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Article

Enantioselective Resolution of (*R,S*)-1-Phenylethanol Catalyzed by Lipases Immobilized in Starch Films

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Lipases de diferentes fontes e dois micélios, na forma livre ou imobilizados em filme de amido de gengibre, foram utilizados como catalisadores na reação do (*R,S*)-1-feniletanol (**1**) com acetato de vinila e outros agentes acilantes. Os efeitos de vários parâmetros na resolução de (**1**) catalisada pela lipase de *Burkholderia cepacia* (LBC) imobilizada em filme de amido de gengibre (tipo do doador acila, razão molar álcool:doador acila, temperatura e solvente orgânico) foram avaliados. A eficiência catalítica da LBC imobilizada em blendas de amido de gengibre e poli (oxido de etileno) (PEO), em diferentes composições, também foi estudada. Os acetato de vinila e o de *iso*-propenila forneceram as maiores conversões (9%) e excessos enantioméricos em (*R*)-éster (> 99%). A razão molar álcool:doador acila e temperatura ótima foram de 1:1 e 28 °C, respectivamente. A mistura de *n*-hexano/glicerol (9:1 v:v) foi a mais adequada para esta reação (conversão 23%, E > 200). A blenda de amido de gengibre/PEO (7:3 m/m) foi reutilizada por seis vezes consecutivas.

Lipases from different sources and two mycelium-bound lipases, in a free or immobilized form, in ginger starch film were screened as biocatalysts in the reaction of (*R,S*)-1-phenylethanol (**1**) with vinyl acetate and other acylating agents. The effect of various reaction parameters in the resolution of (**1**) catalyzed by lipase from *Burkholderia cepacia* (BCL) immobilized in ginger starch film was evaluated (acyl donor type, alcohol:acyl donor molar ratio, temperature and organic solvent). The catalytic efficiency of BCL immobilized in polymeric blends of ginger starch and polyethylene oxide (PEO), in different compositions, was also studied. Vinyl acetate and *iso*-propenyl acetate furnished the highest conversion (9%) and enantiomeric excess (> 99%) of the (*R*)-ester. The alcohol:acyl donor molar ratio and temperature optimum were 1:1 and 28 °C, respectively. The mixture of *n*-hexane/glycerol (9:1 v:v) was the most adequate for this reaction (conversion 23%, E > 200). The ginger starch/PEO (7:3 m/m) blend was successfully reused six times consecutively.

Keywords*:* starch film, immobilization, lipases, enzymatic resolution

Introduction

In recent years, the application of enzymes and microorganisms as biocatalysts in organic synthesis has become a well-known technique.[1,2] Enzymatic procedures to obtain a wide range of products under mild and environmentaly-friendly conditions have become attractive and promising due to the excellent stereoselectivity of enzymes.[3] Lipases (triacylglycerol hydrolases EC 3.1.1.3) are the most widely applied enzymes for regio-, chemo- and enantioselective biotransformations, because they also possess wide substrate specificity, have an excellent ability to recognize chirality, and do not require cofactors.[1,4] They have found wide usage in a growing range of industrial applications, such as in detergents, food processing, biotransformations, waste treatment and bioremediation. They have been used by the pharmaceutical, cosmetics, textile, leather and paper industries and also in medical applications as drugs or biosensors.[5-7] Lipases can catalyze the resolution of several racemic compounds including alcohols,[8] amines,[9] acids,[10] α-hydroxy selenides[11] and tellurides.[12] Due to the poor solubility in water of most of these compounds, the enzymatic resolution is usually performed in non-aqueous media, but enzymes can denature in such media. A practical technique to improve the enzyme efficiency, operational stability and performance in organic solvents is the use of biocatalyst immobilization. Besides the stabilizing effect, the quality of the products can be improved by avoiding by-products or unwanted intermediates and easy separation. Furthermore,

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immobilized enzymes can be reused, making the process economically feasible.[13-16]

Several immobilization methods have been reported, such as covalent binding to a support,[13] magnetic nanoparticles[14] and lipase entrapment in porous polymeric matrices and in gels.[6] Amongst the various immobilization techniques employed, adsorption may have a higher commercial potential because this method is less complex and expensive than other techniques.[17] Physical characteristics, such as surface area, porosity and hydrophobic-hydrophilic balance, are important parameters of the support. They are crucial for enzyme immobilization and will influence the enzyme loading as well as the catalytic behavior.[15,18]

Recently, various biopolymers, such as dextran,[16] chitosan,[19,20] agarose[17] and cellulose,[21] have been used as a support for enzyme immobilization.

Starch is a semi-crystalline biopolymer and is the most abundant storage reserve carbohydrate in plants. It is found in many different plant organs including seeds, fruits, tubers and roots. Many of these starch-storing organs, for example, the grains of maize and rice or the tubers of cassava and potatoes, are staple foodstuffs in the human diet.[22] Starch granules are composed of two types glucose polymers, amylose and amylopectin, which represent approximately 98-99% of the dry weight. The ratio between the two polysaccharides varies according to the botanical origin of the starch.[23] Amylose is a relatively long, linear polymer containing 99% α-1-4 and 1.0% α-1-6 glycoside bonds. Amylopectin contains 95% of short α-1-4 linear chains and 5% α-1-6 linked side chains.[24]

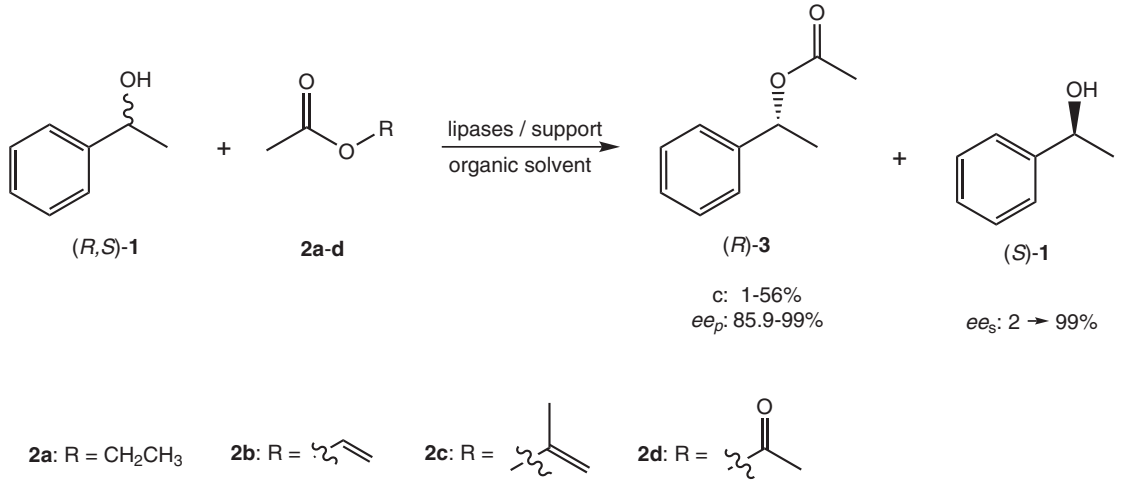
Starch has a broad range of applications both in the food and non-food sectors. For example, it is an important ingredient for the food industry, being used to improve the nutritional and sensory quality.[22-25] Other important fields of starch application include textiles, paper, mining, cosmetics, pharmaceuticals, construction and paints.[26] However, currently one of the main uses is to produce bioplastics with native or modified starch, or in combination with natural or synthetic molecules. These are used in specific industrial applications such as composting bags and sacks, fast food service ware, packaging and others.[26,27]

In this study, starch obtained from ginger was used as a support for lipase immobilization and these systems were employed in the resolution of the (*R,S*)-1-phenylethanol (**1**). Several reaction parameters were evaluated including the type and amount of lipase, alcohol:acyl donor molar ratio, use of different organic solvents and temperature, as well as the influence of lipase immobilization in starch film or in polymeric blends formed of ginger starch/polyethylene oxide (PEO). The recyclability of the immobilized catalyst was also evaluated (Scheme 1).

Experimental

Materials and methods

All chemicals were commercially available and were of reagent grade and used without further purification. *(R,S)*-1-phenylethanol (98%) and 1-butyl-3-methylimidazolium hexafluorophosphate [BMIm][PF_6] (98%) were purchased from Sigma-Aldrich. Polyethylene oxide (PEO) (MM = 300000 daltons) was supplied by Acros Organics. Acetic anhydride (97%), sodium bicarbonate and glycerol (99.5%) were provided from Vetec. Vinyl acetate (99%) and *iso*-propenyl acetate (99%) were obtained from Fluka Chemika. Sulfuric acid was purchased from Quimex. Anhydrous magnesium sulfate and ethyl acetate (99%) were provided by Nuclear. The commercially-available enzyme preparation, lipase from *Candida antarctica* B (CALB, 10000 PLU g^{-1}), was donated by Novozymes A/S. Lipase from *Burkholderia cepacia* previously known as



Scheme 1. Enzymatic resolution of (*R,S*)-1-phenylethanol (**1**) in organic media.

Pseudomonas cepacia (BCL, 30000 U g^{-1}) was donated by Amano Enzymes Inc., and lipase from *Chromobacterium viscosum* (CVL, 5138.7 U mg^{-1}) was provided by Genzyme Biochemicals.

Native lipases from *Aspergillus niger* AC-54 (ANL, 19.4 U mL^{-1}) and *Rhizopus oligosporus* (ROL, 14.9 U mL^{-1}) were kindly donated by Professor Patrícia de Oliveira Carvalho from Universidade São Francisco (USF, Bragança Paulista-SP, Brazil), and were isolated and purified by microorganisms from the region of Bueno Brandão-MG, Brazil.[28] The mycelium-bound lipases isolated from the Amazonian fungi UEA_53 (*Astrocaryum aculeatum*) and UEA_115 (*Colletotrichum* sp) were kindly donated by Professor Sandra Patrícia Zanotto from the Universidade Estadual do Amazonas (UEA, Manaus-AM, Brazil).[29]

The starch (S) was obtained from ginger (G, *Zingiber officiale*) and was extracted and characterized using an Olympus BX40 optical microscope, coupled with a cybernetics cool SNAP-PRO cf color digital camera. The equipment belongs to Laboratório de Microscopia do Departamento de Ciência e Tecnologia de Alimentos, Universidade Federal de Santa Catarina (UFSC, Florianópolis-SC, Brazil), a collaboration with Professor Elisa H. S. Moecke. The grains presented oval or circular shape with diameters ranging from 15-30 μm.[30]

Deuterium chloroform (99.8%) was purchased from Cambridge Isotope Laboratories. All organic solvents were obtained from commercial sources and were of analytical grade.

Infrared spectra were acquired with an ABB Boomer FTLA 2000-100 spectrometer using KBr for solid samples (range 4000-400 cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz, with a Varian 400 Mercury Plus spectrometer using $CDCl_3$ as the solvent and tetramethyl silane (TMS) as the internal standard at Central Analítica, Departamento de Química, UFSC (Florianópolis-SC, Brazil). The specific rotations were determined using a Schmidt + Haensch polarimeter. The pH of the water was measured with a Marte MB10 pH meter.

The progress of the transesterification reaction was followed by thin-layer chromatography (TLC) using *n*-hexane:ethyl acetate (7:3 v/v) as the eluent. The conversion, as well as the enantiomeric excesses of the formed products was determined with a gas chromatograph (GC-14B Shimadzu) equipped with a chiral column (RT – BetaDex – SM, 30 m × 0.32 mm × 0.25 μm, Restek) and H_2 was used as the carrier gas with a pressure of 75 kPa. The injector and detector temperature was 230 ºC. Initial and final column temperatures were 100 and 200 ºC, respectively, and the temperature was increased by 3 ºC min^{-1}. The retention times observed for *S*-(–) and *R*-(+)-1-phenylethanol were 6.1 and 5.8 min, and for the corresponding *S*-(–) and *R*-(+)-acetyl esters they were 5.0 and 5.4 min, respectively. The enantiomeric excess of the reagent (ee_s) and product (ee_p) was defined as $[(S) - (R)]/[(S) + (R)] \times 100\%$, where (*R*) and (*S*) are the peak areas of (*R*) and (*S*) enantiomers, respectively.

Immobilization of lipases in starch film and ginger starch/PEO blends

Enzyme immobilization on ginger starch film was performed by dissolving 1.0 g of ginger starch in 25 mL of distilled water (pH 5.6). This solution was maintained under constant magnetic stirring and mild heating for 1 h until complete dissolution of the polymer. After this period, 0.3 mL of glycerol and 60 mg of lipases (BCL, CVL, ROL or ANL) were added into the solution and the mixture was stirred for more than 20 min. The solvent was evaporated (30-40 °C) forming a stable film, which was then cut into small pieces and maintained in organic solvent. The ginger starch/PEO blends were prepared by varying the composition of the ginger starch polymer and PEO, ranging from 0 to 100% (m/m), the total mass being 1.0 g. The lipase mass was the same, as previously described.

*General procedure for lipase-catalyzed resolution of (R,S)-1-phenylethanol (**1**)*

The lipase-catalyzed resolution of the (*R,S*)-1-phenylethanol in organic media was performed in a 250 mL shake flask. The reaction mixture contained 0.6-6.0 mL (5-50 mmol) of (*R,S*)-**1**, 0.5-4.6 mL (5-50 mmol) of vinyl acetate, 0.6 mL (5 mmol) of *iso*-propenyl acetate, 0.5 mL (5 mmol) of acetic anhydride or 0.5 mL (5 mmol) of ethyl acetate and the lipases (20 or 60 mg) free or immobilized in ginger starch film or in ginger starch/PEO blends. A similar procedure was used for the reactions catalyzed by the mycelium-bound lipases (60 mg). The reaction media was incubated in a shaker using *n*-hexane as a solvent, except in the solvent screening studies, at 22-40 ºC with constant stirring. Aliquots were withdrawn at specified time intervals from the reaction mixture, and analyzed by chiral chromatography to evaluate the percentage of conversion and the enantiomeric excess of reactant and products. Substrate and product peak areas were compared, and the sum of the two was considered as 100%. The enantiomeric ratio (E-value) was calculated from the enantiomeric excess of the product (ee_p), and the conversion degree (c) according to the method described by Chen *et al.*[31]

After purification using silica gel column chromatography (*n*-hexane and ethyl acetate 9:1 v:v) the pure

(*R*)-1-phenylethyl acetate was obtained. This compound was also analyzed by ^{1}H NMR, IR, chiral GC and specific rotation.

Results and discussion

Screening of enzymes

In the first step of this study, three commercially available lipases (from *C. antarctica* B, *B. cepacia* and *C. viscosum*), two natives lipases (from *R. oligosporus* and *A. niger*) free or immobilized in ginger starch film (GS) and two mycelium-bound lipases isolated from the Amazonian fungi UEA_53 (*A. aculeatum*) and UEA_115 (*Colletotrichum* sp) were tested for the resolution of the *(R,S)*-1-phenylethanol (**1**) with vinyl acetate in *n*-hexane at 35 °C (Table 1). In all transesterification reactions catalyzed by free or immobilized lipases, the stereopreference was towards the (*R*)-enantiomer. These data followed the empirical rule proposed by Kazluaskas *et al.* and are in agreement with data reported in the literature which showed that CAL-B and other lipases have a highly pronounced catalytic preference for *R*-enantiomer.[32,33] The acetylation reaction performed in the absence of the biocatalyst led to the recovery of the starting material.

Table 1. Resolution of *(R,S)*-1-phenylethanol with vinyl acetate catalyzed by various free and immobilized lipases

entry	Biocatalyst	c / %	ee_s / %	ee_p / %	E-value[a]
1	ROL	14	14	> 99	> 200
2	CVL	53	89	86	39
3	ANL	56	> 99	98	> 200
4	BCL	55	95	99	> 200
5	CALB	51	97	99	> 200
6	UEA_53	1	2	> 99	> 200
7	UEA_115	4	6	> 99	> 200
8	CVL/GS	6	3	> 99	> 200
9	ROL/GS	1	4	> 99	> 200
10	ANL/GS	10	8	> 99	> 200
11	BCL/GS	9	13	> 99	> 200

Reaction conditions: (*R,S*)-**1** (0.6 mL, 5 mmol), vinyl acetate (0.5 mL, 5 mmol), CVL (20 mg g^{-1} of starch), BCL, ANL, ROL, CALB, UEA_53 and UEA_115 (60 mg g^{-1} of starch), *n*-hexane (25 mL), 35 °C, 72 h; [a]calculated as described in reference 31.

As shown in Table 1, both free and immobilized lipases were able to convert (*R,S*)-**1** to (*R*)-1-phenylethyl acetate (**3**). The highest conversion degrees to (*R*)-**3** were achieved when the lipases CVL, ANL, BCL and CALB were employed in a free form, after 72 h of reaction, these being in the range of 51-56% with the ee_p values of 86-99% (Table 1, entries 2-5). When the lipases CVL, ROL, ANL and BCL were immobilized in ginger starch film, lower conversions into (*R*)-**3** and higher ee_p and E-values were obtained in comparison with free lipases (Table 1, entries 8-11). The conversion degrees ranged from 1-10%, resulting in ee_p > 99% and E-values > 200.

Using the fungi UEA_53 and UEA_115, lower conversion degrees to the product were obtained, these being 1 and 4%, respectively. However, these Amazonian fungi showed a promising enantioselective capacity, since good ee_p (> 99%) and E (> 200) values were obtained (Table 1, entries 6-7). Zanotto *et al.*[29] have also studied the resolution of (*R,S*)-2-octanol via transesterification reactions with vinyl acetate catalyzed by these fungi.

Owing to some advantages of using immobilized lipases, like the simple recovery of the products and reactants, easy separation of enzyme from product and the possibility of continuous and repeated use of the catalyst, the BCL/GS system was chosen as the biocatalyst for the further investigations.

Effect of acyl donor

It is generally described that the acyl group from the acylating agents can affect the reaction rate and the enantioselectivity in a lipase-catalyzed transesterification reaction.[34] Thus, in order to examine the effect of acyl donors, different acyl donors including vinyl esters (vinyl acetate and the *iso*-propenyl acetate), ethyl acetate and acetic anhydride were used in the resolution of the (*R,S*)-1-phenylethanol catalyzed by *B. cepacia* lipase immobilized in the ginger starch film in *n*-hexane. The influence of the reaction time was previously evaluated from 8 to 72 h at 35 °C, and no significant change in the conversion degrees was observed (9-10%). These results may be analyzed considering that for the time studied the conversion degree was relatively low. However, it is worth mentioning that the enantioselectivity of the process was maintained, forming the (*R*)-ester **3** with ee_p > 99% and E-values > 200. Thus, a reaction time of 24 h was selected for the subsequent studies and the results are shown in Table 2.

As observed in Table 2, the selectivity and conversion degrees reached after 24 h reaction were greatly influenced by the nature of the acyl donor. The highest conversion degree (9%) was obtained when vinyl acetate and *iso*-propenyl acetate were used, respectively, forming the (*R*)-ester **3** with ee_p > 99% (Table 2, entries 1-2). Using ethyl acetate and acetic anhydride, the values for the conversion degree to product were 5 and 4%, with ee_p > 99% and 38%, respectively (Table 2, entries 3-4).

These results are also in agreement with those reported in the literature for enzyme-catalyzed esterification and transesterification reactions in organic media, where vinyl

Table 2. Effect of acyl donor on the transesterification of (*R,S*)-**1** with vinyl acetate catalyzed by the BCL/GS system

entry	Acyl donor	c / %	ee_s / %	ee_p / %	E[a]
1	vinyl acetate	9	13	> 99	> 200
2	ethyl acetate	5	13	> 99	> 200
3	*iso*-propenyl acetate	9	17	> 99	> 200
4	acetic anhydride	4	13	38	2.5

Reaction conditions: (*R,S*)-**1** (0.6 mL, 5 mmol), acyl donors (5 mmol), BCL (60 mg g^{-1} of starch), *n*-hexane (25 mL), 35 °C, 24 h; [a]calculated as described in reference 31.

acetate is considered to be a good choice for the acyl donor. In this case, the leaving group is an enol that immediately tautomerizes to a carbonyl compound (acetaldehyde), thereby driving the reaction to completion.[18,35]

Effect of alcohol:acyl donor molar ratio

To evaluate the influence of the (*R,S*)-1-phenylethanol:vinyl acetate molar ratio in the resolution of the (*R,S*)-1-phenylethanol mediated by lipase from *B. cepacia* (BCL) immobilized in ginger starch film (GS) in *n*-hexane, the alcohol:acyl donor molar ratio was varied from 1:1 to 1:10 (Figure 1).

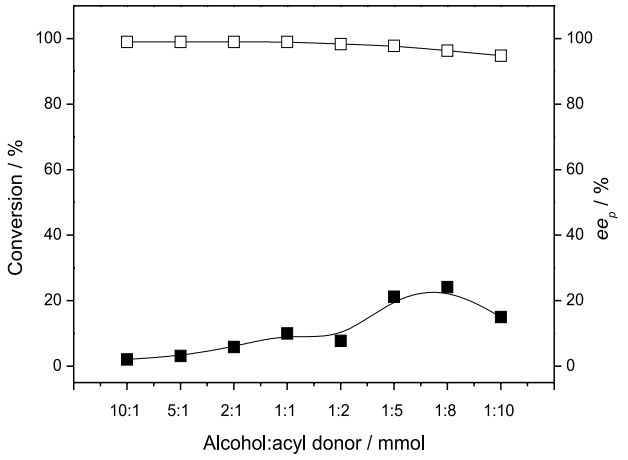


Figure 1. The effect of the (*R,S*)-1-phenylethanol/vinyl acetate molar ratio on the production of (*R*)-1-phenylethyl acetate (**3**) using the BCL/GS as catalytic system. Filled and open symbols (■, □) represent the conversion degrees and enantiomeric excesses (ee_p), respectively. Reaction conditions: (*R,S*)-**1** (0.6-6.0 mL, 5-50 mmol), vinyl acetate (0.5-4.6 mL, 5-50 mmol), BCL (60 mg g^{-1} starch), *n*-hexane (25 mL), 35 °C, 24 h.

It is apparent from Figure 1 that using the catalytic system LBC/GS, the conversion to (*R*)-**3** increased as the (*R,S*)-1-phenylethanol/vinyl acetate molar ratio increased. The highest conversion degrees obtained were 24% using the molar ratio of 1:8. However, there was a small decrease in the enantiomeric excess (*ca.* 95%) with the use of this molar ratio. When the transesterification reactions catalyzed by BCL/GS were carried out with an excess of alcohol (10:1, 2:1) the conversion degree to the product ranged from 2 to 6% with an ee_p value of > 99%. These results show the effect of substrate inhibition caused by the action of the alcohol on the catalyst, causing a decrease in the conversion degree. Ong *et al.*[33] and Trubiano *et al.*[36] reported a similar behavior for the enantioselective esterification of racemic ketoprofen in organic medium and ethyloleate synthesis in a solvent-free system, respectively, both using a commercial immobilized lipase from *C. antarctica B* (Novozyme 435). Considering these results, an alcohol:acyl donor molar ratio of 1:1 was selected for the subsequent experiments.

Effect of temperature

Another key factor which affects the rate of a reaction catalyzed by an enzyme is the temperature. The temperature influences the activity, selectivity and stability of the biocatalyst as well as the reaction equilibrium.[37] To evaluate the influence of the temperature in the resolution of the (*R,S*)-1-phenylethanol with vinyl acetate catalyzed by the system BCL/GS in *n*-hexane, the reaction temperature was varied within the range of 22 to 40 °C. Figure 2 shows the time course for (*R*)-**3** ester formation at various temperatures.

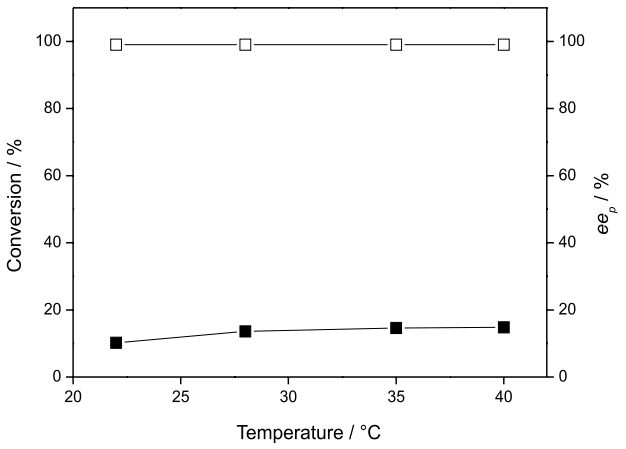


Figure 2. The influence of the temperature on the enzymatic resolution of (*R,S*)-1-phenylethanol mediated by BCL/GS. Filled and open symbols (■, □) represent the conversion and enantiomeric excesses, respectively. Reaction conditions: (*R,S*)-**1** (0.6 mL, 5 mmol), vinyl acetate (0.5 mL, 5 mmol), BCL (60 mg g^{-1} of starch), *n*-hexane (25 mL), 24 h.

The results showed a significant variation in the conversion to (*R*)-ester **3** when the reaction was performed at different temperatures. As the temperature increased from 22 to 28 ºC, the conversion degree increased and a value of 14% was reached at 28 °C in 24 h reaction. A further increase in temperature led to a small decrease in the conversion to (*R*)-**3**. The enantioselectivity of the immobilized LBC was not affected by the change in temperature in the range of 22-40 °C, with ee_p being > 99%.

Based on these results, a temperature of 28 ºC was considered as appropriate to evaluate the effect of the organic solvent as well as the BCL immobilization in ginger starch/PEO blends.

Effect of organic solvent

The most important criteria for solvent selection in biocatalysis are a high substrate and product recovery capacity and biocompatibility, although other characteristics such as chemical and thermal stability, nonbiodegradability, a nonhazardous nature and low market price are also desirable.[38] The most commonly used parameter to classify solvents in terms of biocompatibility is the log*P* value, which is defined as being the partition coefficient of a given compound in a two-phase *n*-octanol and water system.[39]

Thus, in order to study the effect of organic solvents in the resolution of (*R,S*)-phenylethanol catalyzed by *B. cepacia* lipase immobilized in ginger starch film, a series of solvents was chosen covering a wide range of log*P* values (Table 3).

As observed from these results, the percentage of ester formation and selectivity were strongly dependent on the solvent. When the less polar solvents such as *n*-heptane and *n*-hexane (log*P* ≥ 3.5) were employed in the resolution of (*R,S*)-**1**, moderate conversion degrees were obtained, these being 12 and 14%, respectively (Table 3, entries 1-2). However, on using *n*-heptane a lower selectivity was achieved (E = 27). When dichloromethane, ethyl ether, acetonitrile and 1,4-dioxane were used, the conversion degrees were low (< 5%), but the selectivity was high (E > 200) (Table 3, entries 3-6). This data is in agreement with those reported in the literature, where high hydrophobic solvents with log*P* values > 4 are considered the most suitable solvents for use in biocatalytic processes. The solvents with log*P* values between 2 and 4 are moderately effective. Polar solvents with log*P* < 2 are often ineffective,[39,40] and this may be attributed to the minimum distortion of the hydration layer around the enzyme. However, such solvents, due to their high affinity for water, might strip the essential hydration layer around the enzyme which preserve its conformation flexibility to some extent, thus decreasing the enzyme activity.[39,40]

The effect of using co-solvents (ionic liquid and glycerol) in the resolution of (*R,S*)-phenylethanol was also studied. The results showed that when the reaction was performed using a solvent mixture of *n*-hexane/[BMIm][PF_6] (9:1 v:v), the conversion to (*R*)-**3** ester was lower (6%) than that obtained using pure *n*-hexane (14%), while an excellent enantioselectivity was maintained. These results showed that the *n*-hexane/[BMIm][PF_6] (9:1 v:v) mixture has a negative influence on the transesterification reaction of (*R,S*)-**1** with vinyl acetate compared to the reaction using pure organic solvents (Table 3, entry 7).

Hara *et al.*[41] obtained similar results in the acylation of (*R,S*)-1-phenylethanol and other racemic alcohols with vinyl acetate catalyzed by *B. cepacia* immobilized in a sol-gel (BCLxero) and as a CLEA (BCL-CLEA) in organic media and/or in mixtures of organic solvent/ionic liquids. Using BCL-CLEA, the conversion to (*R*)-ester slightly decreased in a mixture of [BMIM][PF_6]/toluene (2:1) (15%) and [BMIM][PF_6]/toluene (1:2) (20%) compared to the pure toluene (50%).

When the solvent mixture of *n*-hexane/glycerol (9:1 v:v) was used, the degree of conversion to the (*R*)-**3** ester was

Table 3. Effect of organic media on the conversion to (*R*)-**3** ester using the BCL/GS catalytic system

entry	Solvents	log*P*[a]	c / %	ee_p / %	E[b]
1	*n*-heptane	4.0	12	91	27
2	*n*-hexane	3.5	14	> 99	> 200
3	CH_2Cl_2	1.5	2	> 99	> 200
4	diethyl ether	0.83	2	> 99	> 200
5	acetonitrile	−0.33	5	> 99	> 200
6	1,4-dioxane	−1.1	4	> 99	> 200
7	*n*-hexane/[BMIm][PF_6][c]	–	6	> 99	> 200
8	*n*-hexane/glycerol[c]	–	23	> 99	> 200

Reaction conditions: (*R,S*)-**1** (0.6 mL, 5 mmol), vinyl acetate (0.5 mL, 5 mmol), BCL (60 mg g^{-1} of starch), 25 mL organic solvent; [a]from reference 39; [b]calculated as described in reference 31; [c]10 mL *n*-hexane:LI or *n*-hexane:gycerol (9:1 v/v), 28 °C, 24 h.

much better, being 23% with an ee_p > 99% (Table 3, entry 8). Andrade *et al.*[42] also observed an improvement in the enzymatic performance of *Aspergillus terreus* and *Rhizopus oryzae* in the enantioselective bioreduction of haloacetophenones using glycerol as a co-solvent. In most cases, glycerol has demonstrated its potential to improve the conversion (up to > 99%) and enantioselectivity (up to > 99%) when compared to reactions in aqueous or other aqueous-organic media (THF, diethyl ether, toluene, DMSO and acetonitrile).

Thus, glycerol was shown to be an excellent alternative for use as a co-solvent in the resolution of (*R,S*)-phenylethanol catalyzed by *B. cepacia* lipase immobilized in ginger starch film. Moreover, the choice of this co-solvent is reinforced by its increasing availability on the market, since it is a by-product of biodiesel production.[42]

Effect of BCL immobilization in ginger starch/PEO blends

As previously mentioned, the immobilization of lipases in solid supports has been a topic of active research for several decades. A variety of supports, including inorganic materials,[18,43] natural[20,44] or synthetic polymers[45] and polymeric blends,[46] has been explored with a view to their use as supports for enzyme immobilization in order to improve the catalytic efficiency and reduce the cost of the process**.**

Thus, herein the catalytic efficiency of BCL immobilized in polymeric ginger starch/PEO blends using different compositions was evaluated in the resolution of (*R,S*)-phenylethanol with vinyl acetate in *n*-hexane and in a mixture of *n*-hexane/glycerol (9:1 v/v) at 28 °C (Figure 3).

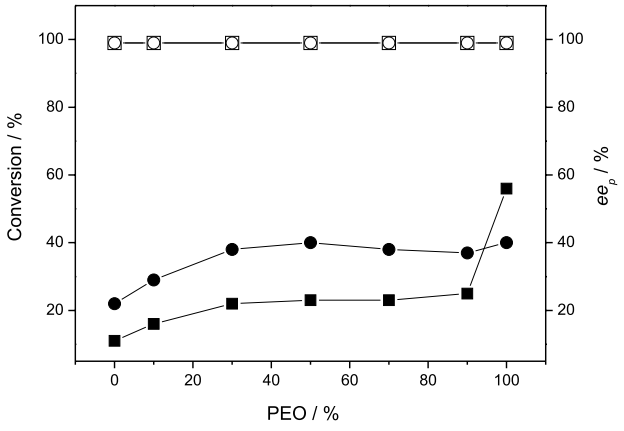


Figure 3. The effect of the PEO content in the ginger starch/PEO blends on the enzymatic resolution of (*R,S*)-1-phenylethanol (**1**) with vinyl acetate in *n*-hexane (■, □) and *n*-hexane/glycerol (9:1 v:v) (●, ○). Filled (■, ●) and open symbols (□, ○) represent the conversion and enantiomeric excess, respectively. Reaction conditions: *(R,S)*-**1** (0.6 mL, 5 mmol), vinyl acetate (0.5 mL, 5 mmol), BCL (60 mg g^{-1} of ginger starch/PEO blends), *n*-hexane (25 mL) or *n*-hexane:gycerol (9:1 v/v) (10 mL), 28 °C, 24 h.

It is evident from Figure 3 that the conversion to (*R*)-ester **3** was influenced by the proportion of PEO in ginger starch/PEO blends and the reaction medium. Using *n*-hexane, when the proportion of PEO increased from 0 to 90%, an increase in the conversion to (*R*)-**3** ester from 14 to 25% was observed. The highest conversion was obtained when BCL was immobilized in a film formed only with PEO, this being of 56%. However, using a mixture of *n*-hexane/glycerol (9:1 v/v), better conversion degrees were achieved regardless of the blend composition, presenting values of 22**-**40%. When BCL was immobilized in a film formed with PEO, the conversion degree was 40%, that is lower than that obtained when the solvent was *n*-hexane, probably due to the presence of glycerol.

Regardless of the blend composition or the reaction media, the ee_p values in all studies were > 99%, which indicated an excellent enantioselectivity of the process. This increase in the product conversion may be related to both the film porosity and the organic media. Recently, Hara *et al.*[47] reported the resolution of (*R,S*)-**1** catalyzed by BCL immobilized in different supports, such as active carbon coal and alumina, among others. The conversion degrees and E-values were highly dependent on the support and ranged from 1-50% with E-values from 7 > 200, respectively. Similar results were obtained by Shah *et al*.,[48] showing the great influence of the support specific surface area and the immobilization technique.

In this study, the SEM micrographs revealed an increase in the porosity as the PEO composition increased (results not presented). Thus, a higher porosity seems to favor the diffusion of reactants and products, thereby favoring the enzymatic catalysis, being in agreement with data reported in the literature.[15,47,49]

Reusability of the LBC immobilized on ginger starch/PEO blend

From an economic point of view, the reuse of an enzyme constitutes one of the main advantages of the biocatalyst immobilization process. Thus, BCL was immobilized in the ginger starch/PEO blend (7:3 m/m) and this system was reused in the resolution of the (*R,S*)-1-phenylethanol with vinyl acetate in *n*-hexane at 28 °C. After 24 h for each run, the immobilized lipase was collected, washed with *n*-hexane in order to remove any substrate or product retained in the support, and reused for 6 successive cycles. The results are shown in Figure 4.

As can be seen in Figure 4, the degree of conversion to (*R*)-**3** ester ranged from 26-16%, presenting an excellent selectivity with ee_p values of > 99% (E > 200) during the reuse. These results can be attributed to BCL, maintaining

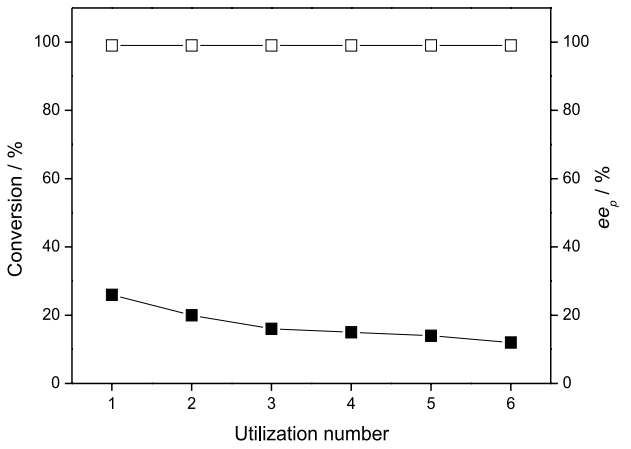


Figure 4. Reusability of immobilized *B. cepacia* lipase in the resolution of (*R,S*)-**1** to afford the (*R*)-**3** ester. Filled and open symbols (■, □) represent the conversion degrees and enantiomeric excesses (ee_p), respectively. Reaction conditions: *(R,S)*-**1** (0.6 mL, 5 mmol), vinyl acetate (0.5 mL, 5 mmol), BCL (60 mg g^{-1} of ginger starch/PEO blend 7:3 m/m), *n*-hexane (25 mL), 28 °C, 24 h.

its catalytic activity when immobilized in this blend, which represents, as expected, another advantage of this process.

Conclusions

Lipases from different sources were immobilized on ginger starch film or ginger starch/PEO blends. These biocatalytic systems were used in the resolution of (*R,S*)-phenylethanol. Lipase from *Burkolderia cepacia* (BCL) was selected to evaluate the influence of various reaction conditions. The conversion degree and the enantioselectivity were shown to be dependent on the acyl donor, molar ratio alcohol:acyl donor, temperature and organic medium. In most cases, the (*R*)-**3** ester was obtained in moderate-to-good conversions (9-40%), but with high selectivity (ee_p > 99%, E > 200). The LBC immobilized on the ginger starch/PEO blend (7:3 m/m) preserved the enzyme activity and was reused for 6 cycles.

Supplementary Information

Supplementary information data (Figures S1-S5) are available free of change at http://jbcs.sbq.org.br as a PDF file.

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