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Supported ionic liquid phase facilitated catalysis with lipase from *Aspergillus oryzae* for enhance enantiomeric resolution of racemic ibuprofen

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ABSTRACT

Supported ionic liquid phase (SILP) was used as a carrier for lipase from *Aspergillus oryzae* (LAO) and used as a biocatalyst for enantiomeric resolution of racemic ibuprofen *via* esterification leading to *(S)*-(+)-ibuprofen ester. Using native form of lipase, outstanding results were achieved, obtaining *(S)*-(+)-ibuprofen propyl ester with enantiomeric excess (*ee*) of 99.9% and high conversion of racemic ibuprofen after 24 h ($\alpha = 34.8\%$) and respectively $ee = 99.9\%$ with $\alpha = 45.2\%$ after 48 h. Several hybrid materials composited with silica and metal-based oxides including magnesium, calcium, and zirconia were evaluated as supports for LAO with various surface characteristics. The selected ionic liquid 1-methyl-3-(triethoxysilylpropyl)imidazolium bis(trifluoromethylsulfonyl)imide was immobilized *via* the covalent bound onto the surface of solid material and in the second step LAO was anchored. Optimized results in enantiomeric resolution of racemic ibuprofen (35.23% conversion of *rac*-ibuprofen after 7 days with 95% *ee* of ester) were obtained for SILP biocatalyst based on $MgO \cdot SiO_2$ (1:1) (ionic liquid loading 6.79%, enzyme loading 3.96%). This is proposed as a generic approach to tailoring supported ionic liquids phase biocatalysts for industrially-relevant reactions, to generate both environmentally and economically sustainable processes.

1. Introduction

Many pharmaceuticals are chiral. They exist as a pair of stereoisomers (or enantiomers) with identical chemical formula, however, geometrically these stereoisomers of the same compound cannot superimpose onto each other after any conformational changes (Nguyen et al., 2022). Industrial production of pharmaceuticals often results initially in a racemate, or a racemic mixture of equal amount of both enantiomers. In most cases, two enantiomers of the same

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compound differ markedly from each other in terms of their therapeutic effects and toxicology. Enantiomeric conversion of chiral pharmaceuticals is, therefore, of significant scientific, industrial, and environmental interest (Wsól et al., 2004; Nguyen et al., 2021; Raczyńska et al., 2021).

As a commonly used over-the-counter medicine to treat pain and fever, ibuprofen is one of the most significant chiral pharmaceuticals. The two enantiomers of ibuprofen *(R)*-(-)-ibuprofen and *(S)*-(+)-ibuprofen exhibits vastly different pharmacological properties and metabolic profiles. *(S)*-(+)-enantiomer has the desired pharmacological activity and is capable of inhibiting cyclooxygenase (COX), while *(R)*-(-)-ibuprofen is not a COX inhibitor and is responsible for the intensification of the inflammatory process. *(S)*-(+)-enantiomer of ibuprofen is 160 times more therapeutically active than *(R)*-(-)-ibuprofen (Adams et al., 1976). More importantly, the *(R)*-(-)-ibuprofen can cause potential side effects (Evans, 2001). Recent studies have focused on the isolation of pure *(S)*-(+)-ibuprofen for faster and more precise therapeutic outcome and lowering the risk of side effects.

Racemic resolution of ibuprofen enantiomers can be achieved *via* kinetic resolution with enzymatic esterification or hydrolysis, high performance liquid chromatography, and diastereoisomeric crystallization (José et al., 2015). In enantiomeric esterification, lipases of fungal sources, such as *Candida rugosa*, *Rhizomucor miehei,* can preferentially catalyze the conversion of *(S)*-(+)-ibuprofen. On the other hand, the lipase B from *Candida antarctica* preferentially converts the *(R)*-(-)-ibuprofen to ester. Other fungal lipases from *Candida* sp, *Aspergillus niger*, *Pseudomona* sp, *Aspergillus terreus*, *Fusarium oxysporum*, *Mucor javanicus*, *Penicillium solitum*, *Rhizopus javanicus, Rhizomucor miehei*, *Rhodothermus marinus* and esterase from *Aeropyrum pernyx* as well as porcine pancreas (José et al., 2015; Wei et al., 2016; Zappaterra et al., 2021; Bachosz et al., 2022; Verri et al., 2016) have been also studied as biocatalysts in enantiomeric esterification but with less success, perhaps with the exception of esterase from *Thermotoga maritima* (Wei et al., 2016).

To increase or even promote the stability, selectivity, specificity and, in certain cases, activity of lipases, these enzymes can be immobilized onto a solid substrate what is important also from the industrial application viewpoint (Zdarta et al., 2022a,b; Bilal et al., 2019; Boudrant et al., 2020; Szelwicka et al., 2020; Markiton et al., 2017; Szelwicka et al., 2019a,b; Mateo et al., 2007a,b). Enzyme immobilization may be coupled to enzyme purification (Dos Santos et al., 2015; Barbosa et al., 2015). The biomolecules immobilization provides also higher resistance against adverse effects of the reaction conditions (Rodrigues et al., 2013). Particularly, a controlled immobilization using porous supports reduces diffusional limitations and partitions as well as enzyme aggregation and its inhibition and prevents against internal conformational changes in enzyme structure (Mateo et al., 2007a,b). Furthermore, multipoint enzyme immobilization reduces leaching of the enzyme from the support and provides additional enzyme rigidization (Garcia-Galan et al., 2011). It should be highlighted that various solid supports were used for enzyme binding including silica, biochar, metal oxides, polymer fibers, and protein-coated microcrystals (Hong et al., 2014; Huang et al., 2015; Jesionowski et al., 2014; Zdarta et al., 2022c; Jiang et al., 2022; Svetozarević et al., 2022). Finally, heterogenization of enzymes enables the easy separation and recycle or use of the flow system (Szelwicka et al., 2019a,b; Baumann et al., 2020).

The current industry challenge in enantiomeric esterification is to obtain the more therapeutically enantiomer at the highest chiral purity possible, defined as enantiomeric excess (*ee*). Formula to calculate *ee*, which is a measurement of purity of a chiral substance is available in the Supplementary Materials. The *ee* for pure ibuprofen ester noted in many works was far below 50% while other previous works only presented medium *ee,* up to 90%. Truly beneficial results of racemic resolution for *ee* higher than 90% are rather rare (José et al., 2015). Very often the full conversion of substrate is not reached due to the detrimental influence of reaction time on *ee*. Reaction times necessary to reach the final results are from several to several dozen hours and were depended on the reactivity of alcohol used for esterification. Very few studies have described near complete conversion of *(S)*-(+)-ibuprofen with high enantioselectivity to *(S)*-(+)-enantiomer of ibuprofen ester using lipase from *Candida rugosa* as a catalyst (Huang et al., 2015; Siódmiak et al., 2012; Mohammadi et al., 2016; Ghofrani et al., 2021). Some important achievements to date are discussed below.

Lipase from *Candida rugosa* was used for the enantioselective esterification reaction of racemic ibuprofen with 1-propanol in cyclohexane as a reaction medium at 30 °C. The highest conversion (44.2% after 140 h), with enantiomeric excess of product equal 68.3% was achieved (Siódmiak et al., 2012). Protein-coated microcrystals prepared from lipase form *Candida rugosa* applied in esterification of racemic ibuprofen with isooctanol in isooctane was extremely active biocatalyst causing the 97.3% *ee* of *(S)*-(+)-ibuprofen ester and total conversion of substrate 49.8% after 8 h. Moreover, studied biocatalyst exhibits good operational stability, retained active in 15 successive batches (Huang et al., 2015). Another example of active system is lipase from *Rhizomucor miehei* immobilized on epoxy-functionalized silica and used for esterification of ibuprofen with 1-propanol in anhydrous isooctane at 0 °C. The obtained conversion was low (around 23.7% after 24 h), but the *ee* was high 92% (Mohammadi et al., 2016). In the recent work lipase from *Candida rugosa* immobilized on silica nanoparticles showed 45% conversion of substrate with 1-propanol and 96% enantioselectivity to *(S)*-(+)-ibuprofen ester at 37 °C after 50 min. Unfortunately, authors did not mention the amount of enzyme immobilized on the surface of silica (Mohammadi et al., 2016). On the other hand, in the only one work describing the enantioselective transport of *(S)*-(+)-ibuprofen through a supported liquid membrane based on ionic liquids, lipase from *Aspergillus oryzae* (Novo) as one of several other enzymes with low activity was tested for comparison reasons. The best result (*ee* 75% for *(S)*-(+)-ibuprofen ester) was obtained for the combination of lipase from *Candida rugosa* and 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide [bmim][NTf_2] (Miyako et al., 2003).

Ionic liquids (ILs) with unique properties (negligible vapor pressure with polar and non-polar regions in the organized 'nanostructures' with hydrogen-bonded polymeric supramolecules) can maintain enzymes in their active conformation

(Itoh, 2017; Zhao, 2015). ILs were used in many biotransformations (Lozano et al., 2017; Drożdż et al., 2015), including several applications in enantiomeric esterification of ibuprofen (Wei et al., 2016; Miyako et al., 2003; Naik et al., 2007). Advantageous results were obtained for thermostable esterase (EST10) from *Thermotoga maritima* with ionic liquid based on $[BF_4]^-$ anion. *(S)*-(+)-ibuprofen ethyl ester was obtained after 10 h of the reaction at 75 °C with high conversion of substrate (47.4%) and high *ee* 96.6% (Wei et al., 2016).

ILs can be integrated with a solid phase carrier to form a supported ionic liquid phase (SILP) system for enzymes in biocatalysis. In the SILP system, ILs form a thin layer on the solid carrier, thus, reducing the amount of IL used in the process. The enzyme can be immobilized within the ILs layer *via* adsorption. These heterogeneous SILP biocatalysts are efficient and have good recyclability (Garcia-Verdugo et al., 2014; Szelwicka et al., 2021a,b; Fernandez-Lafuente et al., 1998; Lee et al., 2018). However, they have not been yet applied for racemic resolution of ibuprofen enantiomers.

In this work, recent advances in enantiomeric esterification of ibuprofen were utilized to develop a robust biocatalytic system based on the lipase from *Aspergillus oryzae* (LAO). Seeking for the best synthetic strategy, biocatalytic reactions were conducted in both homogeneous and heterogeneous conditions, with enzyme adsorbed on IL attached to the surface of various silica-based hybrid materials with covalent bound as SILP, used for the first time in this transformation. The combination of affordable biocatalyst and process advantages leads to a greener alternative, competitive with existent applications.

2. Materials and methods

2.1. Materials

Ibuprofen racemate (≥98.0 wt. %) was purchase from Chemat (Poland). Native lipase B from *Candida antarctica* (CALB) in aqueous-glycerol solution (activity 5000 U L kg^{-1}), native lipase from *Aspergillus oryzae* (LAO) in aqueous-glycerol solution (activity 100,000 U g^{-1}) was purchase from Sigma-Aldrich (Merck Group, Poland) (description of materials can be found in the Supplementary Materials).

2.2. Analysis of materials

Chiral high-performance liquid chromatography (HPLC) was performed on a liquid chromatograph (Alliance, Waters 2690 system) with Waters PDA detector and Chiralcel OD Daicel (250 × 4,6 mm; 10 μm) column. TGA analysis of all obtained materials and biocatalysts were conducted on a thermobalance (Mettler Toledo TGA851e). SEM-EDS images of synthesized supports and biocatalyst were performed applying a Phenom Pro Desktop SEM instrument equipped with an EDS detector (15 kV) (Thermo Fischer Scientific). BET surface area (S_{BET}), average pore size (d_p) and average pore volume (V_p) of obtained materials were determined by means of the BET method and the BJH model, applying low-temperature (−196 °C) nitrogen sorption (ASAP 2020, Micromeritic Instruments Co.) ^{29}Si MAS NMR spectra of SILP materials were recorded at 59.517 MHz using a Bruker HP-WB high-speed MAS probe. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Varian system (400 MHz and 101 MHz, respectively).

2.3. Synthesis of materials

Sol–gel SiO_2 (Stöber et al., 1968; Klapiszewski et al., 2014), ZrO_2·SiO_2 (Degórska et al., 2021; Jankowska et al., 2019), MgO·SiO_2 (Ciesielczyk et al., 2014; Kołodziejczak-Radzimska et al., 2018) materials and 1-methyl-3-(triethoxysilylpropyl) imidazolium chloride ([tespmim][Cl]) (Matuszek et al., 2016) were prepared according to the literature described elsewhere.

2.3.1. Synthesis of heterogeneous biocatalysts

Synthesis of SILP silica-based materials (Kołodziejczak-Radzimska et al., 2018; Valkenberg et al., 2002; Wolny and Chrobok, 2021, 2022): MgO–SiO_2, ZrO_2-SiO_2, SiO_2 or sol–gel SiO_2 (0.3 g) was suspended in a methanol/water system (5 mL; 4:1, *v/v*) in a 25 mL round-bottom flask and then [tespmim][Cl] ionic liquid (0.3 mmol) was added. The reaction mixture was stirred at 90 °C for 24 h. Obtained material was filtered off, washed with 100 mL of methanol and dried under the vacuum on the Schlenk line (6 h, 70 °C).

Anion exchange in SILP-[tespmim][Cl] materials (Fernandez-Lafuente et al., 1998): In a 25 mL round-bottom flask, SILP-[tespmim][Cl] (0.3 g) was suspended in a dichloromethane (3 mL) and then lithium Li[NTf_2] (30% mol. excess *via* [tespmim][Cl] content) dissolved in a 3 mL of deionized water was added dropwise. The reaction mixture was stirred at room temperature for 24 h. The resulted SILP-IL material was filtered off, washed with 100 mL of deionized water (until the absence of chloride — test with $AgNO_3$) and dried under the vacuum on the Schlenk line (6 h, 70 °C).

Lipase immobilization (Szelwicka et al., 2021b; Fernandez-Lafuente et al., 1998): SILP-IL material (0.3 g) and native LAO in aqueous-glycerol solution (2.25 g) were placed in a 25 mL round-bottom flask and then 4 mL of deionized water was introduced. Obtained mixture was stirred in a thermostatic shaker (250 rpm) at 20 °C for 3 h. Then, the biocatalyst was filtered off, washed with deionized water (100 mL) and dried under vacuum on the Schlenk line (24 h, 20 °C).

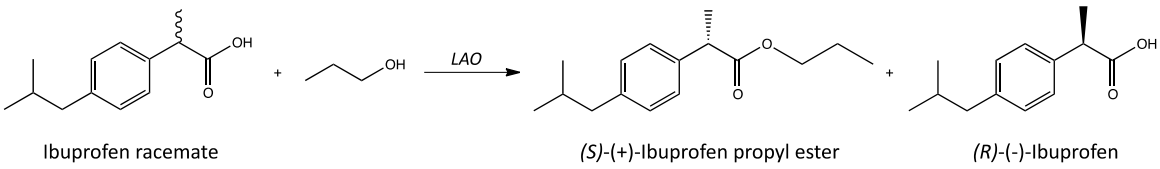

Fig. 1. Enantioselective esterification of racemic ibuprofen with 1-propanol in isooctane.

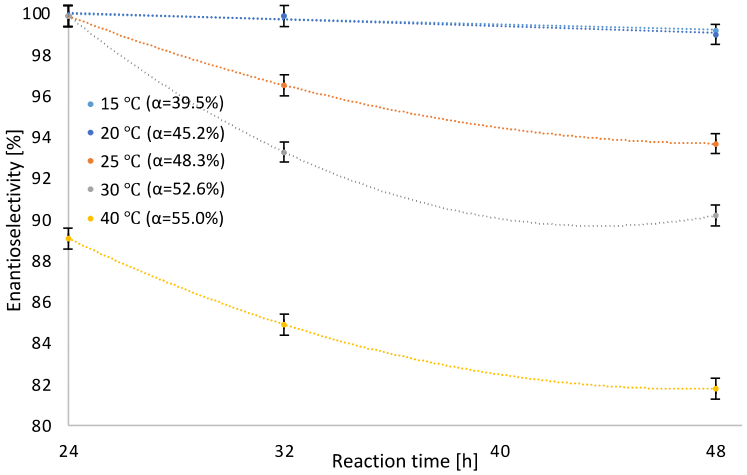

Fig. 2. The influence of temperature on *ee* of *(S)*-(+)-ibuprofen ester. *Reaction conditions: rac*-ibuprofen 1 mmol, 1-propanol 2 mmol, isooctane 2 mL, LAO 1.5 mL, 250 rpm.

2.3.2. Enantiomeric resolution of racemic ibuprofen

General procedure of homogeneous ibuprofen racemate esterification: In a 10 mL vial, ibuprofen racemate (1 mmol) was dissolved in isooctane (2 mL), then 1-propanol (2 mmol) and LAO (1.5 mL) were added. The reaction was performed at 20 °C and stirred in a thermostatic shaker (250 rpm) for 48 h.

General procedure of heterogeneous ibuprofen racemate esterification: In a 3 mL vial, ibuprofen racemate (0.1 mmol) was dissolved in isooctane (0.4 mL), then 1-propanol (0.2 mmol) and 90 mg of SiO_2/Mg(1:1)/[tespmim][NTf_2]/LAO were added. The reaction was performed at 20 °C and stirred in a thermostatic shaker (250 rpm) for 7 days.

Recycle of the heterogeneous biocatalyst: After the reaction, the biocatalyst was filtered off, washed with 25 mL of isooctane, dried under the vacuum on the Schlenk line (6 h, 20 °C) and reused.

3. Results and discussion

3.1. Catalytic activity of native enzyme

In the preliminary studies the optimization of the reaction condition for esterification of *rac*-ibuprofen with two-fold molar excess of 1-propanol as a model alcohol, using isooctane as a solvent in the presence of commercially available, aqueous-glycerol solution of LAO was performed (Fig. 1). The conversion of racemic ibuprofen and enantioselectivity of the process were determined using chiral HPLC (Supplementary Materials Fig.S1).

First, the amount of LAO necessary to convert the *rac*-ibuprofen to ester with high conversion and selectivity was studied (Supplementary Materials Fig.S2). The increasing of the amount of LAO from 0.50 to 1.0 mL causing the increase of reaction rate, but the conversion of (S)-(+)-ibuprofen was not full. Using 1.5 mL of LAO outstanding results were obtained. LAO can convert *rac*-ibuprofen (conversion $\alpha = 34.8\%$, $ee = 99.9\%$) after 24 h and respectively 45.2% and 99.9% after 48 h, $E > 200$, (Supplementary Materials Fig.S2) with high conversion and selectivity. Further increasing of the amount of LAO to 2.0 mL did not affect the yield and selectivity. For comparison the reaction was carried out using the same reaction conditions in the presence of lipase B from *Candida antarctica* using 1.5 mL of enzyme. As the result only 11% of substrate conversion after 48 h was observed (with 26.1% *ee* to *(R)*-(-)-ibuprofen ester).

In order to accelerate the reaction, the temperature was increased from 15 °C to 40 °C. Only slight increase in the reaction rate was observed and the conversion reached almost 50% at 40 °C but the ee_p dropped suddenly up to 82% due to progressive inactivation of the enzyme in higher temperature (Fig. 2 and Supplementary Materials Fig.S3). The reaction carried out at 15 °C slowed down but the *ee* remained as at 20 °C. The most advantageous temperature is 20 °C.

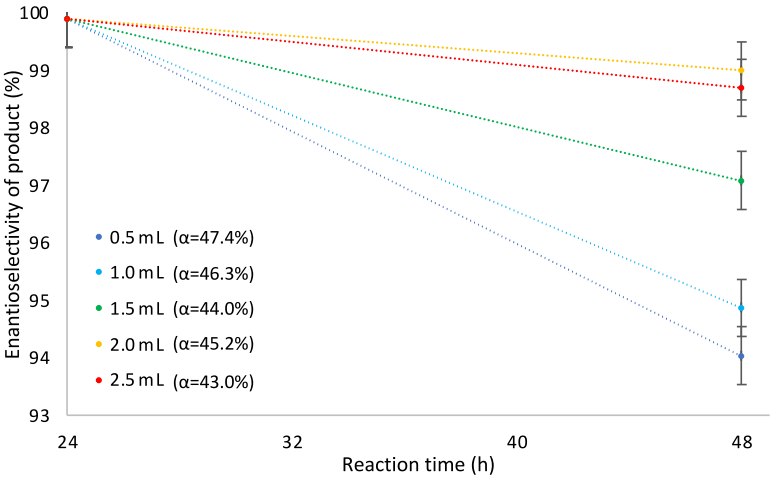

Fig. 3. The influence of the amount of solvent on *ee* of *(S)*-(+)-ibuprofen ester. *Reaction conditions: rac*-ibuprofen 1 mmol, 1-propanol 2 mmol, isooctane, LAO 1.5 mL, 20 °C 250 rpm.

Next, the influence of solvent on the reaction rate was searched. The most commonly used solvents for enantiomeric esterification of ibuprofen were tested (Supplementary Materials Tab.S1). Satisfactory conversion and *ee* (around 90%) were obtained in solvents, like hexane, cyclohexane and heptane. Dichloroethane, acetonitrile and propylene carbonate had an adverse effect on the course of the reaction. The representative of hydrophilic IL [emim][$EtSO_4$] (1-ethyl-3-methylimidazolium ethylsulfate), which dissolve ibuprofen as well as hydrophobic [bmim][NTf_2] not miscible with ibuprofen were also tested. Lack of substrate conversion was observed with the use of [emim][$EtSO_4$] as solvent. The necessity of adding co-solvent (isooctane) to the reaction carried out with [bmim][NTf_2] in order to dissolve substrate influenced the reaction course and the results were promising (conversion 46.9% and 90.4% *ee* after 48 h). Summing up, the best results were obtained in isooctane (conversion 45.2% and 99.9% *ee* after 48 h) where the reaction system was biphasic. The upper phase was composed of solvent and reagents along with LAO created the second phase. Additionally, test concerning the amount of isooctane used for the reaction revealed that the more concentrated reaction mixture is the lower the enantioselectivity is reached (Fig. 3 and Supplementary Materials Fig.S4).

A suitable selection of alcohol moiety (C1–C4) has a significant influence on the conversion and on the enantioselectivity of esterification reaction. The results show that 1-propanol and 1-butanol are excellent substrates for the esterification of ibuprofen, due to the high conversion and enantioselectivity (Fig. 4 and Supplementary Materials Fig.S5). Application of methanol or ethanol for esterification entails the drastic lowering of product purity (*ee* below 88% for methanol). Based on the results, it is essential to apply the C3–C4 alcohols to achieve a high conversion and excellent enantioselectivity when using the LAO as catalyst in enantioselective esterification. Longer alcohol chain and thus increased hydrophobicity of the substrate improve the activity of lipase. This beneficial effect of hydrophobic environment or hydrophobic support's surface on maintaining active conformation of lipase is known as "interfacial activation" (Arana-Peña et al., 2020).

Finally, the influence of molar ratio of ibuprofen:1-propanol was examined in order to evaluate its influence on the reaction parameters (Fig. 5 and Supplementary Materials Fig.S6). The dilution of reaction mixture with alcohol (molar ratio 1:4) caused the slowing down of the reaction, on the other hand the concentration (molar ratio 1:0.5) resulted in lowering of *ee*.

Concluding this part of studies, high-performance of biphasic reaction system: organic reactants/LAO in the process of racemic resolution of ibuprofen was demonstrated. As already mentioned, the focus on LAO was dictated by the drive to find both active and economically attractive system. Next, in this work we set to explore whether the SILP catalysis pathway with LAO may offer an enhancement of catalytic activity of enzyme.

3.2. Catalytic activity of immobilized LAO/SILP system

Superactive biocatalysts play a crucial role in the development of sustainable synthetic processes, while their heterogenization is an important goal for clean technology. The heterogeneous biocatalyst offers many advantages, either used in a fixed bed configuration or simply filtered off or centrifuged from a stirred tank reactor and then reactivated for reuse. Encouraged by the past results (Drożdż et al., 2015; Szelwicka et al., 2021a,b) we decided to develop a SILP dedicated for LAO. By supporting ILs, the required amount of ionic phase can be significantly reduced.

To this aim various silicas and silica-based materials were selected as supports with various surface characteristics (Table 1). Commercially available silica-based supports as well as those fabricated in laboratory, under specific process condition offers unique structural properties including well developed surface area as well surface activity. In order to

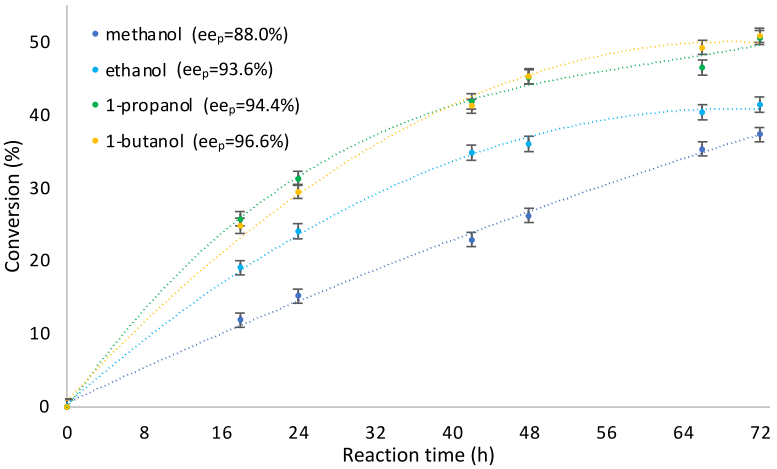

Fig. 4. The influence of the alcohol used for esterification of *rac*-ibuprofen. *Reaction conditions: rac*-ibuprofen 1 mmol, alcohol 2 mmol, isooctane, LAO 1.5 mL, 20 °C 250 rpm.

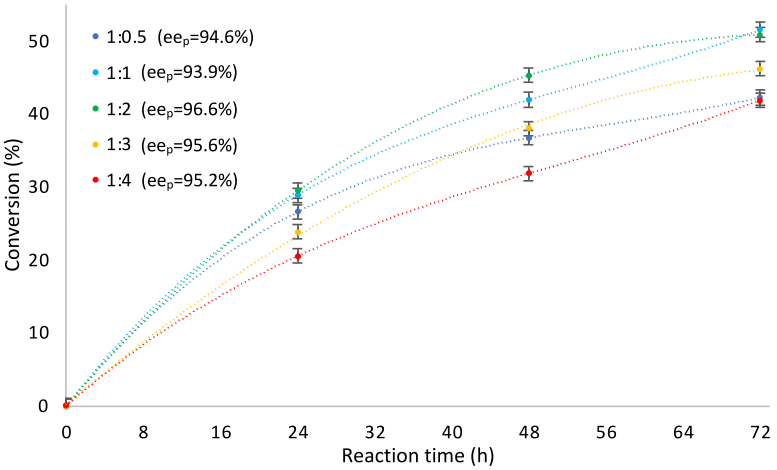

Fig. 5. The influence of the ibuprofen: alcohol molar ratio used for esterification of *rac*-ibuprofen. *Reaction conditions: rac*-ibuprofen 1 mmol, 1-propanol, isooctane, LAO 1.5 mL, 20 °C 250 rpm.

Table 1
Characterization of silica-based supports and biocatalysts.

Matrix	S_{BET} (m^2 g^{-1})	V_p (cm^3 g^{-1})	d_p (nm)	IL, (wt% ± 0.3)[c]	LAO, (wt% ± 0.3)[c]
$MgO{\cdot}SiO_2$ (1:1)	469	0.07	2.1	6.79	3.96
$MgO{\cdot}SiO_2$ (1:5)	289	0.12	2.8	5.05	5.12
$MgO{\cdot}SiO_2$ (5:1)	72	0.05	1.3	7.53	2.64
$CaO{\cdot}SiO_2$ (1:1)	24.9	0.01	1.2	4.29	4.98
$ZrO_2{\cdot}SiO_2$ (1:1)	394	0.10	2.3	–	–
SiO_2[a]	118	0.33	11.1	–	–
SiO_2[b]	200	0.59	11.8	9.4	19.38

[a]Silica used for this study prepared using sol-gel method.
[b]Commercially available silica.
[c]Determined using TGA; the standard deviation of 3 replicate experiments.

enhance structural properties of silicas and highlight other interesting features, they are often combined with metal-based oxides, including among others magnesium oxide, calcium oxide or zirconia. Preparation of such hybrid materials enable to form attractive supports with improved textural and structural properties which predispose them for application in adsorption processes or in immobilization of organic or bioorganic species onto their surface (Degórska et al., 2021). The functionality of as prepared or commercially available silica-based materials results in most cases from the nature of

surface silanol (≡Si-OH), magnesil (-Mg-OH) or others (-Ca-OH, ≡Zr-OH) groups which exhibit relative high affinity, *e.g.* for enzymes and at the same time improve their activity and enantioselectivity, *e.g.* of lipase from *Candida rugosa*, through electrostatic interactions and changes in conformation of enzyme (Salgin and Takac, 2007; Kołodziejczak-Radzimska et al., 2018). Pure commercially available silicas, without modification, and obtained oxide materials were used for comparison reason.

In the next step the ionic liquid was immobilized on the prepared in advance silica-based materials. According to the literature, the efficient way for the anchoring of IL to the surface of silica includes binding cation *via* triethoxysilyl functionality located in the alkyl chain of imidazolium ring (Skoda-Földes, 2014; Valkenberg et al., 2002; Wolny and Chrobok, 2021, 2022). This synthetic route was used in this work (Fig. 7). In the first step, (3-chloropropyl)triethoxysilane was treated with 1-methylimidazole to yield 1-methyl-3-(triethoxysilylpropyl) imidazolium chloride, [tespmim]Cl. Then, the IL was covalently tethered to the silica. The exchange of Cl^- anion for $[NTf_2]^-$ resulted in the final support formation, *e.g.* SiO_2/Mg(1:1)/[tespmim][NTf_2] (Fig. 7). The optimum loading of IL was achieved for experiments with 0.3 mmol of IL for 0.3 g of silica, *e.g.* for MgO·SiO2 (1:1) 6.79 wt%. Increasing the IL amount in immobilization process (from 0.3 mmol to 0.5 mmol) did not increase the IL loading on the matrix remaining it unchanged. However, the decreasing of the IL amount upon the immobilization (from 0.2 mmol to 0.3 mmol) resulted in lowering of its loading (IL loading 4.09 wt%).

TGA, SEM, ^{29}Si NMR and BET analysis were performed to characterize performed process and produced materials. Attachment of the ionic liquid to silica materials was proven by ^{29}Si MAS NMR analysis. MAS-NMR spectra shows the disappearance of the signals assigned to $(SiO)_2Si$-$(OH)_2$ and $(SiO)_3$Si-OH groups, as well as the appearance of the new signal at −66 ppm, what definitely indicates the grafting of the IL to the siliceous surface (Supplementary Materials Fig.S9) (Skoda-Földes, 2014; Valkenberg et al., 2002; Wolny and Chrobok, 2021). Additionally, SEM images of the materials before and after ILs deposition confirmed the presence of irregular shaped particles with slightly bigger tendency to form agglomerate structured in case of ILs coated samples (Supplementary Materials Fig.S15–S17). The MgO·SiO_2 1:1 material is characterized by the highest value of BET surface area of 469 m^2 g^{-1} among tested materials followed by pore volume of 0.07 cm^3 g^{-1} and pore size of 2.1 nm. Changes in the initial precursors ratio resulted in significant drop of all parameters of the porous structure. Surprisingly, the IL loading onto MgO·SiO_2 material determined by TGA did not follow the same trend and varied from 5.05 wt% for MgO·SiO_2 1:5 to 7.53 wt% for MgO·SiO_2 5:1 suggesting that textural properties and structure of the support as well as nature of surface functional groups also affect IL loading. Preparation of hybrid combinations that besides silica, contain also other metal oxides, on the one hand, resulted in improved parameters of the porous structure and on the other hand offers additional functional groups related, *e.g.* with magnesium, which can easily interact with analyzed biomolecules. When immobilization adsorption is considered the amount of active sites onto support surface is of key importance. That is why by selecting support type, the method of its synthesis as well as percentage contribution of specific components, the efficiency of IL loading can be controlled as confirmed, *e.g.* in case of MgO–SiO_2 materials — the more MgO in the material structure the higher amount of IL was bounded. The proposition of the role of magnesium in bounding process was demonstrated on Fig. 7. Silica combination with CaO resulted in 4.29 wt% of IL loading. The highest amount of IL was immobilized on the commercially available silica mainly due to the well-defined porous structure, the presence of a numerous of surface hydroxyl groups and their uniform distribution that reduces steric hindrances and promote efficient binding of significant amount of IL.

The final step in the preparation of biocatalyst included the immobilization of aqueous-glycerol solution of LAO on the surface of support *via* physical adsorption. Native lipase from *Aspergillus oryzae* (LAO) was commercially available product (Sigma-Aldrich) in the form of aqueous-glycerol solution (activity 100,000 U g^{-1}). Additionally, the Lowry protein assay was performed to determine the amount of protein in commercial product that was found to be 43.7 mg/mL. Physical immobilization of LAO was performed in water with a 7-fold mass excess of LAO solution over the SILP carrier and resulted *e.g.* in 3.96 wt% loading of enzyme for SiO_2/Mg(1:1)/[tespmim][NTf_2]/LAO. Increasing the LAO amount during immobilization step (from 7-fold to 10-fold) did not increase the LAO loading, but decreasing the LAO amount to 3-fold resulted in decreasing of its loading (2.09%).

The created biocatalysts, *e.g.* SiO_2/Mg(1:1)/[tespmim][NTf_2]/LAO were analyzed using TGA and SEM analysis (Supplementary Materials Fig.S18), which results confirm effective enzyme deposition. Loading of LAO on supports modified with IL was determined with TGA. The highest amount 19.38 wt% of LAO was tethered on the surface of commercially available silica confirming that this material is the most prone to functionalization by IL that directly facilitate high enzyme loading. Other materials included 2.64–5.12 wt% of LAO. It can be seen that the lowest amount of IL (5.05 wt%) was attached to the MgO–SiO_2 1:5 material and also on this system, the highest amount of enzyme was deposited. This confirms that even low amount of IL provides a sufficient amount of functional groups capable for enzyme binding. These data are also in agreement with hypothesis that the presence of silica in the structure of oxide material facilitates homogeneous distribution of surface functional moieties. For the material SiO_2·Mg(1:1) after anchoring of [tespmim][NTf_2] (SiO_2/Mg(1:1)-IL; S_{BET} 117 m^2 g^{-1}, V_p 0.07 cm^3 g^{-1}, d_p 2.2 nm) and next after immobilization of enzyme (SiO_2/Mg(1:1)-IL-LAO; S_{BET} 83.2 m^2 g^{-1}, V_p 0.04 cm^3 g^{-1}, d_p 2.2 nm) surface characteristics were also determined.

Heterogeneous biocatalysts were tested for racemic ibuprofen resolution using selected above reaction conditions (Fig. 6 and Supplementary Materials Fig.S7). Mass of each biocatalyst was recalculated to obtained biocatalyst with the same content of LAO (3.56 mg/1 mmol of *rac*-ibuprofen). To this aim 0.082 mL of native LAO (aqueous-glycerol solution) was used which contains 3.56 mg of protein. The most active was biocatalyst SiO_2/Mg(1:1)/[tespmim][NTf_2]/LAO causing the 35% conversion of *rac*-ibuprofen after 7 days with 95% *ee* of ester (E = 83, Supplementary Materials Fig.S7).

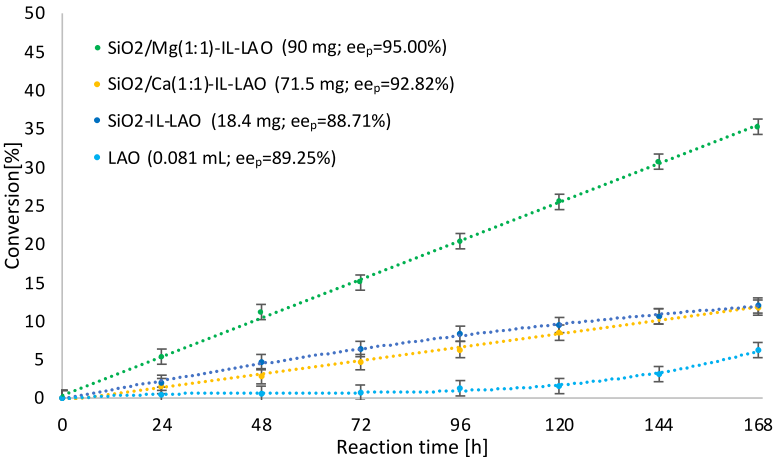

Fig. 6. The influence of the SILP support used for esterification of *rac*-ibuprofen. *Reaction conditions: rac*-ibuprofen 0.1 mmol, 1-butanol 0.2 mmol, isooctane 0.4 mL, 3.56 mg of LAO in native or immobilized form, 20 °C, 250 rpm.

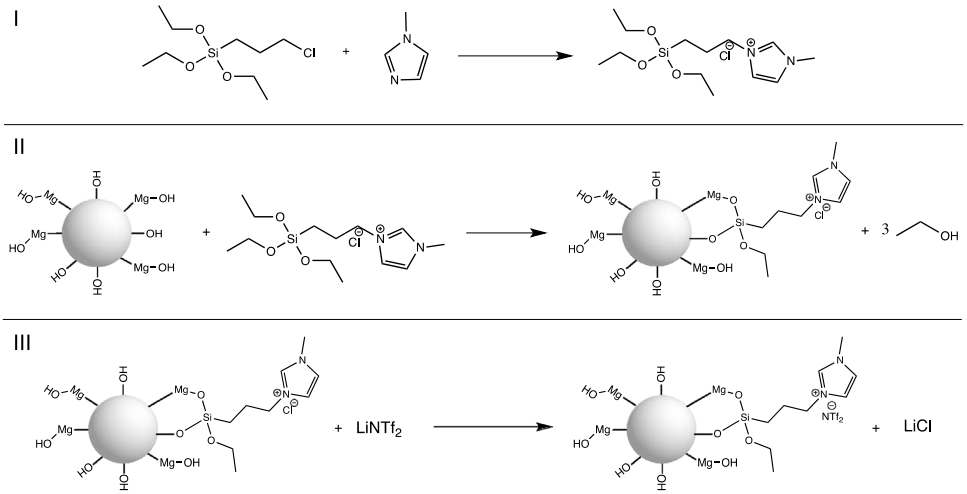

Fig. 7. Immobilization of [tespmim][NTf_2] onto silica surface.

Surprisingly, enrichment (SiO_2·Mg(1:5)) or lowering (SiO_2·Mg(5:1)) of the content of MgO in the support caused the complete deactivation of the final biocatalyst. Probably the amount of MgO in the support (SiO_2·Mg(1:1)) according to the proposed mechanism of the IL immobilization *via* chemical bounding (Fig. 7) was on the proper level, causing the effective immobilization of IL and activating LAO. Although prolongation of process duration did not result in improvement of process yield and its *ee*, it should be highlighted that lower amount of enzyme immobilized on the surface generates higher activity of heterogeneous catalyst (Fig. 6) as well as immobilization improves enzyme stabilization and facilitates its recycle potential. Unfortunately, SiO_2/Ca(1:1)/[tespmim][NTf_2]/LAO was not active enough giving the low conversion. The same phenomenon was observed for the biocatalyst with very high loading of lipase immobilized on the pure surface of silica SiO_2/[tespmim][NTf_2]/LAO. The catalyst did not affect too much the conversion probably due to lipase overloading onto silica surface (agglomeration) that leads to blocking of enzyme active sites and formation of diffusional limitations. Finally, the native LAO used in the same amount (3.56 mg) as was loaded on other heterogeneous biocatalysts was poorly active. It is the proof of the activation of LAO on the surface of Mg-doped silica. The immobilization of lipase on the unmodified MgO–SiO_2 matrix failed (using TGA the protein was not detected). Additionally, bared SILP, without enzyme on the surface was not catalytically active. Only after modification of the surface with IL lipase was able to embed on the support and catalyze the ibuprofen resolution.

The attempts to accelerate the reaction by adding more biocatalysts (40–90 mg) were limited (Fig. 8 and Supplementary Materials Fig.S8). Higher amount of biocatalyst (150 mg) required the increasing of solvent, what was caused with difficulties with stirring of the reaction mixture which was extremity dense. The addition of extra amount of solvent resulted in diluting the reaction mixture and slowing down the reaction rate and lowering the conversion to 19%.

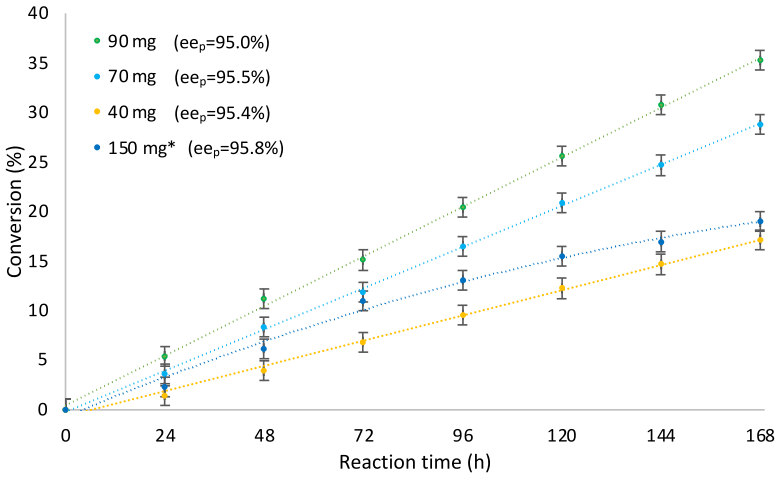

Fig. 8. The influence of the amount of SiO_2/Mg(1:1)/[tespmim][NTf_2]/LAO used for esterification of *rac*-ibuprofen. *Reaction conditions: rac*-ibuprofen 0.1 mmol, 1-butanol 0.2 mmol, isooctane 0.4 mL, SiO_2/Mg(1:1)/[tespmim][NTf_2]/LAO with 3.56 mg of immobilized protein, 20 °C, 250 rpm; * isooctane 1 mL.

Table 2
The comparison of the most important results for enantiomeric resolution of racemic ibuprofen *via* esterification.

Biocatalyst	Reaction conditions	Reaction indicator	Reference
Lipase from *Candida rugosa*	1-propanol, cyclohexane, 30 °C, 140 h	Conversion 44.2%, *ee* 68.3%	Siódmiak et al. (2012)
Protein-coated microcrystals prepared from lipase form *Candida rugosa*	Isooctanol, isooctane 50 °C, 8 h	Conversion 49.83%, *ee* 97.34%	Huang et al. (2015)
Lipase from *Rhizomucor miehei* immobilized on epoxy-functionalized silica	1-propanol, isooctane, 0 °C, 24 h	Conversion 23.7%, *ee* 92%	Mohammadi et al. (2016)
Candida rugosa immobilized on silica nanoparticles	1-propanol, isooctane, 37 °C, 50 min	Conversion 45%, *ee* 96%	Ghofrani et al. (2021)
Lipase from *Aspergillus oryzae*	1-propanol, isooctane, 20 °C, 48 h	Conversion 35%, *ee* 99.9%	This work
Lipase from *Aspergillus oryzae* immobilized on SILP	1-propanol, isooctane, 20 °C, 7 days	Conversion 35%, *ee* 95%	This work

The recyclability of SiO_2/Mg(1:1)/[tespmim][NTf_2]/LAO is demonstrated in Fig. 9. In the first case, the decrease in selectivity in the second cycle of the reaction was from *ee* 96.7% to 88% after 5 days of reaction and from *ee* 95.5% to 85.5% after 7 days of reaction. Although a dilution of the reaction system up to 1 mL caused the decreasing in the reaction rate, it allowed to maintain the same high enantioselectivity (1st cycle *ee* 96.3%, 2^{ed} cycle *ee* 95.4% after 5 days and 1st cycle *ee* 95.8%, 2^{ed} 94.2% cycle after 7 days). The long stability of biocatalyst is due to the stabilization effect of the SILP which is known from their exceptional properties, inter alia, maintenance of the active conformations of the enzymes. Moreover, used IL provides suitable microenvironment for immobilization of lipase that facilitate repeated use of the biocatalysts. The TGA analysis of the biocatalyst after second cycle (experiment with 90 mg of biocatalyst) revealed that the amount of enzyme on the modified surface of silica (SiO2/Mg(1:1)/[tespmim][NTf_2]/LAO) stayed intact (4.04 wt %, Supplementary Materials Fig.S33). Additionally, the experiments with fast catalyst filtration demonstrated no further ibuprofen conversion in the filtrate after catalyst's removal (Supplementary Materials Fig.S34). These are the proofs that the reason of the lower activity is the enzyme inhibition in this long term process (186 h). Even if the excess of catalyst was used (150 mg) the slow inhibition was visible.

Summing up, the developed catalyst is highly active in the resolution of racemic ibuprofen *via* esterification and can compete with catalysts described in the literature what was demonstrated in Table 2.

4. Conclusions

In this work two novel approaches for kinetic resolution of ibuprofen *via* enantiomeric esterification were applied. Lipase from *Aspergillus oryzae* was a very effective enzyme for this purpose. A SILP system was used for designing heterogeneous biocatalyst based on silica-based hybrid carriers. The SILP biocatalytic system showed improved enzyme stability and reusability. Results in this study show *(S)*-(+)-ibuprofen ester *ee* 99.9% with $\alpha = 34.8\%$, after 24 h and respectively *ee* = 99.9% with $\alpha = 45.2\%$ after 48 h using native LAO is competitive with other literature results.

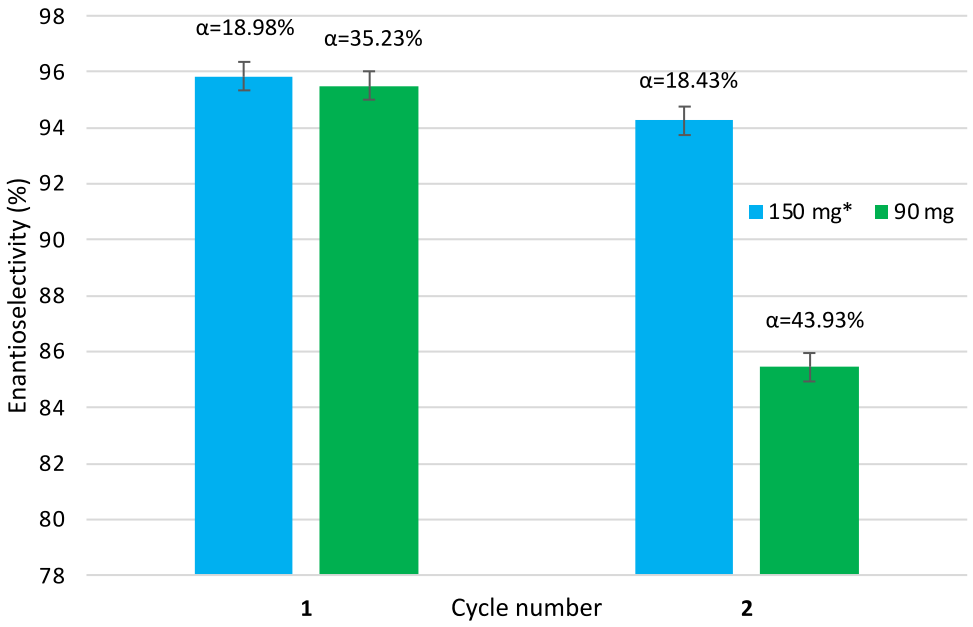

Fig. 9. The recycling of SiO_2/Mg(1:1)/[tespmim][NTf_2]/LAO used for esterification of *rac*-ibuprofen; 168 h; where α means conversion; error bars show the standard deviation of 3 replicate experiments. *Reaction conditions: rac*-ibuprofen 0.1 mmol, 1-butanol 0.2 mmol, isooctane 0.4 mL, MgO·SiO_2-[tespmim][NTf_2]-LAO biocatalyst; * isooctane 1 mL.

Using heterogeneous biocatalyst SiO_2/Mg(1:1)/[tespmim][NTf_2]/LAO (ionic liquid loading 6.79%, enzyme loading 3.96%) the conversion of *rac*-ibuprofen reached 35% after 7 days with 95% *ee* of ester. Although a dilution of the reaction system caused the decreasing in the reaction rate, it allowed to maintain the high enantioselectivity of *(S)*-(+)-enantiomer of ibuprofen in the second cycle. Overall, this work represents a novel alternative for an effective stabilization of LAO in the organic environments.

CRediT authorship contribution statement

Anna Wolny: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft. **Agnieszka Siewniak:** Methodology, Investigation, Formal analysis. **Jakub Zdarta:** Conceptualization, Writing – review and editing. **Filip Ciesielczyk:** Methodology, Investigation. **Piotr Latos:** Methodology, Investigation, Formal analysis. **Sebastian Jurczyk:** Methodology, Investigation. **Long D. Nghiem:** Conceptualization, Writing – review and editing. **Teofil Jesionowski:** Conceptualization, Writing – review and editing, Supervision, Project administration, Funding acquisition. **Anna Chrobok:** Conceptualization, Writing – review and editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.eti.2022.102936. ^{1}H, ^{13}C spectra of ionic liquid and (S)-(+)-ibuprofen, ^{29}Si NMR solid state, TGA, SEM-EDS analysis of synthesized SILPs and biocatalysts (PDF).

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