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Impact of lignin removal on the enzymatic hydrolysis of fermented sweet sorghum bagasse ☆

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HIGHLIGHTS

- Impact of delignification on recalcitrance of FSSB was related to pretreated method.
- NaOH loading is the key factor to affect sodium hydroxide pretreatment efficiency.
- Pretreatment time is the dominant factor for calcium hydroxide pretreatment.
- $Ca(OH)_2$ is more capable of removing surface lignin.

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ABSTRACT

The complete utilization of sweet sorghum stalks including the fermentable sugars and the lignocellulosic faction is necessary to decrease the bioethanol production cost. Moreover, bioethanol yields from lignocellulosic resources depend on the saccharification efficiency of cellulose. Lignin has been considered as an important factor influencing enzymatic hydrolysis of lignocellulose. In this study, the impact of lignin removal on enzymatic hydrolysis was investigated using fermented sweet sorghum bagasse (FSSB) delignified by NaOH or $Ca(OH)_2$ pretreatments. For NaOH pretreated samples, a positive correlation between cellulose conversion rate and lignin removal was found when the lignin removal was from 8.96% to 65.61%. Further delignification of FSSB did not increase the efficiency of enzymatic hydrolysis. For $Ca(OH)_2$ pretreatment, there was no obvious correlation between lignin removal and cellulose conversion rate. More interestingly, the cellulose conversion rate of FSSB pretreated with $Ca(OH)_2$ was significantly higher than that of FSSB pretreated with NaOH when the same amount of lignin was removed. The surface lignin coverage of FSSB pretreated with 10% NaOH was 1.52 times higher than that of FSSB pretreated with $Ca(OH)_2$. These results demonstrated that the impact of lignin removal on enzymatic hydrolysis of FSSB pretreated with NaOH and $Ca(OH)_2$ was different. The lignin removal was the main factor influencing the enzymatic hydrolysis of FSSB pretreated with NaOH, while $Ca(OH)_2$ was more capable of removing surface lignin when the lignin content of the samples was similar.

1. Introduction

In recent decades, many researchers have focused on bioconversion of lignocellulose to ethanol [1–7]. Enzymatic saccharification of cellulose is a critical process for bioethanol production from lignocellulosic resources [8,9]. Saccharification efficiency depends not only on the cellulase activity, but also on the physic-chemical structure of the lignocellulosic substrate [10–12]. In fact, the task of hydrolyzing lignocellulose to fermentable monosaccharides is still technically complex because the digestibility of the cellulose is hindered by many factors, such as, cellulose crystallinity, cellulose degree of polymerization, substrate's available surface area, lignin barrier (content and distribution), hemicellulose content, feedstock particle size, porosity, and cell wall thickness (coarseness) [11]. The pretreatment step is employed in many cases to improve the enzymatic saccharification of cellulose [13]. The ultimate purpose, of whichever pretreatment technologies was used

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in the ethanol production, is to achieve an ideal balance between the ethanol yield and the production cost [14] or the ideal techno-economic index.

Among the leading pretreatment technologies, alkaline pretreatments due to the mild conditions and low inhibitions have attracted extensive attentions in the recent years [15]. When lignocellulosic substrates react with alkalis, the lignin–carbohydrate complex is disrupted efficiently to resulting in the dissolution of partial lignin and hemicellulose in the liquid phase [11]. Lignin is a jelly-like substance embedded in the three-dimension net structure of polysaccharides including cellulose and hemicellulose [16]. This complex structure is the main feature of the plant cell wall of the lignocellulosic biomass. The removal of lignin [17–19] and hemicellulose [20,21] improves the enzymatic hydrolysis of lignocellulose. Moreover, the lower activation energy of alkali delignification of herbaceous biomass compared to that of wood decreased the pretreatment temperature as reported by Kim and Holtzapple [22]. For example, Wu et al. reported the glucan saccharification yield of sweet sorghum bagasse treated with 2.5 M NaOH at room temperature achieved 98.70% [23]. In addition, alkali loading (g NaOH/g dry biomass) rather than alkali concentration (g NaOH/g pretreatment liquid) governed the pretreatment efficiency [24]. Besides NaOH, $Ca(OH)_2$ is also widely used in alkaline pretreatment as its inexpensive (about 6% cost of NaOH), easier to handle and recover [16]. However, the general pretreatment time is several days or even weeks [25] due to its poor solubility in water (1.73 g/L at 20 °C).

Some recent research was conducted to study the effect of alkali delignification on the enzymatic hydrolysis of biomass [26]. Wu et al. found that the 24 h saccharification yield (full enzyme loading) of NaOH pretreated sweet sorghum bagasse (Kyushuko4 and SIL05) was positively and linearly correlated to lignin removal rate regardless of specific treatment conditions [23]. Li et al. [27] reported that there was a clear negative, sigmoidal relationship between lignin content and glucan digestibility. Lignin content of 10–15% appeared to be a "threshold" value that is necessary for either enzyme/water penetration into the cell wall or improved cellulases access into the cell wall to reach cellulose. Yu et al. [28] found that the carbohydrate conversion of ozone delignified loblolly pine (58%) was lower than that of sodium chlorite delignified loblolly pine (80%) while the lignin content of them were 12.8% and 18.0%, respectively. All these findings suggest that currently there is still a need to understand the impact of lignin content on enzymatic hydrolysis more fully.

Sweet sorghum is a very promising energy crop as it is a high photo-synthesis C4 crop, with high biomass production, and high fermentable sugar and rich lignocellulosic content in the stalks. General, the fermentable sugar in the stalks can be converted into ethanol directly by fermentation using microorganisms, such as the yeast, *Saccharomyces cerevisiae* [29]. To be an important feedstock for bio-ethanol production, the cost-efficient bioethanol production from sweet sorghum stalks requires the bioconversion of the lignocellulosic fractions into ethanol [29,30]. To meet the requirement of mild pretreatment temperature, alkaline pretreatment was chosen for this study. Though NaOH and $Ca(OH)_2$ are extensively applied, currently there have not been any reports comparing the impact of NaOH and $Ca(OH)_2$ on lignin removal and enzymatic hydrolysis of fermented sweet sorghum bagasse (FSSB). NaOH and $Ca(OH)_2$ have very different property, and almost all literatures focused on the optimization of pretreatment while ignoring to compare the impact of different alkali on the same substrates. In this paper, we investigated the influence of NaOH and $Ca(OH)_2$ pretreatment on lignin removal and enzymatic hydrolysis of FSSB.

2. Material and methods

2.1. Material

Sweet sorghum, Jitian 2#, was harvested in October 2012, in Huanghua country, Hebei province. Leaves and husks were stripped by hand. Sweet sorghum bagasse was the residual of solid-state fermentation [29] of sweet sorghum stalk. All chemicals used in the study were reagent grade and used directly as purchased. Cellulase enzyme Cellic® CTec3 was generously provided by Novozymes Investment Co. Ltd (Beijing, China). The filter paper enzymatic activity was 213 FPU/mL for Cellic® Ctec3.

2.2. Alkaline pretreatment

2.2.1. Pretreatment condition and utilization rate of pretreated FSSB

An orthogonal array design was used to reduce the total number of experiments needed to explore the relationship between pretreatment condition and utilization rate of FSSB. The statistical software Design Expert, version 8.0.5b was used for the L9 (3^4) orthogonal array design in which 9 pretreatment combinations were derived by altering the three independent variables: alkaline loading, temperature, and time and to analyze the experimental data obtained. The selection of the factorial levels was based on previous studies (data not shown). The parameters for NaOH pretreatment were varied from 25 to 95 °C for temperature, 5–15 g NaOH/100 g dry FSSB for chemical dose, and 10–110 min for pretreatment time. The parameters for $Ca(OH)_2$ pretreatment were varied from 25–95 °C for temperature, 5–15 g $Ca(OH)_2$/100 g dry FSSB for chemical dose, and 1–48 h for pretreatment time. FSSB utilization as determined by the equation below was used to assess the effect of different pretreatment conditions.

$$\text{FSSB utilization} = [(C_1/1.05 + C_2/1.10) + (C_3 + C_4) \times 0.88] \times V/(W/R) \times 100\%$$

C was the reducing sugar concentration of enzymatic hydrolysate analysis by HPLC, which was detailed in Section 2.3. C_1 was concentration of cellobiose; C_2 was concentration of glucose; C_3 was concentration of xylose; C_4 was concentration of arabinose; V was volume of buffer solution used in enzymatic hydrolysis; W was weight of pretreaed FSSB used in enzymatic hydrolysis; R was the solid recovered after pretreatment.

2.2.2. Sugar release and lignin removal of FSSB pretreated with NaOH

Sugar release and lignin removal of FSSB pretreated with NaOH were performed with the following conditions: 5–30 g NaOH/100 g dry FSSB, 95 °C, 0.5 h, 1 g dry FSSB/20 mL water. The pretreated solids were washing to pH 7.0 by deionized water.

2.2.3. Sugar release and lignin removal of FSSB pretreated with $Ca(OH)_2$

Sugar release and lignin removal of FSSB pretreated with $Ca(OH)_2$ were performed with the following conditions: 10 g $Ca(OH)_2$/100 g dry FSSB, 25 °C, 0–48 h, 1 g FSSB/10 mL water. The pretreated solids were washing to pH 7.0 by deionized water.

2.3. Composition analysis

Compositions of samples were determined according to the NREL method [31].

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