Recycling Immobilized Laccase for Lignin Removal from Prehydrolysis Liquor of Kraft-based Dissolving Pulp

Qiang Wang,[a,b,c,]* Tianzhong Yuan,[a] Shanshan Liu,[a,b]* Guihua Yang,[a] Jiachuan Chen,[a] Wenhai Li,[b] Rendang Yang,[b,c] and Pedram Fatehi [a,d]

Laccase treatment of prehydrolysis liquor (PHL) produced in a kraft-based dissolving pulp production may allow for purification of its hemicelluloses. In this work, a magnetic support of magnetic silica particle (Fe_3O_4/SiO_2) was synthesized to immobilize laccase from *Trametes versicolor*. The laccase treatment led to increases in the molecular weight of lignin, which facilitated its removal from PHL. The results showed that laccase activity remained at 65% after seven successive recycle runs. The combination of 10 wt.% fresh immobilized laccase and 90 wt.% recycled immobilized laccase at the overall dosage of 1 U/mL led to 34% lignin removal, irrespective of the recycling runs. The laccase treatment caused 13 wt.% sugar losses from PHL. Based on the results, a process for removing lignin from PHL was proposed based on the application and recovery of an immobilized laccase system.

Keywords: Immobilization; Laccase; Lignin removal; Dissolving pulp

Contact information: a: Key Lab of Paper Science and Technology of Ministry of Education, Qilu University of Technology, Jinan, Shandong Province, 250353, China; b: State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou, Guangdong Province, 510640, China; c: Qifeng New Material Co., Ltd., Zibo, Shandong Province, 255000, China; d: Department of Chemical Engineering, Lakehead University, Thunder Bay, Ontario, Canada P7B 5E1;
** Corresponding author: wangqiang8303@163.com*

INTRODUCTION

The fractionation of lignocellulose for producing value-added products is a goal of the biorefining concept (van Heiningen 2006). The kraft-based dissolving pulp production process fits well into this concept by separating hemicellulose, lignin, and cellulose into different streams (Sixta *et al.* 2013). In this process, wood chips are pretreated with steam or hot water, which removes the hemicelluloses and a part of lignin and dissolves them in prehydrolysis liquor (PHL) (Li *et al.* 2015). The treated wood chips are then subjected to kraft pulping operations for isolating the remaining lignin from the cellulose of wood chips (Duan *et al.* 2015b). The cellulose produced in this process has been used for many products, including cellulose nitrate, cellulose acetate, and cellulose ether productions (Miao *et al.* 2014). The lignin of this process has also been long used commercially for heat recovery in the kraft process (Kong *et al.* 2015). However, the remaining hemicelluloses in PHL have not been well-utilized for value-added applications (Shen *et al.* 2013). The main challenge in using the hemicelluloses of PHL is the presence of lignin.

Recently, laccase treatment was proposed as an effective method for removing lignin from PHL, as it is highly selective and environmentally friendly. Previous studies (Wang *et al.* 2014) showed that lignin removal from PHL could reach 46% at a laccase dosage of 1 U/mL. Jurado *et al.* (2009) also stated that laccase led to the polymerization of phenolic compounds due to the generation of unstable phenoxy radicals. However, the laccase that is dissolved into PHL cannot be recovered for reuse, which may make the enzyme cost of this process prohibitively expensive.

Laccase immobilization technology is a promising method for performing enzyme recycling, improving its stability, and reducing the enzyme cost. In the past, several carriers (Huang *et al.* 2006; Kunamneni *et al.* 2008; Patel *et al.* 2014) have been used for enzyme immobilization, such as chitosan, nanoparticles, alginate, nylon membrane, diatomaceous earth support, and activated carbon. Xu *et al.* (2013) used chitosan/poly composite nanofibrous membranes to immobilize laccase for removing 2,4-dichlorophenol (with 87.6% efficiency), which was higher than that of free laccase (with 82.7% efficiency). Lu *et al.* (2007) employed alginate-chitosan microcapsule immobilized laccase for the decolorization of alizarin red from textile industries and found that 66% decolorization could be achieved in 1 h with the addition of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). Ludwig *et al.* (2013) used Sepabeads® EC-EA as laccase carriers to reduce toxic phenolic compounds in the xylan-rich fraction by polymerization, and found that 72% xylan conversion could be achieved in 1 h.

In contrast, the use of magnets as a carrier for enzymes has attracted attention, as it can be easily recovered from the system after use with the help of an external magnetic field. This feature provides an opportunity for developing a recycling stream for reuse, thus facilitating the practicality of the process by reducing the cost of enzyme use (Deng *et al.* 2008). For example, Wang *et al.* (2010) fabricated new, large-pore, magnetic mesoporous silica nanoparticles (MMSNPs) for laccase immobilization and found that 92% of initial laccase activity remained compared to a complete activity loss for free laccase after 7 weeks of storage in fresh buffer solution. Wang *et al.* (2012) employed magnetic mesoporous silica nanoparticles (MMSNPs-Cu^{2+}-60) to capture laccase from a fermentation broth with a purification rate of 60.6% and an activity yield of 114.6%, offering a robust and inexpensive approach for laccase purification. However, the application of immobilized laccase for treating PHL has not been investigated yet.

The objective of the present study is to evaluate the recyclability and reusability of laccase immobilized *via* the magnetic support of magnetic silica particle (Fe_3O_4/SiO_2) for removing lignin from PHL. The hypothesis was that the laccase immobilized on a magnetic support could be separated from PHL easily and reused readily. According to Wang *et al.* (2010), the immobilized laccase should have better stability and temperature endurance, which could significantly improve laccase application at the industrial scale. In this work, various scenarios are evaluated to remove the maximum amount of lignin from PHL, and a process that incorporates the immobilized laccase treatment into dissolving pulp production is proposed for the first time, which is a novelty of this work.

EXPERIMENTAL

Materials

The prehydrolysis liquor (PHL) was collected from Shandong Sun Paper Industry Company of Rizhao, China, which produces dissolving pulp based on kraft technology and uses mixed hardwood as the raw material. Laccase from *Trametes versicolor* with the activity of 1.07 U/mg was purchased from Sigma-Aldrich (Shanghai, China). Brilliant Blue G was purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). The ferrous chloride tetrahydrate, iron chloride hexahydrate, isopropanol, tetraethoxysilane (TEOS), acetic acid, and sodium acetate were all of analytical grade and purchased from

Kelvin Chemical Company (Jinan, China). The 3-Aminoprogyl triethoxysilane (APTES) of analytical grade was purchased from Aladdin Industrial Corporation (Shanghai, China). Deionized water was used in all of the experiments.

Preparation of Fe_3O_4 /SiO_2 magnetic support and immobilized laccase

In this set of experiments, Fe_3O_4 was synthesized *via* a chemical precipitation method (Kang *et al.* 1996). Briefly, 2.16 g of iron chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) and 0.796 g of ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$) were dissolved in 100 mL of deionized water *via* stirring at 1000 rpm at 80 °C under a N_2 atmosphere in an oil bath. Then, 10 mL of ammonia was added to the reaction medium and the medium was kept for 1 h under constant stirring at 1000 rpm. The resultant Fe_3O_4 particle was collected using external magnets and washed with 50 mL deionized water.

Next, a Fe_3O_4/SiO_2 magnet was fabricated according to the modified sol-gel method (Park *et al.* 2005). Initially, a desired amount of Fe_3O_4 particles was dissolved in pure isopropanol solution, and the pH of the solution was adjusted to 11 using ammonium hydroxide. Then, TEOS was added to the solution (TEOS/Fe_3O_4 of 0.6 wt.%) and reacted for 12 h at 25 °C and 150 rpm. The resultant Fe_3O_4/SiO_2 was separated by an external magnet and washed with 50 mL deionized water.

Thirdly, an amine modified Fe_3O_4/SiO_2 composite was prepared according to the method of Huang *et al.* (2005). In this set of experiments, 1 g of Fe_3O_4/SiO_2 composite was dispersed in 100 mL of ethanol. Afterwards, 2 mL of APTES and 10 mL of deionized water were added into the solution. The reaction was performed at 25 °C and 150 rpm for 12 h. Once completed, the composite was separated by an external magnet and washed with 50 mL deionized water to remove the excess APTES.

Then, the amine modified Fe_3O_4/SiO_2 composite was crosslinked with glutaraldehyde solution (6% concentration) at 25 °C and 150 rpm for 3 h, according to a previously established procedure (Patel *et al.* 2014). Then, the excess glutaraldehyde was washed off with 50 mL deionized water in the presence of an external magnet.

Subsequently, a certain amount of laccase was dissolved in deionized water, and the synthesized magnetic support was added into the solution and kept at 25 °C and 150 rpm for 3 h. The conditions of the treatment were an initial laccase concentration of 0.4 mg/mL and a duration of 3 h. Once the immobilization was completed, the immobilized laccase was separated with an external magnet and washed with deionized water until no protein was detected in the eluate of DI water *via* the Bradford method (Bradford 1976).

Treatment of PHL with immobilized laccase

In this set of experiments, 10 mL of PHL was added into a 100-mL Erlenmeyer flask. Then, a required amount of immobilized laccase was added into the flask, and the reaction was performed at 150 rpm in an incubator shaker (ZWY-240, Zhicheng, Shanghai, China). The temperature and pH were set at 40 °C and 3.6, respectively. The immobilized laccase dosage ranged from 0.25 U/mL to 2 U/mL based on the PHL volume. The treatment time ranged from 0.5 h to 5 h. Once the reaction was completed, the laccase was separated from the PHL with the assistance of an external magnet for recycling. The collected PHL was stored at 4 °C for lignin and hemicellulose analyses.

Methods

The laccase activity was determined by spectrophotometry at 420 nm (ε = 36000 M^{-1} cm^{-1}) as described by Mansfield (2002) at a pH of 4.5 and 20 °C with ABTS (0.5 mM) as the substrate. One activity unit (U/mL) was defined as the amount of enzyme that oxidized 1 μmol of ABTS per min. The loading capacity of laccase onto support was determined by measuring the difference of initial and final concentration in the immobilization medium using the method described by Bradford (1976). A calibration curve established with bovine serum albumin (BSA) was used as the standard for the calculation of protein concentration.

The lignin content of the PHL was analyzed by UV-vis spectrophotometry at 205 nm (Saeed *et al*. 2012). The concentration of sugar in the PHL was determined using an ion chromatography (IC5000+, Thermo Fisher Scientific, Inc., USA) unit equipped with a Dionex CarboPacTMPA20 (3 mm × 150 mm) column made in the USA. Distilled water and 250 mM sodium hydroxide (NaOH) were used as the eluent at a flow rate of 0.4 mL/min. The column temperature was set to 30 ºC. The acid hydrolysis of PHL was performed at 121 °C for 1 h, as described by Yang *et al.* (2012), to convert oligosugars to monosugars, as the ion chromatography can only detect monosugars of PHL. The sugar content of acid hydrolyzed PHL was determined by ion chromatography.

The molecular weight of the lignin of PHL was determined using gel permeation chromatography (GPC; Shimadzu, Shanghai, China). The separated lignin from PHL was acetyl-brominated in a mixture of acetyl bromide and acetic acid (8:92 wt.%) and kept for 72 h in accordance with Iiyama and Wallis (1988) and Wang *et al.* (2014). A filter of 0.22 μm was employed to filter the lignin for GPC analysis and then subjected to molecular weight analysis of lignin based on the GPC method (Agilent 1260).

RESULTS AND DISCUSSION

Impact of Immobilized Laccase on PHL

The synthesized magnetic support (Fe_3O_4/SiO_2) was employed for the immobilization of laccase from *Trametes versicolor*. The support possessed magnetic characteristics, which greatly facilitated the separation process from the liquid phase *via* an external magnetic field. The loading capacity (U/g $_{\text{support}}$) of the magnet support for the laccase was studied as a function of concentration and time in the immobilization buffer. The laccase loading capacity was 60 mg protein/g $_{\text{support}}$ at 0.4 mg/mL laccase concentration for 3 h, and the laccase activity was 60 U/g $_{\text{support}}$.

The immobilized laccase would facilitate the polymerization of lignin in PHL. As stated earlier, the PHL was treated with immobilized laccase under the treatment conditions of pH 3.6, 40 °C, 2 h duration, immobilized laccase dosage of 1 U/mL, and 150 rpm. The molecular weight increase was direct evidence of lignin polymerization in PHL. Therefore, GPC analysis was employed to measure the molecular weight (M_w) changes of lignin in PHL after treatment with immobilized laccase.

The M_w of lignin increased from 1131 g/mol to 21522 g/mol for the untreated and treated PHL, respectively, and the polydispersity increased from 2.21 to 5.35. The lignin in the pretreated PHL was collected from the PHL *via* filtration. A similar phenomenon was observed by Jurado *et al.* (2009) when using laccase for detoxification during ethanol production, and they reported that a new peak with higher molecular mass appeared.

The chemical compositions of the original and treated PHL are listed in Table 1. As expected, xylose and xylan were the major components of PHL, representing 73.8% of the total sugars in the original PHL. The PHL contained 78.7% xylose and xylan in other reports (Yang *et al.* 2013). By treating with immobilized laccase, the xylose loss was only

4.68 g/L (approximately 13.2%). This loss could have been due to the adsorption of sugars onto the magnetic support. According to the report (Liu *et al.* 2011), approximately 33% to 70% of hemicellulose could be adsorbed onto modified activated carbon from the prehydrolysis liquor. It can also be seen that the furfural and acetic acid contents were not affected by the treatment.

Table 1. Chemical Compositions of Original and Treated PHL (g/L) (Conditions were pH 3.6, 40 °C, 2 h, and Immobilized Laccase Dosage of 1 U/mL)

	Original PHL		Yang *et al.* (2013)		Treated PHL in This Work	
	Mono	Oligo	Mono	Oligo	Mono	Oligo
Xylose	20.72	43.19	5.63	46.91	18.72	40.51
Galactose	4.47	4.30	0.79	1.59	3.96	3.67
Arabinose	2.86	ND[a]	1.47	0	1.52	ND[a]
Glucose	0.46	5.06	0.94	4.33	0.30	4.03
Mannose	0.72	4.86	0.43	4.69	0.47	2.05
Total sugars	86.64		66.78		75.23	
Acetic acid	15.98		11.91		15.00	
Furfural	1.01		1.62		0.95	
ND[a]- not detected						

Lignin Removal by Immobilized Laccase Treatment

The effects of immobilization laccase treatment time and dosage on lignin removal are shown in Figs. 1A and 1B. The lignin removal was increased from 19% to 37% by extending the treatment time from 0.5 h to 2 h. The lignin removal further increased to 45% when the duration of the treatment was extended to 5 h. Similarly, the lignin removal rate increased to 48% when the laccase dosage was increased to 2 U/mL. When free laccase was used at 1 U/mL in PHL, 47% lignin removal was achieved. The lower reactivity of laccase in the immobilized state was most probably due to the limited accessibility of laccase in the immobilized state (Bai *et al.* 2006; Sari *et al.* 2006).

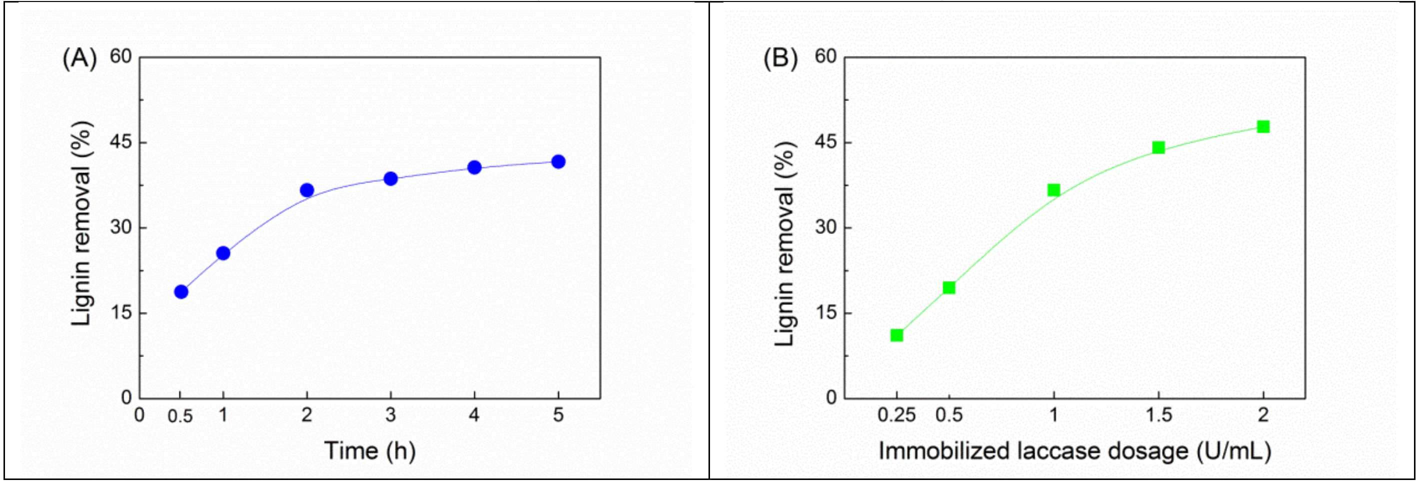

Fig. 1. Lignin removal from PHL by immobilized laccase treatment: (A) Effect of time (at 40 °C, pH 3.6, and a laccase dosage of 1 U/mL); (B) Effect of immobilized laccase dosage (40 °C, pH 3.6, and duration of 1 h)

Recovery of Immobilized Laccase

The laccase activity after a series of recycling runs is shown in Fig. 2. The activity of laccase was 1.07 U/mg in the first run, but it dropped to 70% of its original value after 7 runs. The drop in the activity was attributed in part to the fact that immobilized laccase might become free when reused. Zhu *et al.* (2007) reported that approximately 74% of laccase activity remained after 10 recycling runs when using magnetic mesoporous silica spheres for immobilizing laccase. In another study (Wang *et al.* 2012), approximately 86% of laccase reactivity remained after 10 recycling runs when using large-pore magnetic mesoporous silica nanoparticles as a support.

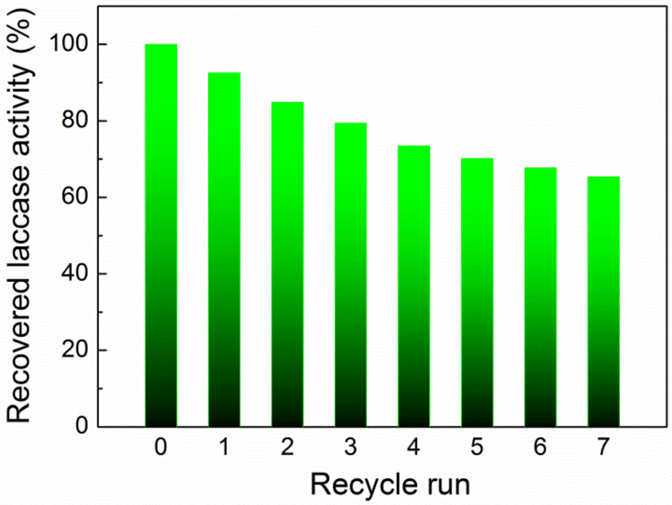

Fig. 2. Recovered laccase activities in seven recycling runs

Reusability of Immobilized Laccase

The reusability of immobilized laccase in lignin removal is presented in Fig. 3.

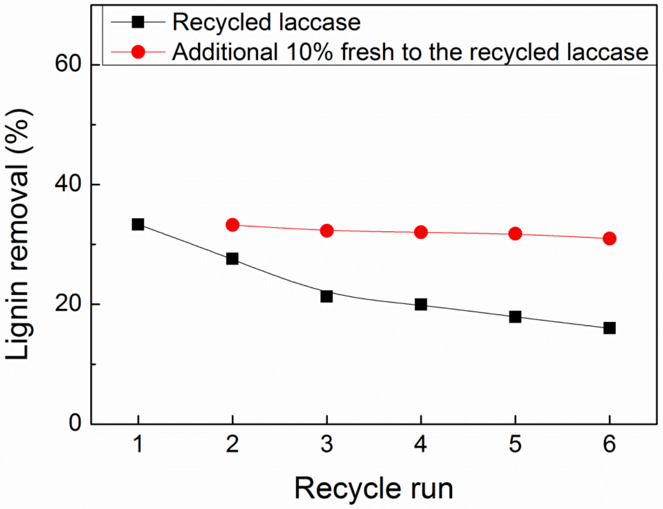

Fig. 3. Lignin removal from PHL by recycled immobilized laccase with or without fresh laccase addition; the experiments were conducted at pH 3.6, 40 °C, 2 h, and an initial immobilized laccase dosage of 1 U/mL

As can be seen, the lignin removal decreased when immobilized laccase was reused. This finding was in agreement with the drop in the laccase activity observed in Fig. 2. By adding 10% fresh immobilized laccase along with recovered immobilized laccase, a consistent lignin removal of 34% after 6 recycling runs was achieved. This analysis shows that a large portion of immobilized laccase can be reused, which is beneficial for large scale implementation.

Proposed Process for Lignin Removal from PHL by Immobilized Laccase

As is well documented (Testova *et al.* 2014; Duan *et al.* 2015a), the prehydrolysis process at 170 °C, a 2 h duration, and a liquid/solid ratio of 4/1 (Wt./ Wt.) is an important process in the removal hemicelluloses from wood chips prior to kraft pulping in the kraft-based dissolving pulp production process. Part of lignin is also dissolved into the PHL along with hemicellulose (Fatehi *et al.* 2013; Liu *et al.* 2013). Shi *et al.* (2012) reported that the presence of lignin in the PHL hampered the production of purified hemicelluloses and their conversion to value-added products. Therefore, lignin removal is a prerequisite for effective valorization of hemicellulose. Based on the results obtained above, a process for treating PHL is introduced in Fig. 4. In this process, the produced PHL is treated with immobilized laccase, in which lignin is polymerized. The lignin with increased particle size could be filtered from the treated PHL. The isolation of lignin aids in the purification of hemicelluloses. The immobilized laccase can then be separated from the PHL *via* applying an external magnetic field and later reused.

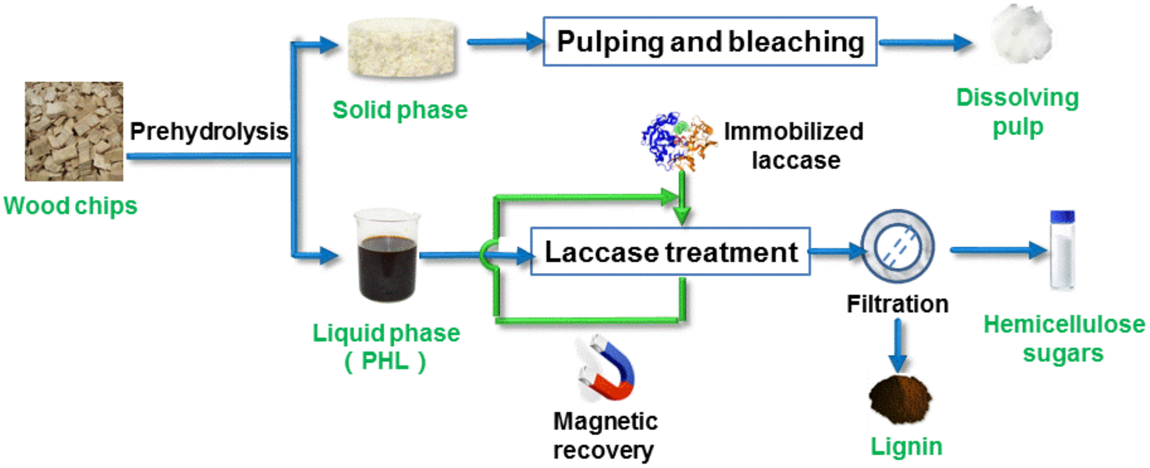

Fig. 4. Proposed process for immobilized laccase treatment of PHL in a kraft-based dissolving pulp production

CONCLUSIONS

1. The recyclability and reusability of immobilized laccase on Fe_3O_4/SiO_2 magnetic support was investigated in this study. The laccase loading capacity was 60 mg protein/g $_{support}$ at 0.4 mg/mL laccase concentration for 3 h. The recovered laccase activity ranged from 65% to 92% in seven successive recycling runs.
2. The recovered immobilized laccase with the addition of 10% of fresh laccase led to a constant 34% lignin removal for 6 recycling runs under the conditions of 1 U/mL of laccase dosage, a 2 h duration, 40 °C, and a pH of 3.6.

3. The total sugar loss was 13.2% for immobilized laccase-treated PHL. The successive removal of lignin and the recycling affinity of immobilized laccase may make this process attractive for large scale implementation.

ACKNOWLEDGMENTS

The authors are grateful for the financial support of the National Key R&D Program of China (2017YFB0307900), the National Science Foundation of China (Grant Nos. 31670584, 31500490), and the Taishan Scholar Project special funds.

REFERENCES CITED

Bai, Y. X., Li, Y. F., and Wang, M. T. (2006). "Study on synthesis of a hydrophilic bead carrier containing epoxy groups and its properties for glucoamylase immobilization," *Enzyme and Microbial Technology* 39(4), 540-547. DOI: 10.1016/j.enzmictec.2005.08.041

Bradford, M. M. (1976). "Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," *Analytical Biochemistry* 72(1-2), 248-254. DOI: 10.1016/0003-2697(76)90527-3

Deng, Y., Qi, D., Deng, C., Zhang, X., and Zhao, D. (2008). "Superparamagnetic high-magnetization microspheres with an Fe_3O_4 @SiO_2 core and perpendicularly aligned mesoporous SiO_2 shell for removal of microcystins," *Journal of the American Chemical Society* 130(1), 28-29. DOI: 10.1021/ja0777584

Duan, C., Li, J., Ma, X., Chen, C., Liu, Y., Stavik, J., and Ni, Y. (2015a). "Comparison of acid sulfite (AS)- and prehydrolysis kraft (PHK)-based dissolving pulps," *Cellulose* 22(6), 4017-4026. DOI: 10.1007/s10570-015-0781-1

Duan, C., Long, Y., Li, J., Ma, X., and Ni, Y. (2015b). "Changes of cellulose accessibility to cellulase due to fiber hornification and its impact on enzymatic viscosity control of dissolving pulp," *Cellulose* 22(4), 2729-2736. DOI: 10.1007/s10570-015-0636-9

Fatehi, P., Ryan, J., and Ni, Y. (2013). "Adsorption of lignocelluloses of model pre-hydrolysis liquor on activated carbon," *Bioresource Technology* 131, 308-314. DOI: 10.1016/j.biortech.2012.12.156

Huang, J., Xiao, H., Li, B., Wang, J., and Jiang, D. (2006). "Immobilization of *Pycnoporus sanguineus laccase* on copper tetra-aminophthalocyanine-Fe_3O_4 nanoparticle composite," *Biotechnology and Applied Biochemistry* 44(2), 93-100. DOI: 10.1042/BA20050213

Huang, J., Zhou, J. Y., Xiao, H. Y., Long, S. Y., and Wang, J. T. (2005). "Study of CuTAPc-Fe_3O_4 nanoparticles and their laccase immobilization," *Acta Chimica Sinica* 63(14), 1343-1347.

Iiyama, K., and Wallis, A. (1988). "An improved acetyl bromide procedure for determining lignin in woods and wood pulps," *Wood Science and Technology* 22(3), 271-280. DOI: 10.1007/BF00386022

Jurado, M., Prieto, A., Martínez-Alcalá, Á., Martínez, Á. T., and Martínez, M. J. (2009). "Laccase detoxification of steam-exploded wheat straw for second generation bioethanol," *Bioresource Technology* 100(24), 6378-6384. DOI: 10.1016/j.biortech.2009.07.049

Kang, Y. S., Risbud, S., Rabolt, J. F., and Stroeve, P. (1996). "Synthesis and characterization of nanometer-size Fe_3O_4 and γ- Fe_2O_3 particles," *Chemistry of Materials* 8(9), 2209-2211. DOI: 10.1021/cm960157j

Kong, F., Wang, S., Price, J. T., Konduri, M. K. R., and Fatehi, P. (2015). "Water soluble kraft lignin-acrylic acid copolymer: Synthesis and characterization," *Green Chemistry* 17(8), 4355-4366. DOI: 10.1039/C5GC00228A

Li, J., Zhang, H., Duan, C., Liu, Y., and Ni, Y. (2015). "Enhancing hemicelluloses removal from a softwood sulfite pulp," *Bioresource Technology* 192, 11-16. DOI: 10.1016/j.biortech.2015.04.107

Liu, H., Hu, H., Jahan, M. S., and Ni, Y. (2013). "Furfural formation from the pre-hydrolysis liquor of a hardwood kraft-based dissolving pulp production process," *Bioresource Technology* 131, 315-320. DOI: 10.1016/j.biortech.2012.12.158

Liu, X., Fatehi, P., and Ni, Y. (2011). "Adsorption of lignocelluloses dissolved in prehydrolysis liquor of kraft-based dissolving pulp process on oxidized activated carbons," *Industrial and Engineering Chemistry Research* 50(20), 11706-11711. DOI: 10.1021/ie201036qrd

Lu, L., Zhao, M., and Wang, Y. (2007). "Immobilization of laccase by alginate–chitosan microcapsules and its use in dye decolorization," *World Journal of Microbiology and Biotechnology* 23(2), 159-166. DOI: 10.1007/s11274-006-9205-6

Ludwig, D., Amann, M., Hirth, T., Rupp, S., and Zibek, S. (2013). "Development and optimization of single and combined detoxification processes to improve the fermentability of lignocellulose hydrolyzates," *Bioresource Technology* 133, 455-461. DOI: 10.1016/j.biortech.2013.01.053

Mansfield, S. D. (2002). "Laccase impregnation during mechanical pulp processing-improved refining efficiency and sheet strength," *Appita Journal* 55(1), 49-53.

Miao, Q., Chen, L., Huang, L., Tian, C., Zheng, L., and Ni, Y. (2014). "A process for enhancing the accessibility and reactivity of hardwood kraft-based dissolving pulp for viscose rayon production by cellulase treatment," *Bioresource Technology* 154, 109-113. DOI: 10.1016/j.biortech.2013.12.040

Park, I., Wang, Z., and Pinnavaia, T. J. (2005). "Assembly of large-pore silica mesophases with wormhole framework structures from α,ω-diamine porogens," *Chemistry of Materials* 17(2), 383-386. DOI: 10.1021/cm048627u

Patel, S. K. S., Kalia, V. C., Choi, J. H., Haw, J. R., Kim, I. W., and Lee, J. K. (2014). "Immobilization of laccase on SiO_2 nanocarriers improves its stability and reusability," *Journal of Microbiology and Biotechnology* 24(5), 639-647. DOI: 10.4014/jmb.1401.01025

Saeed, A., Jahan, M.S., Li, H., Liu, Z., Ni, Y., van Heiningen, A. (2012). "Mass balances of components dissolved in the pre-hydrolysis liquor of kraft-based dissolving pulp production process from Canadian hardwoods," *Biomass and Bioenergy* 39, 14-19. DOI: 10.1016/j.biombioe.2010.08.039

Sari, M., Akgöl, S., Karataş, M., and Denizli, A. (2006). "Reversible immobilization of catalase by metal chelate affinity interaction on magnetic beads," *Industrial and Engineering Chemistry Research* 45(9), 3036-3043. DOI: 10.1021/ie0507979

Shen, J., Kaur, I., Baktash, M. M., He, Z., and Ni, Y. (2013). "A combined process of activated carbon adsorption, ion exchange resin treatment and membrane concentration for recovery of dissolved organics in pre-hydrolysis liquor of the kraft-based dissolving pulp production process," *Bioresource Technology* 127, 59-65. DOI: 10.1016/j.biortech.2012.10.031

Shi, H., Fatehi, P., Xiao, H., and Ni, Y. (2012). "Optimizing the poly ethylene oxide flocculation process for isolating lignin of prehydrolysis liquor of a kraft-based dissolving pulp production process," *Industrial & Engineering Chemistry Research* 51(14), 5330-5335. DOI: 10.1021/ie300141k

Sixta, H., Iakovlev, M., Testova, L., Roselli, A., Hummel, M., Borrega, M., Van Heiningen, A., Froschauer, C., and Schottenberger, H. (2013). "Novel concepts of dissolving pulp production," *Cellulose* 20(4), 1547-1561. DOI: 10.1007/s10570-013-9943-1

Testova, L., Borrega, M., Tolonen, L. K., Penttila, P. A., Serimaa, R., Larsson, P. T., and Sixta, H. (2014). "Dissolving-grade birch pulps produced under various prehydrolysis intensities: Quality, structure and applications," *Cellulose* 21(3), 2007-2021. DOI: 10.1007/s10570-014-0182-x

Van Heiningen, A. (2006). "Converting a kraft pulp mill into an integrated forest biorefinery," *Pulp and Paper Canada* 107(6), 38-43.

Wang, F., Guo, C., Yang, L. R., and Liu, C. Z. (2010). "Magnetic mesoporous silica nanoparticles: Fabrication and their laccase immobilization performance," *Bioresource Technology* 101(23), 8931-8935. DOI: 10.1016/j.biortech.2010.06.115

Wang, F., Huang, W., Guo, C., and Liu, C. Z. (2012). "Functionalized magnetic mesoporous silica nanoparticles: Fabrication, laccase adsorption performance and direct laccase capture from *Trametes versicolor* fermentation broth," *Bioresource Technology* 126(4), 117-122. DOI: 10.1016/j.biortech.2012.09.005

Wang, Q., Jahan, M. S., Liu, S., Miao, Q., and Ni, Y. (2014). "Lignin removal enhancement from prehydrolysis liquor of kraft-based dissolving pulp production by laccase-induced polymerization," *Bioresource Technology* 164, 380-385. DOI: 10.1016/j.biortech.2014.05.005

Xu, R., Zhou, Q., Li, F., and Zhang, B. (2013). "Laccase immobilization on chitosan/poly(vinyl alcohol) composite nanofibrous membranes for 2,4-dichlorophenol removal," *Chemical Engineering Journal* 222(15), 321-329. DOI: 10.1016/j.cej.2013.02.074

Yang, G., Jahan, M. S., Liu, H., and Ni, Y. (2012). "Acid hydrolysis of prehydrolysis liquor produced from the kraft-based dissolving pulp production process," *Industrial and Engineering Chemistry Research* 51(43), 13902-13907. DOI: 10.1021/ie3023059

Yang, G., Sarwar Jahan, M., Ahsan, L., Zheng, L., and Ni, Y. (2013). "Recovery of acetic acid from pre–hydrolysis liquor of hardwood kraft–based dissolving pulp production process by reactive extraction with triisooctylamine," *Bioresource Technology* 138, 253-258. DOI: 10.1016/j.biortech.2013.03.164

Zhu, Y., Kaskel, S., Shi, J., Wage, T., and Pée, K. H. V. (2007). "Immobilization of *Trametes versicolor* laccase on magnetically separable mesoporous silica spheres," *Chemistry of Materials* 19(26), 6408-6413. DOI: 10.1021/cm071265g

Article submitted: August 8, 2017; Peer review completed: September 24, 2017; Revised version received: September 25, 2017; Accepted: September 28, 2017; Published: October 6, 2017.
DOI: 10.15376/biores.12.4.8838-8847