STUDIA UBB CHEMIA, LXIX, 4, 2024 (p. 85-98)
(RECOMMENDED CITATION)
DOI:10.24193/subbchem.2024.4.06

ELECTROSPINNING AS TOOL FOR ENZYME IMMOBILIZATION

Melinda-Emese LÁSZLÓ[a], Matild PAP[a], Gabriel KATONA[a]*

ABSTRACT. Enzyme immobilization (covalent or noncovalent) on solid supports such as nanomaterials, resins or polymers can enhance the enzyme activity-, and selectivity, improving their stability. The present research is focused on the non-covalent immobilization of lipase B from *Candida antarctica* (CaL-B) into polyvinyl alcohol (PVA) nanofibers *via* electrospinning with the aim to prepare a stable and reusable biocatalyst compatible with organic reaction media. Polymer solutions of 8, 10 and 12 *w/w* % concentrations were used to investigate the effect of the polymer concentration on the biocatalyst's activity. The immobilized enzyme amount was determined using the Bradford assay, while structural characterization was performed by transmission electron microscopy. The immobilized enzyme preparates were tested in the enzymatic kinetic resolution of (*rac*)-1-phenylethanol and its halogenated derivatives through transesterification with vinyl acetate in batch mode. The highest conversion was obtained in case of CaL-B entrapped in electrospun nanofibers prepared from 10 *w/w* % PVA solution (noted as 10%–PVA–CaL-B) and its high stability was confirmed in recycling experiments. It was found that after the 5th cycle the biocatalyst maintained 88% of its initial activity.

Keywords: *CaL-B, immobilization, electrospinning, PVA, (rac)-1-phenylethanol, EKR.*

INTRODUCTION

Electrospinning is a voltage-driven versatile method for ultrathin fiber production with diameters ranging from a few nanometers to several micrometers [1]. Morton [2] and Cooley [3] patented the first devices to spray liquids in 1902

[a] *Babeş-Bolyai University, Faculty of Chemistry and Chemical Engineering, 11 Arany János street, RO-400028, Cluj-Napoca, Romania*
* *Corresponding author: gabriel.katona@ubbcluj.ro*

and 1903, and other findings related to this topic were published in the next decades, but despite this early discovery, the procedure was not exploited commercially until the commercialization of electrospun nanofibers for filter applications has been reported for the first time in 1991 [4]. This technique involves the use of high voltage to generate the formation of a liquid jet from a positively charged polymer solution from the emitter, which is solidified by evaporating the solvent to ensure nanofiber formation on the negatively charged collector. Natural and synthetic polymers or polymer alloys are suitable for this process, leading to the production of porous, hollow, core-shell or helical-structured nanofibers. Their quality is influenced by several parameters such as solvent evaporation rate, nature of the polymer, drawing speed and distance between the emitter and collector. The procedure is controllable, parameters can be set and does not involve high costs [5].

Electrospun nanofibers (NFs) are good candidates in numerous fields, the most important being drug delivery [6], filtration [7], tissue engineering [8], biotechnology [9] and green chemistry [10]. Due to their large surface-to-volume ratio and elevated porosity, they are suitable carriers for enzyme immobilization [11-15]. For electronic devices, it is mandatory that the nanofibers produced via electrospinning have controlled fiber structure and ordered fiber orientation [16]. Among biopolymers polycaprolactone, chitosan, polylactic acid and polyvinyl alcohol were reported to be proper for obtaining nanofibers through this technique with applicability in biomedical fields due to their biocompatibility and biodegradability [16-19].

The major advantage of lipases (EC 3.1.1.3) or triacylglycerol ester hydrolases is represented by their extraordinary chemo-, regio- and stereoselectivity. In nature this class of enzymes catalyzes the hydrolysis of triglycerides into glycerol and fatty acids but is also broadly used in esterification reactions. Free lipase is not beneficial in industry because it is difficult to recover for reuse, has poor stability and does not exhibit activity in organic solvents [20], [21]. These inconveniences can be avoided if lipase is immobilized on different types of solid carriers. Over the past years, it has been reported the use of numerous materials [22] which proved to be efficient as support for various lipases immobilization including octyl sepharose [23], Immobead 150 [24] or macroporous resin NKA [25]. The immobilized enzyme should be active and insoluble in the reaction media allowing its reuse in several consecutive catalytic cycles.

Lipase B from *Candida antarctica* yeast belongs to the α/β hydrolase fold family, its catalytic triad consists of serine, histidine and aspartic acid / glutamic acid [26]. The enzyme in its immobilized form exhibits activity in non-polar organic solvents (e. g. toluene, hexane) in broad pH range and temperature. CaL-B is widely utilized in biotransformation, such as enzymatic kinetic resolution (EKR), transesterification, esterification, organic synthesis, and hydrolysis since presents

enantio- and stereo-specificity, a good stability in organic solvents [27-32]. Lipase-catalysed kinetic resolution carried out in organic solvents proved to be effective in obtaining enantiomerically enriched secondary 1-arylethan-1-ols [33], which are important chiral building blocks for the synthesis of a vast number of biologically active compounds. Related to the CaL-B covalent and non-covalent immobilization on solid carriers, a significant number of publications reported the use of different carbon-based nanomaterials such as graphene oxide [34], derivatized single-walled carbon nanotubes [35], core-shell polymeric supports based on polystyrene, methyl polymethacrylate [36], stearic acid-modified nanoparticles [37], different resins [38], [39] or biopolymeric nanofibers such as chitosan [40] [41].

One of the most used commercially available immobilized CaL-B, suitable biocatalyst for biotransformations such as transesterification, is Novozym 435, reported for the first time in 1992. In this case Lewatit VP OC 1600 resin represents the carrier while interfacial activation stands for immobilization method. The active center of the lipases is surrounded by a large hydrophobic pocket, covered by a lid (polypeptide chain), which in its "closed" form inactivates the lipase due to its internal hydrophobic face and interacts with the reaction medium *via* its external hydrophilic face. This immobilization technique may facilitate lipase desorption under specific conditions such as high temperature, in the presence of detergents or organic co-solvents since it is based on hydrophobic interactions. The main disadvantages are linked to the nature of the support's: mechanical fragility under rigorous stirring, moderate hydrophilicity because it can retain hydrophilic by-products such as water and dissolution in certain organic solvents leading to product contamination generated by its degradation [42-44]. In comparison PVA being a water-soluble biopolymer, the electrospun nanofibers produced from its aqueous solution are excellent candidates for non-polar organic reaction media such as hexane and lipase desorption is unlikely to occur due to the entrapment technique used for immobilization by electrospinning. Spelmezan *et al.* reported the immobilization of CaL-B *via* entrapment into polyvinyl alcohol-polylactic acid copolymer [45] and polyvinyl alcohol-chitosan bipolymer electrospun nanofibers [46] while Sóti *at al.* [47] used for the same purpose PVA and PLA nanofibers obtained upon electrospinning.

Herein we propose the fabrication of nanofiber-based reusable biocatalysts by immobilization of CaL-B *via* entrapment into PVA electrospun nanofibers using different polymer concentration solutions, with the aim to determine the effect of polymer concentration on the enzyme's activity while using the same amount of CaL-B in each case. The prepared biocatalysts were tested for EKR of (*rac*)-1-phenylethanol and its halogenated derivatives with vinyl acetate as acylating agent in hexane in batch mode and reusability experiments were also conducted.

RESULTS AND DISCUSSION

Transmission electron microscopy (TEM) served as tool for the structural characterization of the prepared PVA nanofibers and the PVA-based biocatalysts. According to the measurements, PVA nanofibers diameter was found to range between 132 and 176 nm (**Figure 1.A.**, **1.B.**) and structural changes can be observed upon immobilization of lipase by entrapment into NFs (**Figure 1.C.**).

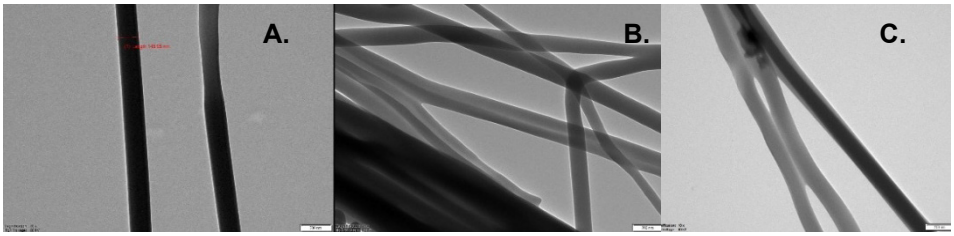


Figure 1. TEM images of PVA nanofibers
(**A.** 10 % PVA solution **B.** 12 % PVA solution; **C.** 10 % PVA–CaL-B biocatalyst

Next the activity and selectivity of the immobilized lipase were evaluated in the kinetic resolution of (*rac*)-1-phenylethanol with vinyl acetate as acyl donor [48]. The selective formation of (*R*)-1-phenyl-ethylacetate was monitored by high performance liquid chromatography (HPLC), using a chiral LUX Cellulose-3 column (250 mm x 4.6 mm x 5 µm). The other parameters were the following: 40 bar column pressure, flow rate 1 mL/min at 25ºC mobile phase hexane:isopropanol 98:2 *v/v*%, detection wavelength was set at 254.16 nm.

First the pure racemic substrate and the pure racemic product separation were performed by HPLC analysis by injecting the racemic mixtures to identify each enantiomer based on their retention time, important parameters for reaction mixture analysis. The retention times in the case of the 1-phenylethyl acetate mixture were found to be 8.6 and 9.5 minutes, respectively. According to literature data [44] retention time of (*R*)-1-phenyl-acetate is 9.5 minutes; therefore, the elution order is *S, R*.

To evaluate the effect of the polymer concentration on the lipase activity and to determine the optimal biocatalyst amount, 9 batch reactions were performed simultaneously. In each case the substrate, acylating agent and solvent volumes were identical, the biocatalyst type (8%–PVA–CaL-B, 10%–PVA–CaL-B, 12%–PVA–CaL-B) and quantity of the enzyme preparate (8 mg, 10 mg, 12 mg) varied, while the setted temperature of the shaker

(1350 rpm) equipped with a heating module was 30 °C. Samples were taken from the reaction mixture at fixed time intervals (after 2, 4, 6, and 12 hours) and analyzed by HPLC. A control experiment was conducted without any biocatalyst. A set of obtained chromatograms are illustrated in **Figure 3** and the results are presented in **Table 1**.

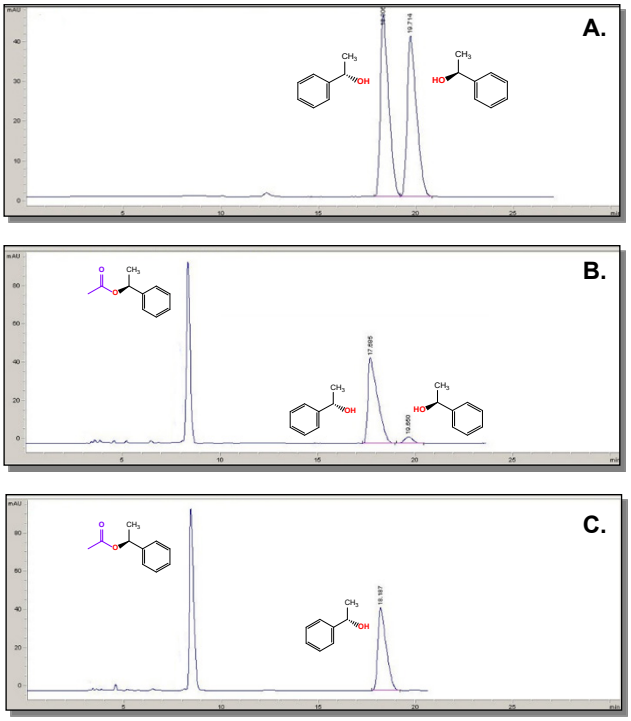


Figure 3. HPLC analysis of reaction samples after 12 hours
A. control reaction; **B.** 12 mg of 8%–PVA–CaL-B biocatalyst (enzyme loading 14 μg/mg); **C.** 12 mg of 10%–PVA–CaL-B biocatalyst (enzyme loading 13.07 μg/mg)

However, in the case of 12%–PVA–CaL-B biocatalyst the maximum conversion was achieved after 12 hours, but not in case of 8%–PVA–CaL-B (**Table 1**, **entry 28-30**). This was analyzed also after 24 hours, when conversion reached 50%.

Based on the recorded chromatograms, after 4 hours the maximum conversion was achieved when 12 mg of 10%–PVA–CaL-B was used (**Figure 4**).

Table 1. PVA–CaL-B biocatalytic activity evaluation (*1st catalytic cycle*)

Reaction time (h)	**C PVA** (*w/w* %)	**m PVA–CaL-B** (mg)	**Enzyme loading** (μg/mg)	**ees** (%)	**Conversion *** (%)
		4	4.7	19	**16**
	8	8	9.3	31	**24**
		12	14.0	41	**30**
		4	4.4	40	**29**
2	**10**	8	7.5	52	**34**
		12	13.1	69	**41**
		4	3.4	31	**24**
	12	8	6.2	45	**31**
		12	10.1	61	**38**
		4	4.7	30	**23**
	8	8	9.3	40	**29**
		12	14.0	58	**37**
		4	4.4	57	**36**
4	**10**	8	7.5	71	**42**
		12	13.1	> 99	***50***
		4	3.4	45	**31**
	12	8	6.2	62	**39**
		12	10.1	81	**45**
		4	4.7	45	**31**
	8	8	9.3	57	**36**
		12	14.0	69	**41**
		4	4.4	71	**42**
6	**10**	8	7.5	88	**47**
		12	13.1	> 99	***50***
		4	3.4	62	**39**
	12	8	6.2	81	**45**
		12	10.1	> 99	***50***
		4	4.7	62	**39**
	8	8	9.3	71	**42**
		12	14.0	88	**47**
		4	4.4	> 99	**50**
12	**10**	8	7.5	> 99	**50**
		12	13.1	> 99	**50**
		4	3.4	> 99	**50**
	12	8	6.2	> 99	**50**
		12	10.1	> 99	**50**

* ***ee***$_P$ > 99% in all cases

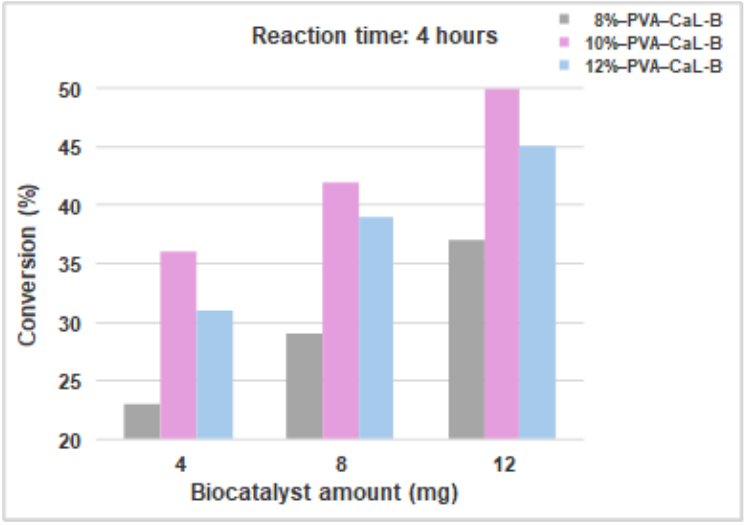


Figure 4. Conversion variation after 4 hours (*1st catalytic cycle*)

For reusability studies, in each case after the first catalytic cycle the biocatalyst was removed from the reaction media, was washed 2 times with isopropanol, 2 times with hexane and was stored in the refrigerator in a sealed glass container until further use regarding reusability.

The second catalytic cycle was performed as previously described. Samples were taken from the reaction mixture at fixed time intervals, analyzed by HPLC and conversion rates were calculated based on the chromatograms. The values indicate no decrease in activity, the 50% conversion rate was achieved after 4 hours using 12 mg of 10%–PVA–CaL-B biocatalyst in this case also. However, in all cases, maximum conversion was observed after 24 hours.

Based on this finding, we decided to test the reusability of the most promising biocatalyst (10%–PVA–CaL-B), starting from the third catalytic cycle, using 12 mg of prepared biocatalyst. The experiments were stopped when conversion dropped below 40% after 4 hours. The results are plotted in **Figure 5**.

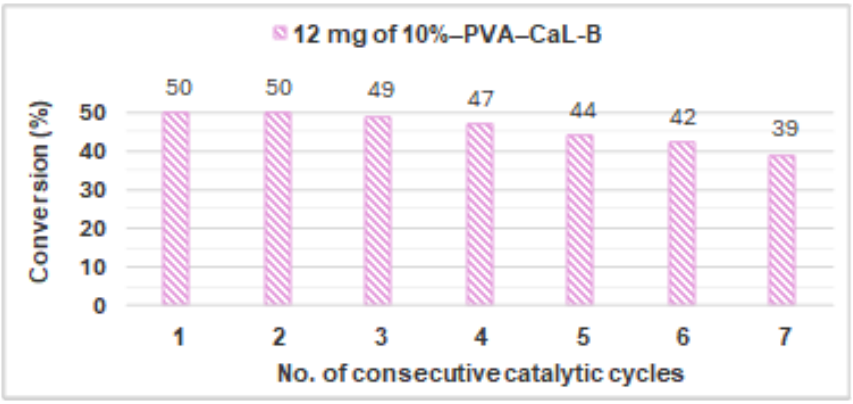


Figure 5. The reusability of 10%–PVA–CaL-B biocatalyst (12 mg) in the EKR of (*rac*)-1-phenylethanol with vinyl acetate after 4 hours

Further, the 10%–PVA–CaL-B biocatalyst was tested on halogenated (*rac*)-1-phenylethanol derivatives as substrates, namely (*rac*)-4-bromo-1-phenylethanol and (*rac*)-4-chloro-1-phenylethanol, under the previously described reaction conditions: substrate/acylating agent *ratio* 1:2, solvent hexane, incubation temperature 30°C, shaking at 1350 rpm with 12 mg biocatalyst (enzyme loading 13.1 μg/mg).

The samples taken from the reaction mixture at fixed time intervals were analyzed by gas chromatography on a Supelco Analytical Astec CHIRALDEX® B-DM Silica capillary column (30m × 0.32mm × 0.12μm) under the following conditions: N_2 as carrier gas, 1 μL injection volume, 100:1 split *ratio*, FID detector and injector temperature 250°C, head pressure 60 psi, operating at 120°C in case of (*rac*)-4-Br-1-phenyl-ethanol and with temperature gradient from 120°C to 160°C with 2.6°/min increment in case of (*rac*)-4-Cl-1-phenylethanol.

Considering the data from **Table 2**, the samples taken from the reaction mixture at fixed time intervals were subjected to GC analysis. Based on the recorded chromatograms, the calculated conversions are summarized in **Table 3**.

Table 2. Chromatographic chiral separation of racemic halogenated 1-phenylethanol derivatives (left) and their acetates (right) [49]

Compound / Enantiomer	Retention time (min) S	R
(*rac*)-4-**Br**-1-phenylethanol	28.3	31.3
(*rac*)-4-**Cl**-1-phenylethanol	11.6	11.3

Compound / Enantiomer	Retention time (min) S	R
(*rac*)-4-**Br**-1- phenylethyl acetate	24.9	26.6
(*rac*)-4-**Cl**-1- phenylethyl acetate	9.4	9.8

Table 3. EKR of racemic halogenated 1-phenyethanol derivatives with vinyl acetate over 12 mg of 10%–PVA–CaL-B biocatalyst in batch mode

Reaction time (h)	Substrate			
	(±)-4-**Br**-phenylethanol		(±)-4-**Cl**-phenylethanol	
	ee$_S$ (%)	***c**** (%)	**ee**$_S$ (%)	***c**** (%)
2	31	**24**	41	**30**
4	45	**31**	> 99	**50**
6	> 99	**50**		

* ee_P > 99% in all cases

The reaction rate was higher in the case of the chlorinated substrate than in the case of the brominated 1-phenylethanol derivative.

CONCLUSIONS

Candida antarctica B lipase was successfully immobilized into polyvinyl alcohol nanofibers *via* electrospinning technique using polymer solutions of different concentrations. The prepared biocatalysts were structurally characterized by transmission electron microscopy which confirmed the fiber formation and structural changes were observable in the case of prepared biocatalysts.

The activity and selectivity upon immobilization were investigated in batch mode in the acylation of racemic 1-phenyletanol with vinyl acetate. In the first catalytic cycle, maximum conversion was obtained after 4 hours in the case of using 12 mg of 10%–PVA–CaL-B noted biocatalyst with enzyme loading equal to 13.07 ug/mg instead of expected 12 mg of 10%–PVA–CaL-B biocatalyst with a higher enzyme loading (14.0 μg/mg). This could be explained with the fact that higher enzyme loading can cause steric hindrance between the protein molecules resulting in a less active biocatalyst.

In the second catalytic cycle the obtained results were in concordance with the previous findings, therefore only 10%–PVA–CaL-B biocatalyst was subjected further to reusability studies. A slight decrease in activity was observed starting with the third cycle. However, after the 7th consecutive catalytic cycle the biocatalyst maintained 78% of its initial activity.

The 10%–PVA–CaL-B biocatalyst proved to be suitable also for EKR of racemic halogenated 1-phenylethanol derivatives as well, returning the highest conversion after 4 hours when (*rac*)-4-chloro-1-phenylethanol was used as substrate.

EXPERIMENTAL SECTION

Materials and methods

Polyvinyl alcohol (molecular weight 130.000), vinyl acetate, (*rac*)-1-phenylethanol and sodium phosphate monobasic hydrate were products of Sigma-Aldrich. (*Rac*)-4-Bromo-1-phenylethanol, (*rac*)-4-chloro-phenyl-ethanol, (*rac*)-4-bromo-phenylethyl acetate and (*rac*)-4-chloro-phenylethyl acetate were synthesized by our colleagues [35] and used as received. Lipase B from *Candida antarctica* was purchased from Chiral Vision; HPLC grade solvents were procured from PromoChem.

For enzyme immobilization into polymeric nanofibers, the Fluidnatek Bioinicia electrospinning system was used. Transmission electron microscopy analyses were conducted on Hitachi H-7650 apparatus, at 80 keV. 460/H Ultrasonic bath operating at 100 W, 40 kHz served for ultrasonication. The shaking and incubation of the enzymatic reactions were performed on a shaker equipped with a heating module (Titramax 1000).

High-performance liquid chromatography analyses were performed with an Agilent 1200 instrument, while gas chromatography determinations were conducted on an Agilent 7890A GC equipped with a flame ionization detector.

The enantiomeric excess values of the substrate (ee_S) and of the product (ee_P) were calculated from the peak areas of HPLC chromato-grams and the conversion (*c)* was determined from the following well-known equation [50]:

$$c\ (\%) = \frac{ee_S}{ee_S + ee_P} * 100$$

1. Immobilization of CaL-B into PVA nanofibers by electrospinning

The first step consisted of the preparation of stock solutions: CaL-B enzyme solution of 2,8 mg/mL concentration in 100 mM phosphate buffer, pH 7.5 and aqueous polyvinyl alcohol solutions of 8%, 10% and 12% concentration, respectively.

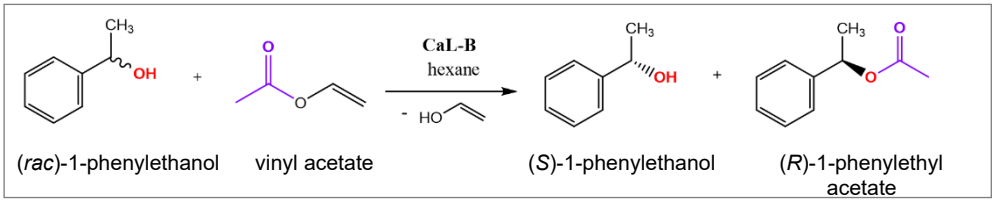
The polymer solution concentration values were chosen based on prior attempts to produce PVA nanofibers since the success of nanofiber production is also viscosity-dependent.

In the second step, the polymer-enzyme mixtures were prepared by adding 200 µL of CaL-B solution to 6 mL polymer solution (c1= 8%, c2= 10%, c3= 12 %) followed by 30 minutes of ultrasonication and 1 h shaking at room temperature for optimal homogenization.

To obtain electrospun nanofibers, the dispensing syringe of the electrospinning equipment was filled with 5 mL of polymer-enzyme mixture and the vertical collector was wrapped with aluminium thin foil to facilitate the collecting of the produced nanofibers afterwards. The sample feeding rate varied between 560-750 µL/h and the applied voltage between 18-19.5 kV depending on the viscosity of the polymer-enzyme mixture. The distance between the emitter and the collector was set to 13 cm in all cases. The as-produced solid biocatalyst (abbreviated further as 8%–PVA–CaL-B, 10%–PVA–CaL-B and 12%–PVA–CaL-B*)* was removed from the aluminium foil using a sharp tweezer, placed in a sealed glass container and stored in the refrigerator until use.

2. Activity and selectivity investigation of the prepared nanofiber-based biocatalysts

The chosen model reaction used for biocatalyst testing is illustrated in **Scheme 1**.



Scheme 1. Enzymatic kinetic resolution of racemic 1-phenylethanol with vinyl acetate mediated by immobilized lipase B from *Candida antarctica*

Batch reactions were performed in 1.5 mL screw-capped glass vials. To 1 mL hexane, 12 µL (*rac*)-1-phenylethanol (1.5 mmol), 36.8 µL vinyl acetate (3 mmol) and nanofiber-based biocatalyst (4 mg, 8 mg, 12 mg) were added. The vials were placed on a thermostated shaker (1350 rpm) at 30ºC. At fixed time intervals (2 h, 4 h, 6 h, 24 h) 50 µL of the reaction mixture was removed, diluted with 450 µL hexane, centrifuged, filtered and injected into HPLC chiral chromatographic column.

3. Activity investigation of the prepared 10%–PVA–CaL-B biocatalyst on p-halogeno-1-phenylethanol derivatives

1.5 mmol racemic *p*-halogeno-phenylethanol derivative, 3 mmol vinyl acetate and 12 mg 10%–PVA–CaL-B were added to 1 mL hexane in a screw-capped glass vial. The vials were placed on a thermostated shaker (1350 rpm) at 30ºC. At fixed time intervals (2 h, 4 h, 6 h, 12 h) 50 µL of the reaction mixture was withdrawn from the reaction media, diluted with 450 µL hexane, centrifuged, filtered and analyzed by gas chromatography.

REFERENCES

1. A. Frenot; I. S. Chronakis; *COCIS*, **2003**, *8*, 64–75.
2. W. J. Morton; Patent no. US 705,691, **1902**.
3. J. F. Cooley; Patent no. US 745,276, **1903**.
4. K. Shaefer; H. Thomas; P. Dalton; M. Moeller; Nano-Fibers for Filter Materials in *Multifunctional Barriers for Flexible Structure*, Springer, **1991**, Chapter 7, pp.125–138.
5. A. A. Babar; N. Iqbal; X. Wang; J. Yu; B. Ding; Introduction and historical overview in *Electrospinning: Nanofabrication and Applications*, William Andrew, **2019**, Chapter 1, pp. 3–20.
6. C. Cleeton; A. Keirouz; X. Chen; N. Radacsi; *ACS Biomater. Sci. Eng.,* **2019**, *5*, 4183–4205.
7. S. Ramakrishna; K. Fujihara; W. E. Teo; T. Yong; Z. Ma; R. Ramaseshan; *Mater. Today*, **2006**, *9*, 40–50.
8. X. Zhang; R. Lv; L. Chen; R. Sun; Y. Zhang; R. Sheng; T. Du; Y. Li; Y. Qi; *ACS Appl. Mater. Interfaces,* **2022**, *14*, 12984–13000.
9. C. Z. Mosher; P. A. P. Brudnicki; Z. Gong; H. R. Childs; S. W. Lee; R. M. Antrobus; E. C. Fang; T. N. Schiros; H. H. Lu; *Biofabrication*, **2021**, *13*, 035049.
10. A. B. J. de Barbosa; C. A. de França; J. J. de S. Gouveia; G. V. Gouveia, M. M. da Costa, H. P. de Oliveira, *J. Appl. Polym. Sci.*, **2019**, *136*, 47479.

11. D. Li; Q. Wang; F. Huang; Q. Wei; Electrospun nanofibers for enzyme immobilization in *Electrospinning: Nanofabrication and Applications*, William Andrew, **2019**, Chapter 26, pp. 765–781.
12. Z. G. Wang; L. S. Wan; Z. M. Liu; X. J. Huang; Z. K. Xu; *J. Mol. Catal. B Enzym.*, **2009**, *56*, 189–195.
13. M. M. Aldhahr; Y. Q. Almulaiky; R. M. El-Shishtawy; W. Al-Shawafi; A. Alngadh; R. Maghrabi; *ACS Omega*, **2018**, *3*, 6346–6350.
14. D. N. Tran; K. J. Balkus Jr.; *Top. Catal.*, **2012**, *55*, 1057–1069.
15. A. Greiner; J. H. Wendorff; *Angew. Chem., Int. Ed.*, **2007**,*46*, 5670–5703
16. Z. Vargas-Osorio; F. Ruther; S. Chen; S. Sengupta; L. Liverani; M. Michálek; D. Galusek; A. R. Boccaccini; *Biomed. Mater.*, **2022**, *17*, 045019.
17. V. Korniienko; Y. Husak; J. Radwan-Pragłowska; V. Holubnycha; Y. Samokhin; A. Yanovska; J. Varava; K. Diedkova; Ł. Janus; M. Pogorielov; *Molecules*,**2022**, *27*, 3343.
18. Y. Samokhin; Y. Varava; K. Diedkova; I. Yanko; Y. Husak; J. Radwan-Pragłowska; O. Pogorielova; Ł. Janus; M. Pogorielov; V. Korniienko; *J. Funct Biomater.*, **2023**, *14*, 414.
19. A. Al-Abduljabbar; I. Farooq; *Polymers*, **2023**, *15*, 65.
20. N. Rao; R. Singh; L. Bashambu; *Mater. Today: Proceedings,* **2021***, 44*, 608–614.
21. P. Adlercreutz, *Chem. Soc. Rev.*, **2013**, *42*, 6406–6436.
22. M. L. E. Gutarra; L. S. M. Miranda; R. O. M. A. de Souza, Enzyme Immobilization for Organic Synthesis in Organic Synthesis Using Biocatalysis, ed. A. Goswami and J. D. Stewart, Academic Press, Amsterdam, **2016**, Chapter 4, pp. 99–126.
23. R. C. Rodrigues; J. J. Virgen-Ortíz; J. C. S. dos Santos; Á. Berenguer-Murcia; A. R. Alcantara; O. Barbosa; C. Ortiz; R. Fernandez-Lafuente; *Biotechnol. Adv.*, **2019**, *37*, 746–770.
24. A. F. de Almeida; C. R. F. Terrasan, C. C. Terrone; S. M. Tauk-Tornisielo; E. C. Carmona; *Process Biochem.*, **2018**, *65*, 71–80.
25. L. de Abreu; R. Fernandez-Lafuente; R. C. Rodrigues; G. Volpato; M. A. Z. Ayub; *J. Mol. Catal. B Enzym.*, **2014**, 99, 51–55.
26. T. Liu; X. Wang; Q. Li; J. Wang; Y. Yan; *J. Mol. Catal. B Enzym.*, **2011**, 71, 45–50.
27. B. Stauch; S. J. Fisher; M. Cianci; *JLR*, **2015**, *56*, 2348–2358.
28. P. Nicolás; V. L. Lassalle; M. L. Ferreira; *Enzyme Microb. Technol.*, **2017**, *97*, 97–103.
29. J. Cassani; H. Luna; A. Navarro-Ocaña; E. Castillo; *Electron. J. Biotechnol.*, **2007**, *10*, 508–513.
30. E. M. Anderson; M. Karin; O. Kirk; *Biocatal. Biotransform.*, **1998**, *16*, 181–204.

31. M. Habulin; Ž. Knez; *J. Mol. Catal. B Enzym.*, **2009**, *58*, 24–28.
32. A. Idris; A. Bukhari; *Biotechn. Adv.*, **2012**, *30*, 550–563.
33. L. F. Solares; R. Brieva; M. Quiros; I. Llorente; M. Bayod; V. Gotor; *Tetrahedron: Asymmetry*, **2004**, *15*, 341−345.
34. C. A. Gál; L. E. Barabás; J. H. Bartha-Vári; M. E. Moisă; D. Balogh-Weiser; L. C. Bencze; L. Poppe; C. Paizs; M. I. Toșa; *React. Chem. Eng.*, **2021**, *6*, 2391–2399.
35. Q. Li; F. Fan; Y. Wang; W. Feng; P. Ji; *Ind. Eng. Chem. Res.*, **2013**, *52*, 6343–6348.
36. L. C. Bencze; J. H. Bartha-Vári; G. Katona; M. I. Toşa; C. Paizs; F. D. Irimie; *Bioresour. Technol.*, **2016**, *200*, 853–886.
37. E. P. Cipolatti; M. C. C. Pinto; J. M. de Robert; T. P. da Silva; T. D. C. Beralto; J. G. F. Santos Jr.; R. Fernandez-Lafuente; E. A. Manoel; J. C. C. D. S. Pinto; D. M. G. Freire; *J. Appl. Polym. Sci.*, **2018**, *135*, 46727.
38. Y. Poojari; S. J. Clarson; *Biocatal. Agric. Biotechnol.*, **2013**, *2*, 7–11.
39. J. Sun; Y. Jiang; L. Zhou; J. Gao; *New Biotechnology*, **2010**, *27*, 53–58.
40. X. Geng; O.-H. Kwon; J. Jang; *Biomater.*, **2005**, *26*, 5427–5432.
41. K. Sun; Z. H. Li; *EXPRESS Polym. Lett.*, **2011**, *5*, 342–361.
42. C. Ortiz; M. L. Ferreira; O. Barbosa; J. C. s. dos Santos; R. C. Rodriguez; Á. Berenguer-Murcia; L. E. Briand; R. Fernandez-Lafuente; *Catal. Sci. Technol.*, **2019**, *9*, 2380–2420.
43. C. José; R. D. Bonetto; L. A. Gambaro; M. P. Guauque Torres; M. L. Foresti; M. L. Ferreira; *J. Mol. Catal. B: Enzym.*, **2017**, *71*, 95–107.
44. C. José; L. E. Briand; *React. Kinet., Mech. Catal.*, **2010**, *99*, 17–22.
45. C. G. Spelmezan; A. Bacoș; G. Katona; *Studia UBB Chemia,* **2023**, *LXVIII, 2,* 53–71.
46. C. G. Spelmezan; G. Katona; L. C. Bencze; C. Paizs; M. I. Toșa; *React. Chem. Eng.*, **2023**, *8*, 1109.
47. P. L. Sóti; D. Weiser; T. Vigh; Zs. K. Nagy; L. Poppe; G. Marosi; *Bioprocess. Biosyst. Eng.*, **2015**, *39*, 449–459.
48. A. Goswami; J. Goswami; *Tetrahedron Lett.*, **2005**, *46*, 4411–4413.
49. *Electronic Supplementary Material (ESI)* for C. A. Gál; L. E. Barabás; J. H. Bartha-Vári; M. E. Moisă; D. Balogh-Weiser; L. C. Bencze; L. Poppe; C. Paizs; M. I. Toșa; *React. Chem. Eng.*, **2021**.
50. C.-S. Chen; Y. Fujimoto; G. Girdaukas; C. J. Sih; *J. Am. Chem. Soc.*, **1982**, *14*, 7294.