(PO22) Laccase immobilization on nanofibrillated cellulose for use in lignin refinery

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INTRODUCTION

The lignin present in the black liquor of the cellulose industries has mostly been used for energy generation [1,2]. However, recent research has shown that the potential of lignin is far beyond low-value fuel. To enhance the applications of lignin it is necessary to overcome its heterogeneity by means of processes of fragmentation, purification or modification of the structure [1,3]. This modification can be promoted by enzymes such as laccase, acting as a biocatalyst to add value to lignin products [1,4–7]. However, the use of enzymes in the free form are subject to chemical, physical and biological factors that limit their useful life during use or storage, making it a high-cost product. Many of these undesirable features may be removed or ameliorated by using the enzymes in the immobilized form [8]. The objective of this work was to immobilize laccase on nanostructured cellulose, aiming at future use in biochemical conversions of Kraft lignin.

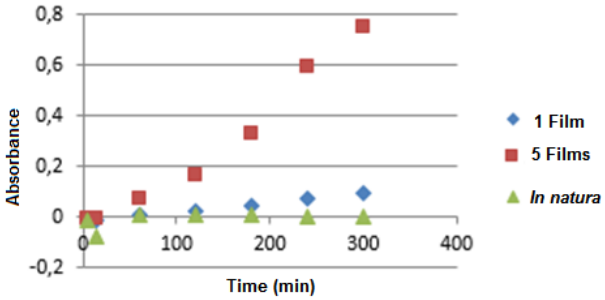
RESULTS AND DISCUSSION

The nanocellulose film (20 $g.m^{-2}$) was prepared from an aqueous suspension of eucalyptus cellulosic pulp previously defibrillated in a colloidal mill. The suspension was filtered through a 60 mesh strainer and nylon mesh, followed by oven drying at 60 °C. The film was oxidized in $NaIO_4$ solution [9]. Immobilization was started incubating 1 g of the oxidized film in sodium acetate buffer (50 mL) containing commercial laccase (0.1 $g.L^{-1}$), for 30 min at 30 °C and 150 rpm, according to Sathishkumar et al. [9]. Subsequent steps of cold incubation and reaction with glutaraldehyde were performed. The films were washed with sodium acetate buffer, left in Tris-HCl buffer, washed again and stored in acetate buffer.

Laccase activity was evaluated for both free and immobilized enzyme using ABTS as a substrate at 30 ° C, spectrophotometer reading at 420 nm. By titration using hydroxylammonium chloride and sodium hydroxide, it was possible to estimate the content of aldehyde groups generated on the films that underwent oxidation. Oxidized films had 18 times more aldehyde groups than those without oxidation, indicating the efficiency of this process for surface preparation prior immobilization.

Analyzing the immobilized laccase on films, the enzymatic activity was calculated to be 0,005 $U.g^{-1}$ of substrate. Despite the low value, it was possible to detect differences between the immobilized films and the original films (*in natura*) – Figure 1. A leakage test was accomplished to discard false positive for immobilized films.

Figure 1. Absorbance *versus* time for immobilized and original films at 420 nm



Considering the activities measured for free laccase solution before and after immobilization, it was possible to estimate the immobilization yield (4.2%). The result was unexpectedly low and some possible reasons could be: low concentration of laccase in the immobilization solution or/and unfavourable characteristics of the film, such as low porosity.

CONCLUSION

Although still insufficient, immobilization of a small fraction of laccase occurred on the nanocellulose film. For greater success, it is proposed to increase the concentration of laccase in the immobilization solution, as well as to use the suspension/gel of nanofibrillated cellulose as substrate, providing a larger surface area.

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(PO23) Conductive Monolithic Polymers for Peroxidase Immobilization

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Keywords: monolithic polymers, polyaniline, peroxidase, immobilization.

INTRODUCTION

Peroxidases are a wide group of enzymes that catalyze the oxidation of a variety of organic and inorganic substrates and can be immobilized into various support[1], the most common of which is polyaniline (PAni), since it presents several advantages over others. However, the PAni has some disadvantages related to its processing as the inability to be processed in high temperatures, since its decomposition temperature is lower than its melting point[2]. One way of solving this and moreover, to improve PAni properties is the incorporation into porous monolithic polymers. In this context, the aim of the present work is to evaluate the immobilization of peroxydase into PAni, incorporated into styrene and divinylbenzene porous monolithic polymers (Sty-DVB)

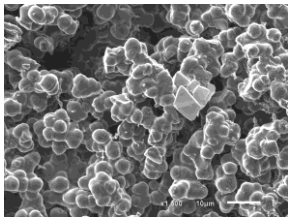
RESULTS AND DISCUSSION

The syntheses of the Sty-DVB monolithic polymers were carried out in cylindrical glass molds by mass polymerization using soybean oil, 1-pentanol and toluene/n-Heptane mixture as diluents under heated conditions. The incorporation of PAni into the polymers was carried out via oxidative polymerization of the aniline in the presence of HNO_3 and HCl. Peroxidase immobilization was performed by the activation of the PAni supported with glutaradehyde followed by direct contact with the crude extract of *Solanum lycocarpum* St. Hil. in different temperature, pH and time conditions. The free and immobilized peroxidase activity was measured under different pH, time and temperature conditions. The polymers were characterized by FTIR-ATR, SEM and measurements of specific surface area, pore volume and average pore diameter.

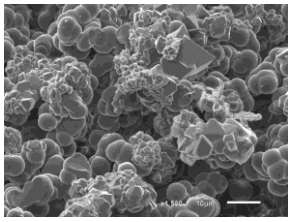
The monolithic polymers with higher pore volume and specific surface area were obtained with high degree of crosslink with soybean oil or toluene /heptane mixture as diluent. The use of HCl as dopant allowed the distribution of PAni only on the surface, whereas the use of HNO_3 allowed the distribution of PAni on the surface and inside the monolithic polymers.

The FTIR-ATR spectra of the different materials showed characteristic bands of all polymers produced. The immobilization time results suggest that 30 minutes are sufficient and longer intervals can cause process efficiency loss. Both free and immobilized enzyme showed similar optimal pH value, 7.0. The immobilization of peroxidase in polymer supported PAni with high porosity and high specific area produced systems with higher catalytic activity (Figure 1). The peroxidase-immobilized into supported Pani on the different monolithic polymers maintained its catalytic activity unchanged for 10 times use.

Figure 1. SEM of monolithic polymers (a) without PAni (b) with PAni.



(a)



(b)

CONCLUSION

The produced Sty-DVB monolithic polymers showed good characteristics for application as a Pani support, such as suitable size and good mechanical strength. After the incorporation of PAni into its structure, the Sty-DVB-PAni presented ideal characteristics for the peroxidase immobilization, once, when immobilized it showed a good catalytic activity.

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