Bioresource Technology 155 (2014) 418–421

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Short Communication

Polymerization reactivity of sulfomethylated alkali lignin modified with horseradish peroxidase

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HIGHLIGHTS

- A new technology to prepare high molecular weight soluble alkali lignin is proposed.
- The molecular weight of lignin increases over 20 times after HRP modification.
- The structure changes of lignin during HRP modification are investigated.
- Sulfonation and HRP modification were mutually promoted.
- HRP modification can improve the adsorption quantity of lignin.

ARTICLE INFO

Article history:
Received 11 October 2013
Received in revised form 2 December 2013
Accepted 4 December 2013
Available online 14 December 2013

Keywords:
Sulfomethylated alkali lignin
Horseradish peroxidase
Polymerization
Reactivity

ABSTRACT

Alkali lignin (AL) was employed as raw materials in the present study. Sulfomethylation was conducted to improve the solubility of AL, while sulfomethylated alkali lignin (SAL) was further polymerized by horseradish peroxidase (HRP). HRP modification caused a significant increase in molecular weight of SAL which was over 20 times. It was also found to increase the amount of sulfonic and carboxyl groups while decrease the amount of phenolic and methoxyl groups in SAL. The adsorption quantity of self-assembled SAL film was improved after HRP modification. Sulfonation and HRP modification were mutually promoted. The polymerization reactivity of SAL in HRP modification was increased with its sulfonation degree. Meanwhile, HRP modification facilitated SAL's radical-sulfonation reaction.

1. Introduction

Alkali lignin (AL), the major byproduct obtained from black liquor in kraft pulping process, accounts for more than 85% of technical lignin in the world. The utilization of AL contributes considerable economic, social and environmental benefits. However, the low reactivity of AL limits its chemical modification and further practical application, owing to the aryl ether linkages cleavage, the disappearance of the reactive functional groups and condensation of polyphenyl propene units during violent kraft pulping process (Sun et al., 2013; Ouyang et al., 2009).

Sulfonation degree and molecular weight are the two main factors influencing the application performance of AL. Extensive studies have focused on improving these two main factors of AL by activation, including chemical, physical or biological modifications (Zhou et al., 2007; Yang et al., 2013). Generally, physical modification process is inefficient and chemical modification is environmentally unfriendly. Moreover, the increase of sulfonation degree and molecular weight of AL by physical or chemical modifications is limited.

Horseradish peroxidase (HRP) is a kind of high catalytic activity peroxidase extracted from the root of horseradish, which is efficient to polymerize phenols, anilines and their derivatives (Kersten et al., 1990; Hong et al., 2006). HRP is also efficient in lignin modification. Grönqvist et al. (2005) discovered that HRP shows a higher catalytic activity than laccase in lignin polymerization. Blinkovsky and Dordick (1993) found that lignin and phenol could be polymerized by HRP to synthetize phenolic resins. However, the reactivity of lignin during HRP modification is barely investigated to date.

The aim of this work is to modify AL by both chemical and biological treatment. AL was first sulfomethylated with sulfite to obtain sulfomethylated alkali lignin (SAL), and then further incubated with HRP to gain HRP polymerized sulfomethylated alkali

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http://dx.doi.org/10.1016/j.biortech.2013.12.017
0960-8524/

lignin (HSAL).The polymerization reactivity and catalytic mechanism of SAL by HRP/H_2O_2 incubation in aqueous system were also investigated.

2. Methods

2.1. Lignin, enzyme and reagents

AL was isolated from pine pulping black liquor in sulfate acid treatment. It was supplied and purified by Jilin Paper Co. Ltd. (Jilin, China). HRP was supplied by Xueman Biotech Co. Ltd. (Shanghai, China). The enzyme activity of HRP (Robert and Bardsley, 1975) was 12,708 U g^{-1}. All other chemicals were of analytical grade.

2.2. Preparation of SAL by sulfomethylation

10 g of AL was firstly dissolved in sodium hydroxide solution (pH 13) in a reaction vessel (Carousel 6, Radleys Corp., England) and the solution was heated to 70 °C. Subsequently, 0.41 g aqueous formaldehyde solution of 37% concentration was added and stirred for 1 h. Then it was heated to 95 °C, to which 0.5–6 g of sodium sulfite was added and stirred for 3 h. Finally, the product was adjusted to pH 6 and filtered by Büchner funnel. SAL was obtained by freeze-drying and SAL1–SAL6 with different sulfonic group contents were obtained by adjusting the dosage of sodium sulfite.

2.3. Preparation of HSAL by HRP modification

1 g of SAL was dissolved in 50 mL phosphate buffer (0.1 M, pH 6.0) in a reaction vessel and the incubation was started by addition of 0.88 mmol L^{-1} H_2O_2 and 6 g L^{-1} HRP. The reaction was maintained at 30 °C and lasted for 2 h. At last, HSAL was collected by freeze-drying. HSAL1–HSAL6 were prepared by HRP modification of SAL1–SAL6 respectively.

2.4. Gel permeation chromatography (GPC)

The molecular weights of lignin samples were determined by aqueous GPC as described by Yang et al. (2013).

2.5. ^1H NMR spectra

The ^1H NMR spectra of lignin samples were determined by DRX-400 (400 MHz ^1H frequency, Bruker Corp., Germany) with 30 mg of each sample dissolved in 0.5 mL DMSO-d_6.

2.6. Functional group content measurements

All samples were purified by ion-exchange before functional group content measurements. The sulfonic group content (Ouyang et al., 2009) and carboxyl group content (Zhou et al., 2012) of lignin samples were measured by automatic potentiometric titrator (905 Titrando, Metrohm Corp., Switzerland). The phenolic hydroxyl content of lignin samples was detected by FC method (de Sousa et al., 2001). The methoxyl content of lignin samples was determined by the head-space gas chromatography (HS-GC) as described by Li et al. (2012).

2.7. Self-assembled film preparation and characterization

The layer-by-layer (LBL) self-assembled films were prepared according to the method of Deng et al. (2010). The adsorption quantity of the assembled film was detected by UV–Vis spectra.

3. Results and discussion

3.1. Sulfonation and polymerization reactivity of SAL during HRP modification

The sulfonation reactivity of SAL during HRP modification was evaluated by the sulfonation degree of corresponding HSAL. With the increasing dosage of sodium sulfite, the sulfonation degree of HSAL increased from 1.51 to 2.87 mmol g^{-1} (Table 1). Under the same dosage of sodium sulfite, the sulfonation degree of HSAL was much higher than that of SAL. These results indicated that the sulfonation reactivity of SAL was both improved by the increasing dosage of sodium sulfite and HRP modification.

The polymerization reactivity of SAL during HRP modification was evaluated by the M_w of corresponding HSAL. After further modification by HRP, the M_w of HSAL is obviously increased, which was over 20 times (Table 1) higher than that of corresponding SAL (expect for HSAL1). Moreover, M_w of HSAL was improved with the increasing dosage of sodium sulfite. It exhibited a linear relationship ($y = 33272x - 31395$; $R^2 = 0.913$) between sulfonic group contents and M_w of HSAL.

Yang's et al. study Yang et al. (2013) showed that laccase modification was able to increase the sulfomethylation reactivity of AL by 35% and promoted its polymerization, but the change of M_w is limited (from 2900 to 3500 Da at maximum). Compared with laccase, HRP modification is more efficient in improving the M_w and sulfonation reactivity of lignin as outlined above. For example, the increase of the M_w and the sulfonation degree of SAL6 were 26.95 times and 39%, respectively. These results were similar to the results reported by Grönqvist et al. (2005).

3.2. ^1H NMR spectra analyses

The ^1H NMR spectra of lignin samples were recorded and the proton signal intensities of lignin samples (Supplementation data Table 2) were benchmarked through the DMSO proton intensity (taken as 1.00).

The proton signal intensity of H, G, S unit (Fan et al., 2008; Jahan et al., 2007) all decreased obviously after HRP modification compared with those of SAL, and it was noteworthy that the signal of G unit decreased from about 1.5–0.8 (Supplementation data Table 2). The drop of H, G and S protons may be due to the destruction of phenyl structure during HRP modification.

Compared with corresponding SAL, all the proton signal intensities of HSAL of β-O-4′, β-1′, β-5′ and β–β′ structures (Jahan et al., 2007; Yang et al., 2013) increased after HRP modification and increased with the increase of sodium sulfite dosage, especially for those of β-O-4′ and β–β′ structures (Supplementation data Table 2). This suggested that SAL formed different inter-unit linkages during HRP modification, most of which was β-O-4 and β–β types.

The proton signal intensities of methoxyl groups (Jahan et al., 2007; Yang et al., 2013) were both weakened after sulfomethylation and HRP modification and they also decreased with the increasing sodium sulfite dosage (Supplementation data Table 2). Moreover, the proton signal intensity of hydrocarbon (Yang et al., 2013; Xu et al., 2006) increased obviously after HRP modification owing to the enhancement of shielding effect caused by the increase of M_w.

3.3. The functional group contents of SAL and HSAL

As shown in Fig. 1a, with the increasing sulfonation degree of SAL samples, the carboxyl group contents of SAL increased while