

Novel biocatalytic systems $\text{Al}_2\text{O}_3\text{-Fe}_3\text{O}_4$ /polyelectrolyte/laccase and $\text{Al}_2\text{O}_3\text{-Fe}_3\text{O}_4$ /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 $\text{Al}_2\text{O}_3\text{-Fe}_3\text{O}_4$ /laccase/polyelectrolytes and $\text{Al}_2\text{O}_3\text{-Fe}_3\text{O}_4$ /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 exhibited 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 $\text{Al}_2\text{O}_3\text{-Fe}_3\text{O}_4$ 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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Possibility of using apple pulp after supercritical extraction in CO₂ 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 CO₂.

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 CO₂ 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 800°C 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 m²/g (600°C) to 1119 m²/g (800°C), with a predominant amount of micropores (less than 2 nm). Sorption of Zn²⁺ ions from an aqueous solution with a concentration of 50 mg/dm³ 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 Zn²⁺ 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-glycosyltransferase 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.

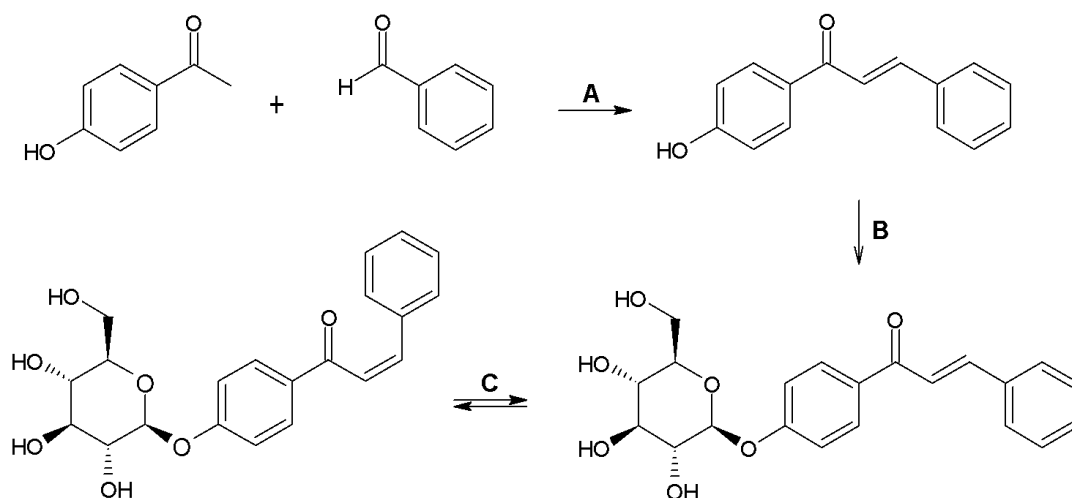


Figure 1. Process of glycoside formation: **A)** chemical synthesis of *trans*-chalcones; **B)** enzymatic glycosylation of 4'-hydroxychalcones; **C)** isomerization process induced by UV radiation.

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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 cysteinylated 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.

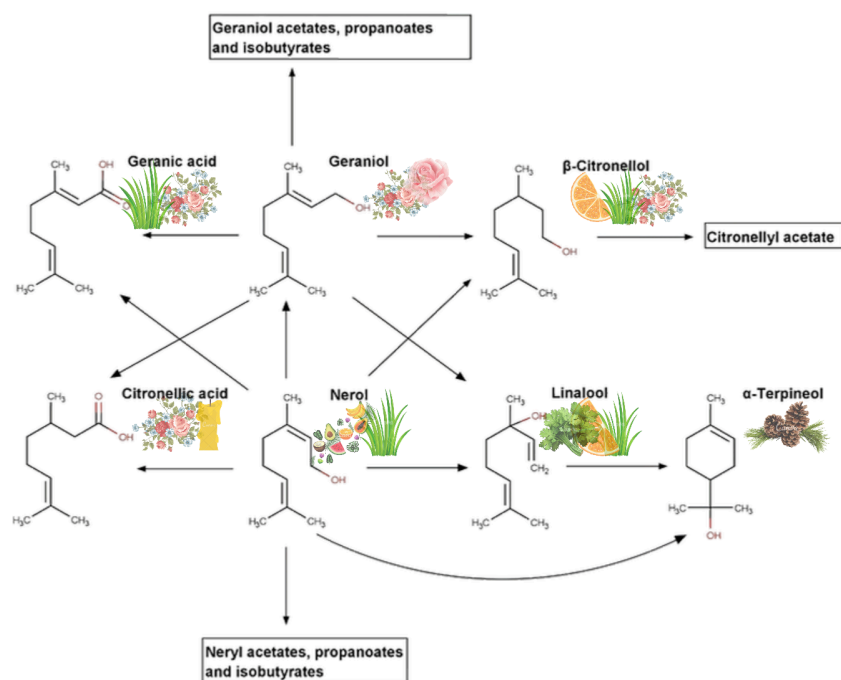


Figure 1. Known biotransformation reactions catalyzed by *S. cerevisiae*. Mentioned esters could undergo further hydrolysis [2].

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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.

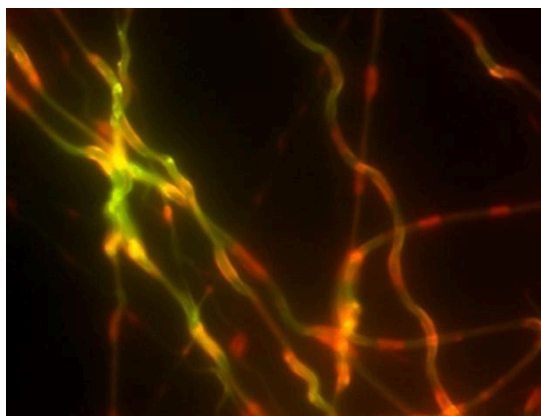


Figure 1. Fluorescent microscope image of core-shell system PVP/PCL with encapsulated enzyme in the core, supported by ionic liquid. Nanofiber layers were stained with the coumarin (core) and Rose Bengal (shell) dyes.

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.

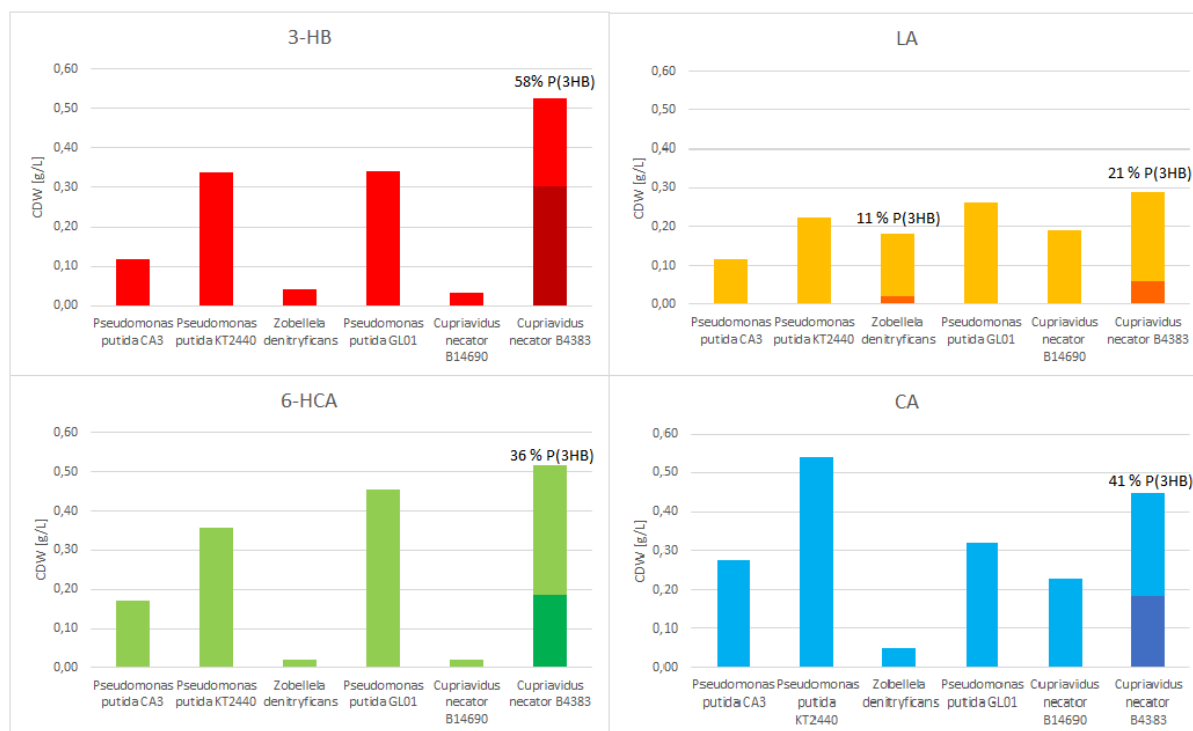


Figure 1. Determination of dry biomass and P(3HB) content in bacterial biomass

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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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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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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.

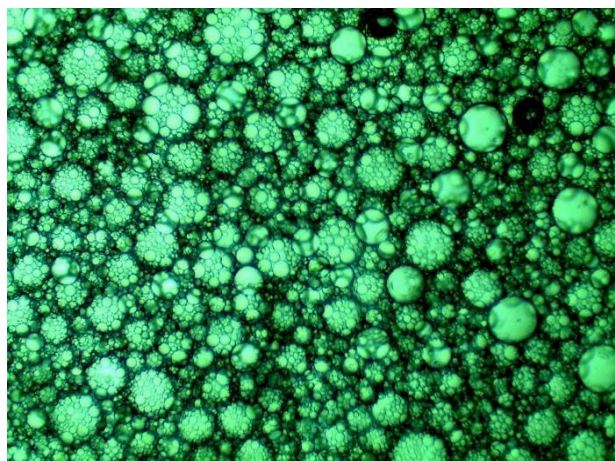


Figure 1. Microscopic photo of saponin based emulsion with urea addition.

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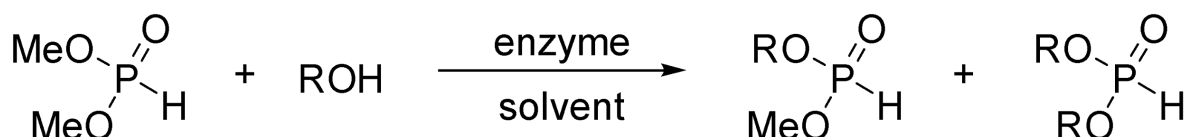
Financial support from the National Science Center of Poland research project (grant no.2022/45/B/ST8/02058) is gratefully acknowledged.

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 phospho-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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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, H₂O₂, 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 H₂O₂, 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).

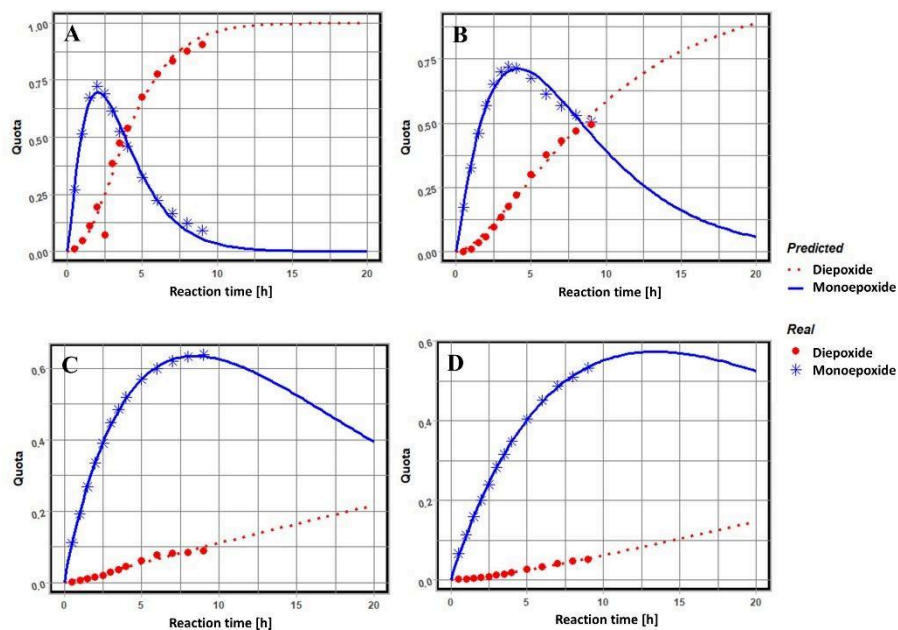


Figure 1. Time dependence of the amount of limonene monoepoxide and diepoxide at the limonene : biocatalyst ratio: $k = 0.2$ (A), $k = 0.5$ (B), $k = 1.0$ (C), and $k = 2.0$ (D)

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).

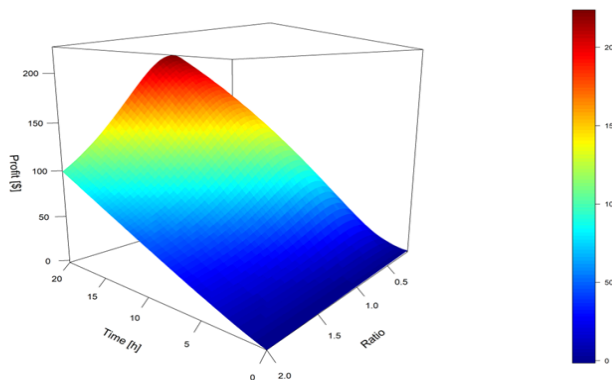


Figure 2. The gain of the reaction depending on the duration of the epoxidation and the quantitative ratio of limonene to the biocatalyst

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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% H₂O₂ 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).

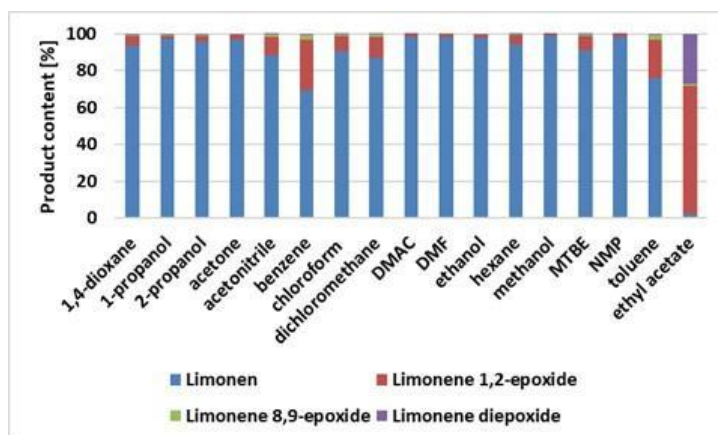


Figure 1. Effect of solvent type on the efficiency of the chemoenzymatic epoxidation of limonene

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).

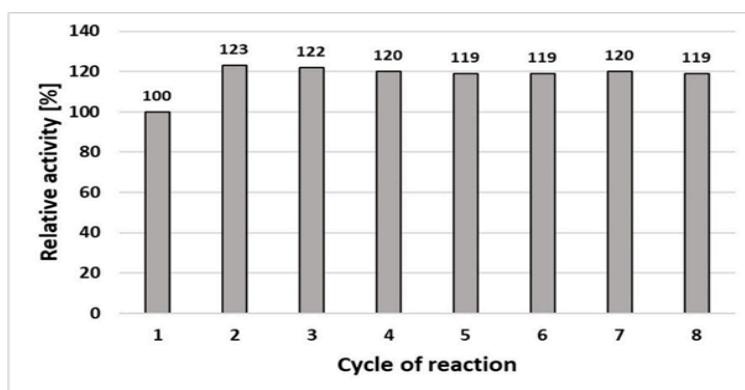


Figure 2. Reusability of *C. cladosporioides* 01 in a chemoenzymatic reaction cycle for the epoxidation of limonene

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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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 (GtHNL-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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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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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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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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