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Comparison of the Performance of the Laccase Bioconversion of Sodium Lignosulfonates in Batch, Continuous and Fed Batch Reactors

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1. Introduction

Wood and food processes generate high quantities of by-products such as lignin, lignosulfonates and free phenols (Rodrigues et al., 2008). These compounds are natural molecules and renewable resources, but they constitute an important source of pollution. However, they can undergo several transformations and processes (hydrolysis, bioconversion and fractionation) to provide fractions with useful properties such as antioxidants, dispersing agent and plasticizer (Benavente-Garcia et al., 2000; Madad et al., 2011; Ouyang et al., 2006; Yang et al., 2008; Zhou et al., 2006). The recovery and development of these by-products are mainly carried out by chemical or physical processes such as thermal decomposition (Jiang et al., 2003), liquid (Correia et al., 2007) or membrane fractionation (Bhattacharya et al., 2005; Ferreira et al., 2005; Venkateswaran and Palanivelu, 2006). The chemical process is often not environmentally friendly and may be expensive. To overcome some drawbacks of the above mentioned processes, enzyme hydrolysis or bioconversion of these raw materials is presented as a promising way (Kobayashi et al., 2001). In fact, the enzymatic processes can be conducted under mild reaction conditions and without using toxic reagents. Moreover, in some cases, they lead to a homogeneous molecular distribution of obtained products and enhanced properties (Gross et al., 1998; Joo et al., 1998; Kobayashi, 1999; Kobayashi et al., 1995; Kobayashi and Uyama, 1998; Kobayashi et al., 2001).

The use of enzymes is firstly applied to the delignification and the removal of free phenols from wastewaters (Dasgupta et al., 2007; Husain, 2010; Nazari et al., 2007; Riva, 2006; Widsten and Kandelbauer, 2008). Recently, the ability of some oxidoreductases and laccas-

es to polymerize phenols have received great attention and applied with success in the field of wood by-products (Ikeda et al., 2001; Jeon et al., 2010; Mita et al., 2003; Reihmann and Ritter, 2006).

Depending on enzyme nature, enzymatic bioconversion of phenols requires either oxygen or hydrogen peroxide. The availability and the concentration of these substrates are essential to these reactions. Ghosh et al. (Ghosh et al., 2008) studied the effect of dissolved oxygen concentration on laccase efficiency during the removal of 2,4-dimethylphenol. These authors experimented several techniques such as dissolution by stirring or bubbling or a high initial saturation of the medium by oxygen. They reported that, whatever the technique used, as long as dissolved oxygen inside the reactor remains high, initial rates of reactions were similar and high compared to a reaction control with a low concentration of oxygen.

The main investigations in the field of enzymatic bioconversion were carried out in batch mode (Ghosh et al., 2008; Kim et al., 2009; Nugroho Prasetyo et al., 2010). However, in this mode, the degree of polydispersity remains high and hydroxyl phenolic groups are often only partially oxidized. This behavior, according to Areskogh et al. (Areskogh et al., 2010a) would be due to the ability of the lignosulfonates to form spherical microgels makes the phenolic groups buried in the core of the gel inaccessible. It could also be explained the inhibition of laccase by formed polymers (Kurniawati and Nicell, 2009). Another explanation is that the bioconversion by laccase is carried out in two ways leading either to C-O-C or to C-C linkages. The last way generates phenolic groups by ionic tautomerisation (Areskogh et al., 2010b). The concentration of the lignosulfonates also seems to influence the conversion rate of the phenolic groups, the polydispersity and the average molecular weight of polymers formed. High M_w were reached with high lignosulfonate concentrations (Areskogh et al., 2010a).

Fed batch and continuous modes are used in chemical bioconversion to control average M_w evolution and polydispersity and could also overcome some drawbacks of batch reactions; because fed batch allows controlling the enzyme and the substrate concentrations in the medium while the continuous system avoids the accumulation of the formed polymers in the medium. In spite of the potential of these two modes of reaction few data are available on their performance in the field of laccase bioconversion of phenols. Wu et al. (Wu et al., 1999) compared phenols removal efficiency by horseradish peroxidase in batch, continuous stirred tank, fed batch and a plug flow reactors. They reported that the plug flow reactor was the most appropriate for this reaction. Areskogh et al. (Areskogh et al., 2010a) compared also the effect of a successive addition of laccase during the lignosulfonates (SLS) bioconversion. They observed only minor differences in the average molecular weight increase which is dependent on the amount of enzyme.

The aim of this paper is to compare the efficiency of lignosulfonate bioconversion by laccase in terms of phenolic OH group consumption, average molecular weight and degree of polydispersity evolution under three modes of reaction conductions: batch with different enzyme/substrate ratio, continuous feed of laccase and lignosulfonates and three alternatives of fed batch feeding. The oxygen consumption was also monitored.

2. Materials and methods

2.1. Enzyme and chemicals

Sodium lignosulfonates (SLS) from (Aldrich, Sweden) : 90 wt. % of SLS, 4 wt. % of reducing sugars and 6 wt% of total impurities. The average molecular weight (M_w), the number molecular weight (M_n) and the polydispersity (Pdi) values are equal to 17800 Da $\pm$ 1500, 2900 Da $\pm$ 400, and 6.2 ± 0.3 , respectively.

Laccase from *Trametes versicolor* (21.4 U/mg) was purchased from Fluka (Sweden).

2.2. Laccase activity assay

The activity of laccase was determined spectrophotometrically by monitoring the oxidation of 2,2'-azinobis-(3-ethylbenzthiazoline)-6-sulfonate (ABTS) to its cation radical as substrate at 436 nm in 50 mM sodium succinate buffer at pH 4.5 and 30 °C using quartz cuvette of path length 10 mm. Enzyme activity was expressed in units (1 U = 1 μ mol ABTS oxidized per min at room temperature).

2.3. Batch operation

Batch operations were performed in a bioreactor with a working volume of 1 L equipped with dissolved oxygen, pH and temperature sensors. The reactor was stirred vigorously at 500 rpm to solubilise SLS at 20°C and throughout the reactions. The lignosulfonates were solubilised in phosphate buffer solution at pH 4.5 and laccase was added to initiate reactions. For the analyses, samples were drawn out from the reactor at different intervals of time and laccase activity was stopped by heating at 90°C for five minutes.

2.4. Fed batch operation

Fed batch reactions were carried out by progressive adding, at different time intervals (every 30 minutes during the first 5 hours), of enzyme alone, substrate alone or both enzyme and substrate. The total amounts of enzyme and substrate for the three fed batch operations were 10 g/L and 30 U/mL of SLS and laccase, respectively. Samples were taken at different time intervals and enzyme activity was stopped by heating to 90°C for five minutes.

2.5. Continuous stirred tank reactor operation

The continuous stirred tank reactor was similar to the one used in batch step. Lignosulfonates (32 g/L) and laccase (63 U/mL) were prepared in two flasks separately and 500 mL of each solution were added progressively at a constant flow-rate into the reactor initially filled with buffered solution (1 L). The reactor was aerated and stirred vigorously at 500 rpm. Samples were taken at different time intervals and the enzyme activity was stopped by heating to 90°C for five minutes.

2.6. Size exclusion chromatography analysis (SEC)

Samples were analysed by Size exclusion chromatography (SEC) (HPLC LaChrom Merck, Germany). The system consists of a pump L-2130, an autosampler L-2200, and a Superdex 200HR 10/30 column (24 mL, 13 µm, dextran/cross linked agarose matrix). Detection was performed using UV detector diode L-2455 at 280 nm. Before analysis, the samples were filtered using regenerated cellulose membrane (0.22 µm) and aliquots of 50 µl were injected into the SEC system. A Buffer Phosphate pH 7, 0.15 M NaCl solution was used as an eluent. The flow rate was 0.4 mL at 25°C and the pressure is maintained at 11 bars. The calibration was performed by using polystyrenes sulfonate (PSS) as a standard to define molecular weight distribution.

Chromatographs were integrated in segments of thirteen second intervals. The number-average molecular weight (M_n), the weight-average molecular weight (M_w), and the polydispersity (Pdi) were calculated as follows (Faix, 1981):

Number average molecular weight

$$M_n = \frac{\sum_{i=1}^n Area_i}{\sum_{i=1}^n \frac{Area_i}{M_i}} \quad (1)$$

Weight average molecular weight

$$M_w = \frac{\sum_{i=1}^n Area_i \times M_i}{\sum_{i=1}^n Area_i} \quad (2)$$

Polydispersity

$$D = \frac{M_w}{M_n} \quad (3)$$

where M_i is the molecular weight and $Area_i$ the area of each segment i .

2.7. Determination of phenolic content

Phenolic content was determined using the method described by Areskog et al. (Areskog et al., 2010a).

Figure 1. OH phenolic residual (a), dissolved O₂ (a), Mw (b) and PDI (b) variations in batchwise operation of reaction carried out with 10 g/L and 30 U/mL of SLS and laccase over time. (■) PDI and (□) Mw.

3. Results and discussion

3.1. Kinetic study of enzymatic bioconversion in batch mode

The performance of the bioconversion reaction of lignosulfonates by laccase can be affected by the ratio of SLS/laccase. To verify this assumption, the reaction of bioconversion was carried out with different ratios SLS/laccase; (1 g/L)/ (3 U/mL), (1 g/L)/ (30 U/mL), (10 g/L)/ (3

U/mL) and (10 g/L)/ (30 U/mL); in a stirred and aerated reactor. For the different assays M_w average, Pdi, phenol OH group content, and oxygen consumption were determined throughout the reaction. The results obtained with the four studied ratios, indicated similar profiles for the consumption of hydroxyl phenolic groups and oxygen. As an illustration, Figure 1 represents the variation of M_w average, Pdi, hydroxyl phenolic groups and oxygen evolution for the reaction with a SLS/laccase ratio equal to (10 g/L) / (30 U/mL). It appears that this reaction is made up of a two distinct steps. The first one is characterized by a rapid decrease of phenol OH group amount, dissolved oxygen, and Pdi value and a high increase of M_w average. The second one shows an increase of the dissolution of the oxygen to reach a plateau near the saturation of the medium, a progressive deceleration in the decrease of Pdi, and in the increase of M_w average and, a stabilisation of phenol OH group content around 0.1 g/L. These profiles could be explained by the fact that the first step consists of the initiation and the propagation of the enzymatic bioconversion. The rapid consumption of the oxygen ensures the formation of the SLS phenoxy radicals via laccase reduction. Thus, the role of the oxygen is important and can become a limiting step. The rapid decrease of dissolved oxygen has already been reported by Ghosh et al. (Ghosh et al., 2008) during the 2,4-dimethylphenol bioconversion by laccase. The second step is rather a combination stage where the need for oxygen is negligible.

The observed increase of the dissolved oxygen while M_w is still growing confirms these assumption. After 24 h of reaction the hydroxyl phenolic groups are not totally oxidized; this is due to the fact that when the reaction of bioconversion is finished, the final obtained structure of polymers contains hydroxyl groups (schema 1) (Areskogh et al., 2010b).

Table 1 reports the conversion rate of phenolic OH groups and the final M_w and Pdi values of batch reactions. These results showed also that regardless of the enzyme concentration, either 3 or 30 U/mL, the highest conversion rate of phenolic groups (73 % and 75 %) is observed at the highest SLS concentration (10g/L). For a given concentration of lignosulfonates, the enzyme concentration slightly affects the conversion rate; this means that a concentration of 3 U/mL of laccase is sufficient to polymerize the concentrations of the lignosulfonates tested in this work. It also appears that whatever the concentration of the enzyme M_w average is significantly improved at high concentrations of lignosulfonates (10 g/L). It increases from 17800 Da to 30600 Da and 31400 Da respectively for 3 U/mL and 30 U/mL of laccase. Pdi decrease approximately to a value of 4, independently of the enzyme and lignosulfonate concentrations. The high conversion yield of phenolic OH groups obtained at 10 g/L of lignosulfonates suggests that higher is generated phenoxy radicals in the reaction media, higher is the consumption of phenolic OH groups and M_w values. This may be due to the fact that the probability of establishing a contact between two phenoxy radicals is increased when their concentration in the medium is high and the C-O-C coupling is also favoured. So, this reaction is under a "kinetic control". The low M_w (26400 Da) observed with 1 g/L suggests that in the presence of a diluted solution and acid pH (4.5), the reaction is under a "thermodynamic control" which promotes C-C linkage.

Scheme 1. Proposed reaction mechanism for the formation of C-O-C and C-C bonds when a lignosulfonates model is oxidized by laccase. (R1) lignin fragment

Reaction	Conversion rate (%)	Final Mw	Final Pdi
1 g/L of S and 3 U/mL of E	47 %	25700	4.6
1 g/L of S and 30 U/mL of E	52 %	26400	4.1
10 g/L of S and 3 U/mL of E	73 %	30600	4.4
10 g/L of S and 30 U/mL of E	75 %	31400	4.6

Table 1. The conversion rate, the specific conversion rate, the final M_w and the final Pdi of reactions carried out in batchwise operation. (S) Substrate; (E) Enzyme

3.2. Kinetic study of enzymatic bioconversion in continuous reactor

The operating conditions for the continuous feeding of the enzyme and lignosulfonates were chosen to add 16 g/L and 32 U/mL of lignosulfonates and laccase respectively and to have the same residence time (24 h) as that used in the batch mode.

The obtained results are summarized in Figure 2 a and 2 b. It appears that phenolic OH group content increases slightly in the medium to reach the same level as that observed at the end of the batch reaction (~ 0.1 g/L) ; while the conversion rate of phenolic OH groups remains constant near 85 % throughout the duration of the reaction. This conversion is higher than that obtained in the batch. Molecular weight average (M_w) of formed polymers increases gradually to 28400 Da during the first four hours and then, as in batch mode, this increase becomes less pronounced. Pdi values decrease quickly to reach a low value (3.7) and remain more or less constant along the time incubation (Figure 2b). The dissolved oxygen (Figure 2a) also decreases over time due to its continuous consumption by the added laccase.

Figure 2. OH phenolic residual (a), dissolved O₂ (a), instantaneous conversion rate (a), M_w (b) and Pdi (b) variations in continuous operation over time. (a) (■) Instantaneous conversion rate (□) OH-Ph residual (b) (■) Pdi and (□) M_w

Although 16 g/L of SLS were added during the 24h of the reaction, the final M_w average is of the same order of magnitude as the batch with 10 g/L of SLS. These results indicate that the increase of M_w average is rather favoured by the conditions allowing a high amount and instantaneous generation of free radicals rather than a progressive feeding of a high quantity of SLS. However, continuous adding of substrate and enzyme allows a low degree of polydispersity to be reached (3.7) compared to the batch (4.6). The low residual phenolic OH groups in the media and their high conversion rate suppose that the continuous mode promotes the C-O-C linkage.

3.3. Kinetic study of enzymatic bioconversion in fed batch operation

In fed batch mode three alternatives of feeding were tested i) with substrate alone, ii) with enzyme iii) or with both enzyme and substrate. For each assay the addition of substrate and enzyme was carried out in stepwise mode 10 times at a rate of 1g or 3000 U or both every 30 minutes during the five first hours of the reaction. Results are shown in figure 3, figure 4 and table 2.

Figure 3. OH phenolic residual and dissolved O₂ variations in fed-batch operations over time. (a) Adding enzyme; (b) Adding substrate; (c) Adding both enzyme and substrate.

Figure 4. M_w (a) and Pdi (b) variations in fed-batch operations over time.(▲) Adding enzyme; (Δ) Adding both enzyme and substrate; (◆) Adding substrate.

Reaction	Conversion rate 5h (%)	Conversion rate 24 h (%)	Final M_w (Da)	Final Pdi
Fed-batch by adding enzyme	72 %	73 %	29400	4.7
Fed-batch by adding both enzyme and substrate	48 %	61 %	28200	4.2
Fed-batch by adding substrate	39 %	44 %	26500	4.2

Table 2. The conversion rate after 5 h and 24 h of reaction, the final M_w and the final Pdi of reactions carried out in fed-batch operations.

Concerning the reaction carried out with enzyme feeding of the reactor (figure 3 a), similar results as batch mode operation were observed for both residual phenolic OH groups and oxygen consumption. In a first step a rapid oxidation of phenolic OH groups and oxygen uptake rate were observed, followed by an increase of the dissolved oxygen in the medium and a low oxidation of phenolic OH groups was observed during a second step. This behaviour confirms that only a low amount of enzyme is needed to oxidise the 10 g/L of SLS and the oxygen consumption occurs only during this first step of free radical generation.

After 24h of reaction, the conversion rate of phenolic OH groups (73 %) was in the same magnitude as that obtained for the batch mode (table 2). As it is indicated in figure 4 and table 2, the M_w average rose gradually during the period of enzyme addition (5h) and then stabilizes around 29000 Da along the remaining time of the reaction. Pdi value decreased rapidly and stabilised more or less at 4.7 until the end of the reaction (24 hours).

For reactions carried out with the addition of substrate or enzyme and substrate, a progressive increase of phenolic OH group content during the first 5 hours (0.2 g/L) then a slight decrease were observed. Moreover, dissolved oxygen decreases and then increases quickly after each addition, in a repetitive way (figure 3b and 3c). The conversion rate of phenolic OH groups after 24 hours is 61 % for enzyme and substrate addition and 44 % for substrate feeding.

As for continuous mode, a progressive increase of M_w was observed to reach 28200 Da and 26400 Da respectively for enzyme and substrate addition and substrate feeding. The Pdi dropped quickly to 4.2 and remained constant throughout the duration of the reaction (Figure 4 and Table 2).

The relatively low final M_w , the conversion rate and the accumulation of phenolic OH groups indicated that similar mechanisms, such as the one observed in batch mode with 1 g/L, occur. This means that these two modes of reaction promote a “thermodynamic control” and then lead to C-C linkages instead of C-O-C coupling.

4. Conclusions

The obtained results in this work indicated that the increase of M_w average and the decrease of the polydispersity depend on the operating conditions. Batch mode with high concentration of SLS (10 g/L), promotes the increase of the M_w and probably the C-O-C coupling route. This seems to be due to the high and instantaneous generation of free radicals, favouring the “kinetic control” of the reaction. The continuous mode also favours the formation of C-O-C bounds and indicates that the increase of M_w is strongly affected by the high amount of phenoxyl radicals generated than the quantity of added substrate. However, continuous feeding of enzyme and substrate leads to a low Pdi. Results for fed batch, carried out with enzyme feeding, is comparable to those obtained for batch with 10 g/L; the enzyme plays a minor role and a low amount is enough to oxidise the tested concentration of SLS.

Substrate adding and enzyme and substrate adding, as a dilute batch system, promotes C-C coupling ("thermodynamic control") and thus a low M_w increase. These results are likely to open new ways to control the enzymatic bioconversion of lignosulfonates. However these assumptions need to be verified by spectroscopy analyses of the formed polymers in order to have a better understanding of the mechanisms of allowing C-C or C-O-C coupling.

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References

- [1] Areskog D, Li J, Gellerstedt G, Henriksson G. 2010. Investigation of the molecular weight increase of commercial lignosulfonates by laccase catalysis. *Biomacromolecules* 11: 904-910.
- [2] Areskog D, Li J, Nousiainen P, Gellerstedt G, Sipila J, Henriksson G. 2010. Oxidative polymerisation of models for phenolic lignin end-groups by laccase. *Holzfor-schung* 64: 21-34.
- [3] Benavente-Garcia O, Castillo J, Lorente J, Ortuo A, Del Rio J. A. 2000. Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves. *Food Chemistry* 68: 457-462.
- [4] Bhattacharya P K, Todi R K, Tiwari M, Bhattacharjee C, Bhattacharjee S, Datta S. 2005. Studies on ultrafiltration of spent sulfite liquor using various membranes for the recovery of lignosulphonates. *Desalination* 174: 287-297.
- [5] Correia P. F. M. M, Ferreira L. M, Reis M. T. A, Carvalho, J. M. R. 2007. A study on the selective recovery of phenol and formaldehyde from phenolic resin plant effluents by liquid-liquid extraction. *Solvent Extraction and Ion Exchange* 25:485-501.
- [6] Dasgupta S, Taylor K. E., Bewtra J. K., Biswas N. 2007. Inactivation of enzyme laccase and role of cosubstrate oxygen in enzymatic removal of phenol from water. *Water Environment Research* 79: 858-867.
- [7] Faix O, Lange W and E.C. Salud 1981. The use of hplc for the determination of average molecular weights and molecular weight distributions of milled wood lignins from *Shorea polysperma*. *Holzforschung* 35: 3-9.

- [8] Ferreira F. C, Peeva L, Boam A, Zhang S, Livingston A. 2005. Pilot scale application of the Membrane Aromatic Recovery System (MARS) for recovery of phenol from resin production condensates. *J Memb Sci* 257: 120-133.
- [9] Ghosh J. P, Taylor K. E, Bewtra J. K, and Biswas N. 2008. Laccase-catalyzed removal of 2,4-dimethylphenol from synthetic wastewater: Effect of polyethylene glycol and dissolved oxygen. *Chemosphere* 71: 1709-1717.
- [10] Gross S. M, Givens R. D, Jikei M, Royer J. R, Khan S, DeSimone J. M, Odell P. G, and Hamer G. K. 1998. Synthesis and swelling of poly(bisphenol a carbonate) using supercritical CO₂. *Macromolecules* 31: 9090-9092.
- [11] Husain Q. 2010. Peroxidase mediated decolorization and remediation of wastewater containing industrial dyes: A review. *Reviews Environ Sci Biotechnol* 9: 117-140.
- [12] Ikeda R, Uyama H, Kobayashi S. 2001. Laccase-catalyzed curing of vinyl polymers bearing a phenol moiety in the side chain. *Polymer J* 33: 540-542.
- [13] Jeon J. R, Kim E. J, Murugesan K, Park H. K, Kim Y. M, Kwon J. H, Kim W. G, Lee J. Y, Chang Y. S. 2010. Laccase-catalysed polymeric dye synthesis from plant-derived phenols for potential application in hair dyeing: Enzymatic colourations driven by homo- or hetero-polymer synthesis. *Microb Biotechnol* 3: 324-335.
- [14] Jiang H, Fang Y, Fu Y, Guo Q. X. 2003. Studies on the extraction of phenol in wastewater. *J Hazardous Mater* 101: 179-190.
- [15] Joo H, Yoo Y. J, Dordick J. S. 1998. Polymers from biocatalysts. *Korean J Chem Engineer* 15: 362-374.
- [16] Kim S, Silva C, Zille A, Lopez C, Evtuguin D. V, and Cavaco-Paulo A. 2009. Characterisation of enzymatically oxidised lignosulfonates and their application on lignocellulosic fabrics. *Polymer Intern* 58: 863-868.
- [17] Kobayashi S. 1999. Enzymatic polymerization: a new method of polymer synthesis. *Journal of Polymer Science, Part A: Polymer Chemistry* 37: 3041-3056.
- [18] Kobayashi S, Shoda S. I, Uyama H. 1995. Enzymatic polymerization and oligomerization. *Advances in Polymer Sci* 121: 1-30.
- [19] Kobayashi S, Uyama H. 1998. Enzymatic Polymerization for Synthesis of Polyesters and Polyaromatics. in *ACS Symposium Series*, Vol. 684, pp. 58-73.
- [20] Kobayashi S, Uyama H, Kimura S. 2001. Enzymatic polymerization. *Chemical Reviews* 101, 3793-3818.
- [21] Kurniawati S, Nicell J. A. 2009. A comprehensive kinetic model of laccase-catalyzed oxidation of aqueous phenol. *Biotechnol Progress* 25: 763-773.
- [22] Madad N, Chebil L, Sanchez C, Charbonnel C, and Ghoul M. 2011. Effect of molecular weight distribution on chemical, structural and physicochemical properties of sodium lignosulfonates. *Rasyan J chem* 4: 189-202.

- [23] Mita N, Tawaki S. I, Hiroshi U, and Kobayashi S. 2003. Laccase-catalyzed oxidative polymerization of phenols. *Macromolecular Bioscience* 3: 253-257.
- [24] Nazari K, Esmaeili N, Mahmoudi A, Rahimi H, and Moosavi-Movahedi A. 2007. Peroxidative phenol removal from aqueous solutions using activated peroxidase biocatalyst. *Enzyme Microb Technol* 41: 221-233.
- [25] Nugroho Prasetyo E, Kudanga T, Ostergaard L, Rencoret J, Gutierrez A, del Rio J. C, Ignacio Santos J, Nieto L, Jimenez-Barbero J, Martinez A. T, Li J, Gellerstedt G, Lepifre S, Silva C, Kim S Y, Cavaco-Paulo A, Seljebakken Klausen B, Lutnaes B. F, Nyanghongo G. S, and Guebitz G. M. 2010. Polymerization of lignosulfonates by the laccase-HBT (1-hydroxybenzotriazole) system improves dispersibility. *Bioresource Technol* 10: 5054-5062.
- [26] Ouyang X, Qiu X, Chen P. 2006. Physicochemical characterization of calcium lignosulfonate-A potentially useful water reducer. *Colloids Surf A* 282-283: 489-497.
- [27] Reihmann M, and Ritter H. 2006. Synthesis of phenol polymers using peroxidases in *Advances in Polymer Science* Vol. 194, pp. 1-49.
- [28] Riva S. 2006. Laccases: blue enzymes for green chemistry. *Trends in Biotechnology* 24: 219-226.
- [29] Rodrigues S, Pinto G. A. S, and Fernandes F. A. N. 2008. Optimization of ultrasound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell powder by response surface methodology. *Ultrasonics Sonochem* 15: 95-100.
- [30] Venkateswaran P, Palanivelu K. 2006. Recovery of phenol from aqueous solution by supported liquid membrane using vegetable oils as liquid membrane. *J Hazardous Mater* 131: 146-152.
- [31] Widsten P, Kandelbauer A. 2008. Laccase applications in the forest products industry: A review. *Enzyme Microb Technol* 42: 293-307.
- [32] Wu Y, Taylor K. E, Biswas N, Bewtra, J. K. 1999. Kinetic model-aided reactor design for peroxidase-catalyzed removal of phenol in the presence of polyethylene glycol. *J Chem Technol Biotech* 74: 519-526.
- [33] Yang D, Qiu X, Pang Y, Zhou M. 2008. Physicochemical properties of calcium lignosulfonate with different molecular weights as dispersant in aqueous suspension. *J. Disper. Sci. Technol.* 29: 1296-1303.
- [34] Zhou M, Qiu X, Yang D, Lou H. 2006. Properties of different molecular weight sodium lignosulfonate fractions as dispersant of coal-water slurry. *J. Dispersion Sci. Technol* 27: 851-856.