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Short Communication

Reducing biomass recalcitrance via mild sodium carbonate pretreatment

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HIGHLIGHTS

- Sodium carbonate was used for pretreating biomass under a mild condition.
- Three feedstocks were tested, including corn stover, *Miscanthus*, and switchgrass.
- The pretreatment substantially removed lignin while keeping most of cellulose.
- The pretreatment increased the cellulose digestibility by 1–3 times.
- Pretreated corn stover gave the highest glucose yield of 95.1%.

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ABSTRACT

This study examined the effects of mild sodium carbonate (Na_2CO_3) pretreatment on enzymatic hydrolysis of different feedstocks (i.e., corn stover, *Miscanthus*, and switchgrass). The results showed that sodium carbonate pretreatment markedly enhanced the sugar yields of the tested biomass feedstocks. The pretreated corn stover, *Miscanthus*, and switchgrass gave the glucose yields of 95.1%, 62.3%, and 81.3%, respectively, after enzymatic hydrolysis. The above glucose yields of pretreated feedstocks were 2–4 times that of untreated ones. The pretreatment also enhanced the xylose yields, 4 times for corn stover and 20 times for both *Miscanthus* and switchgrass. Sodium carbonate pretreatment removed 40–59% lignin from the tested feedstocks while preserving most of cellulose (<5% cellulose loss). Corn stover appeared to be least resistant to breakdown by Na_2CO_3 and enzymatic hydrolysis. Our study indicated that mild sodium carbonate pretreatment was effective for reducing biomass recalcitrance and subsequently improving the digestibility of lignocellulosic biomass.

1. Introduction

Global warming and increasing demand for fossil fuels are the main driving forces for developing biofuels from renewable resources. Lignocellulosic biomass is an abundant, renewable, non-food source for the production of biofuels and bioproducts. The biological conversion platform for the production of biofuels from biomass involves two main stages: (1) hydrolysis of holocellulose (cellulose and hemicellulose) to monosaccharides; and (2) fermentation of monosaccharides into biofuels (e.g., ethanol, butanol). The recalcitrant structure of biomass against the hydrolysis step is the limiting factor for their commercial usage (Taherzadeh and Karimi, 2008). To facilitate biological conversion of lignocellulosic biomass, pretreatment is a crucial step to reduce biomass recalcitrance. The current leading pretreatment methods, such as dilute acid, ammonia fiber explosion, and steam explosion have been studied for different lignocellulosic feedstocks (Alvira et al., 2010; Taherzadeh and Karimi, 2008). Among alkaline pretreatment methods, sodium carbonate (Na_2CO_3) as a pretreatment agent received less attention than other methods.

Sodium carbonate (Na_2CO_3) acts as fairly strong alkali in aqueous solution and has been studied for the pretreatment of lignocellulosic materials (Vaccarino et al., 1987). The pretreatment effectiveness of Na_2CO_3 is similar to that of regular alkaline reagents. Na_2CO_3 pretreatment can remove lignin and various uronic acid substitutes in hemicellulose but cause less degradation of cellulose than acid or hydrothermal pretreatments (Kim et al., 2014). It can potentially overcome the major operational problems with conventional alkaline pretreatments, such as corrosion, extensive neutralization, and environmental hazards. Prior studies reported improved enzymatic digestibility of holocellulose as a result of Na_2CO_3 pretreatment; but most effective prior studies, as summarized in Table 1, were conducted with high pressure

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Table 1
Comparison of studies on Na_2CO_3 pretreatment for improving sugar yields of biomass.

Studies	Feedstocks	Particle size	Na_2CO_3 pretreatment*	Sugar yields
Salehi et al. (2012)	Rice Straw	20–80 mesh	1 g Na_2CO_3/g solid, 180 °C, 120 min	97% glucose yield
Yang et al. (2012)	Rice straw	40–80 mesh	TTA 8%, S/L ratio of 1:6, 140 °C, 60 min **	95% of glucose yield, 76% of xylose yield
Khaleghian et al. (2015)	Rice straw	20–80 mesh	1 g Na_2CO_3/g solid, 100 °C, 180 min	Nearly 100% glucose yield
Jin et al. (2013)	Wheat straw leaf	3–5 cm in length	136.8 mg Na_2CO_3/g, 120 °C, 50 min	74.5% glucose yield
	Wheat straw stem		171.5 mg Na_2CO_3/g solid, 130 °C, 55 min	56.3% glucose yield
Kim et al. (2014)	Corn stover	<80 mesh	600 mg Na_2CO_3/g solid, 160 °C, 20 min	92.5% glucose yield
Present study	Corn stover	20–80 mesh	1 g Na_2CO_3/g solid, 80 °C, 180 min	95.1% glucose yield
	Miscanthus		1 g Na_2CO_3/g solid, 80 °C, 180 min	62.3% glucose yield
	Switchgrass		1 g Na_2CO_3/g solid, 80 °C, 180 min	81.3% glucose yield

* Alkaline loadings as g Na_2CO_3/g solid in this table were calculated based on the original alkaline loading reported in the corresponding citations.
** Pretreatment time was calculated based on the reaction that was held at 80 °C for 30 min and then ramped to 140 °C at a rate of 2 °C/min.

and temperature. Salehi et al. (2012) reported that rice straw pretreated at 180 °C for 120 min gave 97% glucose yield. The sugar yield of 92.5% was reported for corn stover pretreated with Na_2CO_3 at 160 °C for 20 min (Kim et al., 2014). However, there is little research regarding mild Na_2CO_3 pretreatment for breaking down biomass. Also, there is a need to develop a robust Na_2CO_3 pretreatment suited to a broad spectrum of feedstocks.

In this study, sodium carbonate was used to pretreat three herbaceous biomass feedstocks (i.e., corn stover, *Miscanthus*, and switchgrass) under mild conditions. Cellulose digestibility, degradation of cell wall components, and characteristics of pretreated biomass were investigated to reveal the effects of sodium carbonate pretreatment on reducing biomass recalcitrance and subsequent enzymatic hydrolysis.

2. Methods

2.1. Materials

All the biomass feedstocks (i.e., corn stover, *Miscanthus*, and switchgrass) were collected from the Bradford Farm at the University of Missouri in Columbia, Missouri, USA. The raw feedstocks were air-dried and ground through 2 mm screen. The fractions with the particle size of 20–100 mesh (about 85% of the above ground biomass) were used for the experiment. Hydrolytic enzymes (Cellic®CTec 2 and Cellic®HTec 2) were obtained from Novozymes. All the chemicals were of analytical grade and purchased from Fisher scientific (Waltham, MA, USA).

2.2. Pretreatment

Each feedstock (10 g, on a dry basis) was gently mixed in a media storage bottle with 190 ml 0.5 M Na_2CO_3 solution to obtain an alkali loading of 1 g Na_2CO_3/g solid. The pretreatment was conducted in triplicate at 80 °C in a water bath for 3 h (including time for heating up). The bottle was loosely covered with a cap and shaken every 30 min manually. After Na_2CO_3 pretreatment, all the pretreated slurry was collected and centrifuged (8000 rpm, 10 min). The solid fraction was collected, washed with reverse osmosis (RO) water three times, neutralized with HCl until the pH reached 7, and then repeatedly washed with RO water to remove all salts. After washing, the solids of each pretreated feedstock were collected and used for enzymatic hydrolysis and further analysis.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis was performed for both untreated and pretreated samples according to NREL protocol (Dowe and McMillan, 2001). The biomass was added to citrate buffer (with a final molarity of 0.05 M, pH 4.8) in 250 ml Erlenmeyer flask and then hydrolyzed at 50 °C at 120 rpm for 72 h. The working volume was 100 ml with 2.5% (w/w) solid loading. Cellulase (CTec 2) and hemicellulase (HTec 2) were loaded at 30 and 3 mg protein/g solid, respectively. After hydrolysis, a small portion of slurry was boiled for 5 min to deactivate enzymes and then used for sugar analysis using high performance liquid chromatography (HPLC). The reported sugar yields were defined as the percentage of theoretical sugar yields of untreated and pretreated biomass, respectively.

2.4. Analytical methods

Structural carbohydrates and lignin contents of untreated and pretreated biomass were analyzed using two-stage acid hydrolysis according to NREL procedure (Sluiter et al., 2008). In brief, the samples were first hydrolyzed with 72% sulfuric acid at 30 °C for 60 min and then with 4% sulfuric acid at 121 °C for 60 min. The hydrolysate was filtrated to obtain acid insoluble lignin which was determined using a gravimetric method. Acid soluble lignin was determined by UV–Vis spectrophotometer at a wavelength of 320 nm. The sugar contents in the filtrate were analyzed using HPLC equipped with a 410 LC pump, Bio-Rad Aminex HPX-87P column (300 × 7.8 mm), and Shimadzu RID-6A differential refractive index detector (RID). The temperature of the column was kept at 85 °C. The mobile phase was HPLC grade water, eluting at a flow rate of 0.6 ml/min. The removal of lignin, glucan, and xylan was defined as the percentage of the corresponding component reduced after pretreatment, respectively.

Untreated and pretreated samples were analyzed for functional groups by Fourier Transform Infrared (FTIR) spectroscopy. FTIR spectra were acquired using Avatar 380 FTIR spectrophotometer (Thermo Nicolet) with an attenuated total reflection (ATR) probe. Each sample was scanned 64 times from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}.

Crystalline structures of untreated and pretreated samples were analyzed using X-ray diffractometer (Ultima iv, Rigaku, Japan). Scanning was performed at 40 kV and 44 mA at a speed of 2°/min from 5° to 50°. The crystallinity indices (CrI) of the samples were calculated from the height ratio between the intensity of the crystalline peak ($I_{200} - I_{Am}$) and total intensity (I_{200}) (Segal et al., 1959):

$$\mathrm{CrI} = (I_{200} - I_{Am})/I_{200}$$

where I_{200} is the maximum intensity of diffraction peak at 2θ of 22.4–22.5°; I_{Am} is the minimum intensity of amorphous portion at 2θ of 18.0–19.0°.

3. Results and discussion

3.1. Effect of Na_2CO_3 pretreatment on feedstock compositions

The main functions of alkaline pretreatment are lignin removal, alteration of lignin structures, removal of hemicellulose