistance, metabolic plasticity, ease of genetic manipulation or tolerance to unfavorable conditions which makes them suitable for applications in biocatalysis [1, 2]. Cyanobacteria are known for their ability to reduce C=C bonds and carbonyl functional groups, however, their full spectrum of capabilities remains to be probed [3].

In this research, cultures of *Limnospira indica* PCC 8005*, Limnospira maxima* CCALA 27*, Leptolyngbya foveolarum* CCALA 76 and *Nodularia sphaerocarpa* CCALA 114 cultures were deployed as biocatalyst in a biotransformation of epoxyphosphonate (epoxymethyl dimethyl phosphonate). The procedure was executed over a period of 7 days at a stable temperature of 29 °C (±1) under continuous illumination and under stationary conditions. Products were extracted using ethyl acetate and subsequently analyzed by ^{31}P NMR.

After 24 h of the process *L. indica* and *L. maxima* efficiently transformed epoxyphosphonate with a conversion degree exceeding 99%. By contrast, to achieve high conversion rates in reactions mediated by *L. foveolarum* and *N. sphaerocarpa* (96% and 76% respectively), it was indispensable to prolong the biotransformation duration to 7 days. To evaluate the potential toxic impact of phosphonates on cyanobacteria, minimum inhibitory concentrations were determined using AlamarBlue Cell Viability Assay.

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P-24 Sandra Sordon

Exploring the Potential of Flavin-Dependent Halogenases in the Modification of Flavonoids

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Flavonoids are a large group of secondary metabolites widely distributed in plants. They exert numerous important physiological functions in plants, having considerable influence on growth and development of plants, protect them from UV radiation, bacterial and fungal infections and provide color to fruits and flowers [1]. Flavonoids found in food have beneficial effects on human health. They exhibit a diverse spectrum of biological activities such as: antioxidant, antitumor, anti-inflammatory, antiviral, estrogenic and anticancer [2]. Introducing halogens into structures of aromatic natural products could enhance their biological activities and physicochemical properties [3], however data on the biological activity of halogenated flavonoids is scant due to very limited occurrence in nature and difficulty in preparation. Despite the existence of numerous chemical halogenation methods, they often require potentially hazardous reagents, high temperatures and usually, they are characterized by low regio- and stereoselectivity, which is their biggest drawback [4]. Recently, there has been a growing interest in flavin-dependent halogenases (FDHs) as promising biocatalysts that allow for the insertion of halogen atoms into the structures of various compounds with high selectivity and efficiency including those positions in a structure that are electronically unfavorable. Flavin-dependent halogenases (FDH) utilize only oxygen from air, the $FADH_2$ as a cofactor, and halide salts. FDH catalysis occurs in water-based solutions at ambient temperature [5].

In this study, flavin-dependent halogenases: Rdc2 [6] and RadH (with confirmed activity for flavonoids) [7] were employed in the biotransformation process to determine their substrate specificity for a broad panel of flavonoid substrates (various classes of flavonoids), as well as to optimize biotransformation process in order to obtain halogenation products on a preparative scale.

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P-25 Daria Szada

An improved enzymatic approach to the synthesis of (*S*)-ibuprofen; a dynamic kinetic resolution of an ibuprofen methyl ester racemic mixture

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In recent years, the use of biocatalysis has increased rapidly in many industries. A particular application of enzymatic catalysis is in the rapidly growing pharmaceutical industry since biocatalysts are used to synthesize a variety of important pharmaceutically active substances (API). [1]. Several advantages make enzymes effective as biocatalysts for chemical reactions. Firstly, enzyme-catalyzed reactions are characterized by high chemo-, regio-, and stereoselectivity, which can be difficult to achieve with traditional synthesis methods, and is of great significance in the production of APIs. In addition, enzyme catalysts are capable of conducting reactions under mild conditions of temperature, pH, or pressure, thereby reducing process costs. In most cases, enzymatic reactions can be carried out in an aqueous environment, which reduces the risk associated with organic solvents. Furthermore, enzymes can be easily isolated from renewable sources, are biodegradable, non-toxic, and are consistent with Green Chemistry principles [2,3]. Research into enzyme catalysts continues to improve their efficiency to make them even more effective. The immobilization of enzymes improves chemical synthesis processes since it enhances the stability of the enzyme as well as facilitates the separation of the catalyst from the reaction medium and its reuse [4].

Ibuprofen has been widely used throughout the world for many years. Ibuprofen belongs to the nonsteroidal anti-inflammatory drug (NSAID) class. The substance has anti-inflammatory, analgesic, as well as antipyretic effects. Commercially, ibuprofen is mainly available as a racemic mixture of (R)-ibuprofen and (S)-ibuprofen. Nevertheless, it is important to note that both enantiomers possess different pharmacological properties. The pharmacological activity of ibuprofen is mainly manifested by (S)-ibuprofen, which is up to 160 times more active than (R)-ibuprofen. (S)-ibuprofen can inhibit both cyclooxygenase-1 and cyclooxygenase-2 to an equal degree, while the (R)-enantiomer is much weaker in inhibiting COX-1 and COX-2. The dissimilarity of the two enantiomeric forms is also manifested in their metabolic profile, as (R)-ibuprofen becomes involved in lipid metabolism pathways, which is not the case with the (S)-enantiomer of ibuprofen [5,6]. In traditional synthesis methods, ibuprofen is primarily produced as a racemic mixture. The use of enzymatic methods makes it possible to obtain predominantly one, more desirable enantiomer of ibuprofen.

This study aimed to demonstrate the utility of immobilized lipase from *Candida rugosa* as a biocatalyst for the separation reaction of a racemic mixture of ibuprofen methyl ester, ultimately leading to (S)-ibuprofen as the desired product. During the conducted studies, the effect of reaction time was examined, the significance of the type of organic solvent was evaluated, and an attempt was made to demonstrate the differences in the case of changing pH and adding DMSO (kinetic/dynamic kinetic resolution) on the efficiency of biocatalytic conversion. It was shown that the studied parameters have a significant effect on the conversion of the substrate and, on the enantiomeric excess of the obtained (S)-ibuprofen

over the (R)-ibuprofen. The results indicated that isooctane was a more suitable organic solvent, as substrate conversion was higher in its presence than in hexane (for the process conducted at pH = 7, a conversion rate of 48% was obtained for the sample in isooctane and 36% for the sample in hexane). To evaluate the effect of time on enzymatic reaction, samples were taken for testing every 48 hours, and the most favorable results were obtained after 144 hours. As a result of increasing the pH of the reaction and adding DMSO, dynamic kinetic resolution of ibuprofen methyl ester was enabled, and in situ racemization of the unconverted (R)-ester to the (S)-ester was carried out, resulting in a higher conversion rate. For the process carried out in isooctane, the use of NaHCO3-NaOH buffer at pH = 9.5 instead of phosphate buffer at pH = 7 allowed an increase in the substrate conversion rate from 48% to 83%. Moreover, the addition of DMSO made it possible to increase the conversion of ibuprofen methyl ester to 95%.

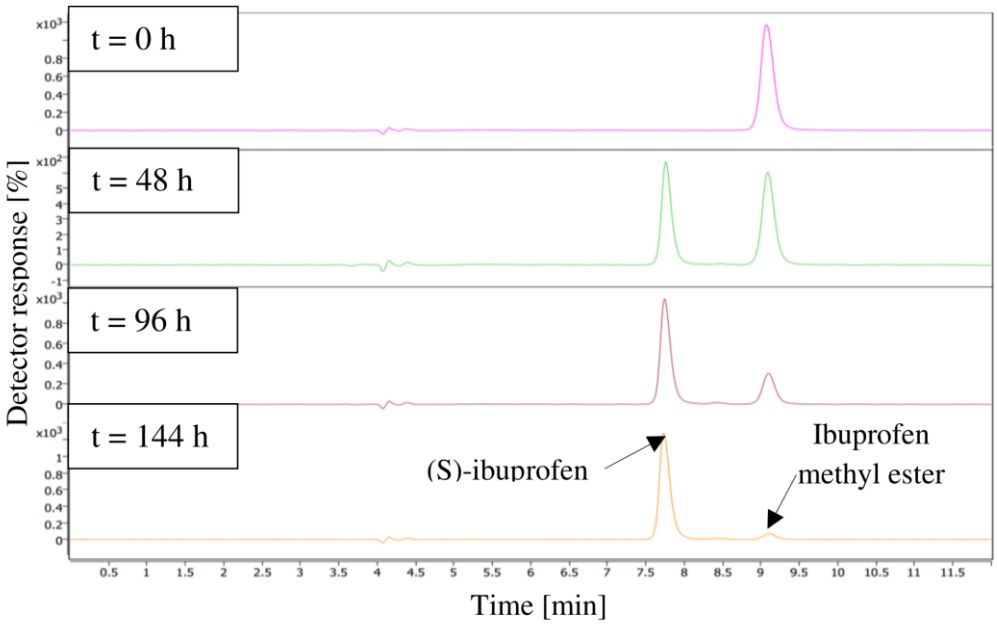

Figure 1. Chromatograms of the process carried out in isooctane and $NaCO_3$-NaOH at pH = 9,5 in the presence of DMSO.

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P-26 Maciej Szaleniec

The Initiation Phase of the Catalytic Cycle in the Glycyl-Radical Enzyme Benzylsuccinate Synthase – Modelling and Experiment

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Benzylsuccinate synthase (BSS) belongs to the family of fumarate-adding enzymes which is itself part of the growing superfamily of glycyl radical enzymes (GRE)[1]. GRE are involved in surprisingly different, but always chemically demanding reactions in anaerobic metabolic pathways of Bacteria, Archaea, and Eukarya. In addition to fumarate-adding enzymes, the currently known families of GRE consist of the pyruvate formate lyases (PFL), type III anaerobic ribonucleotide reductases (ARNR), glycerol or diol dehydratases, hydroxyproline dehydratases, arylacetate decarboxylases, choline or isethionate lyases and phosphonate-cleaving C-P lyases.

The BSS enzyme catalyzes the radical-based addition of toluene to a fumarate cosubstrate. The process is initiated by hydrogen transfer from a conserved cysteine (Cys493) to the nearby glycyl radical (Gly829) in the active center of the enzyme (Fig. 1). Although the BSS mechanism is studied for many years, this first step of the reaction was never properly examined in the context of the already known structure of the enzyme.

In this poster we analyze this step by comprehensive QM:MM modeling, predicting (i) the influence of bound substrates or products, (ii) the energy profiles of forward- and backward hydrogen-transfer reactions, (iii) their kinetic constants and potential mechanisms, (iv) enantiospecificity differences and (v) kinetic isotope effects. We also used microkinetic constants derived from calculations to estimate overall rates of H/D transfer and to propose potential mechanisms of H/D exchange at radical Gly829 [2].

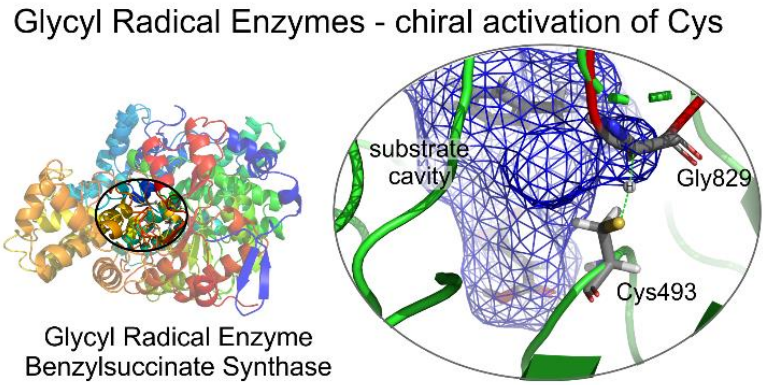

Fig. 1. Catalytic subunit of BSS (left) and a close-up of the BSS active site with substrate cavity holding fumarate and toluene delineated by a blue mesh.

We supported some computational predictions experimentally, providing evidence for predicted H/D-exchange reactions into the product and at the glycyl radical site. We have conducted kinetic experiments analyzing the H/D exchange rate of substrates and products when the reaction was conducted with deuterated substrates or in D_2O. We have also reanalyzed the H/D exchange of the glycyl radical in BSS using EPR spectra after changing the solvent to D_2O and back again to H_2O [3].

Our data indicate that the hydrogen transfer reactions between the active site glycyl and cysteine are principally reversible, but their rates differ strongly depending on their stereochemical orientation, transfer of protium or deuterium, and the presence or absence of substrates or products in the active site. This is particularly evident for the isotope exchange reaction of the remaining protium atom of the glycyl radical to deuterium, which appears to be dependent on substrate or product binding, explaining why it has been observed in some, but not all glycyl-radical enzymes.

Our results show that the radical on Gly829 requires bound substrates or product to enable H-transfer or H/D exchange with Cys493, while these reactions are precluded in apo-BSS. Moreover, the enzyme retains the H- or D-atoms of the glycyl radicals when acting with the preferred stereospecificity but is able to initiate H/D exchange into the glycyl radical at a significantly slower rate (and with even slower reverse D/H exchange) by occasionally reacting in the non-preferential stereospecificity. Such behavior of the BSS enzyme may be valid for all GRE, indicating that substrate or product binding may be a prerequisite for both initiating H-transfer between the active site Gly and Cys residues and the reactions involved in the H/D exchange of the glycyl radical.

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P-27 Marzena Szawara

Searching for selective and safe inhibitors of epoxide hydrolase of juvenile hormone targeting selected pests

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Maintaining biodiversity requires pollinating insects. Worldwide reports of pollinator losses in recent years have major ecological and economic repercussions. Deterioration and loss of habitat are two factors that have a negative impact on the population of these insects. The use of plant protection products by humans, whose primary objective is to lower the pesticides among crops is however, a reason that is becoming more and more prevalent.

We think it's possible to maintain pollinators while also managing insect numbers. It is already known, that JHEH, and enzyme which is crucial for insects' metamorphosis, can be used as a target for insect's control. However, so far developed pesticides were unspecific. In our research we are combining computational and experimental methods used for drug design, to propose highly selective compounds, for selective pests' control.

We aim to explore a unique approach which is based on intramolecular voids analysis and molecular dynamics simulations (MD) in co-solvents which will guide pharmacophore design. Identified differences and similarities between different insect JHEH will help in the design of new pharmacophores and subsequently species-specific inhibitors.

Ultimately, our goal is to conduct an extensive study of selected recombinant proteins from ten organisms to best replicate the taxonomic cross-section of species and experimentally confirm the results of the computational analysis carried out.

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Microbial synthesis of sesamol from piperonylic acid

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Sesamol is an organic compound (derivative of phenol) with wide spectrum important biological activities such as antifungal, anti-inflammatory, antidepressant, neuroprotective, wound healing, and anti-aging [1]. Moreover, it is useful as an antioxidant in edible fats. Currently, sesamol is obtained by extraction from sesame oil or by chemical synthesis from piperonal [2].

This research presents the novel method of obtaining sesamol from piperonylic acid using a biotechnological route. Accordingly to our studies, *Monascus* strains exhibit ability to conduct the biotransormation of piperonylic acid to this highly demanded compound, which constitutes an attractive alternative to synthetic methods. The biotransformation conducted in the prep-scale process (1g/L) led to obtaining sesamol with the yield 36.7%. Additionally, a biological activity of nano-conjugate of sesamol and zinc oxide nanoparticles (ZnONPs) was studied against human dermal fibroblast cell lines.

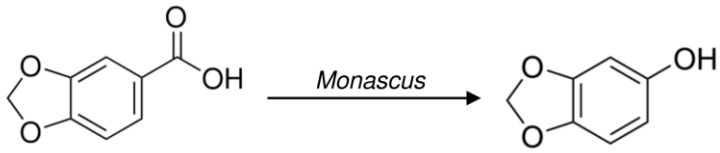

Figure 1. Biotranformation of piperonylic acid to sesamol.

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P-29 Mateusz Tataruch

Thermal inactivation of KSTD1 from *Rhodococcus erythropolis* – preliminary studies

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Steroids are important synthons of many drugs and compounds from the "fine chemicals" group. The wide range of steroid uses results in the continuous development of methods for obtaining these compounds. The challenge is to search for effective enzymatic tools for the green synthesis of this type of compounds and to improve the bioprocesses catalyzed by them. This is based on deepening knowledge about their stabilization and understanding the mechanisms of their inactivation.

3-Ketosteroid delta1-dehydrogenase (KSTD1, PDB ID: 4C3Y) from *Rhodococcus erythropolis* is an FAD-dependent enzyme that catalyses dehydrogenation between C1 and C2 atoms of the steroid ring A of 3-ketosteroid substrates. This enzyme is a high-class biotool for one-step, selective synthesis of steroid compounds, especially desired in the pharmacy industry. The enzyme contains a FAD-binding and catalytic domain, between which the active site is situated, and it is determined to be monomeric in solution. [1,2,3]

The thermal stability of KSTD1 was investigated under storage conditions without stabilizers at temperature range from 25 to 50 °C. Inactivation data were analyzed to assess compliance with the “one step – two steps” mechanism using isotemperature evaluation. Discontinuity observed on the Arrhenius plot indicates that the thermal inactivation of KSTD1 follows a more complex mechanism. Moreover, the influence of potential stabilizing compounds such as glucose, ketosteroid substrate (androst-4-en-3,17-dione) and artificial electron acceptor (2,6-dichloroindophenol) on improving enzyme stability was separately examined.

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P-30 Agata Wawoczny

Enzymatic valorization of lignocellulosic biomass – The influence of deep eutectic solvents and ionic liquids on the activity of cellulolytic enzymes

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The valorization of lignocellulosic biomass has become one of the most frequently discussed topic both in industry and science. Plant waste is not only a renewable source of energy, but also an accessible and affordable source of polysaccharides, which can be transformed to valuable chemicals (e.g. levulinic acid, furfural, glucose) useful in pharmaceutical, food, and fuel industries. Furthermore, the methods for biomass processing are constantly being improved to reduce their impact on natural environment and simultaneously increase their efficiency. One of these methods includes applying cellulolytic enzymes for biomass hydrolysis to monosaccharides. However, one of the main lignocellulose components, lignin, acts as a mechanical barrier for biocatalysts, negatively affecting the processing of cellulose and hemicelluloses [1]. Additionally, lignin can bind non-covalently to applied cellulase, which further reduces the efficiency of hydrolysis [2]. Because of that, it is recommended to pretreat biomass before enzymatic processing, in order to remove part of lignin from the material. One of the methods for such a process is applying deep eutectic solvents (DESs), which are non-toxic, have low volatility, and have low environmental impact [3]. Additionally, they have an excellent ability to dissolve lignin without impact on other lignocellulose components [4]. However, certain DESs might deactivate cellulases, necessitating the removal of the solvent from the material prior to enzymatic hydrolysis. To circumvent this requirement, enzymes exhibiting strong resistance to DESs are recommended for use. While literature does provide examples of biocatalysts with stable activity in the presence of DESs, information regarding cellulolytic enzymes is currently limited [5,6].

In this work, the impact of chosen DESs on the enzymatic activity of cellulases from *Aspergillus niger*, *Trichoderma reesei*, and cellulolytic preparation Viscozyme L is presented. It came out that all of the applied solvents affect negatively the activity of biocatalysts, especially those containing acids (lactic acid and levulinic acid), which excludes the one-pot process of biomass transformation with cellulases and DESs. The experiments showed that Viscozyme L is the most resistant to DESs [7].

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New hydroxylactones obtained by biotransformation of bicyclic halolactones with three methyl groups and their antimicrobial activity

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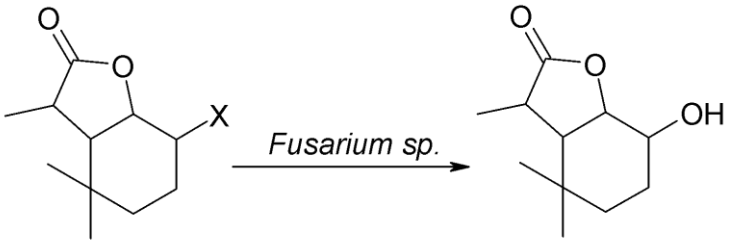
Lactones are a large group of naturally occurring compounds. They are characterized by a variety of biological activities [1-2]. Of particular interest are lactones containing halogen atoms in their structure. Synthetic halolactones show antiproliferative activity [3], antimicrobial activity, cytotoxic activity [4], and the ability to inhibit photosynthesis [5]. In turn, hydroxylactones, which are often isolated from natural sources, show antimicrobial [6-7], fungistatic [8], cytotoxic [9] activity.



X = Cl, Br or I

During our research, halolactones with a gem-dimethylcyclohexane system in the cyclohexane ring and a methyl group in the lactone ring were obtained by chemical synthesis. Halolactones occurring as mixtures of two diasteroisomers were biotransformed using strains of the genus Fusarium, yielding the corresponding hydroxylactones. Knowing that both halo- and hydroxylactones can exhibit antimicrobial activity, compounds obtained by both chemical synthesis and biotransformation were subjected to such tests.

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P-32 Katarzyna Wińska

Carvone biotransformations in carrot cell cultures

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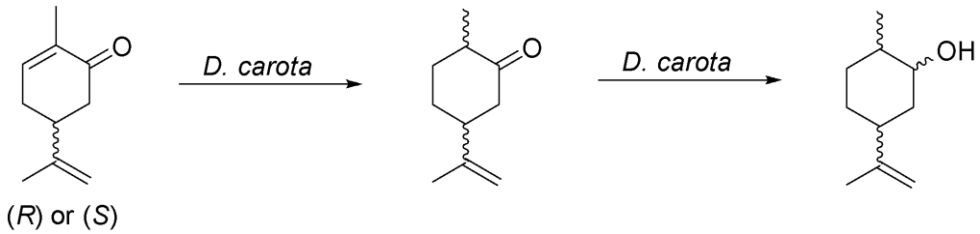
Plant cell cultures have been shown to be effective at performing bioconversion or biotransformation reactions on various organic compounds, including the conversion of ketones into optically pure hydroxy compounds [1]. Several studies have demonstrated that plant cell cultures from different species, such as carrot, tobacco and gardenia [2], can biotransform ketones into the corresponding alcohols [3,4].



Carvone enantiomers - (4R)-(-)-carvone and (4S)-(+)-carvone were substrates in the biotransformation carried out using carrot cell culture. To obtain the starting material, which was carrot callus, two different combinations of growth regulators were used - NAA 2 mg/l, BA 0.2 mg/l and BA 2.0 mg/l, NAA 0.2 mg/l. Biotransformation were carried out for three weeks. It was observed that in the first step the double bond in the cyclohexane ring was reduced and then the carbonyl group was reduced. The final biotransformation products were the corresponding dihydrocarveols.

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Bioinformatic analysis of 3-ketosteroid dehydrogenases family: in between the sequence & substrate specificity

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3-Ketosteroid dehydrogenases (KstDs) are crucial in steroid biotransformation, offering diverse applications across pharmaceutical, biotechnological, environmental, and medical sectors [1]. Due to the ubiquity and diversity of steroid compounds, bacteria have evolved strategies to modify and degrade these compounds in various niches [2]. Consequently, KstDs, key enzymes in steroid degradation, are expressed in numerous variants that differ in structure, biochemical properties, and, most importantly from an application standpoint, substrate specificity[3]. We analyzed over 300 KstD sequences similar to three selected KstDs: AcmB2[4], KstD1 [5], and KstD4 [6]. (Fig. 1).

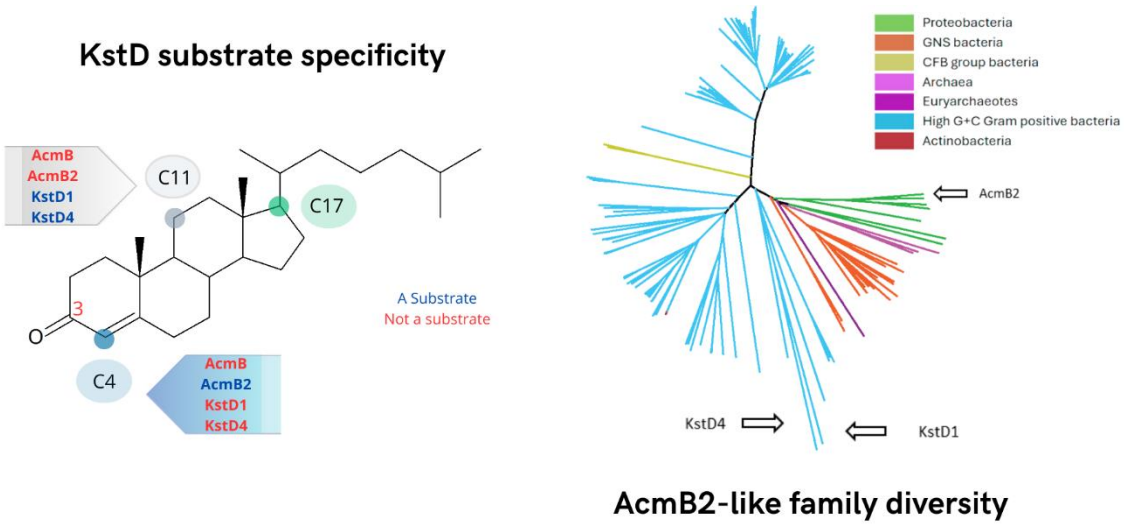

Figure 1. The substrate specificity of selected KstDs: AcmB2 from *Sterolibacterium denitrificans*, KstD4 from *Arthrobacter simplex*, and KstD1 from *Rhodococcus erythropolis.* The structure of cholest-4-en-3-one highlights positions where substituents are allowed (blue) or forbidden (red) to observe 1,2-dehydrogenation activity. Additionally, the diversity of the AcmB-like family is presented as a phylogenetic tree of amino acid sequences, colored by the type of bacteria.

In our work we prepared the analysis for three groups of KstDs: AcmB2 from anaerobic *Sterolibacterium denitrificans*, KstD4 from obligate aerobic *Arthrobacter simplex*, and KstD1 from aerobic *Rhodococcus erythropolis.* The conducted analysis not only identified the type of bacteria producing a specific KstD variant but also determined the habitat of these bacteria and their environmental conditions, whether aerobic or anaerobic. Identifying this latter parameter provides insights into the prevalence of anaerobic bacteria, which have a distinct steroid degradation pathway compared to aerobic bacteria. Such studies can contribute to proposing new candidates for isolation from the environment, with the aim of discovering new

variants of steroid degradation enzymes in addition to KstD, as well as potentially identifying KstDs with specific substrate specificity.

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P-34 Katharina Müller

Investigating the mechanism of acetophenone carboxylase

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Aromatic ketones such as acetone and acetophenone are common intermediates of natural secondary metabolism. As they are chemically inert, these substrates must be activated prior to microbial degradation. Ketone activation proceeds either by oxygenation or by carboxylation [1,2].

Acetone carboxylase (Acx) and acetophenone carboxylase (Apc) first activate both of their substrates (*i.e.*, the ketone and HCO_3^-) by phosphorylation [1]. In Acx, both substrates are phosphorylated at the same activation site. Then, the activated substrates are transferred over a distance of 40 Å from the nucleotide binding site to the catalytic site via an internal channel [3]. In contrast, substrate phosphorylation occurs at two distinct sites in Apc. Phosphorylation sites are 50 Å apart from each other, which precludes direct interaction of the activated substrates. Free diffusion between the activation sites is not possible either as the activated substrates are readily decomposed in water. The Apc core complex ($[\alpha\alpha'\beta\gamma]_2$) moreover lacks internal substrate channels for acetophenone-*enol*-phosphate transport [2]. However, in contrast to Acx, Apc possesses an additional Apcε subunit, which is not part of the Apc complex, but is indispensable for acetophenone carboxylation [4]. The actual function of Apcε is still at issue. It has been hypothesized that Apcε forms a lid which shields the activated substrates from water and allows them to diffuse to the catalytic subunit or induces conformational changes in Apc to bring the substrate binding sites into close proximity [2].

Here, we study the specificity of interactions between Apcε and the core complex. We generate *Aromatoleum spp. ΔapcE* deletion mutants and complement them with heterologous Apcε subunits of high or low sequence identity (*e.g.*, Apcε from *Aromatoleum spp.* or *Nevskia ramosa*). Comparing the growth of these mutants on acetophenone, we evaluate which level of interaction specificity between Apcε and core complex is required to sustain enzyme activity.

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