We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

21

Ionic Liquids: Alternative Reactive Media for Oxidative Enzymes

Oscar Rodriguez, Ana P.M. Tavares,
Raquel Cristóvão and Eugénia A. Macedo
Associate Laboratory LSRE/LCM - Faculty of Engineering, University of Porto. Portugal

1. Introduction

During the last decades, biotechnology has attracted a great interest from academic and industrial environments due to the progress made in the discovery of new and efficient biocatalysts for many different applications. Due to the increasing variety of these applications, the aqueous medium became limiting. Biocatalysis in nonaqueous media offers unique capabilities and thus plays a major role in biotransformation technologies. It is well known that when enzymes are introduced in a non-aqueous medium, a number of factors can alter their native structure (critically at the active centre of the protein) and so alter their biological functions, thus causing reversible or irreversible inactivation of the enzymes. In order to overcome these problems, a high number of papers related to biocatalysis in organic solvents are published every year, in several areas of knowledge, investigating the effects of different organic media in enzyme activity, stability, structure and kinetics. It is important to highlight that good results have been reported for several enzymes, involving many different reaction types and a wide variety of organic solvents (Carrea & Riva, 2000). As in aqueous enzymatic reactions, the enzymology in non-aqueous media also presents specific and unique advantages like: reaction enantioselectivity, resistance to contamination by microorganisms, enhanced thermo-stability of the enzyme. Additionally, the solubility of hydrophobic substrates and/or products can be increased with the right selection of the solvent. The latter decreases diffusional barriers for the reactions, thus improving their yields.

The study of enzymatic reactions in non-aqueous media started more than 100 years ago (Halling & Kvittingen, 1999), but did not receive attention until the 1970s with the pioneering works of Berezin and co-workers (Klyosov et al., 1975; Klibanov et al., 1977; Martinek et al., 1981) and today either water and organic solvents are conventional media for enzymes. Klyosov et al. (1975) studied the hydrolysis of p-nitrophenyl esters by means of α-chymotrypsin and related proteins in four different organic solvents (dimethylsulfoxide, dimethylformamide, formamide, and N-methylacetamide). The work of Klibanov et al. (1977) was based on enzymatic reactions in a "water–*water-immiscible* organic solvent" biphasic system with bovine chymotrypsin using chloroform, benzene, ether, acetone, ethanol, dimethylsulfoxide and dioxane as organic solvents. On the other hand, Martinek et al. (1981) investigated the behaviour of α-chymotrypsin, trypsin, pyrophosphatase,

peroxidase, lactate dehydrogenase and pyruvate kinase in organic solvents (benzene, chloroform, octane, cyclohexane). After these publications, a growing number of articles in this subject can be found in the open literature. The fundamental findings of this field can be found in deeper detail in a series of (recent) review works (Dordick, 1991; Nikolova & Owen, 1993; Halling, 2000; Castro & Knubovets, 2003; Yu et al., 2010).

2. Oxidative enzymes

Oxidative enzymes are enzymes which catalyse oxidation reactions. The most common types of oxidative enzymes are peroxidases, which use hydrogen peroxide for the oxidation of the enzyme, and oxidases (such as laccases and tyrosinases), which use molecular oxygen. These enzymes have broad substrate specificities and can catalyse the oxidation of a wide range of toxic organic compounds and are normally used in the treatment of organic pollutants. In aqueous medium, oxidative enzymes catalyse the transformation of a large number of phenolic and non-phenolic compounds.

Laccase (EC 1.10.3.2, p-benzenediol:oxygen oxidoreductase) is a multicopper belonging to a small group of enzymes denominated blue oxidases (Mayer & Staples, 2002). It is able to catalyse the oxidation of various aromatic compounds (particularly phenols) with the concomitant reduction of oxygen to water. Laccases are extracellular glycoproteins containing normally 4 atoms of copper, which are distributed into three sites and constitute the active site of the enzyme. A scheme of the reaction mechanism is depicted in Figure 1 (Bourbonnais et al., 1998).

It is possible to find much information about the use of oxidative enzymes in several applied areas in the literature (Xu, 2005; Couto & Herrera, 2006). Particularly, laccase can be used for delignification of wood fibres in the preparation of pulp for paper industry, a large variety of bioremediation processes, treatment of industrial wastes, decolourization of dyes for textile industry, enzyme sensors for drug analysis, etc. Besides these direct reactions, laccases can indirectly react with non-phenolic types of substrates. In that case, they need the presence of a redox mediator: the mediator promotes the enzyme action by increasing its oxidation potential (Husain & Husain, 2008). In contrast, an exhaustive effort to explore the more complex aspects of oxidative enzymes in non-aqueous media is still needed.

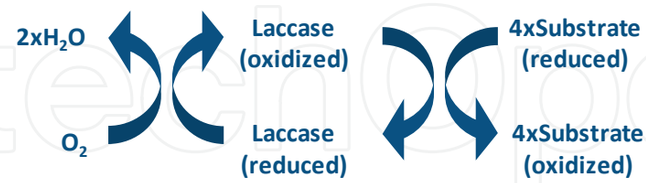


Fig. 1. Scheme of the reaction mechanism (biocatalytic cycle) in laccase-mediated oxidations.

As it comes for peroxidases, also extensive research has been developed towards new possibilities and applications of the enzymes, especially for environmental applications. Peroxidases are heme proteins which use hydrogen peroxide to catalyse one-electron oxidation of a large variety of aromatic compounds (Banci, 1997). A scheme of the reaction mechanism is depicted in Figure 2 (Banci, 1997; Hamid & Rehman, 2009).The most studied peroxidases include: Horseradish peroxidase (HRP, EC 1.11.1.7), Lignin peroxidase (LiP) and Manganese peroxidase (MnP). The most relevant applications for these enzymes are in the removal of recalcitrant phenolic pollutants, the decolourization of Kraft effluents, in

biosensor and for some specific organic syntheses (Conesa et al., 2002). The products of the enzymatic reaction can be further polymerized by downstream processes in order to produce insoluble precipitates. These precipitates can be conveniently and easily separated from the reaction medium by sedimentation or filtration (Karan & Nicell, 1997).

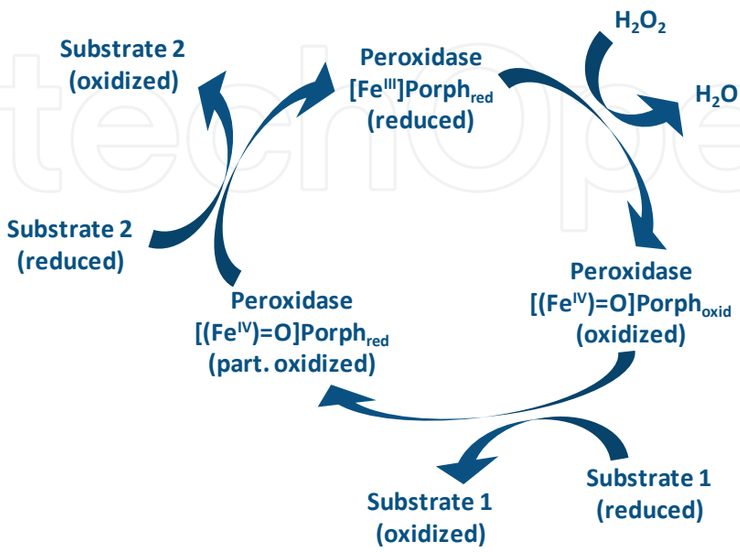


Fig. 2. Scheme of the reaction mechanism (biocatalytic cycle) in peroxidase-mediated oxidations.

Due to the easiness of oxidative enzymes to catalyse the oxidation of phenolic and non-phenolic compounds, and due to the hydrophobicity and low aqueous solubility of many of these chemicals, many researchers have derived their efforts to carry out the reactions in non-aqueous media (organic solvents). Yoshida et al. (1997) studied the oxidation of various phenolics and aromatic amines in organic solvents by means of lignin peroxidase (LiP). It was found that the activity of LiP in organic solvents depends on the nature of solvent and substrate. LiP oxidized the aromatic compounds in the presence of 70% ethylene glycol and aromatic amines were more easily oxidized in 70% aqueous ethylene glycol medium than phenolic compounds. But LiP failed to catalyze the oxidation of veratryl alcohol in the ethylene glycol medium. It was also related to the decrease in the redox potential of LiP in organic solvents. Another study (Azevedo et al., 2001) with horseradish peroxidase (HRP) reported the measurement of enzymatic activity and stability in the presence of organic co-solvents: dimethyl sulfoxide (DMSO), dimethylformamide, dioxan, acetonitrile and tetrahydrofuran. Among the solvents used, DMSO led to the highest activities and stabilities. Additionally, best results were obtained when HRP was immobilized onto silica microparticles. A system based on the use of Manganese Peroxidase (MnP) for the degradation of polycyclic aromatic hydrocarbons (PAHs), where anthracene was selected, was reported by Eibes et al. (2005) with different water miscible organic solvents (acetone, methyl-ethyl-ketone, methanol and ethanol). Comparing the maximum solubilisation of anthracene and the minimum loss of MnP activity, acetone was the best co-solvent for an acetone concentration of 36% (v/v). Other studies show the use of peroxidases for enzyme-catalyzed polymerization reactions due to the advantages over traditional polymerization, namely to improve the control of the polymer structure (Singh & Kaplan, 2002). Further

examples of polymerization reactions for syringaldehyde and bisphenol A by peroxidase (An et al., 2010), 4-[(4-phenylazo-phenyimino)-methyl]-phenol by HRP (Turac & Sahmetlioglu, 2010), cardanol by HPR (Won et al., 2004) and phenols by laccase (Mita et al., 2003) are reported in the literature.

3. Enzymes in ionic liquids

The use of ionic liquids as reaction media for enzymatic catalysis has received a boost of attention within the last decade. The reasons for that are easily understood: biocatalysis in non-aqueous media is a subject of interest to expand the possibilities of enzymes to substrates that are not soluble in water, or enhance the yields and process capacity of those with low solubility. Using ionic liquids, it is possible to separate and reuse the catalyst, obtain higher conversions, higher stabilities, and use polar or hydrophilic substrates (Moniruzzaman et al., 2010a). It is a very promissory and environmental friendly alternative to harmful organic solvents. Room-temperature ionic liquids (electrolytes or salts with a low melting point) are compounds that consist only of ions, have a negligible vapour pressure (for industrial applications), present high chemical, electrochemical and thermal stability, tunable polarity and easier product separation and reutilization (Park & Kazlauskas, 2003; van Rantwijk et al., 2003; Wilkes, 2004). Besides this, ionic liquids have arisen during these years as "green" recyclable solvents, awakening the interest of researchers on virtually all fields of chemical engineering, chemistry (organic synthesis, electrochemistry, and so on), physics, polymer science, nanotechnology, biofuel production, or purification of biomolecules, among others. It is straightforward to combine both ideas and thus get into the field of enzymatic catalysis in ionic liquids.

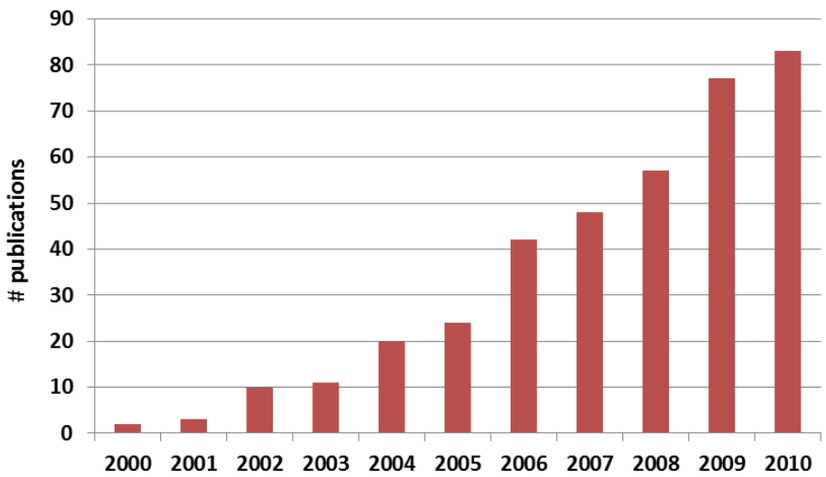


Fig. 3. Number of articles published with keywords "ionic liquid" and either "enzyme" or "biocatalysis". Search done in the Web of Science in January 2011.

The research on enzymatic catalysis in ionic liquids started in 2000 with the work of Erbeldinger et al. (2000). After that, the number of articles found in the open literature regarding "ionic liquid" and "enzymes" or "biocatalysis" increased each year, going up to 377 (search done on January 2011 using the Web of Science©). Just one article could be found before the year 2000, published by Magnuson et al. (1984), where the activity and stability of

alkaline phosphatasein were studied in ethylammonium nitrate [$EtNH_3$][NO_3]. The results of this search are presented in Figure 3, where the increasing rate of publication is evident. This trend indicates that ionic liquids are proving to be an attractive media to carry out enzymatic reactions. Just for comparison, when substituting "ionic liquid" for "supercritical" in the previous search, the result is 620 articles published since 1988. Nearly the double of articles produced in about the double of time. Note that supercritical fluids (namely supercritical CO_2) are other attractive non-conventional solvents for biocatalysis. However, these processes are only economically viable when high added-value products are involved, due to the higher costs of elevated pressure. Despite ionic liquids are still rather expensive solvents, scaling-up the productions will certainly reduce their cost.

As detailed in Figure 3, much work has been done throughout this decade with enzymes in ionic liquid media. Excellent review articles have been published during these years collecting the results produced (Kragl et al., 2002; Sheldon et al., 2002, 2003; van Rantwijk et al., 2003; Park & Kazlauskas, 2003; Zhao, 2005; van Rantwijk & Sheldon, 2007; Yang, 2009; Moniruzzaman et al., 2010b; Lozano, 2010). Nevertheless, it is important to highlight that most of these results refer to lipase biocatalysis: lipases provide fast and convenient enantioselective synthetic routes for esterification and transesterification reactions. Besides, they are well suited for reactions in non-aqueous media. Even so, oxidoreductases (such as laccases and peroxidases) also play an important role in biochemical synthesis. These types of enzymes have received less attention in the literature, and this chapter will focus on the research work performed with laccase and peroxidase biocatalysis using ionic liquid as reaction media.

Most biocatalysis in ionic liquids involved no or low water content (Sheldon et al., 2002). Pugin et al. (2004) reported the interest in reaction systems with an ionic liquid and water (wet ionic liquids) due to the enantioselectivity of the biocatalyst and the importance in the use with ionic liquids when compared with organic solvents. These new reaction media consisting of water/ionic liquid present own and unique properties: they may provide a great potential without significant loss of activity due to their excellent catalytic performance, with turnover numbers even above 10^4.

The effect of ions on the enzyme activity, stability and enantioselectivity is another important factor which must be taken into account. Ions can affect the stability of proteins because of the chemical interactions between the proteins and the ions. According to the work of Vrbka et al. (2006) it seems that the ions in the reaction media interact with the charged amino acid groups of the protein as much as they interact with the corresponding ions in bulk solutions. Ions also may cause competitive or non-competitive inhibition of enzymes. The review of Zhao (2005) reports that anions such as PO_4^{3-}, CO_3^{2-}, or SO_4^{2-} (kosmotropic anions) and cations such as Cs^+, Rb^+, or K^+ (chaotropic cations) stabilize enzymes, while chaotropic anions and kosmotropic cations destabilize them. However, the influence of ionic liquids on the enzyme is complex especially when ionic liquids are present as nearly anhydrous solvents. As ionic liquids are composed only by ions, the knowledge about their influence on proteins is fundamental for choosing the most suitable as solvent for a given enzymatic reaction. Specific properties of ionic liquids also affect the enzymatic stability, such as polarity and viscosity (Park & Kazlauskas, 2003). Depending on the structures of cations and anions, ionic liquids can be hydrophobic or hydrophilic (Huddleston et al., 2001). Those with hydrophilic anions such as chloride and iodide are miscible and those with hydrophobic anions (eg. PF_6^- or NTf_2^-) are immiscible in water. The content of water and the hydrophobicity/hydrophilicity are also related to the cation/anion

substitution (or functionalization) and is a significant factor considering the applications as reaction medium of a particular ionic liquid. It is important to recall that this substitution/functionlization of the ions allows for modifying (or controlling) their hydrophobicity/hydrophilicity, which can be tuned making ionic liquids the so called "designer solvents".

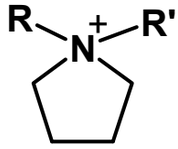
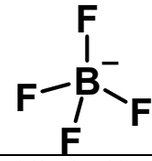
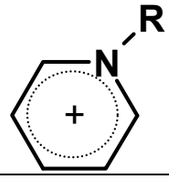
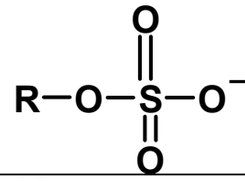
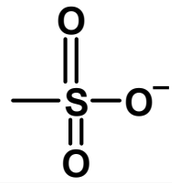
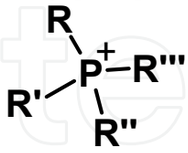
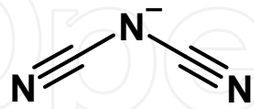
Cations	**Anions**
1.3-dialkylimidazolium 	Hexafluorophosphate 
1.1-dialkylpyrrolidinium 	Tetrafluorophosphate 
Alkylpiridinium 	Alkylsulfate 
Tetraalkylammonium 	Methanesulfonate 
Tetraalkylphosphonium 	Dicyanamide 
	Halides **Cl^-, Br^-, I^-**

Table 1. Anions and cations of ionic liquids commonly used in biocatalysis.

4. Enzyme activity in ionic liquids: Comparison with aqueous media

Most studies involving enzymatic catalysis are performed by ionic liquids which can be miscible and immiscible with water depending on the suitable choice of the anion and the cation, as described above. This characteristic allows ionic liquids to be designed for specific enzymatic reactions and substrates. In Table 1 it is possible to find the most common

structures of ionic liquids used in biocatalysis. In general, ionic liquids consist of a salt where one or both ions are large, and the cation should be (highly) unsymmetrical. These factors tend to reduce the lattice energy of the crystalline form of the salt, and hence lower the melting point (Olivier-Bourbigou, 2010). The review article written by Rantwijk & Sheldon (2007) is focused in the structures and properties of ionic liquids used in biocatalysis. The choice of the cation and the anion is very important in order to avoid the complete denaturation of the enzyme. Either the anion or the cation may deactivate the active centre of the enzyme by interactions with positively/negatively charged residues in the enzyme structure (Park & Kazlauskas, 2003).

Peroxidases and oxidases are very attractive biocatalysts for selective oxidative transformations in ionic liquids. The enzymatic reactions with laccase, horseradish and soybean peroxidases in ionic liquids were started by Hinckley et al. (2002). In their study, 4-methyl-N-butylpyridinium tetrafluoroborate, [4-mbpy][BF_4], and 1-butyl-3-methyl imidazolium hexafluorophosphate, [bmim][PF_6], were used as reaction media at different concentrations for the antracene oxidation by laccase. The previous assay on the catalytic activity of laccase for syringaldazine oxidation showed that the enzyme tolerates moderate concentrations of [4-mbpy][BF_4]. At high concentrations of this ionic liquid (above 50%), laccase activity decreased and then the enzyme precipitated. Similar results of laccase precipitation in ionic liquids were observed with 1-ethyl-3-methylimidazolium 2-(2-methoxyethoxy) ethylsulfate, [emim][$MDEGSO_4$], 1-ethyl-3-methylimidazolium ethylsulfate, [emim][$EtSO_4$], and 1-ethyl-3-methylimidazolium methanesulfonate, [emim][$MeSO_3$] at concentrations of 75% (v/v) or above (Tavares et al., 2008). For the tests with peroxidases Hinckley et al. (2002) observed significant activity when guaiacol was used as substrate, in the presence of 25% (v/v) of the water-miscible ionic liquid [4-mbpy][BF_4]. A similar behavior has been reported (Sgalla et al.,. 2007) for HRP in 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF_4]) and water mixtures. Here, also high activity was obtained for ionic liquid concentrations up to 25%, depending on the pH value. The enzymatic reactions were carried out with non-soluble phenolic compounds with up to 75% of [bmim][BF_4], presenting high yields of dimeric species for 4-phenylphenol (85% dimers). Again, the polymerization of phenol by HRP in [bmim][BF_4] presented a yield of polymerization of 100% with ionic liquid content of 60% (Zaragoza-Gasca et al., 2010). Other study with HRP and [bmim][BF_4] showed the importance of water content in the ionic liquid for the maintenance of the enzyme catalytic activity (Wang et al., 2007): No HRP activity was achieved in the presence of anhydrous ionic liquid, however, this deactivation was reversible with little addition of water. The authors explain that the enzyme structure is dependent on the hydrogen bonding, ionic, hydrophobic, and van der Waals interactions. Consequently, in non-aqueous media these interactions can be broken, which are fundamental for the maintenance of the enzyme activity.

Okrasa et al. (2003) have studied the particular interest in the oxidation products of sulfides using non-aqueous enzymatic reactions with peroxidase and glucose oxidase (GOD), with 1-butyl-3-methyl imidazolium hexafluorophosphate ([bmim][PF_6]). In this study, an interesting transformation of thioanisole to sulfoxide was carried out by the bi-enzymatic system with GOD/peroxidase with 10% of water in ionic liquid. The hydrogen peroxide is produced in situ by GOD (to transform the glucose in gluconic acid) and it is used by peroxidase to oxidize the sulfides.

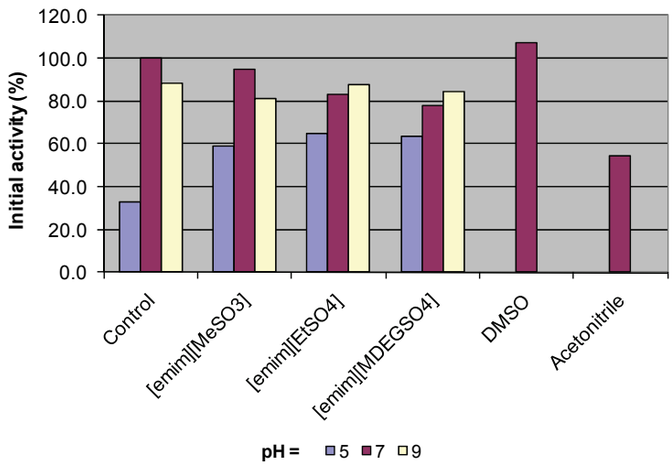


Fig. 4. Comparison of laccase initial activity in aqueous buffer solution (control), ionic liquids, dimethyl sulfoxide and acetonitrile at 50% (v/v) and different pH values.

Up to date, some papers are published in the literature regarding to the enzymatic reactions of laccase in ionic liquids. Most of them are related to the development of biosensors containing laccase and ionic liquids (Liu et al., 2007; Franzoi et al., 2009a,b; Brondani et al., 2009). Tavares et al. (2008) optimized the conditions (pH, ionic liquid concentration) for the activity of commercial laccase (DeniLite base), using ABTS as substrate, in different water-soluble ionic liquids: 1-ethyl-3-methylimidazolium 2-(2-methoxyethoxy) ethylsulfate, [emim][$MDEGSO_4$], 1-ethyl-3-methylimidazolium ethylsulfate, [emim][$EtSO_4$], and 1-ethyl-3-methylimidazolium methanesulfonate, [emim][$MeSO_3$]. As an example, Figure 4 presents a comparison of laccase activity in these ionic liquids (50% v/v) at different conditions. The comparison is extended to an aqueous buffer solution (named control) and organic solvents (dimethyl sulfoxide and acetonitrile, also at 50% v/v). Activities are presented as percentage relative to the initial activity of the enzyme in the buffer with pH 7. Laccase was most active at pH 7.0 - 9.0. The different solvents have little effect in laccase activities at pH 7.0 or 9.0 (both ionic liquids and organic solvents) at the lower concentrations (10 and 25% (v/v)), with a certain reduction in enzyme activity at higher concentrations. Carneiro et al. (2009) also studied the peroxidase activity in two of the previous ionic liquids, [emim][$MDEGSO_4$] and [emim][$EtSO_4$], using once again ABTS as substrate. Promising results of peroxidase activity were obtained at pH 7 for moderate concentrations of ionic liquid (5 and 10 % (v/v)) and good initial stability was obtained when compared to the control sample (an aqueous buffer medium). From both works of the same research group, it is possible to compare the enzymes activities between the buffer solution and ionic liquid media. Figure 5 shows a comparison of initial activities of both enzymes at 50% (v/v) concentration of ionic liquids and different pH values. The initial activity is presented relative to that in the pure aqueous buffer solution (control) for each enzyme. The high activities for laccase and peroxidase show the potential of these enzymes for catalytic reactions in ionic liquids.

Non-traditional ionic liquid media, based on micro emulsions with the nonionic surfactant Triton X-100, have been applied to improve the activity of laccase and lignin peroxidase (LiP) with 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim][PF_6]) and water (Zhou et al., 2008). The results in pure or water-saturated [bmim][PF_6] revealed that both LiP and laccase had insignificant catalytic activity, thus showing the negative effects of [bmim][PF_6]. However, good activities of both enzymes were obtained for [bmim][PF_6]-

based microemulsions and this was attributed to the TX-100 interfacial membrane, as it separates the enzyme from the [bmim][PF_6] bulk phase. In addition, the apparent viscosity of the medium decreases, which reduces diffusional barriers.

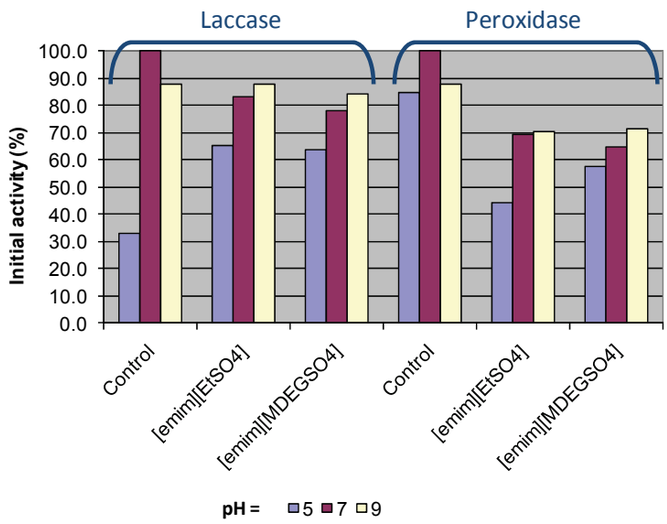


Fig. 5. Comparison of laccase (left side) and peroxidase (right side) initial activities in aqueous buffer solution (control) and ionic liquids, at 50% (v/v) and different pH values.

5. Ezymatic stability in ionic liquids

Enzyme stabilization deserves special attention because of the increasing number of enzyme applications. Enzymatic stability is essential for the enzyme to work at its full potential as catalyst for long periods, and consequently the economic feasibility of applying such enzyme in an industrial process. In addition, stable enzymes permit the use of higher temperatures in biochemical processes, which may have beneficial effects on reaction rates and/or reactant solubilities, while at the same time reduce the risk of microbial contamination. The enzyme stability (storage stability in ionic liquids) is obtained by incubation of the enzyme in an ionic liquid with convenient control of the temperature, and the measurement of the residual activity at certain time intervals. Different methodologies to improve enzyme stability with respect to their use in aqueous (or other non-aqueous) environments have been given in the literature, such as optimization of the reaction medium, chemical modifications of the enzymes, and enzyme immobilization. As discussed above, much of the current focus in enzyme technology involves enhancement of enzyme activity and stability into non-conventional media. The non-aqueous medium such as ionic liquids brings several advantages as enhanced solubility of non-polar substrates and/or products. However, these advantages would often be limited by a lower stability of biocatalysts in these systems. Just as an example, the use of hydrophilic ionic liquids can capture the water molecules which are fundamental to keep the enzyme 3D structure and consequently, its activity. This activity loss is related to the interactions established between the enzyme and the ions, and the rupture of those with water molecules. To overcome these problems, existing strategies for organic solvents have been employed to enhance enzyme stability in ionic liquids with significant results. Below are presented some examples of oxidative enzymes which have demonstrated to provide high stability in different ionic liquids.

Wang et al. (2007) described the stability of free and immobilized HRP in aqueous mixture of 1-butyl-3-methylimidazolium tetrafluroborate ([bmim][BF_4]). The activity was improved by HRP immobilization in agarose hydrogel retaining 80.2% of its initial activity for 10.5 h.
The stability of peroxidase in aqueous mixture of [emim][$MDEGSO_4$] and [emim][$EtSO_4$] was well maintained when compared to a phosphate buffer reference medium, for at least seven days of incubation, at optimized conditions: pH 7.0, ionic liquid 10% v/v, room temperature (Carneiro et al., 2009). Chloroperoxidase was highly stable in 1,3-dimethylimidazolium methylsulfate [mmim][$MeSO_4$] or 1-butyl-3-methylimidazolium methylsulfate [bmim][$MeSO_4$] (30%(v/v) co-solvent/citrate buffer), whereas it was deactivated within of 3 hours in the presence of *t*-butyl hydroperoxide or acetone (Sanfilippo et al., 2004).
To improve the stability of HRP in ionic liquids, Das et al. (2007) investigated various anions. Initially, tests with [bmim][Cl], [bmim][CF_3CO_2] and the non-conventional [bmim][$H_2NCH(CH_3)CO_2^-$] with a small amount of water (5% v/v) were evaluated. HRP lost 50% of its initial activity within 24 h in [bmim][Cl] or [bmim][$CF_3CO_2^-$]. On the other hand, [bmim][$H_2NCH(CH_3)CO_2^-$] promoted total deactivation of HRP in only one hour of incubation indicating that the presence of the aminoacid in the ionic liquid structure did not favor the stability of HRP. Further, the water content in ionic liquids was also investigated and HRP activity loss increased with the decrease in water content from 70% to 5%. In more common ionic liquids, a good stability of HRP, for at least 30h of incubation, even at 0% water, was obtained for [bmim][CF_3SO_3] and a similar stability for [bmim][BF_4] was obtained just for high water content (50-70%).
The stability of laccase was also studied in different water soluble ionic liquids [emim][$MDEGSO_4$], [emim][$EtSO_4$], and [emim][$MeSO_3$] at different water contents for up to ten days of incubation. The stabilities were compared with two organic solvents, acetonitrile and dimethyl sulfoxide and with an aqueous buffer solution. The enzyme maintained a high stability at pH 9.0 for all ionic liquids tested, particularly for [emim][$MDEGSO_4$], presenting small activity loss after the incubation for an ionic liquid content of 10%. Increasing this content resulted in a decrease of the enzyme activity for all ionic liquids and organic solvents. Just as an example, laccase activity is presented in Figure 6 as a function of time the

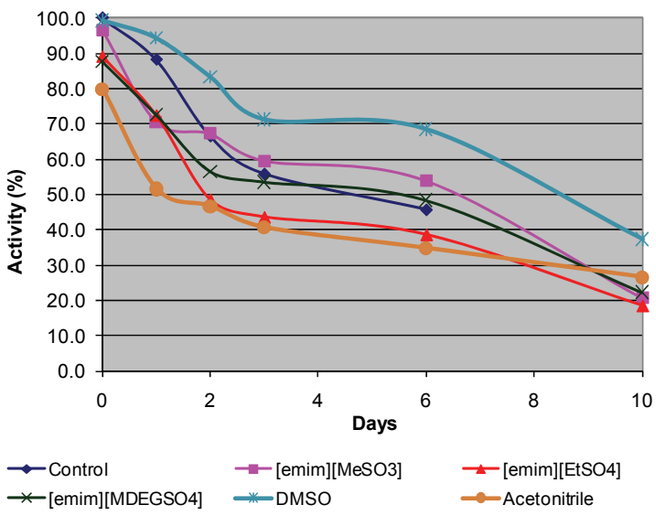


Fig. 6. Evolution of laccase activity with time: initial activity after incubation of the enzyme in aqueous solutions of ionic liquids or organic solvents (25 % v/v; pH = 7). Activities are relative to the initial control value.

above mentioned ionic liquid and organic solvents at 25 % (v/v) and pH 7. All activities are relative to that in pure aqueous buffer solution (named control) whose initial activity is considered 100%. It is clear that all ionic liquids present an initial decrease larger than the control solution (until day 3), but then the decrease is reduced and similar to the control. [emim][$MeSO_3$] provided the best behaviour. Compared to the organic solvents, acentonitrile performed worse but DMSO better than all ionic liquids and control.

6. Kinetics

Most of the previous studies discussed above were concerned with the measurement of activity or stability of enzymes and its reactions, without focusing on how ionic liquids affect the kinetic mechanisms. However, clarification of the role of ionic liquids in the catalytic mechanisms of enzymes is an important factor for the development of efficient biocatalytic processes utilizing ionic liquids as solvents. Many papers regarding enzyme-catalyzed reactions follow the traditional Michaelis–Menten equation, because of its simplicity and easiness to interpret the physical meaning of the equation parameters. The reaction rate can be described on the basis of the concentration of the substrate and of the enzymes. For a single enzyme and single substrate, the Michaelis-Menten parameters can be obtained by non-linear regression, based on the Michaelis-Menten equation:

$$v_0 = \frac{V_{\max} \cdot [S]}{K_m + [S]} \tag{1}$$

where v_0 is the initial reaction rate, $V_{\max}$ is the maximum reaction velocity, K_m is the Michaelis-Menten constant and [S] the substrate concentration. K_m is the value of substrate concentration at which the reaction rate reaches half of its maximum value ($V_{\max}/2$), and can be understood as a measurement of the enzyme affinity for the substrate. Thus, low K_m values indicate that the enzyme attains its maximum catalytic efficiency at a lower substrate concentration. Moreover, the ratio ($V_{\max}/K_m$) can be interpreted as a measurement of the enzyme catalytic efficiency.

The kinetic parameters of laccase were determined in the presence of 4-methyl-N-butylpyridinium tetrafluoroborate, [4-MBP][BF_4], 25% (v/v) and 1-butyl-3-methyl imdizaolium hexafluorophosphate, [bmim][PF_6], both saturated with water, and compared with tert-butyl hydroperoxide (20%v/v) and an aqueous buffer (Hinckley et al., 2002). The authors indicate that the kinetic data revealed a simultaneous decrease of $V_{\max}$ and increase of K_m in the presence of the two ionic liquids. This points to a dramatic reduction in the catalytic efficiency (the $V_{\max}/K_m$ ratio mentioned above). Correspondingly, the catalytic activity of laccase in [bmim][PF_6] was much lower than in aqueous media. These first results of laccase kinetics suggested that the optimization of reaction conditions in ionic liquids may be necessary to obtain higher catalytic activities. Another work can be referred with laccase (Tavares et al., 2008) but different ionic liquids. The ionic liquids used were based on sulfate and sulfonate anions, rather than the tetrafluoroborate: [emim][$MDEGSO_4$], [emim][$EtSO_4$] and [emim][$MeSO_3$], previously referred. Despite the value of $V_{\max}$ decreased for all ionic liquids, that of K_m also decreased (in a lower extent), thus diminishing the reduction in the catalytic effieciency.

Enzymatic inhibition is another point that should be considered in kinetics. An inhibitor is any substance that reduces the reaction rate. If an inhibitor binds reversibly to the same site

than the substrate, the inhibition will be competitive: the inhibitor blocks the active site. Non-competitive inhibition occurs when an inhibitor binds to the enzyme-substrate complex, so the inhibition cannot be overcomed by increasing the concentration of substrate. The inhibitor does not compete with the substrate for the active site of the enzyme, and thus substrate concentration has no influence on the degree of inhibition of the enzyme. The Michaelis-Menten equation can be used to provide evidences of this kind of inhibition: the inhibitor decreases $V_{\max}$ but has no effect on K_m. That is, the inhibitor binding does not affect the enzyme-substrate affinity (K_m), both the substrate (S) and the inhibitor (I) can simultaneously bind to the enzyme, but the resultant E-S-I complex is catalytically inactive. As a result, the inhibitor (I) can hide the adequate positioning of the catalytic center. In that case the reaction of the noncompetitive inhibitor is irreversible and the substrate cannot overcome the inhibitor's impact on the enzyme. Very few studies regarding ionic liquids as possible inhibitors are published in the literature.

An example of such study can be found for peroxidase (Carneiro et al., 2009) with the ionic liquids [emim][$EtSO_4$] and [emim][$MDEGSO_4$]. The Michaelis-Menten parameters for the enzymatic reactions in the presence and absence (buffer solution) of these ionic liquids were determined for a set of different concentrations. The results indicate that K_m values in the absence and in the presence of [emim][$MDEGSO_4$] (for all compositions studied) present close values (in the range 0.009-0.012 mM) while in the presence of [emim][$EtSO_4$] (for all compositions studied) the result was a bit lower (0.0064-0.0079 mM). Nevertheless, the presence of either ionic liquid had a much clearer effect in the values of V_{max}. As the ionic liquid concentration was increased, the V_{max} obtained decreased. The effect of [emim][$EtSO_4$] concentration was higher than that of [emim][$MDEGSO_4$], and so V_{max} was reduced from 3.10 mM/min in the pure buffer to 2.19 mM/min for the first ionic liquid (a reduction of 29 %) and 2.35 mM/min for the latter (a reduction of 24 %). The V_{max} values obtained decrease as the ionic liquid concentration increases. According to these results, K_m value can be considered approximately constant for all concentrations, while V_{max} gradually decreased with the increase in ionic liquid. These facts indicate a non-competitive inhibition mechanism, thus the ionic liquids do not affect the apparent substrate binding to the enzyme but indeed affect the rate of the reaction. The reaction rate was calculated using the following equation:

$$v_0 = \frac{V_{\max}[S]}{\left([S] + K_m\right)\left(1 + \frac{[I]}{K_i}\right)} \tag{2}$$

where [I] is the concentration of the inhibitor (here, the IL) and K_i is the inhibitory constant. Equation (2) allowed to represent the dependency of the reaction rate on the concentration of both substrate (ABTS) and ionic liquid, with fair agreement with experimental data.

7. Applications of enzymes in ionic liquids

As discussed in the previous sections, performing biocatalytic reactions in ionic liquids can be promising with regards to activity, selectivity and stability towards the target substrates, even presenting a short history. Indeed, the use of enzymes in ionic liquids opens up new possibilities for enzymology in non-aqueous media. Ionic liquids have added benefits for performing different kinds of biotransformations. A large number of biocatalysts have been reported to be active in ionic liquids, and the application of oxidative enzymes has played

an important role. The first application of oxidative enzymes in ionic liquids was reported by Hinckley et al. (2002), using laccase, horseradish and soybean peroxidases for veratryl alcohol, anthracene and guaiacol oxidations in the presence of [4-mbpy][BF_4] containing 25% (v/v) of aqueous buffer. Since then, reports have been published using oxidative enzymes. A summary of these applications is depicted in Table 2.

In recent years, the main application found for laccase in ionic liquids is related to the construction of biosensors. As examples, the determination of rutin (Franzoi et al., 2009a) or rosmarinic acid (Franzoi et al., 2009b) using biosensors constructed with laccase and 1-alkyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([$C_x$mim][NTf_2], x = 4, 10, 14), and [bmim][PF_6] or [bmim][BF_4], respectively. The determination of adrenaline in pharmaceutical formulations, using a biosensor constructed with 1-butyl-3-methyl-imidazolium hexafluorophosphate [bmim][PF_6] and laccase, has also been carried out (Brondani et al., 2009).

Enzyme	Ionic Liquid	Application	Reference
Laccase	[4-mbpy][BF_4]	Oxidation of anthracene, veratryl alcohol and guaiacol	Hinckley et al., 2002
Laccase	([$C_x$mim][NTf_2]	Determination of rutin	Franzoi et al., 2009a
Laccase	[bmim][PF_6]; [bmim][BF_4]	Determination of rosmarinic acid	Franzoi et al., 2009b
Laccase	[bmim][PF_6]	Determination of adrenaline	Brondani et al., 2009
Peroxidase	[bmim][NTf_2] [bmim][PF_6]	Oxidation of guaiacol	Laszlo & Compton, 2002
HRP	[bmim][BF_4]	Detection of H_2O_2	Liu et al., 2005; Wang et al., 2007
HRP	[C_2mim]Cl; [C_4mim]Cl; [C_6mim]Cl; [C_8mim]Cl;	Enzyme extraction system	Cao et al., 2008
HRP	[bmim][BF_4]	Polymerization of phenolic compounds	Sgalla et al., 2007; Zaragoza-Gasca et al., 2009
Oxidase-peroxidase	[bmim][PF_6]	Sulfoxidation of thioanisoles	Okrasa et al., 2003
Cloroperoxidase	[mmim][$MeSO_4$]; [bmim][$MeSO_4$]; [bmim][Cl]; [bmim][BF_4]	Oxidation of 1,2-dihydronaphthalene	Sanfilipo et al., 2004
D-amino acid oxidase	[bmim][BF_4]; [mmim][Me_2PO_4]	Deamination of amino acid	Lutz-Wahl et al., 2006
Soybean peroxidase	[bmim][BF_4]; [bmpy][BF_4]	Polymerization of phenols	Eker et al., 2009

Table 2. Examples of application of oxidative enzymes in ionic liquids.

More applications are found for reactions with peroxidases: Immobilized HRP was applied in non-aqueous biosensing for the detection of H_2O_2 with [bmim][BF_4] (Wang et al., 2007). HRP was also used in amperometric biosensing devices, and the extension of the technique to entrapment in a [bmim][BF_4] sol-gel matrix has recently been demonstrated for the detection of H_2O_2 (Liu et al., 2005). A different application for HRP was reported (Cao et al., 2008) showing the enzyme partition behavior in aqueous biphasic systems with the following ionic liquids: [C_2mim]Cl; [C_4mim]Cl; [C_6mim]Cl and [C_8mim]Cl. HRP was also applied for oxidative coupling of water insoluble phenolic compounds in [bmim][BF_4] water mixtures (Sgalla et al., 2007; Zaragoza-Gasca et al., 2009). Besides, soybean peroxidase (SBP) has been used to catalyze the polymerization of phenols in [bmim][BF_4] and 1-butyl-3-methylpyridinium tetrafluoroborate [bmpy][BF_4] (Eker et al., 2009).
The work of Okrasa et al. (2003) demonstrated the oxidase–peroxidase-catalyzed sulfoxidation of thioanisoles to sulfoxide in [bmim][PF_6]. Cloroperoxidase was reported (Sanfilipo et al., 2004) to catalyze the oxidation of 1,2-dihydronaphthalene to (1R,2R)-(+)-dihydroxy-tetrahydro-naphthalene in buffer/ionic liquid mixtures using [mmim][$MeSO_4$], [bmim][$MeSO_4$], [bmim][Cl], and [bmim][BF_4]. The oxidative deamination of the amino acid phenylalanine and cephalosporin-C were catalized by immobilized d-amino acid oxidase in the presence of [bmim][BF_4] and 1,3-dimethylimidazolium dimethylphosphate [mmim][Me_2PO_4] (Lutz-Wahl et al., 2006).

8. Conclusion

Throughout this chapter a revision of the research performed with oxidative enzymes, namely laccase and peroxidase, in ionic liquids has been provided. The focus has been centered in the studies of enzymatic activity and stability as these are critical properties for biocatalytic processes. The results presented allow to conclude that enzyme activity may diminish in the presence of ionic liquids, but such reduction may be balanced with the increase in poorly-soluble substrates and/or products, leading to better performances of teh global bioprocess. In general, the use of ionic liquids as co-solvents to enhance substrate solubility demonstrate the feasibility of this combination, but the type of ionic liquid and its concentration need to be carefully optimized. Works presenting the direct use of an oxidative enzyme in the pure ionic liquid (with very limited water content) are scarce. This fact indicates that direct dissolution of the enzyme in ionic liquids does not seem to be an option. Alternatively, the use of immobilized oxidative enzymes in ionic liquids has received little attention so far. Considering the excellent results that have been obtained with other immobilized enzymes, namely lipases (Lozano, 2010), this approach may be a suitable solution and an excelent path for future research.

9. References

An, E.S.; Cho, D.H.; Choi, J.W.; Kim, Y.H. & Song, B.K. (2010). Peroxidase-catalyzed copolymerization of syringaldehyde and bisphenol A. *Enzyme and Microbial Technology*, Vol. 46, 287-291.

Azevedo, A.M.; Prazeres, D.; Cabral J. & Fonseca, L. (2001). Stability of free and immobilised peroxidase in aqueous–organic solvents mixtures. *Journal of Molecular Catalysis B: Enzymatic*, Vol. 15, 147-153.

Banci, L. (1997). Structural Properties of Peroxidases. *Journal of Biotechnology*, Vol. 53, 253-263.

Bourbonnais, R.; Leech, D. & Paice, M.G. (1998). Electrochemical analysis of the interactions of laccase mediators with lignin model compounds. *Biochimica et Biophysica Acta,* Vol. 1379, 381–390.

Brondani, D.; Scheeren, C.W.; Dupont, J. & Vieira, I.C. (2009). Biosensor based on platinum nanoparticles dispersed in ionic liquid and laccase for determination of adrenaline, *Sensors and Actuators, B: Chemical*, Vol. 140, 252-259.

Cao, Q., Quan, Li; He, C.; Li, Na.; Li, K. & Liu, F. (2008). Partition of horseradish peroxidase with maintained activity in aqueous biphasic system based on ionic liquid. *Talanta*, Vol. 77, 160–165.

Carneiro, A.P.; Rodríguez, O.; Mota, F.L.; Tavares, A.P.M. & Macedo, E.A. (2009). Kinetic and stability study of the peroxidase inhibition in ionic liquids. *Industrial & Engineering Chemistry Research*, Vol.48, 10810-10815.

Carrea, G. & Riva, S. (2000). Properties and synthetic applications of enzymes in organic solvents. *Angewandte Chemie,* Vol. 39, 2226-2254.

Castro, G.R. & Knubovets, T. (2003). Homogeneous biocatalysis in organic solvents and water-organic mixtures. *Critical Reviews in Biotechnology*, Vol. 23, 195-231.

Conesa, A.; Punt, P.J. & van den Hondel, C.A.M.J.J. (2002). Fungal peroxidases: molecular aspects and applications. *Journal of Biotechnology,* Vol. 93, 143–158.

Couto, S.R. & Herrera, J.L.T. (2006). Industrial and biotechnological applications of laccases: A review. *Biotechnology Advances*, Vol. 24, 500–513.

Das, D.; Dasgupta, A. & Das, P. (2007). Improved activity of horseradish peroxidase (HRP) in 'specifically designed' ionic liquid. *Tetrahedron Letters*, Vol. 48, 5635–5639.

Dordick, J.S. (1991). Non-aqueous enzymology. *Current Opinion in Biotechnology*, Vol. 2, 401-407.

Eibes, G.; Lú-Chau, T.; Feijoo, G.; Moreira, M.T. & Lema, J.M. (2005). Complete degradation of anthracene by Manganese Peroxidase in organic solvent mixtures. *Enzyme and Microbial Technology*, Vol. 37, 365-372.

Eker, B.; Zagorevski, D; Zhu, G.; Linhardta, R.J & Dordick, J.S. (2009). Enzymatic polymerization of phenols in room-temperature ionic liquids. *Journal of Molecular Catalysis B: Enzymatic*, Vol. 59, 177–184.

Erbeldinger, M.; Mesiano, A.J. & Russel, A.J. (2000). Enzymatic catalysis of formation of Z-aspartame in ionic liquid – an alternative enzymatic catalysis in organic solvents. *Biotechnology Progress*, Vol. 16, 1129–1131.

Franzoi, A.C.; Migowski, P.; Dupont, J. & Vieira, I.C. (2009-a). Development of biosensors containing laccase and imidazolium bis(trifluoromethylsulfonyl)imide ionic liquid for the determination of rutin. *Analytica Chimica Acta*, Vol. 639, 90-95.

Franzoi, A.C.; Dupont, J.; Spinelli, A. & Vieira, I.C. (2009-b). Biosensor based on laccase and an ionic liquid for determination of rosmarinic acid in plant extracts. *Talanta*, Vol. 77,1322-1327.

Halling, P. & Kvittingen, L. (1999). Why did biocatalysis in organic media not take off in the 1930s?. *Trends in Biotechnology*, Vol. 17, 343-344.

Halling, P. (2000). Biocatalysis in low-water media: understanding effects of reaction conditions. *Current Opinion in Chemical Biology,* Vol. 4, 74–80.

Hamid, M. & Rehman, K. (2009). Potential applications of peroxidases. *Food Chemistry,* Vol. 115, 1177–1186.

Hinckley, G.; Mozhaev,V.V.; Budde, C. & Khmelnitsky, Y.L.(2002). Oxidative enzymes possess catalytic activity in systems with ionic liquids. *Biotechnology Letters,* Vol. 24, 2083–2087.

Huddleston, J.G.; Visser, A.E.; Reichert, W.M.; Willauer, H.D.; Broker, G.A. & Rogers, R.D. (2001).Characterization and comparison of hydrophilic and hydrophobic room temperature ionic liquids incorporating the imidazolium cation. *Green Chemistry*, Vol., 3, 156–164.

Husain, M. & Husain, Q. (2008). Applications of Redox Mediators in the Treatment of Organic Pollutants by Using Oxidoreductive Enzymes: A Review. *Critical Reviews in Environmental Science and Technology*, Vol. 38, 1–42.

Karam, J. & Nicell, J.A. (1997). Potential Applications of Enzymes in Waste Treatment. *Journal of Chemical Technology & Biotechnology*, Vol. 69, 141-153.

Klibanov, A.M. (1986). Enzymes that work in organic solvents. *Chemtech*, Vol. 16, 354-359.

Klibanov, A. M.; Samokhin, G.P.; Martinek, K. & Berezin, I. (2000) A new approach to preparative enzymatic synthesis. *Biotechnology and Bioengineering*, Vol.19, 1351-1361 (1977). Special Issue: Special 40th Anniversary Issue: *Biotechnology and Bioengineering,* Vol. 67, 737–747.

Klyosov, A.A.; Van Viet,N. & Berezin, I.V. (1975). The Reactions of a-Chymotrypsin and Related Proteins with Ester Substrates in Non-aqueous Solvents. *European Journal of Biochemistry*, Vol. 59, 3-7.

Kragl, U.; Eckstein, M. & Kaftzik, N. (2002). Enzyme catalysis in ionic liquids. *Current Opinion in Biotechnology*, Vol. 13, 565–571.

Laszlo, J.A. & Compton, D.L. (2002). Comparison of peroxidase activities of hemin, cytochrome c and microperoxidase-11 in molecular solvents and imidazolium-based ionic liquids. *Journal of Molecular Catalysis B: Enzymatic,* Vol. 18, 109–120.

Liu, Y.; Shi, L.; Wang, M.; Li, Z.; Liu, H. & Li, J. (2005). A novel room temperature ionic liquid sol–gel matrix for amperometric biosensor application. *Green Chemistry*, Vol. 7, 655-658.

Liu, Y; Huang, L. & Dong, S. V. (2007). Electrochemical catalysis and thermal stability characterization of laccase-carbon nanotubes-ionic liquid nanocomposite modified graphite electrode. *Biosensors and Bioelectronics,* Vol. 23, 35-41.

Lozano, P. (2010). Enzymes in neoteric solvents: From one-phase to multiphase systems. *Green Chemistry*, Vol. 12, 555–569.

Lutz-Wahl, S.; Trost, E.-M.; Wagner, B.; Manns, A. & Fischer, L. (2006). Performance of d-amino acid oxidase in presence of ionic liquids. *Journal of Biotechnology,* Vol. 124, 163–171.

Magnuson, D.K.; Bodley, J.W. & Evans, D.F. (1984). The activity and stability of alkaline phosphatase in solutions of water and the fused salt ethylammonium nitrate. *Journal of Solution Chemistry*, Vol.13, 583–587.

Martinek, K.; Levashov, A.V.; Klyachko, N.L.; Pantin, V.I. & Berezin, I.V. (1981). The principles of enzyme stabilization. VI. Catalysis by water-soluble enzymes entrapped into reversed micelles of surfactants in organic solvents. *Biochimica et Biophysica Acta*, Vol. 657, 277-294.

Mayer, A.M. & Staples, R.C. (2002). Laccase: new functions for an old enzyme. *Phytochemistry,* Vol. 60, 551-565.

Mita, N.; Tawaki, S.; Uyama, H. and Kobayashi, S. (2003). Laccase-Catalyzed Oxidative Polymerization of Phenols. Macromolecular Bioscience, Vol. 3, 253–257.

Moniruzzaman, M.; Kamiya, N. & Goto, M. (2010a). Activation and stabilization of enzymes in ionic liquids. *Organic and Biomolecular Chemistry* Vol. 8, 2887–2899.

Moniruzzaman, M.; Nakashima, K.; Kamiya, N. & Goto, M. (2010b). Recent advances of enzymatic reactions in ionic liquids. *Biochemical Engineering Journal,* Vol. 48, 295–314.

Nikolova, P. & Ward, O.P. (1993). Whole cell biocatalysis in nonconventional media. Journal of Industrial Microbiology, Vol. 12, 76-86.

Okrasa, K.; Guibé-Jampel, E. & Therisod, M. (2003). Ionic liquids as a new reaction medium for oxidase–peroxidase-catalyzed sulfoxidation. *Tetrahedron: Asymmetry,* Vol. 14, 2487–2490.

Olivier-Bourbigou, H.; Magna, L. & Morvan, D. (2010). Ionic liquids and catalysis: Recent progress from knowledge to applications. *Applied Catalysis A: General*, Vol. 373, 1–56.

Park, S. & Kazlauskas, R.J. (2003). Biocatalysis in ionic liquids –advantages beyond green technology. *Current Opinion in Biotechnology*, Vol., 14, 432–437.

Pugin, B.; Studer, M.; Kuesters, E.; Sedelmeier, G. & Feng, X. (2004). Mixtures of ionic liquids and water as a medium for efficient enantioselective hydrogenation catalyst recycling. *Advanced Synthesis and Catalysis*, Vol. 346, 1481–1486.

Rantwijk, F. & Sheldon, R.A. (2007). Biocatalysis in Ionic Liquids. *Chemical Reviews,* Vol. 107, 2757-2785.

Sanfilippo, C.; D'Antona, N. & Nicolosi, G. (2004). Chloroperoxidase from *Caldariomyces fumago* is active in the presence of an ionic liquid as co-solvent. *Biotechnology Letters,* Vol.26, 1815–1819.

Sgalla, S.; Fabrizi,G.; Cacchi, S.; Macone, A.; Bonamore, A. & Boffi, A. (2007). Horseradish peroxidase in ionic liquids Reactions with water insoluble phenolic substrates. *Journal of Molecular Catalysis B: Enzymatic* Vol. 44, 144–148.

Sheldon, R.A.; Lau, R.M.; Sorgedrager, M.J.; van Rantwijk, F. & Seddon, K.R. (2002). Biocatalysis in ionic liquids. *Green Chemistry*, Vol. 4, 147–151.

Sheldon, R.A.; Lau, R.M. & van Rantwijk, F. (2003). Biocatalytic transformations in ionic liquids. *Trends in Biotechnology*, Vol. 21: 131-138.

Singh, A. & Kaplan, D.L. (2002). Enzyme-Based Vinyl Polymerization. *Journal of Polymers and the Environment,* Vol. 10, 85-91.

Tavares, A.P.M.; Rodriguez, O. & Macedo, E.A. (2008). Ionic liquids as alternative co-solvents for laccase: Study of enzyme activity and stability. *Biotechnology and Bioengineering,* Vol. 101, 201-207.

Turac, E. & Sahmetlioglu, E. (2010). Oxidative polymerization of 4-[(4-phenylazo-phenyimino)-methyl]-phenol catalyzed by horseradish peroxidase. *Synthetic Metals,* Vol. 160, 169-172.

van Rantwijk, F.; Lau, R.M. & Sheldon, R.A. (2003). Biocatalytic transformations in ionic liquids. *Trends in Biotechnology*, Vol. 21, 131–138.

van Rantwijk, F. & Sheldon, R.A. (2007): Biocatalysis in Ionic Liquids. *Chemical Reviews*, Vol. 107, 2757-2785.

Vrbka, L.; Jungwirth,P.; Bauduin, P.; Touraud, D. & Kunz, W. (2006). Specific Ion Effects at Protein Surfaces: A Molecular Dynamics Study of Bovine Pancreatic Trypsin Inhibitor and Horseradish Peroxidase in Selected Salt Solutions. *The Journal of Physical Chemistry B*, Vol. 110, 7036-7043.

Wang, S-F.; Chen, T.; Zhang, Z-L & Pang, D-W. (2007). Activity and stability of horseradish peroxidase in hydrophilic room temperature ionic liquid and its application in non-aqueous biosensing. *Electrochemistry Communications,* Vol. 9, 1337–1342.

Wilkes, J.S. (2004). Properties of ionic liquid solvents for catalysis. *Journal of Molecular Catalysis A: Chemical*, Vol. 214, 11–17.

Won, K.; Kim, Y.H.; An, E.S.; Lee, Y.S. & Song, B.K. (2004). Horseradish Peroxidase-Catalyzed Polymerization of Cardanol in the Presence of Redox Mediators. *Biomacromolecules*, Vol. 5, 1–4.

Xu, F. (2005). Applications of oxidoreductases: Recent progress. *Industrial Biotechnology*, Vol. 1, 38-50.

Yoshida, S.; Watanabe, T.; Honda, Y. & Kuwahara, M. (1997). Effects of water-miscible organic solvents on the reaction lignin peroxidase of *Phanerochaete chrysosporium. Journal of Molecular Catalysis B: Enzymatic,* Vol. 2, 243-251.

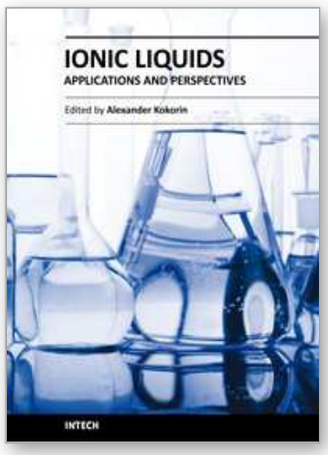
Yang, Z. (2009). Hofmeister effects: an explanation for the impact of ionic liquids on biocatalysis. *Journal of Biotechnology*, Vol. 144, 12–22.

Yu, H-L; Ou, L.; Xu, J-H. (2010). New Trends in Non-Aqueous Biocatalysis. *Current Organic Chemistry*, Vol. 14, 1424-1432.

Zaragoza-Gascaa, P.; Villamizar-Galveza,O.J; García-Arrazolaa, R.; Gimenoa,M. & Bárzana, E. (2010). Use of ionic liquid for the enzyme-catalyzed polymerization of phenols. *Polymers Advance Technologies,* Vol. 21, 454–456.

Zhao, H. (2005). Effect of ions and other compatible solutes on enzyme activity, and its implication for biocatalysis using ionic liquids. *Journal of Molecular Catalysis B: Enzymatic,* Vol. 37, 16–25.

Zhou, G-P.; Zhang, Y.; Huang, X-R.; Shi, C-H.; Liu, W-F.; Li, Y-Z.; Qu, Y-B. & Gao, P-J. (2008). Catalytic activities of fungal oxidases in hydrophobic ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate-based microemulsion. *Colloids and Surfaces B: Biointerfaces*, Vol. 66 146-149.



Ionic Liquids: Applications and Perspectives

Edited by Prof. Alexander Kokorin

ISBN 978-953-307-248-7
Hard cover, 674 pages
Publisher InTech
Published online 21, February, 2011
Published in print edition February, 2011

This book is the second in the series of publications in this field by this publisher, and contains a number of latest research developments on ionic liquids (ILs). This promising new area has received a lot of attention during the last 20 years. Readers will find 30 chapters collected in 6 sections on recent applications of ILs in polymer sciences, material chemistry, catalysis, nanotechnology, biotechnology and electrochemical applications. The authors of each chapter are scientists and technologists from different countries with strong expertise in their respective fields. You will be able to perceive a trend analysis and examine recent developments in different areas of ILs chemistry and technologies. The book should help in systematization of knowledges in ILs science, creation of new approaches in this field and further promotion of ILs technologies for the future.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen