

Extraction of Lignin from Wheat Straw by Catalysts in 1,4-Butanediol Medium under Atmospheric Pressure

Yunzheng Li¹, Huizhen Jia¹, Rouzimaimaiti Ruxianguli, Hongquan Yin,* and Qingshan Zhang

Extraction of lignin from lignocellulosic materials in an autoclave using 1,4-butanediol medium has been examined by researchers. However, there has been no research on the extraction of lignin in a 1,4-butanediol medium at atmospheric pressure and no extraction of HBS lignin from wheat straw has been investigated. In this paper, lignin was isolated from wheat straw in 1,4-butanediol media (from 50% to 100%, v/v) that was catalyzed by H₂SO₄ or NaOH while refluxing under atmospheric pressure. Reaction time, liquid-solid ratio, the volume percentage of 1,4-butanediol, as well as catalysts were optimized to improve the lignin yield. The optimum extraction conditions were: 12-to-1 liquor-to-straw ratio of 80% 1,4-butanediol, 1.67% of H₂SO₄ or 0.83% of NaOH, and 3 h reaction time. The optimum yield of lignin was found to be 60.64%. Analysis with Fourier transform infrared spectroscopy, gel permeation chromatography, and thermogravimetry showed that the lignin obtained using the NaOH catalyst had fewer unconjugated C=O groups, fewer =C-O-C bonds, lower molecular weight, and was more thermally unstable than lignin obtained using the H₂SO₄ catalyst.

Keywords: Lignin extraction; 1, 4-butanediol; Wheat straw; Atmospheric pressure

Contact information: Department of Applied Chemistry and Pharmaceutics, School of Chemical Engineering and Environment, Beijing Institute of Technology, Beijing 100081 P. R. China; 1: these authors contributed to this work equally;

* Corresponding author: yinhongquan2008@yahoo.com

INTRODUCTION

Lignin, as the second most abundant polymer only to cellulose in nature, is a three-dimensional network polymer that is found in the cell walls of plants; it is the glue that adheres organic tissue together. The somewhat “incompatible” three components—cellulose, hemicelluloses, and lignin—comprise lignocellulosic materials and are partly connected to one another by covalent bonds in the cell wall (Goyal *et al.* 2006; Hubbe and Lucia 2007). Nowadays, lignin has been widely used for adhesives (Oiihares *et al.* 1988), adsorption agents (Cotoruelo *et al.* 2007), drugs (Reztsova 2007), antioxidant (Ma *et al.* 2013), and phenolic resins (Kuroe *et al.* 2013). But because of the recalcitrant nature of lignin, it is difficult to separate it from lignocellulose. Many organosolv pulping methods such as methanol (Bland and Menshun 1965), ethanol (El Hage *et al.* 2010; Cybulska *et al.* 2012), *n*-propanol and 1-butanol (Pereira *et al.* 2007), aqueous acetic acid (Li and Qin 2006), kraft (Yu *et al.* 1989), alkali (Chen *et al.* 2009), and ionic liquid (Sidik *et al.* 2012) have been used to extract lignin from botanical tissues. Steam explosion is commonly used in the pretreatment of lignocellulose to decrease the content of lignin, but this approach does not separate the lignin from the material (Chen and Liu 2007; Cui *et al.*

2012; Xing *et al.* 2012). Enzymatic hydrolysis lignin is often used to illustrate the structure of the proto-lignin owing to there being little structure modification, yet it has a low lignin yield (Xing *et al.* 2012; Zeng *et al.* 2013). And when compared to ethanol and propanol medium, 1,4-butanediol (BDO) medium is well suited for the separation of lignin from biomass because of its high boiling point, good lignin solubility, and low pressure in the autoclave reactor during cooking (Wang *et al.* 2011a, b, 2014).

In previous studies, lignin was extracted from biomass by high boiling solvent (HBS) cooking in an auto-clave reactor with catalyst. For instance, Cheng *et al.* (2004) extracted HBS lignin from Masson Pine at 220 °C for 3 h with catalyst in an autoclave with lignin yield up to 33.5%. After that, Chen and Cheng (2008) reported that rice husk lignin can be extracted in a BDO medium with a small amount of acid catalyst in an autoclave with lignin yield up to 23.8%. Then, Jia *et al.* (2013) separated lignin from pine-nut at 200 °C for 2 h, using 90% (v/v) BDO as the cooking medium with 6% catalyst. Recently, Wang *et al.* (2014) studied the extraction process of HBS lignin from bagasse, with 57 to 70% lignin isolated from the spent liquor. However, these studies mentioned above were all carried out in autoclaves with high pressure, and no researchers have done extraction attempts of lignin in BDO medium under atmosphere pressure. In addition, the agricultural byproduct wheat straw is of great interest nowadays (Xu *et al.* 2010; Gasser *et al.* 2014; Rossberg *et al.* 2014), and no extraction of HBS lignin from wheat straw (WS) has been investigated. In this paper, lignin was isolated from WS in BDO media (from 50% to 100%, v/v) in a system catalyzed by H₂SO₄ or NaOH while refluxing under atmospheric pressure. Experiments were conducted to optimize lignin output extracted in BDO medium under atmosphere pressure. Reaction time, liquid-solid ratio, the volume percentage of BDO medium, as well as catalysts were optimized to improve lignin yield. In addition, characterization of lignin extracted from WS powder was carried out with Fourier transform infrared (FT-IR) spectroscopy, gel permeation chromatography (GPC), and thermogravimetric analysis (TGA) to compare the effects of the different treatment conditions.

EXPERIMENTAL

Materials

The chemicals were used as received without further purification and were of analytical reagent grade. Ethanol, toluene, BDO, hydrochloric acid, sulphuric acid, dimethyl sulfoxide (DMSO), *N,N*-dimethylformamide (DMF), and tetrahydrofuran (THF) were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Sodium hydroxide, potassium bicarbonate, citric acid, sodium citrate, dioxane, and dichloromethane were purchased from Beijing Chemical Plant (China). Cellulase (> 30 U/mg) was purchased from Beijing Dongxu biotechnology Co., Ltd. (China). WS (60 mesh) was purchased from Shanxi Jin He Agriculture Industry Co., Ltd. (China), and was dried at 100 °C for 3 h prior to use.

Methods

Determination of Klason lignin content in WS powder

The Klason lignin content in the WS powder was determined according to the standard method TAPPI T222 om-02 (2002). Generally, WS (2.0 g) was treated with an

ethanol-toluene mixture (v/v = 1:2) in a Soxhlet extractor for 12 h to remove resin, wax, and fat. Next, the solid was washed with ethanol four times and dried at 100 °C for 4 h. Afterwards, dry WS (1.0 g) was placed into a beaker and mixed with 72 wt.% H₂SO₄ (15 mL) gradually in an ice-bath while stirring. After 2 h at room temperature, the mixture was diluted with deionized water to a final 3 wt. % of H₂SO₄, boiled and refluxed for 4 h, and cooled to room temperature. The solution was left undisturbed overnight and then filtered. The filtered residue was washed with deionized water (DIW) until the pH was 7. The lignin residual was collected and dried under vacuum at 60 °C for 12 h. Klason lignin measurements were performed in triplicate.

Preparation of enzymatic treatment-acid hydrolysis lignin (EAL) (Argyropoulos *et al.* 2002; Zeng *et al.* 2013)

First step (enzymatic treatment): 15 g of oven dried WS was treated with an ethanol-toluene mixture (v/v = 1:2) in a Soxhlet extractor for 12 h. Then the solid was washed with ethanol three times and air-dried. Next 10 g of the dried residue was weighed and mixed with 0.4 g cellulase (> 30 U/mg) in 500 mL 50 mM of sodium citrate buffer (pH 5.0) in a water bath at 50 °C with mechanical agitation for 72 h. After the enzymatic hydrolysis, the partly-digested lignocellulosic material was recovered as an insoluble residue through filtrating. The residual was rinsed with DIW until the pH reached 7, then dried in a vacuum oven at 60 °C with pressure less than 133 Pa.

Second step (moderate acid hydrolysis): The dried residual recovered from the first step was mixed with 100 mL of 0.05 mM HCl solution in 85% dioxane (v/v) in a 250 mL round bottom flask, and was refluxed at 110 °C under nitrogen atmosphere for 2 h. After that, the mixture was filtered and rinsed with 10 mL dioxane three times. The filtrate and the washings were collected together and were neutralized with potassium bicarbonate solution. The neutralized solution was diluted with three-fold DIW (v/v), and hydrochloric acid was added to adjust pH to 2. The precipitated lignin was isolated by filtration followed by freeze-drying. Then, the dried lignin was washed with dichloromethane (3 × 30 mL) to remove the existing extractives. Finally, the lignin was air-dried for 24 h in a fume hood at room temperature. The enzymatic treatment-acid hydrolysis lignin (EAL) prepared by the above two steps was obtained

Extraction of lignin from WS by homogeneous catalysts

Ten grams of oven-dried wheat straw was added to a 500 mL 3-neck boiling flask and mixed with 100 to 140 g of BDO (80%, v/v) medium and 0.5 to 3 g of H₂SO₄ (98% wt.), with a reaction time for 3 h. Or else, 5 g of oven-dried wheat straw was added to a 250 mL 3-neck boiling flask and mixed with 50 to 100 g of BDO (from 50% to 100%, v/v) medium and 0.1 to 0.8 g of NaOH, reaction time for 1 to 5 h. The flasks were all heated in an oil bath at 160 °C under reflux conditions. Afterwards, the contents were filtered hot to separate the solid residues from the medium. The residue was washed with 100 °C BDO (80% (v/v), pH = 10) once, and sequentially washed with 80 °C DI water (pH = 10) three times. The filtrates from the flask and the washings were mixed together and diluted three-fold with DIW (v/v). Hydrochloric acid was added to regulate the pH to 2. This diluted mixture was stirred for 12 min to precipitate thoroughly the high boiling solvent lignin (HBSL). The mixture was left to stand for 1 h, and then decanted to separate the supernatant. The residual portion was heated in a thermostatic water bath at 50 °C for 30 min to coagulate the lignin. After cooling, the residual portion was filtered

and washed with DIW until the filtrate was colorless. Finally, the residual was dried for 6 h at 60 °C in a vacuum drying oven with the vacuity less than 133 Pa, which resulted in a brown powder. The powder was washed by dichloromethane three times (3×30 mL). Each extraction process was replicated for three times. The flow chart of the extraction process is shown in Fig. 1.

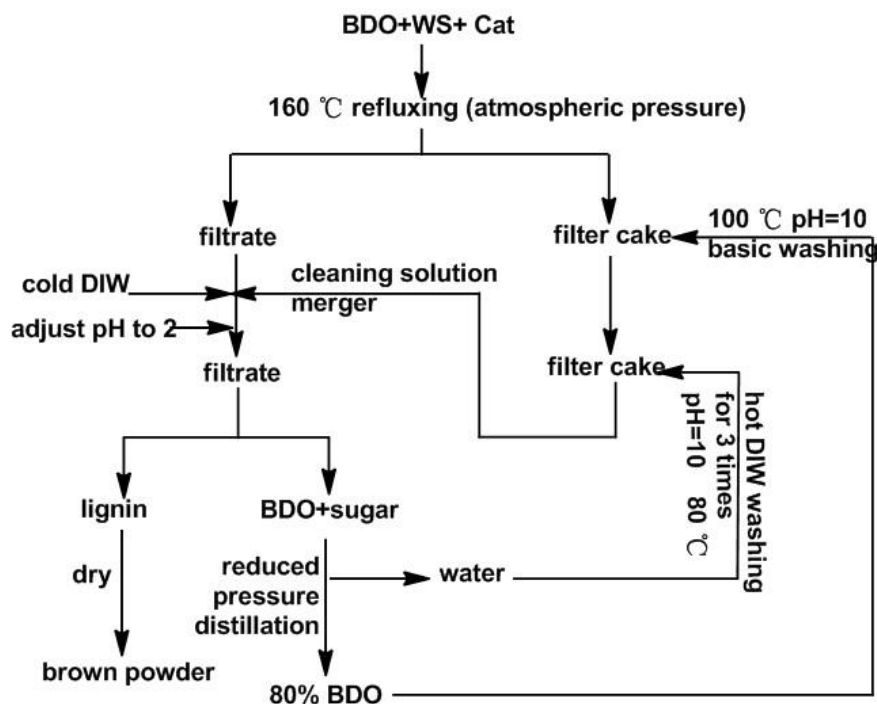


Fig. 1. Extraction of lignin from WS by homogeneous catalysts (WS: wheat straw; DIW: deionized water; BDO: 1, 4-butandiol; Cat: H₂SO₄ or NaOH; + denotes mixing)

Solubility of lignin extracted by homogeneous catalysts in some organic solvents

Lignin extracted in BDO medium catalyzed by H₂SO₄ or NaOH under the optimum conditions that were obtained in the experiments was dried at 60 °C in a vacuum oven with the vacuity less than 133 Pa. Then 1 g of dried HBSL-acid lignin (high boiling solvent lignin catalyzed by sulfuric acid) was weighed in triplicate and mixed with 25 mL DMSO, 25 mL DMF, and 25 mL THF in three 50 mL beakers, respectively. And all three mixtures were stirred for 1 h at room temperature. After that, the three mixtures were filtered, respectively. The HBSL-base lignin (high boiling solvent lignin catalyzed by NaOH) was dealt with in the same manner as the HBSL-acid lignin.

Gel permeation chromatography

The molecular weight distribution of the extracted lignin was obtained through gel permeation chromatographic measurements. GPC analysis was carried out at 40 °C on a HLC-8320GPC (TOSOH, Japan) instrument using DMSO as eluent at a flow rate of 0.3 mL/min. All of the GPC data were calibrated using polystyrene (PS) standards.

Fourier transform infrared spectroscopy

Lignin extracted by the two methods was characterized by FT-IR (Nicolet iS10, Thermo Scientific, US). The characterization was achieved by smart attenuated-total reflectance infrared spectroscopy (Smart aTR-IR) by direct transmittance in a diffuse-reflection ATR System, using OMNIC software (Nicolet iS10, Thermo Scientific). Spectra data were obtained through 40 scans over a wavenumber range of 4000 to 0 cm^{-1} and a wavenumber resolution of 4 cm^{-1} .

Thermogravimetric analysis

Thermogravimetric analysis was performed using a TG/DTA 6200 LAB SYS (Perkin Elmer, US) in a temperature range of 30 to 800 $^{\circ}\text{C}$ with a heating rate of 10 $^{\circ}\text{C}/\text{min}$. Lignin (4 to 5 mg) was analyzed under a nitrogen flow rate of 100 mL/min.

RESULTS AND DISCUSSION

Determination of Lignin Content in WS Powder

The lignin content in WS powder was measured following the TAPPI standard method T 222 om-02 (2002). The lignin content in the WS was calculated by Eq. 1. The mean lignin content in the WS powder was $19.13 \pm 0.07\%$,

$$X = (A/W) \times 100\% \quad (1)$$

where X is the lignin content in WS, A is the weight of lignin recovered, and W is the oven-dry weight of WS.

$$Y = (A'/W') \times 100\% \quad (2)$$

In Eq. 2, Y is the lignin yield in lignin extraction process, A' is the weight of lignin recovered, and W' is the weight of lignin theoretical output.

Effect of Reaction Conditions on Lignin Recovered by Catalysts

As noted earlier, lignin was difficult to separate from the WS powder because of the recalcitrant properties of the lignin. However, it can be extracted from the WS powder under appropriate conditions. Here, a series of experiments were conducted to improve the yield of lignin catalyzed by H_2SO_4 or NaOH .

The effect of liquid-solid ratio (the ratio of the mass weight of BDO medium (80%, v/v) to WS) on the lignin yield in H_2SO_4 -catalyzed extraction is illustrated in Fig. 2 [a]. The extracted lignin yield increased first, reached a maximum value 29.27% when the liquid-solid ratio was 12, and then decreased. This may be due to low solubility of lignin when the volume of the BDO medium was inadequate. Alternatively, when the volume of BDO medium was excessive, the mass concentration of H_2SO_4 was too low to cleave bonds between lignin and cellulose, and less lignin dissolved in the BDO medium, leading to a low yield after the lignin extraction process. As a result, the optimum liquid-solid ratio in H_2SO_4 -catalyzed extraction was 12-to-1 (w/w).

Figure 2 [b] shows the effect of liquid-solid ratio (the ratio of the mass weight of BDO medium (80%, v/v) to WS) in NaOH -catalyzed extraction on lignin recovery, which was different from Fig. 2 [a]. Lignin recovery increased rapidly as the liquid-solid

ratio increased to 12, and it reached a value of 60.64%; when the liquid-solid ratio was beyond 12, the amount of lignin recovered increased very slowly, and it did not boost the amount of lignin recovered (lignin yield increased to 66.91% when the liquid-solid ratio was 20). This may be because the mass concentration of NaOH on lignin extraction was not considerable when the mass ratio of NaOH to WS was certain.

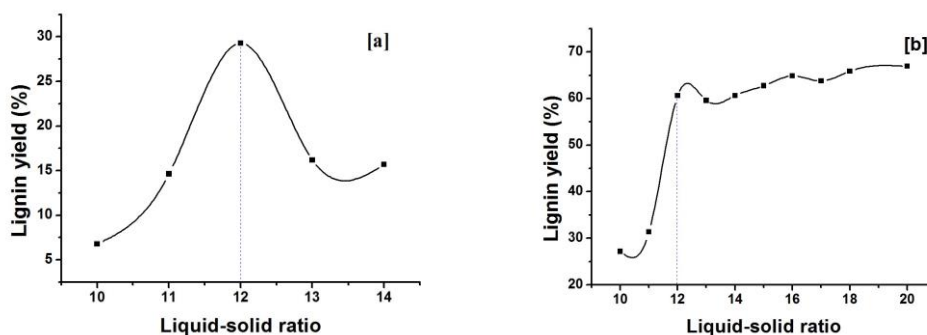


Fig. 2. [a] Effect of liquid-solid ratio (the ratio of the mass weight of BDO medium (80%, v/v) to WS) on the lignin extracted from WS powder catalyzed by H₂SO₄. Conditions: 10 g WS, 2 g H₂SO₄ (98% wt.), 160 °C, and extraction time of 3 h; [b] Effect of liquid-solid ratio (the ratio of the mass weight of BDO medium (80%, v/v) to WS) on the lignin extracted from WS catalyzed by NaOH. Conditions: 0.5 g NaOH, 5 g WS, 160 °C, and extraction time of 3 h

When the liquid-solid ratio was less than 12, BDO medium was inadequate to dissolve lignin fragments separated from lignocellulose tissue. When the liquid-solid ratio was increased beyond 12, more C-O bonds in the remainder were cleaved under the catalytic action of NaOH as lignin fragments dissolved completely in BDO medium, which resulted in a little more lignin fragments becoming dissolved in the BDO medium. Relatively, 60 g of BDO was more economically reasonable. The optimum liquid-solid ratio in NaOH-catalyzed extraction was 12-to-1 (w/w), the same value as when H₂SO₄ was used as the catalyst.

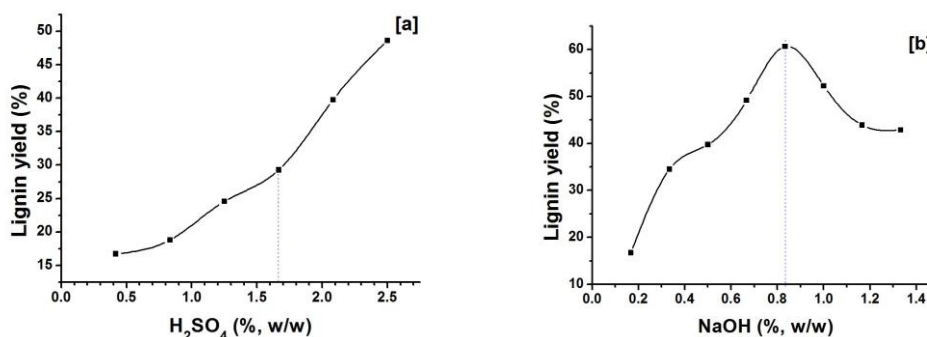


Fig. 3. [a] Effect of the mass concentration of H₂SO₄ on the lignin extracted from WS catalyzed by H₂SO₄. Conditions: 10 g of WS, 120 g of 80% BDO medium, 160 °C, and extraction time for 3 h; [b] Effect of the mass concentration of NaOH on the lignin extracted from WS catalyzed by NaOH. Conditions: 5 g of WS, 60 g of 80% BDO medium, 160 °C, and extraction time for 3 h

The effect of the dosage of H₂SO₄ on lignin recovery is shown in Fig. 3 [a]. An obvious trend is shown in the graph, where increasing the acid catalyst dosage increased the amount of lignin output; the maximum extracted lignin yield was 48.61%. However, when the concentration of H₂SO₄ was up to 2.0% ($W_{H_2SO_4(98\%, w/w)}/W_{BDO(80\%, v/v)}$), the

extracted lignin was viscous and therefore difficult to weigh accurately. This may be because the excessive dosage of H_2SO_4 resulted in extensive degradation of cellulose, which formed coke and tar, or viscous solutions. These effects were more likely to arise from high concentrations of high molecular weight products, consistent with the high yield obtained at high concentrations of sulfuric acid and the high molecular weight observed by GPC in Fig. 6. Thus, the optimum mass concentration of the H_2SO_4 catalyst was fixed at 1.67% (lignin yield of 29.27%).

When compared to H_2SO_4 -catalyzed BDO extraction, NaOH-catalyzed BDO extraction tended to be more efficient at recovering lignin from WS. As shown in Fig. 3 [b], lignin recovery increased as the dosage of NaOH catalyst increased, and it reached its maximum value of 60.64% when 0.83% ($W_{\text{NaOH}}/W_{\text{BDO}}$ (80%, v/v)) of NaOH was used. Furthermore, lignin recovery decreased when the dosage of NaOH was excessive (*e.g.*, greater than 0.83%). This may be due to excess NaOH contributing to the excessive condensation of lignin, which resulted in less lignin being recovered. Thus, 0.83% (w/w) NaOH was determined to be the optimum catalyst mass concentration.

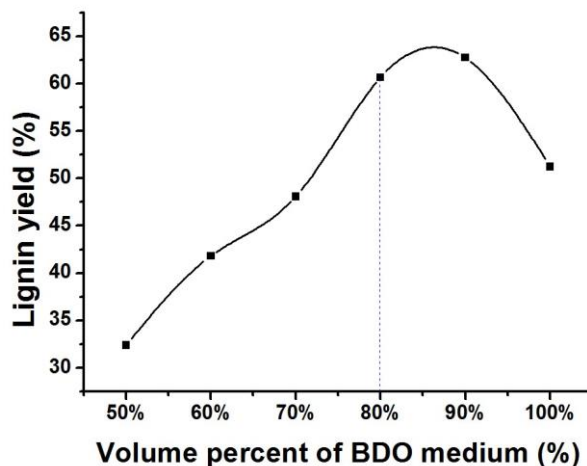


Fig. 4. Effect of volume percent of BDO on the lignin extracted from WS catalyzed by NaOH. Conditions: 0.5 g of NaOH, 5 g of WS, 60 g of BDO medium, 160 °C, and extraction time of 3 h

To investigate the effect of the BDO medium concentration on lignin recovery, conditions with 12-to-1 liquor-to-straw ratio of different volume percentage (from 50% to 100%) BDO media were used to conduct a series of experiments. The results are depicted in Fig. 4. Lignin recovery increased as the volume amount of BDO in the extraction medium increased from 50% to 90%, and reached a maximum value of 62.73% at a HBS concentration of 90% (v/v). However, when the volume of BDO was higher than 90%, the amount of lignin recovered was lower. This result indicated that a small amount of water in the BDO extraction medium was advantageous to the extraction of lignin. This may be because of the insolubility of lignin when water content in BDO medium (*i.e.*, 50%, 60%, and 70% BDO) was too high. Also, when the BDO medium contained high levels of BDO (beyond 90%), the medium was too viscous, which resulted in low lignin recovery. Lignin yield was of little difference when 80% BDO or 90% BDO medium was used. Given to the economic value of BDO recovery, the optimum volume percentage of BDO in the extraction medium was determined to be 80%.

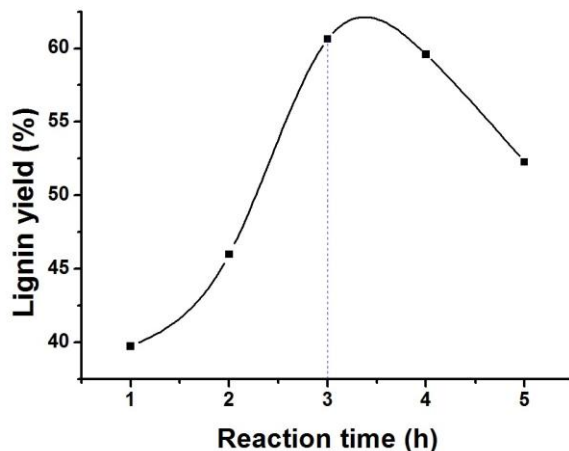


Fig. 5. Effect of reaction time on the lignin extracted from WS catalyzed by NaOH. Conditions: 0.5 g of NaOH, 5 g of WS, 60 g of 80% BDO medium, and 160 °C

The effect of reaction time on lignin recovery of the HBSL-base system is shown in Fig. 5. In terms of reaction time, the amount of lignin that was obtained increased to a maximum yield value 60.64% when the extraction time was increased up to 3 h. Prolonging the extraction above 3 h resulted in lower amounts of lignin recovered. This may be because at a reaction time less than 3 h, the rate of C-O bonds cleavage between lignin and cellulose/hemicellulose was faster than the rate of recondensation of the separated lignin fragments. At a 3 h reaction time, the two rates were nearly equivalent. However, after a 3 h reaction time, the amount of alkali was depleted and the lignin fragments could undergo condensation reaction with the lignin which was still trapped in the fiber, or condense with the carbohydrates. The lignin condensation reactions could account for lower lignin yields at the longer extraction times. Thus, the optimum reaction time was fixed at 3 h.

In summary, in the HBSL-acid method, an extraction time of 3 h was set. Thus, the optimum lignin extraction conditions were: 10 g of WS mixed with 120 g of BDO medium (80%, v/v) and 1.67% (w/w) of H₂SO₄, 160 °C, reaction time for 3 h. While in NaOH-catalyzed reaction, the optimum conditions for lignin extraction of WS powder were: 5 g of WS, 0.83% (w/w) of NaOH, 60 g of BDO medium (80%, v/v), 3 h extraction time, and 160 °C.

GPC Analysis of Lignin Extracted by the BDO Medium

Figure 6 shows the gel permeation chromatographs of lignin isolated from wheat straw by HBS methods and EAL method. The structure of lignin isolated by EAL method was little modified compared to enzyme hydrolysis lignin (Argyropoulos *et al.* 2002). In gel permeation chromatography, molecules with higher molecular weight outflow first. As shown in Fig. 6, firstly, lignin extracted without catalyst (No cat) has the lowest molecular weight compared to lignin prepared by the other five methods. This is to say that only small molecule lignin fragments dissolved in the BDO medium without any catalyst, and NaOH or H₂SO₄ catalyzed the cleavage of C-C/C-O bonds of lignin or C-O bonds between lignin and cellulose, separating lignin fragments with higher molecular weight compared to No cat. Secondly, the molecular weight of lignin isolated for 3 h

reaction time under NaOH catalyst (B3) was lower than that of lignin isolated for 1 h reaction time under NaOH catalyst (B1). Likewise, the molecular weight of lignin isolated for 3 h reaction time under H₂SO₄ catalyst (A3) was lower than that of lignin isolated for 1 h reaction time under H₂SO₄ catalyst (A1), yet higher than No cat. This manifested that as reaction time extended, lignin fragments were split into smaller pieces under the catalytic effect of NaOH or H₂SO₄. Thirdly, the molecular weight of B1 or B3 was lower than A1 or A3, and what's more, the yield of B1 or B3 was higher than A1 or A3, which illustrated the fact that the catalytic effect of NaOH on C-O/C-C cleavage was more severe than H₂SO₄.

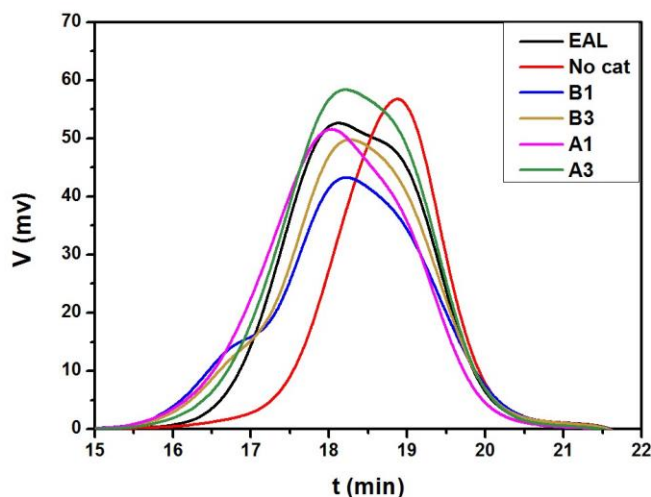


Fig. 6. Gel permeation chromatographs of lignin isolated from wheat straw by HBS methods and EAL method (EAL: enzymatic treatment-acid hydrolysis lignin; No cat: lignin extracted in 80% (v/v) BDO medium without any catalyst, refluxing in 160 °C oil bath for 3 h; B1: lignin isolated in the same condition as “no cat”, except reaction time for 1 h and with 0.83% (w/w) NaOH catalyst; B3: lignin isolated in the same condition as “B1”, except reaction time for 3 h; A1: lignin isolated in the same condition as “B1”, except with 1.67% (w/w) H₂SO₄ catalyst; A3: lignin isolated in the same condition as “B3”, except with 1.67% (w/w) H₂SO₄ catalyst).

FT-IR Spectroscopy of Lignin Extracted by the BDO Medium

The infrared absorption peaks (Fig. 7) of the extracted lignin by HBSL-acid, HBSL-base or EAL process were determined. Corresponding bonds were assigned on the basis of Shen *et al.* (2010), Hu *et al.* (2013), and Boeriu *et al.* (2004). The stretching band at 3421 cm⁻¹ is generated by -OH group stretching. Peaks at 2939 cm⁻¹ are predominantly arising from C-H stretching in methyl and methylene as well as aromatic methoxy groups. Lignin prepared by this three methods at these peaks had similar shapes, values, and intensities. And the C=O stretching absorption peak at 1716 cm⁻¹ is generated by non-conjugated ketones, carbonyls, and ester groups of lignin. Compared to HBSL-acid lignin, the peak at 1716 cm⁻¹ of the HBSL-base lignin had weak values, wider peak shapes, and decreased intensity, while EAL had sharpened shapes and strong values, which indicated that the number of unconjugated C=O bonds in the HBSL-base lignin were less than that in the HBSL-acid lignin or the EAL. Also, EAL had more unconjugated C=O bonds than HBSL-acid lignin. This suggested that the lignin was more deoxygenized in the HBSL-base method than that in the HBSL-acid method. Peaks at 1595 cm⁻¹ and 1506 cm⁻¹ were due to the aromatic skeleton vibrations in lignin compounds. This peak of the

HBSL-base lignin was reduced when compared to the other lignins, which suggested that the C-O-C bonds in HBSL-base lignin had been more extensively cleaved than the other two lignins. The stretching absorption peak at 1267 cm^{-1} , 1225 cm^{-1} , and 1034 cm^{-1} is generated by C-O-C stretching of aryl ether (O links directly with aromatic ring through single bond) in lignin (Shen *et al.* 2010). HBSL-base lignin at this peak had weaker values and reduced intensity compared to HBSL-acid lignin or EAL, and HBSL-acid lignin at this peak had weaker values than EAL, indicating that lignin extracted by HBSL-base method had the least =C-O-C group, HBSL-acid lignin the second, and EAL the most.

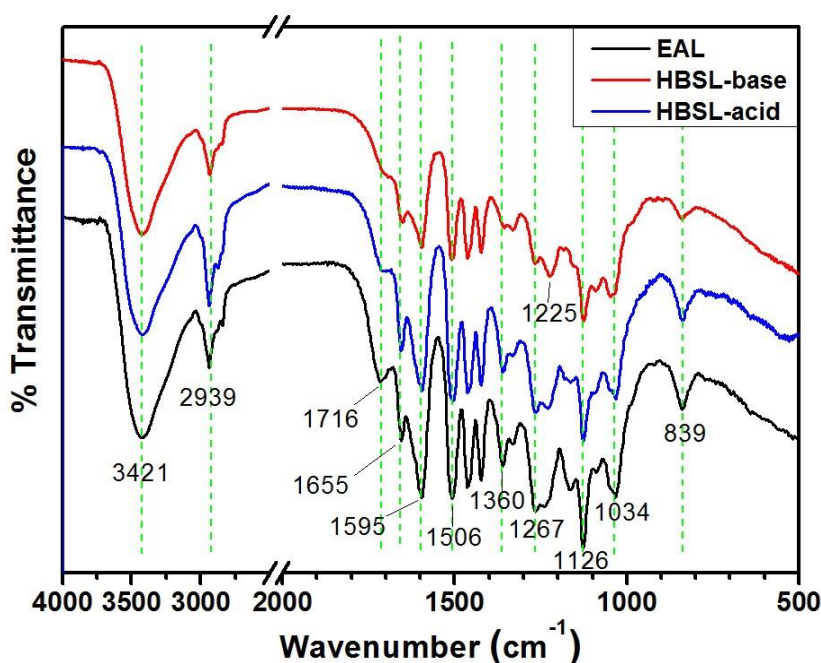


Fig. 7. Infrared spectra of HBSL-acid lignin, HBSL-base lignin and EAL (HBSL-acid lignin: lignin extracted in 80% (v/v) BDO medium with 1.67% (w/w) H_2SO_4 catalyst, refluxing in $160\text{ }^\circ\text{C}$ oil bath for 3 h; HBSL-base lignin: lignin extracted in 80% (v/v) BDO medium with 0.83% (w/w) NaOH catalyst, refluxing in $160\text{ }^\circ\text{C}$ oil bath for 3 h; EAL: enzymatic treatment-acid hydrolysis lignin).

Thermal Stability of Lignin Extracted by the BDO Medium

Thermal stability is a significant attribute with regards to polymer chemistry and depolymerization of lignin. 4.24 mg of EAL, 4.32 mg of HBSL-base lignin, and 4.30 mg of HBSL-acid lignin were taken to do thermogravimetric analysis with a range from 30 to $800\text{ }^\circ\text{C}$ at a constant heating rate of $10\text{ }^\circ\text{C}/\text{min}$ according to the literature (Yang *et al.* 2007; Wang *et al.* 2008). As shown in Fig. 8, when the temperature reached $800\text{ }^\circ\text{C}$, 1.47 mg (34.67%) of EAL, 1.25 mg (28.94%) of the HBSL-base lignin, and 1.35 mg of the HBSL-acid lignin (31.40%) remained. With respect to the TG curve, the initial weight loss before $200\text{ }^\circ\text{C}$ was mostly due to water loss. The weight loss that occurred from 200 to $450\text{ }^\circ\text{C}$ was due to the primary decomposition of organic matter of lignin. After $450\text{ }^\circ\text{C}$, weight loss was still occurring, but at a much slower rate. The DTG curve reflected the rate of weight loss of the samples. Evidently, the DTG curves illustrated that the rate of HBSL-base weight loss was faster than that of HBSL-acid and EAL. Moreover, the

maximum weight loss of the EAL, HBSL-acid, and the HBSL-base lignins took place at 372, 376, and 304 °C, respectively. This result agreed with the literature (Wörmeyera *et al.* 2011; Wang *et al.* 2014) insofar as the maximum weight loss temperature for lignin occurred between 300 and 400 °C. Besides, it has reported that cleavage of the β -O-4 linkage occurred in the temperature range from 250 °C to 350 °C, and the cleavage of carbon-carbon occurred after the temperature region of 350 °C (Kim *et al.* 2013; Chu *et al.* 2013). Thus, the results indicated that there were more β -O-4 bonds in HBSL-base lignin and less carbon-carbon bonds than the other two lignins. What's more, the weight loss of HBSL-base lignin was faster than EAL and HBSL-acid lignin. These results manifested that the thermal stability of HBSL-base lignin was relatively lower than EAL and HBSL-acid lignin. From this analysis, as well as the molecular weight analysis of isolated lignin shown in Fig. 6, it is reasonable to surmise that HBSL-base process resulted in a lignin that is further depolymerized into smaller fragments with lower stability than HBSL-acid lignin.

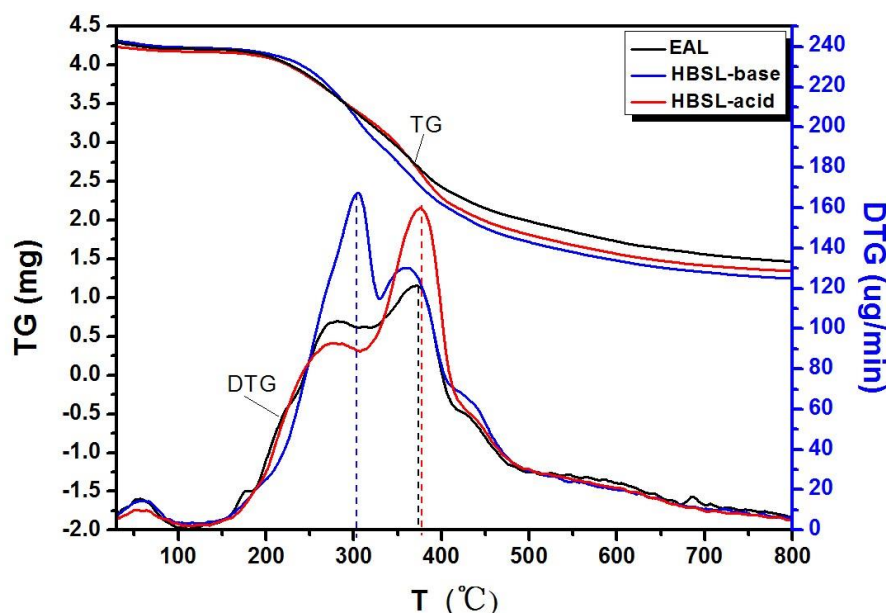


Fig. 8. Thermogravimetric (TG and DTG) analysis of lignin prepared by HBSL-acid, HBSL-base and EAL methods

CONCLUSIONS

1. In the HBSL-acid method, the optimum conditions were: 3 h extraction time, 10 g of WS, 120 g of BDO medium (80%, v/v), and 1.67% (w/w) of H₂SO₄ (98%, wt. %) catalyst at 160 °C under refluxing conditions. The optimum yield of lignin was 29.27% (Eq. 2). For the HBSL-base method, the optimum yield of lignin was 60.64% (Eq. 2), which was higher than the HBSL-acid method. The optimum conditions for the HBSL-base method were: 3 h extraction time, 5 g of WS, 60 g of BDO medium (80%, v/v), and 0.83% (w/w) of NaOH catalyst at 160 °C under refluxing conditions.

2. The FT-IR spectra of lignins extracted by the three methods (HBSL-acid method, HBSL-base method and EAL method) confirmed that the HBSL-base lignin structure had fewer unconjugated C=O groups and =C-O-C bonds than the EAL and HBSL-acid lignin.
3. The GPC results showed that the molecular weight of the HBSL-base lignin was lower than the HBSL-acid lignin, but higher than No cat. This suggested that the HBSL-base lignin was more depolymerized into smaller fragments than HBSL-acid lignin, and the catalytic effect of NaOH or H₂SO₄ separated lignin fragments with higher molecular weight compared to No cat.
4. Additionally, TGA showed that the HBSL-base lignin had lower stability than EAL and HBSL-acid lignin.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the funding support from Anhui Sealong Biotechnology Co., Ltd (China).

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Article submitted: August 25, 2014; Peer review completed: October 19, 2014; Revised version received: December 5, 2014; Accepted: December 8, 2014; Published: December 18, 2014.