

Applications of Ionic Liquids to Increase the Efficiency of Lipase Biocatalysis

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1. Introduction

Stabilization of enzymes is a key issue to develop more efficient biocatalysts for industrial, environmental, or biomedical applications (Péter, 2005). In the last decades, important research potential has been focused on enzyme immobilization, explainable by the limited reusability of the native enzymes as industrial catalysts. Enzyme immobilization emerged as a valuable tool to overcome such a drawback, as it allows enzyme recycling and facilitates separation and recovery of the product from the reaction medium. In addition, could often result in increased thermal and operational stability of biocatalysts, thus allowing their employment in wider experimental conditions, compared to the native enzymes (Bucholz et al., 2005; Cao, 2005). Enzyme engineering by immobilization techniques was demonstrated to be compatible with other chemical or biological approaches to improve the enzyme function (Reetz & Jaeger, 1998).

Lipases are enzymes widely used in organic chemistry as biocatalysts for a large number of synthetic reactions. They showed an interesting combination of broad range substrate specificity with high regio- and enantioselectivity, which made them particularly useful in chiral resolution of organic compounds (Whittall & Sutton, 2010). Immobilization of lipases to increase the biocatalytic efficiency and reduce the costs is very well documented (Christensen et al., 2003; Sheldon, 2007; Mateo et al., 2007). The most frequently used lipase immobilization techniques are:

- physical adsorption
- covalent attachment
- entrapment in polymeric matrixes
- cross-linking of enzyme molecules

All these methods target to gather the advantages of immobilization (enhanced stability, repeated or continuous use, easy separation from the reaction mixture, possible modulation of the catalytic properties, prevention of protein contamination in the product), without any significant decline of the enzyme catalytic activity. In the last decades, the utilization of new enzyme carriers was, besides exploitation of the increasing knowledge regarding enzyme structure and mechanism, the most important scientific trend in enzyme engineering. (Bornscheuer, 2003).

In this respect, encapsulation in sol-gel matrices has been proved as one of the most efficient immobilization methods, considering both activity and biocatalyst stability. Due to their nano- or microporous structure, sol-gel materials exhibit valuable properties, such as high surface to volume ratio, large surface area and porosity (Pirozzi et al., 2009). In addition, the sol-gel process allows high compositional and morphological flexibility, by utilization of alkoxyde-type silane precursors holding one or two nonhydrolyzable organic functional groups and various additives, to yield organic-inorganic hybrid matrices (Reetz et al., 2003). Sol-gel matrices are formed by hydrolytic polymerization of the silane precursors, mainly alcoxides. The first step is hydrolytic, followed by condensation reactions to yield silica. Silica particles grow progressively as condensation proceeds, leading to the formation of colloidal solutions and gels. Dried at room temperature, the gels form a porous network of hydrated amorphous silica. Conducting the sol-gel process in the presence of a biomolecule will result in its entrapment by the obtained matrix (Pierre, 2004).

Lipase immobilization by the sol-gel method was subject of numerous recent publications (Kiss et al., 2007; Hara et al., 2008; Kawakami et al., 2009; Zarcuła et al., 2009; Tomin et al., 2010). The main benefit of this method is the enzyme inclusion throughout the gelation phase, at low synthesis temperature and not adverse reaction medium for the catalytic activity (Ciriminna & Pagliano, 2006). Another important advantage is the possibility to combine the entrapment with adsorption, to obtain supported sol-gel polymers with improved properties. Diverse carriers, as Fe₃O₄ magnetic nanoparticles, non-woven fabric (Chen & Lin, 2003), or Celite diatomaceous earth (Kawakami & Yoshida, 1996; Zarcuła et al., 2009), have been tested with promising results.

As protection of the enzyme during the encapsulation process is very important to prevent inactivation caused by gel shrinkage throughout maturation and drying, or by inadequate pore size resulting in slow diffusion rate, several compounds have been tested as immobilization additives. Sol-gel glasses have the ability to entrap large amounts of such additives, resulting in preservation or even enhancement of enzyme activity and selectivity. Most of the tested compounds were of polyhydroxylic nature: sugar, polyethylene glycols with different molecular weights, glycerol, cyclodextrins, polyvinyl alcohol, but also crown ethers, nonionic surfactants, or even proteins have been investigated (Reetz et al., 1996; Reetz et al., 2003; Péter et al., 2005; Cao et al., 2009).

Unexpectedly, ionic liquids (ILs) also proved to be efficient immobilization additives, although they are mainly known as substitute reaction media of volatile organic solvents. Ionic liquid usually contain a bulky cation and a small anion, and are available in large varieties. They gained constantly increasing attention as possible green reaction media of the future, based on their lack of vapour pressure, thermal stability, non-flammability and widely tunable physicochemical properties through the appropriate modification of the cation and/or anion (Van Rantwijk et al., 2003).

ILs with 1,3-dialkylimidazolium cations are generally recognized as the most valuable for biocatalytic applications. Their physical properties cover a broad range of values, but the catalytic properties are mainly influenced by their polarity, hydrophobicity and solvent miscibility. The polarity of ILs based on imidazolium cation is in the range of lower alcohols and formamide, and slightly decreases with increase of the length of alkyl chain linked to the imidazolium ring. However, it is difficult to correlate the IL polarity with the reaction rate of a specific substrate because other parameters, like viscosity, are also influential

(Moniruzzaman et al., 2010). Based on their water miscibility, ILs can be classified as hydrophobic and hydrophilic. This miscibility seems to be influenced by the ability of their anions to form hydrogen bonds, not by the overall polarity of the molecule. From the series of ILs with the same (1-butyl-3-methylimidazolium) cation, those with hexafluorophosphate ($[\text{PF}_6]^-$), bis(trifluoromethyl)-sulfonylimide ($[\text{Tf}_2\text{N}]^-$), or perchlorate ($[\text{ClO}_4]^-$) anions are water-immiscible, whereas those with tetrafluoroborate ($[\text{BF}_4]^-$), bromide ($[\text{Br}]^-$), or octylsulphate ($[\text{OcSO}_4]^-$) anions are water-miscible (Gorke et al., 2007). The explanation is probably the stronger hydrogen-bond accepting ability of water-miscible IL's anions. For biocatalytic purposes, the hydrophobic ILs are more interesting, as synthetic reactions are usually performed in non-aqueous solvents. Esterification, acylation, or polymerization reactions catalyzed by lipases in hydrophobic ILs have been reviewed (Moon et al., 2006; Sureshkumar & Lee, 2009). Unexpectedly, the hydrophobic ILs are not inherently miscible with hydrophobic organic solvents. The water-immiscible 1-butyl-3-methyl-imidazolium tetrafluoroborate is also immiscible with hexane and toluene, but miscible with acetone (Poole, 2004). Even in case of miscibility, the miscibility gaps of ILs with organic solvents are highly asymmetrical. The IL can dissolve appreciable amount of the solvent, but the solubility of IL in the molecular solvent is low (Weingärtner, 2008). Consequently, it is difficult to presume the solubility of various substrates and reaction products in a given ionic liquid, and experiments could run in a different way as it was expected. Monophasic reaction systems are commonly employed in biocatalytic reactions in IL media, with the main disadvantage of a supplementary product extraction step at the end of reaction. This extraction step could be eliminated using biphasic or multiphasic systems, but the IL and the organic components (product, unreacted substrates) must represent effectively immiscible phases, without negative effect on the reaction yield and rate, an objective not easy to achieve (Fehér et al., 2007).

Beyond their increasing potential as green reaction media in biocatalytic reactions, the ILs also demonstrated to be valuable additives during the sol-gel immobilization process of lipases. Nevertheless, identifying the exact role of ILs in the preparation of xerogel-type materials and the effect of their presence during immobilization on the entrapped enzyme properties is not an easy task. The main issues emerged from the relative scarce number of publications focused on this subject are:

- ILs can protect the enzyme against inactivation by the released alcohol and shrinking of gel during the maturation and drying step of the sol-gel immobilization process (Lee et al., 2006).
- ILs could totally replace the solvents used in the immobilization process, allowing the formation of a structure similar to aerogels in terms of pores size and volume, without supercritical drying, but this technique was not tested in the presence of an enzyme (Dai et al., 2000).
- The presence of ILs as additives increased the gelation time and influenced the gel structure, increasing the average pore radius and resulting in a narrower pore size distribution (Karout & Pierre, 2007).
- ILs can act as a template to form a wormlike mesoporous silica framework, based on hydrogen bond formation between the IL anion ($[\text{BF}_4]^-$) and the silanol groups of the silica gel (Zhou et al., 2004), but the formation of such a structure in the presence of an enzyme was not yet demonstrated.

Our work was focused on both possible applications of ionic liquids in lipase biocatalysis: as immobilization additives and as reaction media for the biocatalytic reaction.

Enantioselective acylation reactions of racemic 2-octanol have been used as test reactions for the characterization of the immobilized lipase biocatalysts. Chiral 2-octanol is an important building block for the preparation of liquid crystal materials, as well as an essential intermediate of many optically active pharmaceuticals (Dai & Xia, 2006). The precursor composition was finely tuned to obtain the best catalytic efficiency, by using secondary or tertiary silane mixtures with alkyl- or aryl nonhydrolyzable groups, and various ionic liquids as additives. Ionic liquids were tested as well as reaction media, compared with organic solvents. Operational stability of the obtained biocatalysts was studied in several reuse cycles and thermal stability was also tested at temperatures up to 80°C.

2. Experimental

2.1 Materials

Lipases from *Pseudomonas fluorescens* (Amano AK) and *Burkholderia cepacia* (Amano PS) have been purchased from Aldrich. *Candida antarctica* B was produced by C-Lecta (Leipzig, Germany). Methyl-trimethoxysilane (MeTMOS), tetramethoxysilane (TMOS), acetone, n-hexane, 2-propanol, acetonitrile, tetrahydrofuran (THF), toluene, *t*-butanol, sodium fluoride, vinyl acetate, and 2-octanol were products of Merck. Octyl- (OcTMOS), and phenyl-trimethoxysilane (PhTMOS), were obtained from Fluka. All the specified reagents have been purchased at analytical grade and used as purchased. Dodecane (99%, Merck) and decane (>99%, Aldrich) were used as internal standards for quantitative gas-chromatographic analysis. Ionic liquids 1-ethyl-3-methyl-imidazolium tetrafluoroborate [Emim]BF₄, 1-ethyl-3-methyl-imidazolium trifluoroacetate [Emim]COOCF₃, 1-hexyl-3-methyl-imidazolium tetrafluoroborate [Hmim]BF₄, 1-butyl-3-methyl-imidazolium hexafluorophosphate [Bmim]PF₆, were purchased from Merck at the highest available purity. 1-Octyl-3-methyl-imidazolium tetrafluoroborate [Omim]BF₄ was product of Fluka.

2.2 Sol-gel immobilization method

A microbial lipase suspension (120 mg/mL) in TRIS/HCl 0.1 M, pH 8.0 buffer, was stirred at 700 rpm for 30 min, centrifuged, and the supernatant used for immobilization. In a 4 mL glass vial, 1 mL of this lipase solution was mixed (magnetic stirrer) with 200 µL ionic liquid or PEG 20,000, followed by addition of 100 µL 1M NaF solution, and 200 µL isopropyl alcohol. This mixture was kept for 30 min under continuous stirring for homogenization, and subsequently a binary or tertiary mixture of silane precursors (total 6 mmoles) was added. The mixture was stirred at room temperature until start of gelation. The obtained gel was kept for 24 h at room temperature to complete polymerization. The bulk gel was washed with isopropyl alcohol (7 mL), distilled water (5 mL), isopropyl alcohol again (5 mL) and finally hexane (5 mL), filtered, dried at room temperature for 24 hrs, and in a vacuum oven at room temperature for another 24 hrs. Finally, it was crushed in a mortar and kept in refrigerator.

2.3 Immobilization by sol-gel entrapment combined with adsorption

The immobilization protocol was identical as described for the simple sol-gel entrapment, until the start of gelation, when 0.5 g Celite 545 was blended with the gelling mixture. Subsequently, the obtained solid preparate was worked-up as described in Subchapter 2.2. A simplified scheme of both immobilization procedures is presented in Fig. 1.

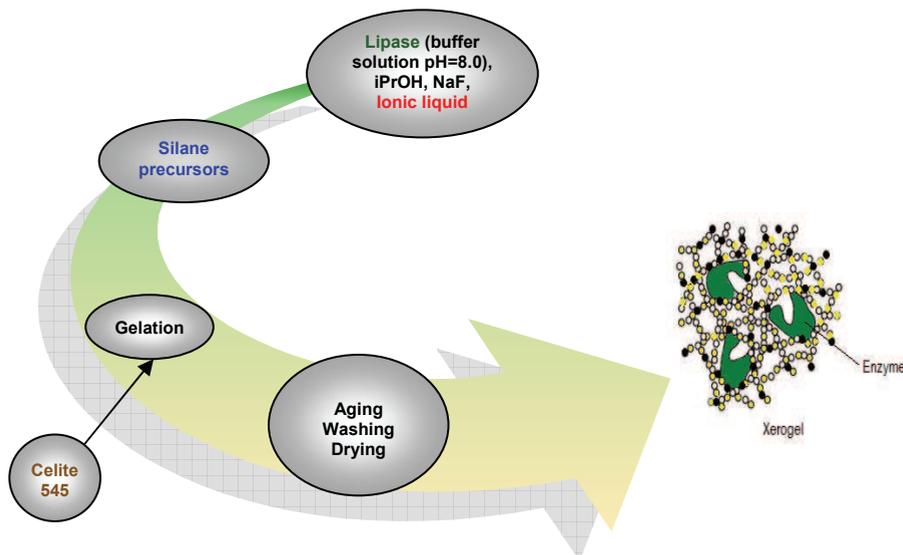


Fig. 1. Immobilization scheme of sol-gel entrapped lipase, simple or combined with adsorption on Celite 545.

2.4 Lipase-catalyzed acylation of 2-octanol

The procedure was the same as previously described (Zarcuła et al., 2010). Acylations were performed in 4 mL capacity glass vials, charged with a mixture of 2-octanol (1 mmole), vinyl acetate (3 mmole), reaction medium (organic solvent or ionic liquid, 2 mL) and free (10 mg) or sol-gel immobilized (50 mg) lipase. The solvents, alcohols and ILs used were separately equilibrated to 0.328 water activity at 25°C over saturated $MgCl_2$ solution for 48 hrs, as described in the literature (Bell et al., 2001). The mixture was incubated using an orbital shaker (MIR-S100, Sanyo, Japan) at 300 strokes/min and 40°C (ILW 115 STD incubator, Pol-Eko-Aparatura, Poland). The conversion and enantiomeric excess of the product were assayed by gas-chromatography, on a Varian 450 instrument (Varian Inc., USA) equipped with flame ionization detector, using a 30 m x 0,25 mm Elite-Cyclosil B chiral column with 0.25 mm film thickness (Perkin-Elmer, USA). The analysis conditions were: oven temperature: 50° to 120°C with 10°C/min heating rate, injector temperature 240°C, detector temperature 280°C, carrier gas (hydrogen) flow 1.2 mL/min. The reactions were usually run for 24 hrs. Conversions have been calculated based on the internal standard method. When ILs were used as reaction media, the obtained reaction mixture was extracted 4-times with hexane to isolate the product, the extracts merged and analyzed by gas-chromatography in the same conditions.

Transesterification activities were calculated at 24 hrs reaction time and expressed as the average 2-octyl-acetate amount (in micromole) synthesized per hour by 1 mg of free or immobilized enzyme. The control reaction without enzyme did not give any product in the same conditions. To characterize the overall efficiency of the immobilization process, total activity yield was calculated as % of the total enzymatic activity recovered following immobilization, divided by the total activity of the lipase subjected to immobilization. The enantiomeric excess of the resulted ester product (ee_p) was determined from the two

enantiomers peak area, and the enantiomeric ratio (E) values were calculated based on conversion and ee_p values (Chen et al., 1982).

All experiments and samplings were run in duplicate, and the calculated average values are presented in the tables and figures, as the differences between the measured values for the same assay were less than 2% for conversion and 1% in case of enantiomeric excess.

2.5 Enzyme recycle and thermal stability study

The enzyme recycle study was performed at 40°C. The initial reaction system was set up as described for the acylation study (Subchapter 2.3). At the end of every reaction cycle, the product (upper liquid phase of the reaction mixture) was removed with a pipette, the remained solid phase (native or immobilized lipase) was washed two times with 2 mL hexane, centrifuged at 15°C and 5,000 rot/min, and the supernatant decanted. Subsequently, the same amounts of reagents (2-octanol, vinyl acetate, and hexane) as for the initial reaction were added to the reused enzyme, and the reaction run in the same conditions.

For thermal stability, 5 mg native or 25 mg immobilized lipase was incubated in hexane, at specified temperatures for a definite time period. Then, the lipase activity was determined in the acylation reaction of 2-octanol, as described in Subchapter 2.3.

3. Results and discussion

3.1 Influence of the silane precursor ratio in binary and ternary mixtures with ionic liquid additive on the immobilized biocatalyst efficiency

Lipases from three microbial species, *Burkholderia cepacia* (Amano PS), *Pseudomonas fluorescens* (Amano AK) and *Candida antarctica* (CALB-Lecta) were immobilized in sol-gel matrices obtained from binary and ternary silane mixtures in the presence of an IL additive, using our methodology presented in the Experimental part. The test reaction was the enantioselective acylation of 2-octanol by vinyl acetate, in hexane medium. The enantioselectivity in this reaction was based on kinetic discrimination, all studied lipases being (R)-selective (Fig. 2), which means that the (R)-2-octyl-acetate enantiomer was the fast reacting enantiomer, following the empirical Kazlauskas rule (Kazlauskas et al., 1991; Rottici et al., 1998).

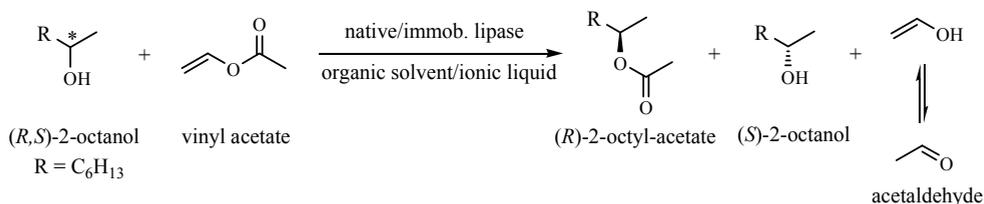


Fig. 2. Reaction scheme of 2-octanol enzymatic acylation

Conversions and product enantiomeric compositions of 2-octanol acylation reactions were measured by chiral GC at 24 hrs reaction time, and used to calculate the immobilized lipase activity, total activity recovery yield, enantiomeric excess (e.e.), and enantiomeric ratio (E) values.

Our previous results demonstrated that the catalytic efficiency of *Pseudomonas fluorescens* lipase in the acylation reaction of secondary alcohols could be enhanced by the presence of

hydrophobic alkyl groups in the hybrid organic-inorganic sol-gel matrix, and utilization of ILs as nonstructural template compounds (Péter et al., 2008; Zarcula et al., 2010). Recently, ternary silane mixtures, containing alkyl-triethoxysilanes, phenyl-triethoxysilane and tetraethoxysilane, have been reported with higher catalytic efficiency than the corresponding binary silane mixtures, for the sol-gel entrapment of Celite-supported *Pseudomonas fluorescens* lipase (Tomin et al., 2010). Based on these results, we used in the immobilization protocol binary and ternary mixtures of methyl-trimethoxysilane (MTMOS), phenyl-trimethoxysilane (PhTMOS), and tetramethoxysilane (TMOS), as well as [Emim]BF₄ or [Omim]BF₄ as additive. It must be pointed out that the total silane precursor amount was the same in all immobilization experiments (6 mmoles).

The immobilization efficiency was excellent for *Burkholderia cepacia* and *Candida antarctica* lipases, as the total activity recovered following immobilization was about 10-fold higher than the total activity of the enzyme subjected to immobilization (Fig. 3). Even in the case of the lowest-performing *Pseudomonas fluorescens* lipase, the recovered total activity value of the entrapped lipase was enough good, up to 90% related to the native lipase.

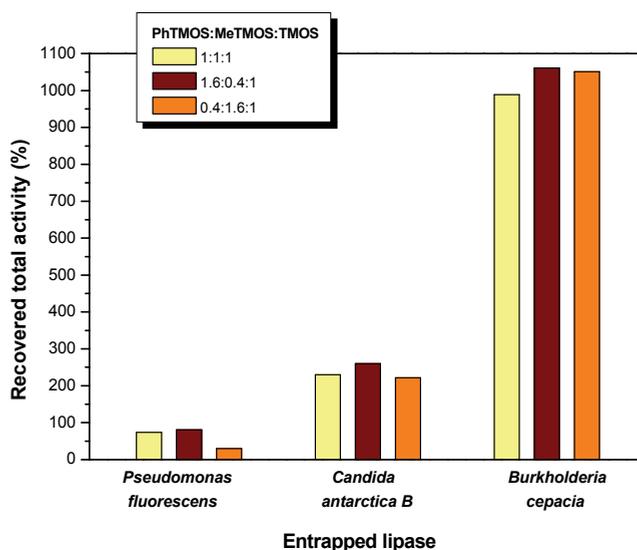


Fig. 3. Influence of silane precursors molar ratio on the relative total activity recovered after sol-gel immobilization of lipases from *Burkholderia cepacia*, *Pseudomonas fluorescens* and *Candida antarctica B*.

Sol-gel entrapped *Burkholderia cepacia* lipase showed higher activity and enantioselectivity, when the immobilization was performed using ternary silane precursor systems, compared to binary mixtures of the same precursors (Table 1).

It is obvious that fine tuning of the sol-gel matrix composition allows maximizing the catalytic efficiency of entrapped enzyme. Among ternary silane precursor mixtures, the highest activity was observed at lower phenyl-group concentration in the precursor silane mixture, but associated with a slightly lower enantioselectivity. The highest enantioselectivity was exhibited by the prepate obtained with equimolar mixture of the

Silane precursors ¹ (molar ratio)	Additive	Conversion ² (%)	Activity ³	e.e. (%)	E
native enzyme	-	23	0.953	74	8
A:C (1:1)	[Omim]BF ₄	38	0.329	79	14
B:C (1:1)	[Omim]BF ₄	13	0.114	78	9
A:B:C (1:1:1)	[Omim]BF ₄	61	0.493	62	19
	[Emim]BF ₄	12	0.105	68	6
A:B:C (1.6:0.4:1)	[Omim]BF ₄	53	0.433	73	16
	[Emim]BF ₄	20	0.165	73	8
A:B:C (0.4:1.6:1)	[Omim]BF ₄	67	0.538	49	15
	[Emim]BF ₄	11	0.091	66	5

¹ A = phenyl-trimethoxysilane; B = methyl-trimethoxysilane C = tetramethoxysilane

² at 24 hrs reaction time

³ transesterification activity of 2-octanol substrate, expressed as ($\mu\text{mole h}^{-1} \text{mg catalyst}^{-1}$)

Table 1. Catalytic efficiency and enantioselectivity of *Burkholderia cepacia* lipase, immobilized by sol-gel entrapment with binary and ternary silane precursor mixtures in different molar ratios, using an ionic liquid as additive.

three silane precursors, and [Omim]BF₄ as ionic liquid additive. The enantiomeric ratio (E) value of this biocatalyst was more than double, compared to the native enzyme. Therefore, we consider the equimolar mixture of the three silane precursors as the best to obtain high catalytic efficiency of sol-gel entrapped lipase. The presence of a more hydrophobic alkyl chain (octyl vs. ethyl) in the cationic part of the IL yielded an immobilized lipase with higher activity and enantioselectivity, irrespective of silane molar ratio used.

Changing the immobilization technique to sol-gel entrapment combined with adsorption, the same ternary silane precursor mixture yielded the highest activity in different conditions as registered for the simple sol-gel entrapment (Table 2). The most active prepare was obtained at increased phenyl group concentration in the sol-gel matrix, and using the IL with shorter alkyl chain (ethyl) in the imidazolium moiety. The explanation should be related to the physical properties of the adsorption support, Celite 545 in our case. As hydrophobicity of the matrix is essential for the catalytic efficiency of the immobilized lipase, it is strongly influenced by physical characteristics of the support used for adsorption, overtaking the influence of alkyl chain length in the IL cationic part. The enantioselectivity slightly increased following immobilization, and in this case the same prepare showed the best values for both activity and enantioselectivity. Using the combined method and fine tuning the silane precursor ratio we obtained an immobilized biocatalyst with excellent performances, as the effective transesterification activity for the 2-octanol substrate was 44% compared to the native enzyme (very high, considering the significant enzyme dilution in the matrix), and the total activity next to immobilization showed a more than 20-fold increase.

The sol-gel entrapped lipase from *Pseudomonas fluorescens* (Amano AK) exhibited the lowest activity and enantioselectivity, considering the investigated enzymes. Using a ternary precursor mixture was also in this case beneficial to the catalytic performance of the immobilized enzyme, compared to binary mixtures of the same silanes (Table 3). Similar to *Burkholderia cepacia* lipase, an equimolar phenyl and methyl group concentration in the silica matrix resulted in higher activity of the sol-gel entrapped enzyme, but at this optimal

precursor composition we found no significant difference associated with the chain length of alkyl group from the imidazolium moiety of the IL used. The low enantioselectivity of this enzyme for the racemic 2-octanol substrate was not changed by immobilization.

Silane precursors ¹ (molar ratio)	Additive	Conversion ² (%)	Activity ³	e.e. (%)	E
native enzyme	-	23	0.953	74	8
A:B:C (1:1:1)	[Omim]BF ₄	29	0.246	76	10
	[Emim]BF ₄	27	0.233	76	10
A:B:C (1.6:0.4:1)	[Omim]BF ₄	40	0.341	75	11
	[Emim]BF ₄	47	0.421	76	15
A:B:C (0.4:1.6:1)	[Omim]BF ₄	9	0.076	62	5
	[Emim]BF ₄	12	0.100	69	6

¹A = phenyl-trimethoxysilane; B = methyl-trimethoxysilane C = tetramethoxysilane

²at 24 hrs reaction time

³transesterification activity of 2-octanol substrate, expressed as ($\mu\text{mole h}^{-1} \text{mg catalyst}^{-1}$)

Table 2. Catalytic efficiency and enantioselectivity of *Burkholderia cepacia* lipase immobilized by sol-gel entrapment combined with adsorption on Celite 435, using ternary silane precursor mixtures in different molar ratios, and an ionic liquid as additive.

Silane precursors ¹ (molar ratio)	Additive	Conversion ² (%)	Activity ³	e.e. (%)	E
native enzyme	-	47	1.910	53	5
A:C (1:1)	[Omim]BF ₄	8	0.065	60	4
B:C (1:1)	[Omim]BF ₄	22	0.191	64	5
A:B:C (1:1:1)	[Omim]BF ₄	45	0.372	53	5
	[Emim]BF ₄	21	0.179	56	4
A:B:C (1.6:0.4:1)	[Omim]BF ₄	41	0.343	56	5
	[Emim]BF ₄	43	0.349	57	5
A:B:C (0.4:1.6:1)	[Omim]BF ₄	21	0.176	53	4
	[Emim]BF ₄	23	0.191	53	4

¹A = phenyl-trimethoxysilane; B = methyl-trimethoxysilane C = tetramethoxysilane

²at 24 hrs reaction time

³transesterification activity of 2-octanol substrate, expressed as ($\mu\text{mole h}^{-1} \text{mg catalyst}^{-1}$)

Table 3. Catalytic efficiency and enantioselectivity of *Pseudomonas fluorescens* lipase, immobilized by sol-gel entrapment with binary and ternary silane precursor mixtures in different molar ratios, and an ionic liquid as additive.

Combining the sol-gel entrapment with adsorption on Celite 545, the influence of relative molar ratio of the three silane precursors showed the same tendency, the highest activity being exhibited by the preparate obtained at equimolar phenyl and methyl group concentration in the matrix. Contrary to the influence observed for *Burkholderia cepacia* lipase, in this case the preparates obtained with [Omim]BF₄ as additive were more active compared to those obtained with [Emim]BF₄, suggesting a more important role of the IL during the combined enzyme entrapment and adsorption (Table 4).

Silane precursors ¹ (molar ratio)	Additive	Conversion ² (%)	Activity ³	e.e. (%)	E
native enzyme	-	47	1.910	53	5
A:B:C (1:1:1)	[Omim]BF ₄	37	0.304	58	4
	[Emim]BF ₄	2	0.015	27	2
A:B:C (1.6:0.4:1)	[Omim]BF ₄	36	0.295	57	5
	[Emim]BF ₄	15	0.131	55	4
A:B:C (0.4:1.6:1)	[Omim]BF ₄	10	0.082	42	3
	[Emim]BF ₄	3	0.025	16	1

¹A = phenyl-trimethoxysilane; B = methyl-trimethoxysilane C = tetramethoxysilane

²at 24 hrs reaction time

³transesterification activity on 2-octanol substrate, expressed as ($\mu\text{mole h}^{-1} \text{mg catalyst}^{-1}$)

Table 4. Catalytic efficiency and enantioselectivity of *Pseudomonas fluorescens* lipase immobilized by sol-gel entrapment combined with adsorption on Celite 435, using ternary silane precursor mixtures in different molar ratios, and an ionic liquid as additive.

The third investigated lipase, from *Candida antarctica* B, showed the highest enantioselectivity in the kinetic resolution of 2-octanol, and an important improvement related to the native enzyme. Regardless to the immobilization method, silane precursor composition, or IL additive used, the reaction reached around 50% conversion in 24 hrs reaction time, at more than 93% enantiomeric excess of the (*R*)-2-octyl acetate product. For this reason, in Table 5 only the results of the combined immobilization method are presented.

Silane precursors ¹ (molar ratio)	Additive	Conversion ² (%)	Activity ³	e.e. (%)	E
native enzyme	-	17	0.705	89	21
A:B:C (1:1:1)	[Omim]BF ₄	51	0.427	95	201
	[Emim]BF ₄	51	0.426	95	201
A:B:C (1.6:0.4:1)	[Omim]BF ₄	51	0.434	95	201
	[Emim]BF ₄	51	0.431	93	114
A:B:C (0.4:1.6:1)	[Omim]BF ₄	51	0.418	95	201
	[Emim]BF ₄	47	0.394	95	104

¹A = phenyl-trimethoxysilane; B = methyl-trimethoxysilane C = tetramethoxysilane

²at 24 hrs reaction time

³transesterification activity on 2-octanol substrate, expressed as ($\mu\text{mole h}^{-1} \text{mg catalyst}^{-1}$)

Table 5. Catalytic efficiency and enantioselectivity of *Candida antarctica* B lipase immobilized by sol-gel entrapment combined with adsorption on Celite 435, using ternary silane precursor mixtures in different molar ratios, and an ionic liquid as additive.

Based on these results, the CALB lipase was selected for the solvent and stability study, as the more serious candidate for a possible industrial application. Although the fine tuning of silane precursor composition and IL additive allows finding the optimal immobilization conditions for every enzyme, the equimolar ratio of the three silanes with [Omim]BF₄ as additive can be considered with generic validity for the sol-gel immobilization of microbial lipases.

3.2 Solvent engineering of 2-octanol acylation reaction catalyzed by sol-gel immobilized *Candida antarctica* B lipase

Although many enzymatic synthetic reactions are performing well in organic solvents, they have the disadvantage to be harmful for the environment. Room-temperature ILs are a possible solution to replace volatile organic solvents with an environment-friendly reaction medium, as they are non-volatile, can be used at higher temperatures, and recycled. Besides the environmental advantages, also improvements of reaction rates, conversion, enantioselectivity and regioselectivity have been reported for various biocatalytic reactions (Moniruzzaman et al., 2010). However, it was not possible to correlate the IL structure with the solvent properties, and a careful selection is needed to find the appropriate IL for every enzyme and application. We investigated the enantioselective acylation of 2-octanol in common organic solvents and different ILs, using CALB lipase immobilized by sol-gel entrapment as catalyst. The reactions were carried out at 40°C. As the employed ILs were not miscible with hexane, the product was recovered by repeated extraction with this compound. Such a procedure has a further advantage, the possibility to easily recycle the catalyst and IL which remain in the non-organic phase, but the utilization of an organic solvent invalidates the environment-friendly nature of the process. To overcome such a drawback, extraction with supercritical CO₂ or evaporation at low pressure should be a solution for syntheses at larger scale, which will be investigated in a future work.

Solvent	Conversion ¹ (%)	Activity ² ($\mu\text{mol h}^{-1} \text{mg}^{-1}$)	e.e. (%)	E
[Emim]BF ₄	54	0.457	83	46
[Hmim]BF ₄	51	0.437	89	58
[Omim]BF ₄	51	0.440	93	114
[Bmim]PF ₆	47	0.402	86	30
[Emim]COOCF ₃	2	0.015	64	5
<i>tert</i> -Butanol	48	0.402	95	113
Acetone	51	0.431	95	201
Tetrahydrofuran	61	0.529	63	20
Acetonitrile	51	0.433	95	201
Toluene	51	0.424	94	146
<i>iso</i> -Octane	51	0.435	95	201
Hexane	51	0.410	95	201

¹ at 24 hrs reaction time

² transesterification activity on 2-octanol substrate, at 24 hrs reaction time

Table 6. Influence of reaction medium on enzymatic acylation of 2-octanol by vinyl acetate, catalyzed by CALB lipase immobilized with PhTMOS:MTMOS:TMOS precursors (1:1:1 molar ratio), and [Omim]BF₄ IL additive

The sol-gel immobilized CALB lipase proved to be once more an excellent catalyst for the investigated reaction, showing high enantioselectivity in all medium- and low-polarity organic solvents tested. This behavior is different as we previously registered with lipase from *Pseudomonas fluorescens*, which had a significantly lower enantioselectivity in acylation reactions of secondary alcohols and showed a strong influence of the solvent (Zarcula et al, 2010). In ILs, the influence of reaction medium was much more significant. The conversion

was very low in [Emim]COOCF₃, the single water-miscible of the tested ILs. Higher conversions in water-immiscible than in water-miscible ILs were also reported by other authors for lipase-catalyzed transesterification reactions (Gorke et al, 2007). ILs with BF₄⁻ anion were more efficient as reaction media than [Bmim]PF₆, considering both activity and enantioselectivity. From this group of ILs with BF₄⁻ anion, the best results were obtained in case of a more hydrophobic alkyl chain (octyl) presence in the imidazolium cationic moiety. Previously, we obtained for 2-hexanol acylation with *Pseudomonas fluorescens* lipase in the same reaction media an inverse effect of the IL polarity (Zarcu et al, 2010). The polarities of all ILs tested are in a close range, between 0.67-0.71 on the Reichardt's scale (Reichardt, 2005), and we presume that other properties, like the IL influence on lipase active conformation and/or miscibility with reagents and reaction products, is more important than the intrinsic polarity of the IL. Therefore, are unlikely to predict the solvent behavior of an IL based on its structure, and the selection of the appropriate reaction medium must be done on experimental basis. For the investigated reaction, the enantioselective acylation of 2-butanol, the optimal reaction medium was 1-octyl-3-methyl-imidazolium tetrafluoroborate, yielding the same results as the best organic solvents.

3.3 Operational and thermal stability of sol-gel entrapped lipases with ionic liquid as immobilization additive

The most important enzyme property which can be improved through immobilization is stability. However, such improvement is not an intrinsic result of immobilization, as in some cases the enzyme stability may decrease after immobilization for different reasons, like undesired interactions between the support and the enzyme. It is now generally accepted that tailor-made immobilization protocols are needed to obtain the best catalytic performances. As for industrial application the main requirement is reuse of the enzyme in as many as possible reaction cycles, to make the process economically feasible, the operational stability of the immobilized enzyme is an essential issue. Sol-gel entrapment not only allows an easier separation of the catalyst at the end of every reaction cycle, but also stabilizes the lipase molecules against aggregation and proteolysis due to possible presence of proteases in the crude enzyme. However, depending on pores size, distribution, and location of the enzyme inside the porous matrix, the enzyme stability could also deteriorate, generated by pore collapsing throughout use or colmation caused by different impurities, resulting in increased steric hindrance.

ILs can improve the operational stability of the sol-gel immobilized lipase by two mechanisms:

- increasing the stability of the enzyme itself;
- acting as non-structural templates during the immobilization process.

This study was performed with *Candida antarctica* B lipase (CALB-Lecta), immobilized by sol-gel entrapment in a matrix obtained from a ternary precursor mixture of PhMTOS:MTMOS:TMOS (1.6:0.4:1 molar ratio), using [Omim]BF₄ as immobilization additive. The same enzyme, immobilized by sol-gel entrapment combined with adsorption on Celite, was also studied, using as reference the native lipase (Fig. 3). The reactions were carried out in hexane, at 40°C, for 24 hrs.

As results from Fig. 3, the immobilized biocatalysts showed a remarkable operational stability, as the relative activities (related to the activity measured in the initial reaction with the same catalyst) were above 0.9 for the sol-gel entrapped lipase and 0.8 for the lipase

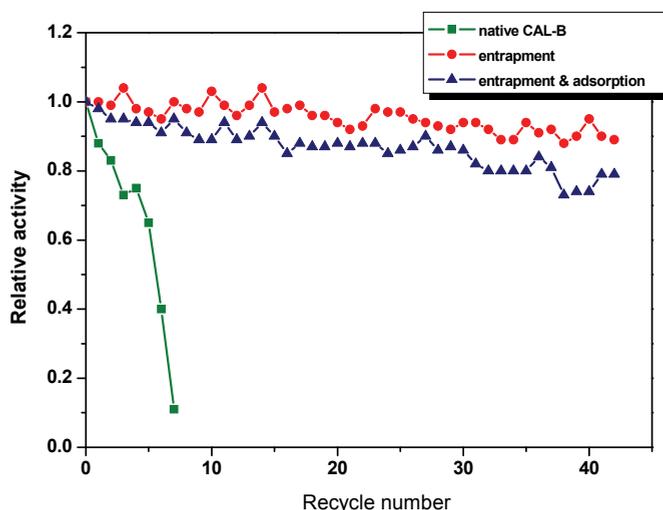


Fig. 3. Operational stability of *Candida antarctica* B lipase, immobilized by sol-gel entrapment and sol-gel entrapment combined with adsorption.

immobilized by the combined method, for more than 40 reuse cycles. In the same time, the native lipase exhibited a very early decline, to less than 20% of the initial activity after only 6 reuse cycles. Such an important difference can be explained by the protective effect of the sol-gel network and IL incorporated in the matrix against any kind of mechanical or chemical inactivation of the enzyme during the chemical reaction and enzyme recovery steps. The enantioselectivity did not change during the whole enzyme recycle process, the enantiomeric excess of the product remaining at the same values, 94-95% (data not shown). Although the sol-gel entrapment does not influence the intrinsic structural stability of the lipase, it results in increased operational stability and manifold reuse possibility.

Thermal stability is another important property of an industrial enzyme, as in many processes a higher temperature than usually employed in enzymatic reactions is needed, to enhance the reaction rate. Thermal inactivation is typically related to unfolding of enzymes, and immobilization could prevent the conformational changes involved in this type of inactivation. The presence of ILs in the immobilization matrix should induce a more prominent stabilizing effect by maintaining the active structure of the enzyme through specific interactions. Additionally, immobilized enzymes are better protected against local temperature increase in the reactor zone close to the heating surface.

Our research was focused on thermal stability of the same immobilized lipases used in the operational stability study. The biocatalysts were maintained at different temperatures for 1 hr, in hexane, and subsequently used for the acylation reaction of 2-octanol by vinyl acetate, in the same solvent, at 40°C.

The results from Table 7 show an excellent thermal stability of immobilized lipases in the organic solvent in the studied temperature range, regardless to immobilization method or lipase nature. Small variation of activity values can be attributed to differences in distribution of the enzyme in the matrix, even if the samples have been run in duplicate.

Temperature (°C)	Activity ¹ ($\mu\text{mole h}^{-1} \text{mg catalyst}^{-1}$)					
	<i>Pseudomonas fluorescens</i> lipase		<i>Burkholderia cepacia</i> lipase		<i>Candida antarctica</i> B lipase	
	SG ²	SGA ³	SG ²	SGA ³	SG ²	SGA ³
40	0.343	0.295	0.433	0.341	0.429	0.434
45	0.338	0.299	0.438	0.286	0.418	0.414
50	0.316	0.250	0.395	0.274	0.423	0.406
55	0.315	0.286	0.398	0.289	0.411	0.397
60	0.316	0.282	0.393	0.315	0.424	0.404
65	0.325	0.297	0.413	0.285	0.426	0.413
70	0.335	0.298	0.412	0.291	0.420	0.425
75	0.331	0.310	0.411	0.273	0.424	0.428
80	0.326	0.319	0.415	0.286	0.419	0.430

¹transesterification activity on 2-octanol substrate, at 24 hrs reaction time

²immobilized by sol-gel entrapment

³immobilized by sol-gel entrapment with adsorption

Table 7. Influence of temperature on the activity of sol-gel entrapped lipases. The biocatalyst samples were incubated 1 hr in hexane, at temperatures between 40-80°C.

Based on the high thermal stability resulted from this study, the experiments were continued for *Candida antarctica* B lipase in more severe temperature conditions, by incubation in hexane at 80°C, for 5 days. Even in these conditions, the immobilized lipase exhibited remarkable stability, the activity values remaining practically unchanged, while the native enzyme lost about 50% of the initial activity after 5 days (Fig. 4). It was no

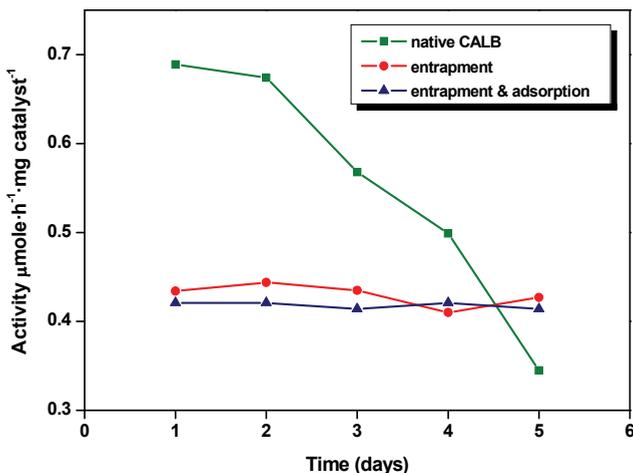


Fig. 4. Influence of temperature on the activity of native and sol-gel entrapped *Candida antarctica* B lipase. The biocatalyst samples were incubated in hexane, at 80°C, and used subsequently for the acylation of 2-octanol.

significant activity difference between the lipase immobilized by simple sol-gel entrapment and entrapment by the combined method. The enantioselectivity of lipase was also not affected, remaining at the same value of 94-95% (*R*)-2-octyl-acetate enantiomeric excess. High thermal stability of lipase from *Candida cylindracea* entrapped in sol-gels from TMOS and propyl-trimethoxysilane deposited on Celite, using a different immobilization protocol, was previously reported (Kawakami & Yoshida, 1996), but the incubation time was not extended beyond 1 hr. Our results demonstrate the possibility to maintain the catalytic properties of sol-gel immobilized enzymes intact for a much longer time period.

4. Conclusions

Sol-gel encapsulation of lipases is an emerging method to obtain biocatalysts with high activity and enantioselectivity for possible industrial applications. Utilization of specific ionic liquids as immobilization additives make possible the design of a more adequate sol-gel matrix to prevent enzyme leakage during operation and preserve the catalytic activity. The fine tuning of silane precursor mixture composition and ionic liquid structure allows developing the best biocatalyst for a specific application. For enantioselective acylation of 2-octanol, the most effective immobilization protocol involved a ternary mixture of PhTMOS:MTMOS:TMOS in equimolar ratio and [Omim]BF₄ as IL additive. Combining the sol-gel entrapment with adsorption on a porous support did not cause any damage of enzyme catalytic properties or stability, moreover we registered a very important (up to 20-fold) increase of the total immobilized activity related to the total activity of native lipase. Among three microbial lipases investigated, *Burkholderia cepacia* lipase showed the highest activity and *Candida antarctica* B lipase the highest enantioselectivity in the studied reaction. The more hydrophobic [Omim]BF₄ was also the best reaction medium among several ILs tested, with performances similar to organic solvents, but having the advantage of avoiding the operation with toxic volatile compounds in the synthetic process. The obtained biocatalysts demonstrated excellent operational and thermal stability properties, recommending them for scale-up.

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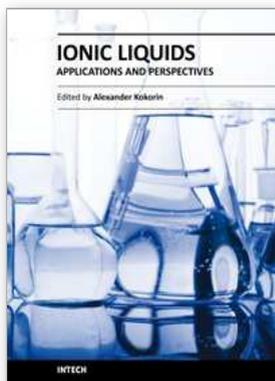
5. References

- Bell, G., Halling, P.J., May, L., Moore, B.D., Robb, D.A., Ulijn, R. & Valivety, R.H. (2001). Methods for Measurement and Control of Water in Nonaqueous Biocatalysis in: *Methods in Biotechnology: Enzymes in Nonaqueous Solvents*, Vulfson, E.N., Halling, P.J., Holland H.L. (Eds.), 105-126, Humana Press, Totowa (N.J.)
- Bornscheuer, U.T. (2003). Immobilizing enzymes: how to create more suitable biocatalysts, *Angewandte Chemie, International Edition*, 42, 3336 - 3337.
- Buchholz, K., Kasche V. & Bornscheuer, U.T. (2005). *Biocatalysts and enzyme technology*, Wiley-VCH Verlag, Weinheim.
- Cao, L. (2005). *Carrier-bound immobilized enzymes: principles, applications and design*, Wiley-VCH Verlag, Weinheim.

- Cao, X., Yang, J., Shu, L., Yu, B. & Yan, Y. (2009). Improving esterification activity of *Burkholderia cepacia* lipase encapsulated in silica by bioimprinting with substrate analogues, *Process Biochemistry*, 44, 177-182.
- Chen, C.-S., Fujimoto, Y., Girdauskas, G. & Sih, C.J. (1982). Quantitative analyses of biochemical kinetic resolutions of enantiomers, *Journal of the American Chemical Society*, 104, 7294-7299.
- Chen, J.-P. & Lin, W.-S. (2003). Sol-gel powders and supported sol-gel polymers for immobilization of lipase in ester synthesis, *Enzyme and Microbial Technology*, 32, 801-811.
- Christensen, M.W., Andersen, L., Husum, T.L. & Kirk, O. (2003). Industrial lipase immobilization, *European Journal of Lipid Science & Technology*, 105, 318-321.
- Ciriminna, R. & Pagliano, M. (2006). Recent Uses of Sol-Gel Doped Catalysts in the Fine Chemicals and Pharmaceutical Industry *Organic Process Research & Development*, 10, 320-326.
- Dai, S., Ju, Y.H., Gao, H.J., Lin, J.S., Pennycook, S. J. Barne & C. E. (2000). Preparation of silica aerogel using ionic liquids as solvents, *Chemical Communications*, 243-244.
- Dai, D.-Z. & Xia L.-M. (2006). Resolution of (R,S)-2-octanol by *Penicillium expansum* PED-03 lipase immobilized on modified ultrastable-Y molecular sieve in microaqueous media, *Process Biochemistry*, 41, 1455-1460.
- Fehér, E., Major, B., Bélafi-Bakó, K. & Gubicza L. (2007). On the background of enhanced stability and reusability of enzymes in ionic liquids, *Biochemical Society Transactions*, 35, 1624-1627.
- Gorke, J.T., Okrasa, K., Louwagie, A., Kazlauskas, R.J. & Srienc, F. (2007). Enzymatic synthesis of poly(hydroxyalkanoates) in ionic liquids, *Journal of Biotechnology*, 132, 306-313.
- Hara, P., Hanefeld, U. & Kanerva, L.T. (2008). Sol-gels and cross-linked aggregates of lipase PS from *Burkholderia cepacia* and their application in dry organic solvents, *Journal of Molecular Catalysis B: Enzymatic*, 50, 80-86.
- Karout, A. & Pierre, A.C. (2007). Silica xerogels and aerogels synthesized with ionic liquids, *Journal of Non-Crystalline Solids*, 353, 2900-2909.
- Kazlauskas, R.J. Weissfloch, A.N.E., Rappaport, A.T., & Cuccia L.A. (1991). A rule to predict which enantiomer of a secondary alcohol reacts faster in reactions catalyzed by cholesterol esterase, lipase from *Pseudomonas cepacia*, and lipase from *Candida rugosa*, *Journal of Organic Chemistry*, 56 (8), 2656-2665.
- Kawakami, K. & Yoshida, S. (1996). Thermal stabilization of lipase by sol-gel entrapment in organically modified silicates formed on Kieselguhr, *Journal of Fermentation and Bioengineering*, 82 (3), 239-245.
- Kawakami, K., Takahashi, R., Shakeri, M. & Sakai S. (2009). Application of a lipase-immobilized silica monolith bioreactor to the production of fatty acid methyl esters, *Journal of Molecular Catalysis B: Enzymatic*, 57, 194-197.
- Kiss, C., Zarcu, C., Csunderlik, C. & Péter, F. (2007). Enantioselective acylation of secondary alcohols by biocatalysis with sol-gel immobilized *Pseudomonas fluorescens* lipase, *Revista de Chimie (Bucharest)*, 58 (8), 799-804.
- Lee, S.H., Doan, T.T.N., Ha, S.H. & Koo, Y.-M. (2006). Using ionic liquids to stabilize lipase within sol-gel derived silica, *Journal of Molecular Catalysis B: Enzymatic*, 45, 57-61.

- Mateo, C., Palomo, J.M., Fernandez-Lorente, G., Guisan, J.M. & Fernandez-Lafuente R. (2007). Improvement of enzyme activity, stability and selectivity via immobilization techniques, *Enzyme and Microbial Technology*, 40, 1451-1463.
- Moniruzzaman, M., Nakashima, K., Kamiya, N. & Goto, M. (2010). Recent advances of enzymatic reactions in ionic liquids, *Biochemical Engineering Journal*, 48 (3), 295-314.
- Moon, Y.H., Lee, S.M., Ha, S.H. & Koo Y.-M. (2006). Enzyme-catalyzed reactions in ionic liquids, *Korean Journal of Chemical Engineering*, 23(2), 247-263.
- Péter, F. (2005). Biotransformări enzimatică, Editura Politehnica, Timișoara.
- Péter, F., Poppe, L., Kiss, C., Szócs-Biró, E., Preda, G., Zarcu, C., Olteanu, A. (2005). Influence of precursors and additives on microbial lipases stabilized by sol-gel entrapment, *Biocatalysis and Biotransformation*, 23 (3-4), 251-260.
- Péter, F., Zarcu, C., Kakasi-Zsurka, S., Croitoru, R., Davidescu, C., Csunderlik, C. (2008). Solid-phase lipase biocatalysts for kinetic resolutions, *Journal of Biotechnology*, 136S, S374.
- Pierre, A.C. (2004). The sol-gel encapsulation of biocatalysts, *Biocatalysis and Biotransformation*, 22, 145-170.
- Pirozzi, D., Fanelli, E., Aronne, A., Pernice, P. & Mingione, A. (2009). Lipase entrapment in a zirconia matrix: Sol-gel synthesis and catalytic properties, *Journal of Molecular Catalysis B: Enzymatic*, 59, 116-120.
- Poole, C.F. (2004). Chromatographic and spectroscopic methods for the determination of solvent properties of room temperature ionic liquids, *Journal of Chromatography A*, 1037, 49-82.
- Reetz, M.T., Zonta, A. & Simpelkamp J. (1996). Efficient immobilization of lipases by entrapment in hydrophobic sol-gel materials, *Biotechnology and Bioengineering*, 49, 527-534.
- Reetz M.T. & Jaeger K.-E. (2003). Overexpression, immobilization and biotechnological application of *Pseudomonas* lipases, *Chemistry and Physics of Lipids*, 93, 3-14.
- Reetz, M.T., Tielmann, P., Wiesenhöfer, W., Könen, W. & Zonta, A. (2003). Second generation sol-gel encapsulated lipases: robust heterogeneous biocatalysts, *Advanced Synthesis & Catalysis*, 345, 717-728.
- Reichardt, C. (2005). Polarity of ionic liquids determined empirically by means of solvatochromic pyridinium N-phenolate betaine dyes, *Green Chemistry*, 7, 339-351.
- Rotticci, D., Häffner, F., Orrenius, C., Norin, T., & Hult, K. (1998). Molecular recognition of *sec*-alcohol enantiomers by *Candida antarctica* lipase B. *Journal of Molecular Catalysis B: Enzymatic*, 5, 267-272.
- Sheldon R.A. (2007). Enzyme immobilization: the quest for optimum performance, *Advanced Synthesis & Catalysis*, 349, 1289-1307.
- Sureshkumar, M. & Lee, C.-K. (2009). Biocatalytic reactions in hydrophobic ionic liquids. *Journal of Molecular Catalysis B: Enzymatic*, 60, 1-12.
- Tomin, A., Weiser, D., Hellner, G., Bata, Zs., Corici, L., Péter, F., Koczka, B. & Poppe, L. (2010). Fine-tuning the second generation sol-gel lipase immobilization with ternary alkoxysilane precursor systems, *Process Biochemistry*, doi:10.1016/j.procbio.2010.07.021.
- Van Rantwijk, F., Lau, R.M. & Sheldon, R.A. (2003). Biocatalytic transformations in ionic liquids, *Trends in Biotechnology*, 21, 131-138.

- Weingärtner, H. (2008). Understanding ionic liquids at the molecular level: facts, problems, and controversies, *Angewandte Chemie, International Edition*, 47, 654-670.
- Whittall, J. & Sutton, P., Editors (2010). *Practical methods for biocatalysis and biotransformations*, John Wiley&Sons, Chichester.
- Zarcula, C., Croitoru, R., Corici, L., Csunderlik, C. & Péter, F. (2009). Improvement of lipase catalytic properties by immobilization in hybrid matrices, *International Journal of Chemical and Biomolecular Engineering*, 2(3), 138-143.
- Zarcula, C., Kiss, C., Corici, L., Croitoru, R., Csunderlik, C. & Péter, F. (2009). Combined sol-gel entrapment and adsorption method to obtain solid-phase lipase biocatalysts, *Revista de Chimie (Bucharest)*, 60, 922-927.
- Zarcula C., Corici L., Croitoru R., Ursoiu A. & Péter F. (2010) Preparation and properties of xerogels obtained by ionic liquid incorporation during the immobilization of lipase by the sol-gel method, *Journal of Molecular Catalysis B: Enzymatic*, 65 (1-4), 79-86.
- Zhou, Y., Schattka, J.H., Antonietti, M. (2004). Room-temperature ionic liquids as template to monolithic mesoporous silica with wormlike pores via a sol-gel nanocasting technique, *Nano Letters*, 4 (3), 477-481.



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