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Reactivity improvement of cellulolytic enzyme lignin via mild hydrothermal modification

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ABSTRACT

Isolated by the cellulolytic enzyme lignin (CEL) process, water-alcohol (1:1, v/v) was introduced as co-solvent in the process of the hydrothermal treatment. The modification parameters such as reaction temperature and time, solid-to-liquid ratio, and catalysts (NaOH and $NaOAlO_2$) have been investigated in terms of the specific lignin properties, such as the phenolic hydroxyl content (OH_{phen}), DPPH free radical scavenging rate, and formaldehyde value. The CELs were also characterized by GPC, FT-IR and ^{1}H NMR spectroscopy, and Py-GC/MS. The key data are under optimal lignin modification conditions (solid-to-liquid ratio of 1:10 (w/v) and a temperature of 250 °C for 60 min) are: OH_{phen} content: 2.50 mmol/g; half maximal inhibitory concentration (IC_{50}) towards DPPH free radicals: 88.2 mg/L; formaldehyde value: 446.9 g/kg). Both base catalysts decrease the residue rate, but phenol reactivities of the products were also detracted. Py-GC/MS results revealed that modified lignin had a higher phenolic composition than the CEL did, especially the modified lignin without catalyst (ML), which represented 74.51% phenolic content.

1. Introduction

Industrial lignin is a typical by-product or waste material of the papermaking industry [1] and the ethanol fuel industry include cellulosic ethanol [2]. The natural aromatic structure of lignin has received increasing attention from the field of biomass material utilization [3], which is more promising as a simple burning for energy production. Industrial lignin is also suitable for production of phenol-formaldehyde resin [4], epoxy resin [5,6], polyurethane foams [7], and ion-exchange resin [8]. The lignin-based polymer materials can partly replace petrochemical products [9–13].

However, there are many limitation problems during the synthesis and preparation of lignin-based polymer materials. Because natural lignin is an amorphously biological macromolecule with high steric hindrance and few reactive sites, the phenol reactivity of lignin towards other chemical reagents is limited [14]. To improve the phenol reactivity of lignin, previous studies have reported various methods [15] with chemical and biological modification proposed as the two most common methods [16–20]. The common ground of those methods is that lignin macromolecules are degraded into low-molecular and high-reactive phenol compounds. Hydrothermal modification [10,21,22] was universally used for lignin degradation. Frequently, some alcohol was added to make the system more homogeneous [23,24]. An apparent advantage is that the solvents of the hydrothermal method are mostly non-toxic and recyclable alcohols that improve the phenolic reactivity of lignin. Consequently, water-alcohol co-solvents played an important role in this process [25–27].

In this study, the cellulolytic enzyme lignin (CEL), extracted from residues of the ethanol fuel industry, was degraded and isolated in water-alcohol co-solvents. Generally, the fermentation process for ethanol production is very mild and could not change lignin chemical structure much. The phenolic structure of lignin could be remained in CEL, so that CEL is more suitable to be utilized as a phenolic resource. After hydrothermal modification, the phenol reactivity of lignin, including phenolic hydroxyl content, half maximal inhibitory concentration (IC_{50}) value, and reactivity towards formaldehyde, were conducted via the Folin-Ciocalteu (F-C) method, 1,1-diphenyl-2-picryhydrazyl (DPPH) free radical scavenging activity, and potentiometric titration. Effects of base catalysts on the phenol reactivity of lignin were also discussed. Furthermore, main phenolic compositions of the lignin samples were analysed by Py-GC/MS method.

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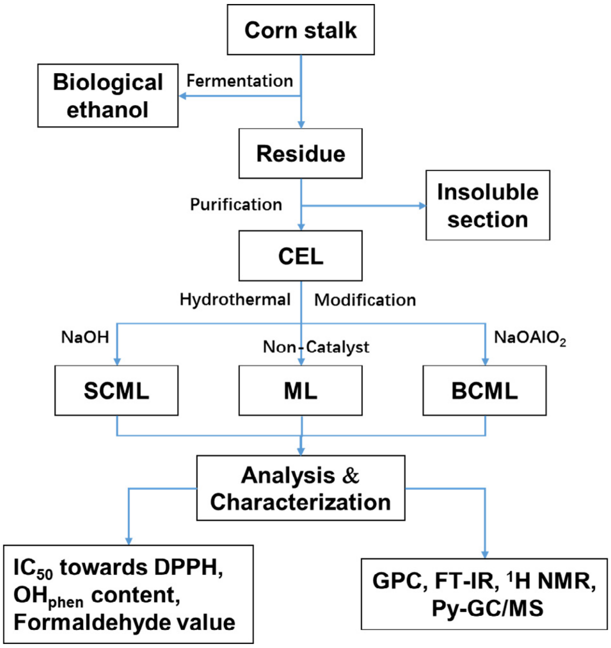
2. Materials and methods

2.1. Materials and methods

As shown in Scheme 1, the CEL was extracted from solid residue of cornstalk fermentation for bio-ethanol. The cornstalks were harvested from Zhaodong County, Heilongjiang Province, China. Prior to the simultaneous saccharification and fermentation (SSF) treatment [28], the air-dried cornstalks were cut into 5–10 cm and then treated to stream explosion at 1.7 MPa and 205 °C for 6 min. The cornstalk underwent SSF, and most carbohydrates were converted into fuel ethanol. CEL was the main component (62.18%) in the residue. Others were unused carbohydrates (27.91%) and ashes (9.91%). In order to remove the influence of impurities, the residue was purified via alkali-solution and the acid-isolation method [29]. 100 g of residue was dispersed in 1000 mL 1 M NaOH aqueous solution and stirred for 1 h. The suspension solution was separated using a centrifuges in 8000 RPM for 20 min. The solution was collected and adjusted to pH of 2 with dilute sulfuric acid. Resulting precipitate was separated by filtration and washed repeatedly with distilled water and vacuum-dried at 40 °C for 12 h, CEL was obtained with 1.82% ashes and 4.34% carbohydrates. The solid base ($NaAlO_2$, Aluminium-oxide sodium) was provided by Shandong Taide Chemical Process Technology Co. Ltd.

2.2. Hydrothermal modification of CEL

The hydrothermal modification experiments of the CEL were conducted in a 50 mL stainless steel autoclave reactor lined with p-polyphenylene (PPL). Defined solid-to-liquid ratios (1:5, 1:10, 1:15, and 1:20 g/mL) of both CEL and co-solvent (alcohol:water = 1:1, v/v) were loaded into the reactor. The sealed reactor was heated to the four specified reaction temperatures (150, 200, 250, and 300 °C) for the indicated reaction time (30, 60, 90, and 120 min), respectively. The amount of catalysts (including solid base and sodium hydroxide) were 10% of the sample mass. Subsequent to the completion of the hydrothermal CEL treatment, the reactor was immediately cooled down to room temperature. The mixture of solids and liquids were separated via a Buchner funnel under vacuum. The insoluble solids were oven-dried at 105 °C and weighed to calculate the residue rate, the mass percentage of the insoluble residue to the CEL. The liquid product was evaporated to recover solvent, and dried under vacuum at 40 °C for 12 h to obtain powder product, which was further used to analysis and characterization. The hydrothermal treatment and analytical procedures for the modification product of CEL are shown in Scheme 1.



Scheme 1. Outline of the process diagram for the CEL hydrothermal modification and analysis.

2.3. DPPH radical scavenging activity

The determination of DPPH free radical scavenging activity was performed as described by Aadil et al. [30] with slight modifications: The lignin sample was dissolved in a dioxane-water co-solvent (4:1, v/v) to configure series of different concentration gradients for the sample solution (0.8, 0.6, 0.4, 0.2, 0.1, 0.05, and 0.025 mg/mL). 1 mL of the sample solution was mixed with the 0.4 g/L DPPH ethanol solution (4 mL) and then incubated at room temperature and in the dark for 30 min. Absorbance values were measured using a TU-1900 double beam ultraviolet-visible spectrophotometer (Persee, China) at 517 nm. The activity of DPPH free radical scavenging (*ARS*) was calculated as Eq. (1).

$$ARS\,(\%) = \frac{A_0 - A_1}{A_0} \times 100\% \tag{1}$$

where A_0 and A_1 are the absorbance values of the DPPH solution + dioxane-water mixture and the DPPH solution + lignin solution mixture at ratio of 4:1 (v/v), respectively. When the *ARS* value reached 50%, the concentration was recorded as half maximal inhibitory concentration (IC_{50}).

2.4. Phenolic hydroxyl content of lignin

The phenolic hydroxyl content is the most intuitive measure for the phenol reactivity of lignin. There are many methods for the estimation of the total phenolic content [31], among which, the F-C method is fast and convenient [32–35]. The F-C reagent triggered an oxidation-reduction reaction during which the phenolic hydroxyl groups show blue colour. The absorbance of the mixture solution was conducted using the TU-1900 ultraviolet-visible double beam spectrophotometer at 760 nm. A linear, positive correlation between the concentration of phenolic hydroxyl groups and the absorbance value was developed with phenol used as standard substance. And then, the phenolic hydroxyl content of lignin was calculated according to its absorbance value.

2.5. Reactivity towards formaldehyde (formaldehyde value)

To comprehensively reveal the phenol reactivity of lignin, the reactivity towards formaldehyde (formaldehyde value) was determined. First, 1.00 g of the lignin sample was dissolved into 10 mL 0.2 M sodium hydroxide solution in a 20 mL test tube, before 1.00 mL of 37% formaldehyde solution was loaded. The whole system was sealed with a rubber plug and kept in a water bath at 80 °C for 3 h. After the experiment finished, the free formaldehyde content of the mixture solution was determined via the hydroxylamine hydrochloride method (referred to **ISO 11402-2004 (2004)**). During this determination, the free formaldehyde reacted with hydroxylamine hydrochloride and generated formaldoxime (C=NOH). At the same time, hydrochloric acid (HCl) was released and neutralized the standard sodium hydroxide (NaOH) solution. The formaldehyde value (*FV*) was calculated in accordance with the following Eq. (2).

$$FV\,(g/g) = \frac{0.03c(V - V_1)}{m} \tag{2}$$