



## Case Study

## Enhanced enzymatic digestibility of mixed wood sawdust by lignin modification with naphthol derivatives during dilute acid pretreatment

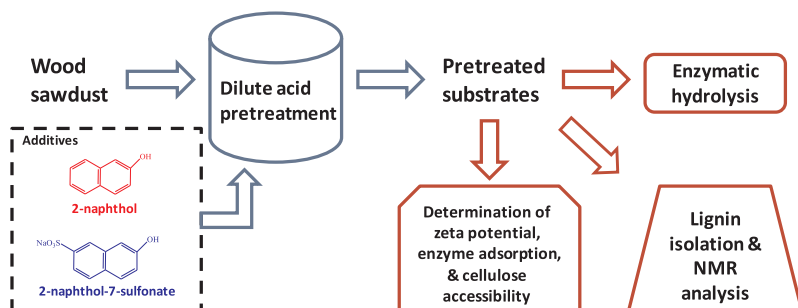
Chenhuan Lai<sup>a,b,1</sup>, Bo Yang<sup>b,1</sup>, Juan He<sup>b</sup>, Caoxing Huang<sup>b</sup>, Xin Li<sup>b</sup>, Xiangyang Song<sup>b</sup>, Qiang Yong<sup>a,b,\*</sup>

<sup>a</sup> Co-Innovation Center of Efficient Processing and Utilization of Forest Resources, Nanjing Forestry University, Nanjing 210037, China

<sup>b</sup> College of Chemical Engineering, Nanjing Forestry University, Nanjing 210037, China



## GRAPHICAL ABSTRACT



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## ABSTRACT

Effects of the addition of 2-naphthol and 2-naphthol-7-sulfonate on the dilute acid pretreatment of mixed wood sawdust were investigated, respectively. Compared to 2-naphthol, 2-naphthol-7-sulfonate was more effective to enhance delignification and facilitate the enzymatic hydrolysis. The 72 h hydrolysis yield was improved by 47.8% for 2-naphthol-7-sulfonate, while only 9.1% was observed for 2-naphthol. The surface charges, enzyme adsorption, and cellulose accessibility of dilute acid pretreated substrates with or without naphthol derivatives were examined. The improved enzymatic hydrolysis by adding 2-naphthol-7-sulfonate was ascribed to the higher negative surface charges, the lower enzyme non-productive binding, and the higher cellulose accessibility of pretreated substrates. Additionally, the HSQC NMR and <sup>31</sup>P NMR analysis were carried out on both decomposed lignins and residual bulk lignins. It indicated that the addition of the naphthol derivatives during pretreatment could suppress the lignin repolymerization, which further mitigated the inhibition of residual lignins on enzymatic hydrolysis.

## 1. Introduction

Lignocellulosic biomass, the most abundant renewable resources, has gained considerable interest to be used as the feedstocks for the

production of fuels and chemicals (Demirbaş, 2001). Wood sawdust as one of the major and low-value by-products of wood manufacturing facilities has the potential to be the feedstock for the lignocellulose biorefinery (Pielhop et al., 2015). However, the recalcitrant structure of

\* Corresponding author at: Co-Innovation Center of Efficient Processing and Utilization of Forest Resources, Nanjing Forestry University, Nanjing 210037, China.  
E-mail address: [swhx@njfu.com.cn](mailto:swhx@njfu.com.cn) (Q. Yong).

<sup>1</sup> Bo Yang contributed equally to this work, regarding as the first author.

lignocellulose hinders the efficient bioconversion. In order to destroy the compact lignocellulose matrix, various pretreatment methods have been developed to enhance the bioconversion efficiency (Alvira et al., 2010; Galbe and Zacchi, 2012). Among them, dilute acid pretreatment has been widely applied due to its low cost and simplicity. It can effectively remove hemicelluloses, thus making the cellulose more accessible to the enzyme attack (Dien et al., 2006). Nevertheless, this method hardly removes the lignins in the pretreated materials. Furthermore, the residual lignins in dilute acid pretreated biomass showed a stronger inhibition on enzymatic hydrolysis than the original lignins in the untreated biomass (Li and Zheng, 2017; Nakagame et al., 2011). This is closely related to the significant structural changes on lignins during the dilute acid pretreatment (Pielhop et al., 2016). Therefore, it is essential to understand the changes and alleviate the negative effects by controlling the changes on lignin structure during pretreatment.

Nuclear magnetic resonance (NMR) analysis of lignins gives an insight to elucidate the lignin structural changes. It has been reported that dilute acid pretreatment leads to an obvious decrease of  $\beta$ -O-4 ether linkages in lignins, suggesting the occurrence of lignin depolymerization (Li et al., 2007; Moyle and Tyner, 1953). Meanwhile, the number of C–C condensed structures increased, which implied that the lignin repolymerization/condensation happened simultaneously with the lignin depolymerization (Li et al., 2007). A potential reaction scheme proposed by Li et al. showed that there was a competition between lignin depolymerization and repolymerization reaction (Li et al., 2007). Both types of reactions had a common intermediate, a carbonium ion. Under the acidic condition, a carbonium ion was formed from benzyl alcohol structure. It could lead to the cleavage of  $\beta$ -O-4 linkages, resulting in the lignin depolymerization, and generating the Hibbert ketone structure together with a new phenolic end group. Whereas, the carbonium ion could also react with other adjacent aromatic ring with electron-rich carbon, to form a stable carbon-carbon linkage in the lignin repolymerization.

Besides the structural changes, the association between the condensed lignin structure and its inhibition on enzymatic hydrolysis has been investigated as well. The more the condensed syringyl and guaiacyl phenolic units existed in the residual lignins from autohydrolysis pretreated aspen, the more enzyme non-productive binding occurred (Sun et al., 2016). This might be due to the enhanced hydrophobic interactions between the condensed lignins and enzymes. A similar result has been reported that the negative effects of condensed lignin on enzymatic hydrolysis were intensified by the addition of resorcinol in autohydrolysis, which was known to promote lignin condensation reaction (Pielhop et al., 2015). Based on these observations, it can be concluded that the suppression of lignin condensation could lower the adverse effects of condensed lignins on enzymatic hydrolysis.

Recently, carbonium ion scavengers like 2-naphthol have been applied to suppress the lignin condensation (Li et al., 2007; Pielhop et al., 2015; Pielhop et al., 2016). Since 2-naphthol could function as a strong nucleophile, it competed with the adjacent lignin fraction to react with the positively charged carbonium ion. Moreover, 2-naphthol only underwent a single electrophilic substitution, thereby suppressing lignin repolymerization reactions. However, due to the low water-solubility, 2-naphthol was not able to disperse uniformly in the dilute acid pretreatment liquor, which limited its application. Therefore, carbonium ion scavengers with the good water-solubility seem to be a better choice to increase its efficiency. On the other hand, lignosulfonate has been recently demonstrated as an effective enzymatic hydrolysis enhancer (Wang et al., 2013; Zhou et al., 2013). The incorporation of sulfonyl groups could increase the hydrophilicity of lignins, leading to the decreased enzyme non-productive binding. As a result, the enzymatic hydrolysis was improved.

Herein, 2-naphthol-7-sulfonate, which possesses the similar reactivity with 2-naphthol and the higher water solubility, was applied in the dilute acid pretreatment of mixed wood sawdust. It was hypothesized that the addition of 2-naphthol-7-sulfonate could not only relieve

the lignin condensation reaction, but also introduce sulfonyl group on lignin subunits to potentially improve the enzymatic hydrolysis. To verify our hypothesis, the dilute acid pretreatment of wood sawdust was carried out with 2-naphthol and 2-naphthol-7-sulfonate, respectively. The effects of the naphthol derivatives on chemical composition, enzymatic hydrolysis, enzyme adsorption, and cellulose accessibility of the obtained pretreated biomass were examined. The structural changes on the residual lignins were also elucidated by NMR analysis in the pretreated biomass with the naphthol derivatives.

## 2. Materials and methods

### 2.1. Materials and enzymes

Mixed wood sawdust with the size of  $0.5 \times 0.5$  cm ( $L \times W$ ) collected from Xuzhou, Jiangsu province, China, was used in the dilute acid pretreatment experiments. Two naphthol derivatives, including 2-naphthol and 2-naphthol-7-sulfonate, purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), were used as lignin repolymerization blockers in the dilute acid pretreatment.

Commercial cellulase (UTA-8) was kindly provided by Youtell Biochemical Co., Ltd. (Hunan province, China). Its filter paper activity and  $\beta$ -glucosidase activity were 70.6 FPIU/mL, and 27.5 IU/mL, respectively. Commercial  $\beta$ -glucosidase (BG188) with 205.4 IU/mL of  $\beta$ -glucosidase activity was provided by Novozymes (Beijing, China). Cellulase (UTA-8) was mixed with  $\beta$ -glucosidase (BG188) in a filter paper activity to  $\beta$ -glucosidase activity ratio of 1:1. This enzyme blend was applied in the enzymatic hydrolysis and enzyme adsorption experiments.

### 2.2. Dilute acid pretreatment of wood sawdust

The pretreatment experiments were carried out in a rotary cooking system with ten 1.25-L stainless-steel bomb reactors and an electrically heated oil bath. Wood sawdust (80 g, dry weight) was loaded in the bomb reactor with 1% sulfuric acid (based on biomass) in a solid to liquid ratio of 1:10. To limit the lignin repolymerization, the naphthol derivatives (2-naphthol and 2-naphthol-7-sulfonate) with the final concentration of 28 mM in pretreatment liquor were added prior to the pretreatment. The amount of naphthol derivatives was chosen according to a previous study, in which 4% (w/w) (based on the dry weight of biomass) 2-naphthol was applied, equaling to the addition amount in this study (Pielhop et al., 2016). Then the wood sawdust mixture was pretreated at 180 °C for 60 min. At the end of the pretreatment, the bomb reactors were cooled in a cold water bath. The pretreated substrate was collected by filtration, and washed with tap water until the pH closed to neutrality. The dilute acid pretreatment of wood sawdust without the naphthol additives was also carried out as a control. The obtained dilute acid pretreated substrate without the naphthol additives was assigned as DA. While the dilute acid pretreated substrates with the addition of 2-naphthol and 2-naphthol-7-sulfonate were assigned as DA-N and DA-NS, respectively.

### 2.3. Compositional analysis of biomass

The chemical composition analysis of samples was conducted according to the NREL protocol (Sluiter et al., 2011). Firstly, the extractives contents of the pretreated biomass were determined by ethanol extraction. Then, the extractive-free samples were hydrolyzed by 72% sulfuric acid at 30 °C for 1 h, and followed by 4% sulfuric acid at 121 °C for 1 h. The concentrations of glucose released during acid hydrolysis were analyzed by HPLC, and were used to calculate the cellulose contents in the substrates. The concentrations of mannose, xylose and arabinose were determined as well to calculate the hemicellulose contents in the substrates. Finally, the acid-insoluble lignin contents were determined by weighing the solid residues remained after the acid

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