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mini review

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Non-Aqueous Biocatalysis in Heterogeneous Solvent Systems

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Summary

Biocatalysis has become a useful alternative to chemical transformations for the production of a range of compounds with applications in the food, feed, chemical and pharmaceutical industries. However, it is not necessarily an easy task to obtain the desired levels of performance in terms of rate, yield and selectivity of the reaction. One strategy for optimizing biocatalyst performance is to use non-conventional media, such as non-aqueous heterogeneous systems. In this article, we highlight some of the current trends in biocatalysis in such systems, focusing on reverse micelles, supercritical fluids and ionic liquids.

Key words: biocatalysis, non-conventional media, reverse micelles, supercritical fluids, ionic liquids

Introduction

In biocatalysis, the term non-conventional media refers to systems that use solvents other than water or the addition of components to aqueous systems with the intention of favoring specific properties of the biocatalyst or the reaction catalyzed by it. Non-conventional media can be used for biocatalysis with either enzymes or whole cells.

The solvent can cause modifications in the conformation of the enzyme, altering either its catalytic efficiency or specificity. For example, through the rational use of non-conventional media, it may be possible to increase the enantioselectivity of the reaction catalyzed by the biocatalyst. Non-aqueous reaction media may also increase the stability of the enzyme (*1–3*). A further advantage is that the risk of microbial contamination is lower than is the case in aqueous systems. Non-conventional media are of special interest for hydrolases since low water contents can be used in order to favor synthesis reactions and, in the case of lipases, to provide better solubility of hydrophobic substrates.

The water-restricted organic systems used in biocatalysis can be classified as heterogeneous and homogeneous. Homogeneous systems are reviewed by Torres and Castro (*4*). Heterogeneous systems include liquid--liquid macro-heterogeneous systems, in which water represents 1 to 5 % of the reaction medium, liquid-solid

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macro-heterogeneous systems and micro-heterogeneous systems. This mini-review highlights some of the recent developments in the use of heterogeneous non-conventional media for biocatalysis, such as reverse micelles, supercritical fluids and ionic liquids.

General Considerations about Biocatalysis in Non-Conventional Heterogeneous Media

In macro-heterogeneous systems there is a visible separation of phases, such as systems that use immobilized enzymes or powdered enzymes. In micro-heterogeneous systems the separation of phases can only be observed microscopically, such as is the case with reverse micelles. In reverse micelles, the amount of water in the system is generally expressed in terms of the parameter W_0 ([H_2O]/[surfactant]), while in other systems it is expressed in terms of the water activity (a_w) (*5–8*). In the case of reactions catalyzed by hydrolases, the amount of water can be manipulated to favor either synthesis or hydrolysis reactions. In the reversed micellar system it also affects the size of the micelles and the conformation of the enzyme within the micelles.

Unfortunately, despite the advantages that biocatalysis in organic solvent-based systems can bring, the catalytic activities of enzymes in these systems are typically much lower than activities in aqueous solutions (*9*). Further, protein stability is lower in water-miscible solvents (–2.5<log P<0), such as acetone and ethers, than in hydrophobic solvents (2<log P<4), such as alkanes or haloalkanes. Hydrophobic organic solvents do not strip off the crucial bound water from the enzyme surface (*10–12*), while hydrophilic organic solvents strip this water from the enzymes, leading to the unfolding of the molecule (*13*). As a result, acceptable stability of bacterial and fungal enzymes in hydrophilic organic solvents is rare. Two enzymes that are reasonably stable in hydrophilic organic solvents are a lipase of *Pseudomonas mendocina* PK12CS (*14*), which maintained an 83 % residual activity after 2.5 h incubation in 100 % ethanol, and a lipase of *Bacillus megaterium* CCOC-P2637, which was not only stable but also showed activation at ethanol and acetone volume fraction as high as 80 % and maintained its activity in pure isopropanol (*15*). However, the general lack of stability in hydrophilic solvents represents a problem for the use of hydrolases, such as in reactions involving the esterification of sugars during the production of biosurfactants, given that in these reactions the medium contains polar solvents such as 2-metil-2-butanol (*16*).

Successful biocatalysis in non-conventional media has been reported, the main applications being in ester and peptide synthesis, the resolution of chiral building blocks and the production of cocoa butter substitutes (*17*). Four of the fifteen industrial processes using hydrolases cited by Krishna (*18*) are carried out in the presence of organic solvents: the synthesis of chiral amines and alcohols in MTBE-ethylmethoxy acetate, the synthesis of an anti-cholesterol drug in toluene, the generation of an intermediate in the synthesis of Diltiazem in a water-toluene mixture and the synthesis of isopropyl palmitate and myristate in 2-propanol.

Reverse Micelles

The low water content necessary to favor synthesis reactions in organic media by hydrolases can be achieved by micro-encapsulation of the biocatalyst within reverse micelles. Reverse micelles are relatively ordered structures that consist of a water pool surrounded by a surfactant layer, with the hydrophobic moieties of the surfactant molecules interacting with the bulk hydrophobic solvent (Fig. 1). Since they are dynamic structures, the micelles can exchange their constituents (biocatalyst, water, substrates and products) between each other and also with the bulk organic solvent. The reversed micellar system is particularly attractive for biocatalysis because it mimics the »natural« environment that many enzymes experience within cells. Many surfactants and solvents can be used, the AOT (sodium bis-2-ethylhexyl sulfosuccinate)/isooctane system being one of the most suitable systems (*19–22*), since the reverse micelles formed by this surfactant are very stable over a wide range of concentrations in the absence of co-surfactants.

The reversed micellar system is especially interesting for lipases, which are activated in the presence of an interface, since this system provides a high interfacial area, with the enzyme anchoring on the aqueous side of the AOT interface. In addition, lipase-catalyzed reac-

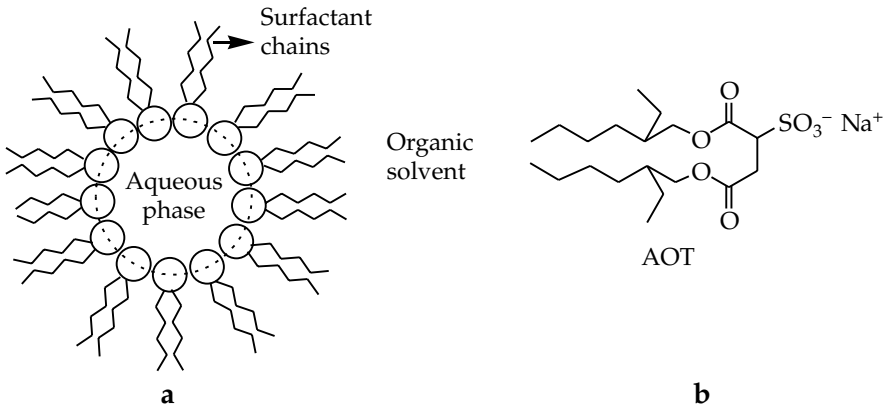


Fig. 1. (a) Schematic representation of a reverse micelle, and (b) the chemical structure of AOT (sodium bis-2-ethylhexyl sulfosuccinate)

Table 1. Recent examples of reactions catalyzed by enzymes in reversed micellar systems

Source (Enzyme)	Surfactant	Organic solvent	Reaction	Reference
Thermomyces lanuginosa (lipase)	AOT	Isooctane	Synthesis of ethyl-laurate	*25*
α-chymotrypsin	AOT	*n*-heptane	Hydrolysis of 2-naphthyl acetate	*26*
Bacillus megaterium (lipase)	AOT	*n*-heptane	Hydrolysis of *p*NPP	*15*
Rhizopus delemar (lipase)	AOT	Isooctane	Hydrolysis of triolein	*27*
Mucor javanicus (lipase)	AOT	Isooctane	Acylation of doxorubicin	*28*
Horse heart (Microperoxidase-11)	AOT	Isooctane	Oxidative decolorization of azo and anthraquinone dyes	*29*
Candida lypolytica (lipase)	AOT	Isooctane	Esterification of octanoic acid with 1-octanol	*30*

Key: AOT (sodium bis-2-ethylhexyl sulfosuccinate), *p*NPP (*p*-nitrophenylpalmitate)

tions often involve hydrophobic substrates, which are readily soluble in the bulk organic phase. Further, some enzymes are highly stable in reverse micelles, such as α-chymotrypsin and the lipase of *Chromobacterium viscosum* (*23*).

Due to the complexity of the reversed micelle system, with the addition of different enzymes, buffers, salts, substrates and products influencing the size and shape of the micelles formed, it is difficult to make predictions as to how the system will perform in a specific case. The selection of the reversed micelle system is typically based on the successes reported in the literature for similar enzymes and reactions (*23,24*). Table 1 reports some such reactions, with potential applications in the food, pharmaceutical and chemical industries and also in the environmental area (*15,25–30*).

The results reported for reversed micellar systems for the synthesis of esters with applications in the food sector, such as ester-based aromas, are better, in terms of overall conversion and reaction rate, than those reported for other water-restricted media. For instance, for ethyl-laurate synthesis by *Thermomyces lanuginosa* lipase, a yield of 90 % was obtained after 60 min in an AOT--isooctane reversed micellar system (*25*), whereas 168 h was required for a 95 % yield of ethyl-caproate when lyophilized powder of the esterase of *Bacillus licheniformis* was added directly to *n*-heptane (*31*) and 72 h was required for a 95 % yield of isoamyl acetate using an immobilized lipase of *Rhizopus miehei* in isooctane (*32*).

Before reversed micellar systems can be effectively applied at industrial scale, there are some issues to be solved. It is difficult to recover the product from reverse micelles at large scale due to the presence of the surfactant and other components of the system, such as protein and water. A possible solution is continuous operation in membrane reactors in which an ultrafiltration membrane retains the micelles and therefore also the enzyme, while the small molecules of substrate and products pass freely. Such membrane bioreactors have been used for ester synthesis and for peptide synthesis (*33, 34*). The main problem of continuous membrane bioreactors is that the enzyme needs to be stable for long periods, which is not always the case. Recently, ester synthesis in such reactor was optimized at laboratory scale, using cutinase (a small hydrolase that acts either as an esterase or as a lipase) as the biocatalyst. A stable 60 % conversion to the ester was achieved over five weeks of continuous operation, during which the enzyme lost only 20 % of its initial activity (*23*). Another problem of continuous membrane bioreactors was noted: the surfactant passes the membrane in the form of small aggregates or as monomers, contaminating the product stream. This makes the separation and purification of the product more difficult, especially when it is to be used in food or pharmaceuticals or other products that require either non-toxic or highly pure products (*23*).

Supercritical Fluids (scF)

Enzymes can express activity in supercritical and near-supercritical fluids, such as carbon dioxide, freons (CHF_3), hydrocarbons (ethane, ethene, propane) or inorganic compounds (SF_6, N_2O). The most commonly used system is supercritical carbon dioxide ($scCO_2$), which is probably explained by the fact that its critical point of 73.8 bar and 31.1 °C makes equipment design and reaction set-up relatively simple (*17*). Supercritical fluids, which represent a state between the gaseous and liquid phases of the compound, exhibit properties similar to those of hydrophobic solvents such as hexane, so it is likely that the activities and stabilities of enzymes in these systems will be similar to those presented in hydrophobic solvents. Although the use of supercritical fluids is not restricted to hydrolases, the use of this class of enzymes, especially lipases, dominates (Table 2) (*35–57*).

Small changes in the temperature or pressure of a supercritical fluid may result in great changes in its viscosity and of the diffusivity and solubility of compounds dissolved within it. This may allow control of the rate and enantioselectivity of enzyme-catalyzed reactions. The main advantages of the system are the high diffusion rates (one or two orders of magnitude higher than in common solvents), which facilitate transport phenomena and can increase the bioconversion rate. In some cases a high diffusion rate can also facilitate product separation. Further, supercritical fluids such as carbon dioxide are non-toxic and can be removed easily after the reaction. The main drawback of supercritical reaction media is that the process requires reactors and auxiliary equipment that can withstand high pressures, which increases process costs. In addition, the use of supercritical carbon dioxide can have adverse effects on enzymes, for example, by decreasing the pH of the microenvironment of the enzyme, by the formation of carbamates due to covalent modification of free amino groups at the surface of the protein and by deactivation during pressurization/depressurization cycles (*17,41*).

Table 2. Different types of reactions catalysed by enzymes in supercritical fluids (scFs) and in ionic liquids (ILs)

Enzyme/Source	System	Reaction	Reference	Comments
Free and immobilized lipases: *Rhyzomucor meihei*, *P. fluorescens*, *Rhizopus javanicus*, *R. niveus*, *C. rugosa*, porcine pancreas	$scCO_2$ and scPropane	Ester synthesis: oleyl oleate	*35*	Economy of the process is given
Free and immobilized lipases: *C. antarctica*, *Mucor miehei*	$scCO_2$ and ILs	Synthesis of glycidyl esters: kinetic resolution of *rac*-glycidol	*36*	Activity up to 95-times enhanced in ILs. Succesful combination of scF and ILs
Recombinant free epoxide hydrolase	ILs	Stereoselective hydrolysis of epoxides	*37*	Reaction rates and stereoselectivity comparable with those in buffer solution
Lipase: *C. antarctica* Novozyme 435	scMethanol, scEthanol, and $scCO_2$	Synthesis of biodiesel	*38*	Complete conversion for scMethanol, scEthanol; 30 % conversion for $scCO_2$
Lipase: *C. antarctica* Novozyme 435	$scCO_2$ with ethanol	Hydrolysis of retinyl palmitate and α-tocopheryl acetate	*39*	Optimized conditions applied to fat-soluble vitamin determination
Immobilized lipase: *M. miehei* (Lipozyme)	$scCO_2$	Hydrolysis of blackcurrant	*40*	Simultaneous extraction and hydrolysis of the oil
Immobilized lipase: *C. antarctica*	$scCO_2$	Butyl butyrate synthesis	*41*	Combines scF with membrane technology
Immobilized lipase: *P. cepacia*	BMIM-BF_6	Resolution of racemic alcohols	*42*	The addition of triethylamine to ILs enhanced the rate of reaction
Immobilized *C. antarctica B* lipase	ILs (several)	Amine synthesis	*43*	Enantioselective acylation of 1-phenylethylamine in BDMIM-TFMS
Lipase: *C. rugosa*	BMIM-BF_4, HMIM-BF_4, BMIM-BF_6	Enantioselective hydrolysis	*44*	ILs as co-solvents markedly enhanced enantioselectivity
Immobilized *C. antarctica B* lipase	BMIM-BF_6 EMIM-BF_4	Enantioselective acylation	*45*	Increased reaction rate, decreased enantioselectivity
Lipase: *C. rugosa*	ILs (several)	Esterification of 2-chloropropanoic acid with 1-butanol	*46*	Highest conversion for BMIM-BF_6
Immobilized esterases: *Bacillus stearothermophilus* and *B. subtilis*	ILs (several)	Transesterification of 1-phenylethanol	*47*	Higher stability in ILs as compared to organic solvents
Lipase: *C. rugosa*	BMIM-BF_6 ONIM-PF_6	Esterification of 2-substituted-propanoic acids and 1-butanol	*48*	Higher enantioselectivity than in *n*-hexane
Glucose oxidase: *Aspergilus niger* Peroxidase: *Coprinus cinereus*	BMIM-BF_6	Oxidation of sulfides	*49*	High operational stability of the enzymes. Recycling of mixture IL–enzymes possible
Lipase: *C. rugosa*	BMIM-BF_6, MOEMIM-BF_6	Acylation of glycosides	*50*	Higher reaction rates and selectivity than in conventional organic solvents
Immobilized and free lipases: *P. cepacia*	BMIM-BF_6, BMIM-BF_4	Transesterification of 2-hydroximethyl-1,4--benzodioxane	*51*	The enzyme-IL mixture could be recycled for several runs
Lipase: *C. antarctica* Novozyme 435	BDMIM-BF_4	Transesterification using vinyl-acetate as acyl donor	*52*	The lipase was reused for 10 times without losing enantioselectivity and reactivity
Immobilized lipase: *P. cepacia*	BMIM-BF_4, BMIM-BF_6	Hydrolysis and alcoholysis of 3,4,6-tri-O-acetyl-D-glucal	*53*	High regioselectivity
Immobilized *C. antarctica B* lipase and α-chymotrypsin	ILs (several)	Transesterification reactions	*54*	All ILs improved the thermal stability of both enzymes

Table 2. (Continued)

Enzyme/Source	System	Reaction	Reference	Comments
Whole cells: baker's yeast	BMIM-BF_6/buffer	Reduction of ketones	*55*	Maintenance of activity in presence of a small aqueous phase
Whole cells: *Rhodococcus* R312	BMIM-BF_6/water	Biotransformation of 1,3-dicyanobenzene	*56*	Specific activity of the biocatalyst in the ILs-water system greater than in the water-toluene system
Mandelate racemase: *Pseudomonas putida*	MMIM-$MeSO_4$, BMIM-$OcSO_4$	Kinetic resolution of mandelic acid	*57*	Reaction rate strongly influenced by the a_w

Key: BDMIM-BF_4: 1-butyl-2,3-dimethylimidazolium tetrafluoroborate
BDMIM-TFMS**:** 1-butyl-2,3-methylimidazolium trifluormethane sulphonate
BMIM-BF_4, BMIM-BF_6: 1-butyl-3-methylimidazolium tetrafluoroborate and hexafluoroborate
BMIM-$OcSO_4$: 1-butyl-3-octylsulphate
EMIM-BF_4: 1-ethyl-3-methylimidazolium tetrafluoroborate
HMIM-BF_4: 1-hexyl-3-methylimidazolium tetrafluoroborate
MMIM-$MeSO_4$: 1,3-dimetylimidazolium methylsulphate
MOEMIM-BF_6: 1-methoxyethyl-3-methylimidazolium hexafluoroborate
ONIM-PF_6: 1-octyl-3-nonyl-imidazolium hexafluorophosphate

Ionic Liquids (ILs)

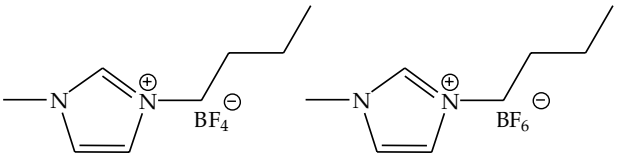
The most exciting recent development in biocatalysis in non-conventional media is the use of ionic liquids (ILs) to improve the activity, stability and selectivity of enzymes. Ionic liquids are low melting point salts that are liquid at room temperature. They are composed entirely of ions and are considered to be highly polar solvents (see Table 3) (*58*,*61*). They possess negligible vapor pressures, which can be taken advantage of for the separation of volatile products, such as water, driving the reaction equilibrium towards product formation (*43*). One of the main advantages of using ionic liquids as reaction media is that, due to the wide range of possible counter-ions, it is possible to tune their solvent properties, so that they can dissolve many different compounds used in chemical and biochemical synthesis reactions (*58*). Further, ionic liquids are claimed to contribute to »green chemistry« since they are non-volatile at room temperature, in contrast to organic solvents.

The application of ionic liquids as non-aqueous reaction media in biocatalysis is still in its infancy. Until 2002 (*54*), only three papers described the use of ionic liquids for hydrolase-catalyzed reactions, all of them quite successful. Various articles have appeared since then, including three excellent reviews about their application in biocatalysis (*59–61*). Also, the review by Gordon (*58*) deserves a mention because it covers chemical and biochemical aspects of catalysis in ionic liquids.

Although some groups have reported contradictory results regarding enzymatic activity in ionic liquids (*60*), many biocatalyzed reactions show advantages when carried out in ionic liquids. Table 2 presents some recent examples of processes that were carried out in ionic liquids, using enzymes and whole-cells. Claims have been made for improved reaction rates (*42*,*45*,*46*,*50*), increased selectivity (*37*,*44*,*48*,*50*,*53*), improved enzyme stability (*47*,*49*,*54*) and easier recycling of the biocatalyst (*49*,*51*, *52*,*58*).

As with supercritical fluids, many of the processes carried out in ionic liquids have involved lipases, due to their tolerance of organic solvents and their ability to catalyze a broad range of reactions. With lipases, the focus is typically on the synthesis of pure enantiomers or on the resolution of racemates (Table 2). There are also reports of the use of ionic liquids with other enzymes, such as mandolases (*57*), peroxidases and glucose oxidases (*49*) and with whole-cells (*59*,*61*).

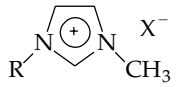
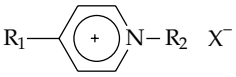
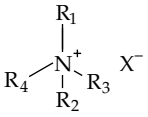
The first ionic liquid, reported in 1914, was $EtNH_3 \times NO_3$ (ethylammonium nitrate) but nowadays the most common ionic liquids for biocatalysis are imidazolium-based ionic liquids, such as BMIM-BF_4 and BMIM-BF_6 (1-butyl-3-methylimidazolium tetrafluoroborate and hexafluoroborate) (Fig. 2) (*18*,*60*,*62*). As the application of ionic liquids in biocatalysis is still at an early stage, there is not yet a well-established theoretical basis for predicting the solvent properties of ionic liquids, which vary greatly with the nature of the counter-ion. For instance, BMIM-BF_4 is water miscible, whereas BMIM-BF_6 is not. The miscibility behavior of ionic liquids and organic solvents is not very well known, although it is known that supercritical carbon dioxide does not mix with ionic liquids (*61*). In fact, the possibility of having a solvent like an ionic liquid, which does not mix well either with water or with non-polar solvents, can be taken advantage of by the use of two-phase systems. One successful example was presented by Lozano *et al*. (*36*). They were able to simultaneously catalyze their reaction within the ionic liquid and remove the product by extraction into supercritical carbon dioxide, thereby shifting the equi-



BMIM-BF_4 BMIM-BF_6

Fig. 2. Common ionic liquids in biocatalysis: 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM-BF_4) and hexafluoroborate (BMIM-BF_6) structures

Table 3. General structure of ionic liquids (ILs) (*58,61*)

General structure	Abbreviation	Anion (X^-)	Radical (R)
 1-Alkyl-3-methylimidazolium cations	[MMIM][$MeSO_4$]	$CH_3OSO_3^-$	CH_3
	[EMIM][BF_4]	BF_4^-	C_2H_5
	[EMIM][Tf_2N]	$(CF_3SO_2)_2N^-$	
	[BMIM][BF_4]	BF_4^-	n-C_4H_9
	[BMIM][BF_6]	BF_6^-	
	[BMIM][TfO]	$CF_3SO_3^-$	
	[BMIM][Tf_2N]	$(CF_3SO_2)_2N^-$	
	[BMIM][$MeSO_4$]	$CH_3OSO_3^-$	
	[BMIM][$EtSO_4$]	$C_2H_5OSO_3^-$	
	[BMIM][NO_3]	NO_3^-	
	[BMIM][lactate]	$CH_3CH(OH)COO^-$	
	[HMIM][BF_6]	BF_6–	n-C_6H_{13}
	[OMIM][BF_4]	BF_4^-	n-C_8H_{17}
	[OMIM][BF_6]	BF_6^-	
	[MOEMIM][BF_4]	BF_4^-	$CH_3OCH_2CH_2$
	[PPMIM][BF_6]	BF_6^-	$C_6H_5CH_2CH_2CH_2$
 Alkylpyridinium cations	[Epy][TFA]	CF_3COO^-	R_1: H R_2: C_2H_5
	[BMPy][BF4]	BF_4^-	R_1: CH_3 R_2: n-C_4H_9
 Alkylammonium cations	[$EtNH_3$][NO_3]	NO_3^-	R_1: C_2H_5 R_2, R_3, R_4: H
	[Et_3MeN][$MeSO_4$]	$CH_3OSO_3^-$	R_1, R_2, R_3: C_2H_5 R_4: CH_3

librium position of the reaction towards synthesis. The possibility of reusing the mixture of ionic liquid and enzyme was also shown.

The rule for enzyme activity in ionic liquids seems to be »there is no rule«, since the performance in a particular ionic liquid appears to vary significantly from enzyme to enzyme. Ionic liquids probably affect enzymatic activity in much the same way as the commonly used solvents do (*61*).

Regarding stability in ionic liquids, it appears that when enzymes actually dissolve in an ionic liquid they are totally inactivated (*60*). To remain active in ionic liquids, enzymes must remain in a powder suspension, so that the system can be classified as a heterogeneous one. Ionic liquids do not inactivate enzymes as hydrophilic solvents do, which makes it possible to use ionic liquids for the synthesis of compounds that involve polar substrates, such as glucose, maltose or ascorbic acid. For instance, ascorbic acid acylation in ionic liquids was enhanced, compared to that in organic solvents, which Park and Kazlauskas (*60*) attributed to the higher solubility of the substrate in the ionic liquids. The counter-ion can also affect stability. For lipases, methylsulfate, nitrate and lactate counter-anions seem to render the enzyme inactive (*61*).

As is the case with reverse micelles, ionic liquids have not yet been used in full-scale industrial processes. Two factors may determine whether the use of ionic liquids will be viable at large scale: the ability to reuse the biocatalyst without a decrease in its activity and the ability to separate the products efficiently. Indeed, one of the main drawbacks of the ionic liquid technique is the difficulty of recovery of non-volatile or low volatility products such as phenylethanol and carbohydrates. In such cases, the recovery of the product has been attempted by pervaporation (*46*) and nanofiltration (*59*). Extraction of the product with supercritical carbon dioxide in ionic-liquid/supercritical-CO_2 mixed systems has also been proposed (*36*). Finally, there is a major difficulty to be overcome: ionic liquids are about 800 times more expensive than organic solvents (*60*), rendering them viable only when the product is of high value.

Concluding Remarks

In this article we have shown some of the current advances in the field of non-aqueous biocatalysis in heterogeneous solvent systems. Further developments in this field are guaranteed by the ever-increasing interest in new compounds, especially chiral compounds, since the medium used can have significant effects on the stereoselectivity of the reaction. However, many questions still remain to be answered, especially concerning product recovery and the cost not only of equipment but also of non-conventional media components.

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References

1. Y. Y. Linko, M. Lämsa, A. Huhtala, O. Rantanen, *J. Am. Oil Chem. Soc. 72* (1995) 1293–1299.
2. N. A. Turner, E. N. Vulfson, *Enzyme Microb. Technol. 27* (2000) 108–113.
3. Z. Knezevic, S. Bobic, A. Milutinovic, B. Obradovic, L. Mojovic, B. Bugarski, *Process Biochem. 38* (2002) 313–318.
4. S. Torres, G. Castro, *Food Technol. Biotechnol. 42* (2004) 271–277.
5. Y. L. Khmelnitsky, A. V. Levashov, N. L. Klyachko, K. Martinek, *Enzyme Microb. Technol. 10* (1988) 710–724.
6. R. Verger, *Trends Biotechnol. 15* (1997) 32–38.
7. S. D. Jonathan, *Enzyme Microb. Technol. 11* (1989) 194–211.
8. G. A. Sellek, J. B. Chaudhuri, *Enzyme Microb. Technol. 25* (1999) 471–482.
9. G. Pencreac'h, J. C. Baratti, *Enzyme Microb. Technol. 28* (2001) 473–479.
10. A. Zaks, A. M. Klibanov, *J. Biol. Chem. 263* (1988) 3194–3201.
11. H. Sztajer, H. Lünsdorf, H. Erdmann, U. Menge, R. Schmid, *Biochim. Biophys. Acta, 1124* (1992) 253–261.
12. S. Hazarika, P. Goswami, N. N. Dutta, *Chem. Eng. J. 85* (2002) 61–68.
13. A. M. Azevedo, D. M. F. Prazeres, J. M. S. Cabral, L. P. Fonseca, *J. Mol. Catal. B-Enzym. 5* (2001) 147–153.
14. U. K. Jinwal, U. Roy, A. R. Chowdhury, A. P. Bhaduri, P. K. Roy, *Bioorg. Med. Chem. 11* (2003) 1041–1046.
15. V. M. G. Lima, N. Krieger, D. A. Mitchell, J. C. Baratti, I. Filippis, J. D. Fontana, *J. Mol. Catal. B-Enzym. 31* (2004) 53–61.
16. S. Soultani, J.-M. Engasser, M. Ghoul, *J. Mol. Catal. B-Enzym. 11* (2001) 725–731.
17. P. Adlercreutz: Biocatalysis in Non-Conventional Media. In: *Applied Biocatalysis*, A. J. J. Straathof, P. Adlercreutz (Eds.), Harwood Academic Publishers, Amsterdam (2000) pp. 295–316.
18. S. H. Krishna, *Biotechnol. Adv. 20* (2002) 239–267.
19. N. Krieger, M. A. Taipa, M. R. Aires-Barros, E. H. M. Melo, J. L. Lima-Filho, J. M. S. Cabral, *J. Chem. Technol. Biotechnol. 69* (1997) 77–85.
20. N. Krieger, M. A. Taipa, E. H. M. Melo, J. L. Lima-Filho, M. R. Aires-Barros, J. M. S. Cabral, *Bioprocess Eng. 20* (1999) 59–65.
21. C. M. L. Carvalho, J. M. S. Cabral, *Biochimie, 82* (2000) 1063–1085.
22. J. R. S. Alves, L. P. Fonseca, M. T. Ramalho, J. M. S. Cabral, *Biochem. Eng. J. 15* (2003) 81–86.
23. E. P. Melo, M. R. Aires-Barros, J. M. S. Cabral, *Biotechnology Annual Review, Vol. 7*, Elsevier (2001) 87–129.
24. N. Krieger, M. A. Taipa, E. H. M. Melo, J. L. Lima-Filho, M. R. Aires-Barros J. M. S Cabral, *Appl. Biochem. Biotechnol. 67* (1997) 87–95.
25. M. L. M. Fernandes, N. Krieger, A. M. Baron, P. P. Zamora, L. P. Ramos, D. A. Mitchell *J. Mol. Catal. B-Enzym. 30* (2004) 43–49.
26. R. D. Falcone, M. A. Biasutti, N. M. Correa, J. J. Silber, E. Lissi, E. Abuin, *Langmuir, 20* (2004) 5732–5737.
27. K. Naoe, S. Awatsu, Y. Yamada, M. Kawagoe, K. Nagayama, M. Imai, *Biochem. Eng. J. 18* (2004) 49–55.
28. D. H. Altreuter, J. S. Dordick, D. S. Clark, *Enzyme Microb. Technol. 31* (2002) 10–19.
29. S. Okazaki, S. Nagasawa, M. Goto, S. Furusaki, H. Warishi, H. Tanaka, *Biochem. Eng. J. 12* (2002) 237–241.
30. G. W. Zhou, G. Z. Li, J. Xu, Q. Sheng, *Colloid Surf. A-Physicochem. Eng. Asp. 194* (2001) 41–47.
31. E. Alvarez-Macarie, J. Baratti, *J. Mol. Catal. B-Enzym. 10* (2000) 377–383.
32. S. H. Krishna, B. Manohar, S. Divakar, S. G. Prapulla, N. G. Karanth, *J. Biotechnol. 87* (2001) 191–201.
33. M. L. M. Serralheiro, D. M. F. Prazeres, J. M. S. Cabral, *Enzyme Microb. Technol. 31* (1999) 507–513.
34. C. M. L. Carvalho, M. R. Aires-Barros, J. M. S. Cabral, *Biotechnol. Bioeng. 72* (2001) 136–143.
35. Z. Knez, M. Habulin, *J. Supercrit. Fluids*, 23 (2002) 29–42.
36. P. Lozano, T. Diego, D. Carrié, M. Vaultier, J. L. Iborra, *J. Mol. Catal. A-Chem. 214* (2004) 113–119.
37. C. Chiappe, E. Leandri, S. Lucchesi, D. Pieraccini, B. D. Hammock, C. Morisseau, *J. Mol. Catal. B-Enzym. 27* (2004) 243–248.
38. G. Madras, C. Kolluru, R. Kumar, *Fuel, 83* (2004) 2029–2033.
39. C. Turner, M. Persson, L. Mathiasson, P. Adlercreutzb, J. W. King, *Enzyme Microb. Technol. 29* (2001) 111–121.
40. H. Sovová, M. Zarevúcka, *Chem. Eng. Sci. 58* (2003) 2339–2350.
41. P. Lozano, G. Víllora, D. Gómez, A. B. Gayo, J. A. Sánchez-Conesa, M. Rubio, J. L. Iborra, *J. Supercrit. Fluids, 29* (2004) 121–128.
42. M. Rasalkar, M. K. Potdar, M. M. Salunke, *J. Mol. Catal. B-Enzym. 27* (2004) 267–270.
43. R. Irimescu, K. Kato, *J. Mol. Catal. B-Enzym. 30* (2004) 189–194 a.
44. S. S. Mohile, M. K. Potdar, J. R. Harjani, S. J. Nara, M. M. Salunke, *J. Mol. Catal. B-Enzym. 30* (2004) 185–188.
45. R. Irimescu, K. Kato, *Tetrahedron Lett. 45* (2004) 523–525.
46. K. Bélafi-Bakó, N. Dörmõ, O. Ulbert, L. Gubicsa, *Desalination, 149* (2002) 267–268.
47. M. Persson, U. T. Bornscheuer, *J. Mol. Catal. B-Enzym. 22* (2003) 21–27.
48. O. Ulbert, T. Fráter, K. Belafi-Bakó, L. Gubicza, *J. Mol. Catal. B-Enzym. 31* (2004) 39–45.
49. K. Okrasa, E. Guimbé-Jampel, M. Therisod, *Tetrahedron: Asymmetry, 14* (2003) 2487–2490.
50. M. J. Kim, M. Y. Choi, J. K. Lee, Y. Ahn, *J. Mol. Catal. B-Enzym. 26* (2003) 115–118.
51. S. J. Nara, J. R. Harjani, M. M. Salunke, *Tetrahedron Lett. 43* (2002) 2979–2982.
52. T. Itoh, Y. Nishimura, N. Ouchi, S. Hayase, *J. Mol. Catal. B-Enzym. 26* (2003) 41–45.
53. S. J. Nara, S. S. Mohile, J. R. Harjani, P. U. Naik, M. M. Salunke, *J. Mol. Catal. B-Enzym. 28* (2004) 39–43.
54. P. Lozano, T. Diego, D. Carrié, M. Vaultier, J. L. Iborra, *J. Mol. Catal B-Enzym. 21* (2003) 9–13.
55. J. Howarth, P. James, J. F. Dai, *Tetrahedron Lett. 42* (2001) 7517–7519.
56. S. G. Cull, J. D. Holbrey, V. Vargas-Mora, K. R. Seddon, G. J. Lye, *Biotechnol. Bioeng. 69* (2000) 227–233.
57. N. Kaftzik, W. Kroutil, K. Faber, U. Kragl, *J. Mol. Catal. A-Chem. 214* (2004) 107–112.
58. C. M. Gordon, *Applied Catal. A-Gen. 222* (2001) 101–117.

59. U. Kragl, M. Eckstein, N. Kaftzik, *Curr. Opin. Biotechnol. 13* (2002) 565–571.

60. S. Park, R. Kazlauskas, *Curr. Opin. Biotechnol. 14* (2003) 432–437.

61. F. van Rantwijk, R. M. Lau, R. A. Sheldon, *Trends Biotechnol. 21* (2003) 131–138.

62. R. Fortunato, C. A. M. Afonso, M. A. M. Reis, J. G. Crespo, *J. Membr. Sci. 242* (2004) 197–209.

Nevodena biokataliza u heterogenim sustavima otapala

Sažetak

Biokataliza je postala korisna alternativa kemijskim transformacijama za proizvodnju niza spojeva što se primjenjuju u prehrambenoj, kemijskoj i farmaceutskoj industriji. Međutim, nije tako jednostavno postići zadovoljavajuću provedbu s obzirom na brzinu, iskorištenje i selektivnost reakcije. Jedna od mogućnosti optimiranja biokatalitičkih provedaba je korištenje nekonvencionalnih otapala, kao što su nevodeni heterogeni sustavi. U radu su prikazani neki od suvremenih tendencija u biokatalizi, provedeni u takvim sustavima s posebnim osvrtom na reverzne micelije te superkritične i ionske tekućine.