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Mixing alkali pretreated and acid pretreated biomass for cellulosic ethanol production featuring reduced chemical use and decreased inhibitory effect

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ABSTRACT

Alkali and acid pretreatment based processes for lignocellulosic biofuel production typically consume substantial amount of alkali and acid. In addition, alkali pretreatment generates compounds with high toxicity rendering washing/detoxification inevitable. In this work, alkali (AL) and dilute acid (DA) pretreatment based processes were compared side by side for ethanol production from corn stover (CS) using commercial enzymes and *Zymomonas mobilis* 8b strain. Next, AL and DA pretreated CS slurries (without removing liquid stream) were mixed to neutralize for enzymatic hydrolysis and ethanol fermentation. The digestibility and fermentability of the mixed pretreated biomass were systematically studied and compared to unmixed pretreated biomass. Results showed that mixed biomass process saved 40–50% alkali/acid use and enhanced fermentability compared to AL-CS. This work suggests that it could be beneficial to have both alkali and acid pretreatments operating in a biorefinery, which could accommodate various feedstocks.

1. Introduction

Lignocellulosic materials are the most abundant biomass on the earth. Development of biorefineries to convert lignocellulosic biomass to fuels and chemicals, which could help alleviate the issues of energy security and global warming, has attracted much attention. Due to the recalcitrance of lignocellulosic biomass, pretreatment is needed to open up the cell wall structure and increase the accessibility of cellulose. Among the existing pretreatment methods, alkali (AL) pretreatment using sodium hydroxide and dilute acid (DA) pretreatment using sulfuric acid have been commonly used in previous studies because of their high effectiveness (Kapoor et al., 2015). AL pretreatment breaks the linkages between lignin and carbohydrate fractions, increases porosity of biomass, sometimes changes cellulose configuration and decreases cellulose crystallinity. In addition, AL pretreatment also leads to the swelling of cell wall material, which increases the internal surface of the lignocellulosic biomass, and make biomass more digestible (Karp et al., 2014; Li et al., 2016). Nevertheless, AL pretreatment degrades some lignin and produces phenolic compounds, which can inhibit subsequent enzymatic hydrolysis and fermentation. In addition, additional acidic reagents are needed to neutralize pH after AL pretreatment, which introduces salts with potential inhibitory effects. The use of chemical reagents before and after pretreatment increases the cost of the system and, at the same time, may have negative environmental effects.

DA pretreatment is also a promising pretreatment which effectively solubilizes and hydrolyzes hemicellulose into sugars (xylose, glucose and soluble oligomers) (Karimi et al., 2006; Sun and Cheng, 2005), and thus improves cellulose digestibility. The monomeric sugars formed can be further decomposed during pretreatment into various toxic products such as furfural, 5-hydroxymethyl furfural (HMF) and levulinic acid, resulting in strong inhibition on enzymes and fermentation microorganisms. In addition, a small fraction of lignin can also be degraded into phenolics that are inhibitory to the subsequent enzymatic hydrolysis and fermentation.

Many previous studies have used either AL pretreatment or DA pretreatment to increase the digestibility of biomass. For instance, AL pretreatment was found to be efficient on common reed (*Phragmites australis*) (Tozluoglu, 2018), corn husk (Sharma et al., 2018), peach tree prunings (Buratti et al., 2018), etc., and DA pretreatment was proven efficient on corn stover (Avci et al., 2013), oil palm empty fruit bunches (Jung et al., 2013), switchgrass (Garlock et al., 2011), etc. However, both AL and DA pretreatment need extra acid or alkali to neutralize pretreatment slurry, which increases the total cost. In addition, high

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concentrations of inhibitors in pretreated slurry, especially in the AL pretreated slurry, limit the efficacy of enzymatic hydrolysis and fermentation. Therefore, washing/detoxification is inevitable after AL pretreatment, which requires a large amount of water or chemicals. Considering pH differences between DA and AL pretreatments, one alternative is to combine AL and DA pretreated biomass slurries, which can reduce the consumption of acid/base for neutralization. In addition, mixing the pretreated biomass slurries help dilute the inhibitors present in both pretreatment slurries and facilitates the following enzymatic hydrolysis and fermentation. Moreover, since AL pretreatment and DA pretreatment have their own advantages on different biomass, it would be beneficial to have both pretreatments on a biorefinery if various biomass would be used as feedstock.

In this work, we compared the effectiveness of AL pretreatment, DA pretreatment side-by-side on corn stover (CS) and also for AL pretreated biomass mixed with DA pretreated biomass. Then the pretreated biomass materials were used as substrates for enzymatic hydrolysis and fermentation with *Zymomonas mobilis* 8b strain to produce ethanol. This strategy (mixing alkali and acid pretreated biomass slurries) alleviated the inhibitory effect of soluble fractions in the slurry on fermentation and also minimized the usage of chemicals used during the process.

2. Materials and methods

2.1. Biomass and enzyme

Air-dried corn stover was harvested in Lianyungang, Jiangsu province, China. The corn stover was knife milled using a laboratory mill with 4 mm particle size interior sieve. The moisture content of the corn stover was 10.0%.

2.2. Microorganism and medium

Z. mobilis 8b strain (capable of metabolizing both glucose and xylose to ethanol) was used for hydrolysate fermentation. Before each fermentation, seed culture was prepared in a 50 mL flask with 40 mL medium containing 10 g/L yeast extract, 2 g/L potassium dihydrogen phosphate, 5 g/L peptone, 50 g/L glucose and 20 g/L xylose. A frozen stock was used as inoculum, and seeds were cultured at 150 rpm, 30 °C for 24 h. *Z. mobilis 8b* seeds were then transferred to fresh medium and cultured for another round under the same conditions for 20–24 h in 50 mL flasks. The inoculum was then centrifuged at 4000 rpm for 10 min. The resulting cell pellet was used for inoculation of fermentation medium/hydrolysate. The initial cell optical density (OD) at 600 nm was 2.0 for fermenation (Uppugundla et al., 2014).

2.3. Biomass pretreatment

Two thermochemical pretreatments were used to pretreat the corn stover, including AL pretreatment and DA pretreatment under optimized pretreatment conditions. AL pretreatment was performed at 10% (w/w) solids loading using 2% (w/w) NaOH at 121 °C for 20 min in an autoclave (Karp et al., 2014; Park and Kim, 2012). DA pretreatment was performed in a 2 L reactor at 160 °C for 10 min with sulfuric acid loading of 1% (w/w) and total solids loading of 10 wt.% (Dien et al., 2006; Na et al., 2016). After pretreatment, the pH of the pretreated slurry was adjusted to 7.0 with hydrochloric acid or NaOH. The pretreated slurry was then dried to a moisture content of around 20% in an oven with air circulation at 60 °C and stored in refrigerator at 4 °C before use. The slurry was dried for easier storage and handling.

2.4. Mixing alkali pretreated biomass and dilute acid pretreated biomass

After pretreatment by AL and DA, the two pretreated slurries (without neutralization) were mixed at ratios of 5:7.3 and 1:2.5, based on dry weight. The pH values of these mixtures were 7.0 and 4.6, respectively. Mixing the two pretreated biomass slurries saved chemical use for pH adjustment.

Table 1
Composition analysis of corn stover pretreated by AL and DA[a].

	Pretreatment methodology		
	None	AL	DA
Composition of biomass after dry (%)[b]			
Glucan	37.19 ± 0.66	28.19 ± 0.65	31.22 ± 1.30
Xylan	19.20 ± 0.02	13.59 ± 0.28	16.76 ± 0.23
Klason Lignin	19.45 ± 0.07	10.43 ± 1.02	19.04 ± 0.45
Ash	3.91 ± 0.48	5.68 ± 0.01	5.53 ± 0.43
Soluble sugars in pretreated biomass(g/100 g dry weight)			
Glucose monomer	NA	2.70 ± 0.07	5.73 ± 0.09
Glucose oligomer (DP > 1)	NA	0.76 ± 0.04	0.71 ± 0.00
Xylose monomer	NA	0.55 ± 0.01	15.19 ± 0.31
Xylose oligomer (DP > 1)	NA	3.21 ± 0.26	0.32 ± 0.00

All experiments were done in triplicates and each value is expressed as mean ± S.D.

[a] AL: alkali pretreatment, DA: dilute acid pretreatment.

[b] Pretreated biomass slurry was neutralized, dried and then two-step acid hydrolysis was performed for composition analysis.

2.5. Enzymatic hydrolysis

Pretreated biomass was enzymatically hydrolyzed using CTec2 (30 mg protein/g glucan) at 3% w/w glucan loading (pH 4.8, 50 °C, 250 rpm) in a baffled shake flask. Samples were taken at 4, 8, 12, 24, 36, 48 and 72 h and the concentrations of glucose and xylose were determined by HPLC. After 72 h of hydrolysis, the overall mass balances were determined as described previously (Lau and Dale, 2009).

2.6. Fermentation

The sterile filtered hydrolysate from enzymatic hydrolysis was fermented after pH adjustment to 6.0 using a recombinant strain *Z. mobilis 8b*. Fermentation was carried out in a 150 mL flask with a total reaction volume of 60 mL. Fermentation was initiated by inoculating *Z. mobilis 8b* strain to reach OD_{600} of 2.0. Yeast extract (5 g/L) and tryptone (10 g/L) were supplemented as nutrients before fermentation. Samples were taken at 8, 16, 24, 96 and 120 h and the concentrations of glucose, xylose and ethanol were determined by HPLC.

2.7. Analysis

Biomass composition, soluble oligomeric sugars and mass balances were carried out according to NREL methods and our previous studies (Jin et al., 2010; Lau and Dale, 2009).

The concentrations of glucose, xylose, acetic acid, ethanol, HMF and furfural were determined using a Shimadzu HPLC system equipped with an Aminex HPX-87H column maintained at 60 °C and Shimadzu refractive index detector (RID). Sulfuric acid (5 mM) was used as an eluent at 0.6 mL/min. Injection volume was 15 μL (Uppugundla et al., 2014).

The total phenol concentrations were analyzed through Folin-Ciocalteu Phenol Assay (Chen et al., 2015). Sample (100 μL) was added into a glass tube and then mixed with 3 mL of water. 250 μL Folin-Ciocalteu reagent, 750 μL Na_2CO_3 solution (20% w/w) and 900 μL water were then added to bring the volume to 5 mL. The tube was incubated at 22 °C for 2 h. Then, the concentration of total phenol was determined by measuring the absorbance at 760 nm.

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