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The fate of lignin during atmospheric acetic acid pretreatment of sugarcane bagasse and the impacts on cellulose enzymatic hydrolyzability for bioethanol production

Xuebing Zhao [a, *], Jialong Wen [b], Hongmei Chen [a], Dehua Liu [a]

[a] Key Laboratory for Industrial Biocatalysis, Ministry of Education of China, Institute of Applied Chemistry, Department of Chemical Engineering, Tsinghua University, Beijing 100084, China
[b] Beijing Key Laboratory of Lignocellulosic Chemistry, Beijing Forestry University, Beijing 100083, China

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ABSTRACT

Cellulose enzymatic hydrolyzability and fermentability of sugarcane bagasse was well improved to produce bioethanol by H_2SO_4-catalyzed atmospheric acetic acid (AA) pretreatment, which was mainly ascribed to delignification and modification of lignin structure. Crude bagasse milled lignin (CBML) was further used to investigate the structural changes of lignin macromolecule. Results showed that lignin-carbohydrate complex (LCC) underwent significant cleavage by acid hydrolysis as revealed by the reduction of polysaccharide content after AA treatment. The cleavage of β-O-4′ aryl ether bond was the predominant reaction responsible for lignin depolymerization. After AA treatment the negative effects of CBML were greatly weakened mainly due to the acylation of hydroxyl groups. The phenolic hydroxyl group mediated non-productive adsorption of cellulases was primarily attributed to hydrogen bonding interaction for endoglucanase and cellobiohydrolase, but both hydrogen bonding and electrostatic attraction played important roles for the adsorption of β-glucosidase on lignin.

1. Introduction

Bioconversion of lignocellulosic biomass, typically available as forestry and agricultural residues, to biofuels and chemicals in a conception of biorefining has been considered as a sustainable process for substitution of current fossil resource-based refining system. Green processing based on biomass conversion is also an important topic for the development of modern chemical engineering. However, the naturally-evolved complex cell wall structure has become the main barrier to efficient release of sugars from lignocellulose for the second generation bioethanol production, which is known as the biomass recalcitrance [1,2]. Therefore, the raw lignocellulosic biomass usually must be pretreated in order to increase cellulose digestibility [3]. A great number of pretreatment processes have been developed, and among the chemical pretreatments, mineral acid-catalyzed acetic acid (AA) pretreatment appears to be a promising technology, due to several reasons. Firstly, lignin fragments can be well dissolved in AA solution because the Hildebrand solubility parameter (δ_1 value) of AA is around that of lignin (δ_2 value) [4]; secondly, delignification can be performed under atmospheric pressure under the catalysis of mineral acid catalysts [5,6]; thirdly, the AA formed by deacetylation of hemicelluloses can be a supplement of the solvent; and fourthly, AA can be easily recovered by simple distillation. AA pretreatment of lignocellulosic biomass can be performed at higher temperature without addition of mineral acid; however, reaction temperature can be significantly reduced to <110 °C when H_2SO_4 is used as a catalyst. Previous kinetic studies have revealed that the rates of delignification and solubilization of polysaccharides are greatly enhanced by addition of H_2SO_4 [6,7].

Lignin has been found to be an important limitation to cellulose accessibility. This is because lignin not only plays as a physical barrier to protect cellulose, but also can non-productively adsorb proteins. Both hydrolysis of hemicelluloses and delignification contribute to the increase in cellulose accessibility, while delignification seems to be more important to expose cellulose surface, especially under mild pretreatment conditions [8,9]. Therefore, AA pretreatment can simultaneously achieve a fractionation of hemicelluloses, lignin and cellulose by a “one-pot” process. Some reported works have demonstrated that AA pretreatment of

* Corresponding author.
E-mail address: zhaoxb@mail.tsinghua.edu.cn (X. Zhao).

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lignocellulosic biomass combined with some post-treatment such as alkaline saponification can efficiently improve the enzymatic cellulose saccharification [10–13]. Some works on the structural features of lignins isolated by Acetosolv pulping of various feedstocks also have been reported [14–16]. As found by Pinheiro et al. [16], the isolated Acetosolv lignins exhibited higher thermal stability, lower molar weights, higher content of *p*-hydroxyphenyl units, higher relative phenolic and total hydroxyl contents, and lower relative methoxyl group content, compared to Kraft lignin. However, few works on the fate of lignin during acid-catalyzed atmospheric AA pretreatment were reported. Therefore, the objective of this work is to study the delignification mechanism of AA pretreatment as well as the structural changes of lignin and corresponding impacts on enzymatic hydrolysis of cellulose, in order to illustrate the mechanisms of AA pretreatment to improve cell wall cellulose accessibility and the correlation between lignin structure and cellullase non-productive adsorption. To achieve the objective, this work contained several sections as described in supplementary Figure S1. First, sulfuric acid (SA) catalyzed AA pretreatment under atmospheric pressure was employed to pretreat sugarcane bagasse, and the enzymatic digestibility of the pretreated samples and substrate structural features were analyzed, which corroborated that delignification played an important role to increase cellulose digestibility. Second, crude sugarcane bagasse milled lignin (CBML) was isolated from sugarcane bagasse and treated by AA to study the chemical structure changes during AA treatment to investigate the mechanism of SA catalyzed AA delignification. Third, the CBML and AA treated lignins were added to the enzymatic hydrolysis of pure cellulose in order to illustrate the impacts of lignin structure modification by AA treatment on the non-productive adsorption of cellulases.

2. Materials and methods

2.1. Materials and chemicals

The lignocellulosic biomass, sugarcane bagasse, was collected from Guangxi, China. The biomass contained 42.1 ± 1.7% glucan (cellulose), 26.4 ± 1.1% xylan, 0.8 ± 0.1% galactan, 1.9 ± 0.3% mannan, 1.5 ± 0.3% arabinan, 2.4 ± 0.1% acetyl group, 23.7 ± 1.1% acid-insoluble lignin, and 1.0 ± 0.1% acid-soluble lignin. The cellulase formula (Cellic-CTec2) was kindly provided by Novozymes (Beijing branch, China). The chemicals used in the experiments were chemical-pure and purchased locally. All the standard chemicals, including glucose, xylose etc. were purchased from Sigma-Aldrich (Shanghai branch, China). Crude bagasse milled lignin (CBML) was isolated according to the procedure described by Sun et al. [17].

2.2. AA pretreatment process

The AA pretreatment of sugarcane bagasse was carried out according to our previous work [6]. 30 g of air-dried bagasse was packed in a 1000-ml three-neck glass flask followed by addition of 300 ml 80% AA solution containing 0.3 wt% SA as the catalyst. The mixture was then heated by electric jacket to the boiling point of the solution at atmospheric pressure (~107 °C). One neck of the flask was connected with a condenser to condense the evaporated AA. After pretreatment, the solid was filtered and washed with 80% fresh AA prior to washing by water to neutrality. For the alkaline post-treatment to removal acetyl group introduced by AA pretreatment, 1–4 wt% (based on initial sugarcane bagasse) NaOH or lime was added to the water washed solid and heated at 120 °C for 1 h. To further study the chemical and structural changes of lignin macromolecule during AA pretreatment, 1 g of CBML was dissolved in 10 ml AA solution with addition of 0-0.3 wt% SA as a catalyst in a 50 ml round-bottom flask, followed by heating to the atmospheric boiling point of the AA solution (~107 °C) for 0.5–1.5 h. After the treatment, the solution was cooled down as quickly as possible in a cold water bath. Lignin was then precipitated by adding 300 ml deioned water and centrifuged. The precipitated lignin was washed by deioned water until neutrality and then lyophilized under vacuum.

2.3. Enzymatic hydrolysis of pretreated substrates and microcrystal cellulose

The enzymatic hydrolysis of pretreated cellulosic substrates were performed with cellulase loading of 15 FPU/g solid at 50 ± 0.5 °C and pH 4.8 (0.1 M sodium acetate buffer) in an air-bath shaker at 130 rpm for 120 h. The initial solid consistency was 2.5% (g/ml). The enzymatic digestibility was described as enzymatic cellulose conversion (*ECC*) defined as the percentage of the cellulose converted to glucose. The reported datum was the average of at least duplicate tests for each sample. It should be noted that determination of the components of pretreated substrates and enzymatic hydrolysis of the substrates were performed by duplication again, which indicated that quadruple tests actually were performed for each sample obtained under the same pretreatment condition. To study the effects of AA-treated CBML on cellulose hydrolysis, the treated lignin samples were added into the hydrolysis system of pure cellulose (microcrystal cellulose, MCC). Samples were then taken at regular time to analyze the produced glucose concentration.

2.4. Analytic methods

The main components of raw bagasse and pretreated substrates, FTIR spectroscopic analysis, scanning electron microscope (SEM), analysis of elemental composition, functional groups and average molecular weights of the lignin samples were performed following the procedures described in previous work [8,15]. Quantitative 2D-HSQCNMR spectra were recorded on a Bruker AVIII 400 MHz spectrometer at 25 °C in DMSO-d_6 as described in the work of Wen et al. [18].

3. Results and discussion

3.1. Effects of delignification by AA pretreatment on cellulose enzymatic digestibility

As shown in Fig. 1, lignin and hemicelluloses (xylan) were continually removed as the pretreatment proceeded, while glucan (cellulose) content increased gradually. The delignification kinetics demonstrated in Fig. 1A indicated that most of lignin was removed in the first hour, which could be termed as bulk delignification stage (BDS). Lignin content was reduced from 24.7% of raw materials to 12.3% of the pretreated substrate in this stage, with a degree of delignification (*DD*) of 71.6%. Prolonging reaction time did not result in a further significant increase in *DD*, known as the residual delignification stage (RDS). Removal of xylan showed similar tendency, but the degree of xylan removal was smaller than that of delignification, mainly due to the relatively low SA concentration (0.3 wt%). To understand whether removing xylan under the conditions of this work could significantly contribute the cellulose hydrolyzability, SA pretreatment was used to selectively remove hemicelluloses. It was found that pretreatment with 2% SA at 121 °C for 2 h hydrolyzed 85% of hemicelluloses and removed 16% of lignin, resulting in an *ECC* of 2 only 0.5%. It seems that delignification might be more important to expose cellulose surface under