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Sustainable biobutanol production using alkali-catalyzed organosolv pretreated cornstalks

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ABSTRACT

Biobutanol is an important alternative fuel source, but current processing methods suffer from low yields. This study aimed to develop and optimize a pretreatment protocol for biobutanol production from cornstalks. Fractionation of cornstalks into carbohydrate (cellulose and hemicellulose) and lignin was performed by alkali-catalyzed organosolv pretreatment (ACOS). After optimization of the process parameters, more than 80% of the total lignin was removed, with minimal hemicellulose degradation, at 110 °C, 4% (w/w dry cornstalk) NaOH, 90 min reaction time, and 60% (v/v) ethanol. After enzymatic hydrolysis, the maximum recovery of total monosaccharide was 83.7% (85.0% cellulose, 82.0% hemicellulose). In acetone-butanol-ethanol (ABE) fermentation, a slightly higher total ABE concentration (12.8 g/L *vs.* 11.9 g/L) was produced from the enzymatic hydrolysate, compared with that from a glucose control. The physical structure and chemical properties of alkali-catalyzed organosolv lignin (ACOSL) showed higher phenolic group content and antioxidant capacity compared with alkali lignin. ACOS pretreatment is an economical method for the production of fermentable monosaccharide and high-value lignin, for use in biofuel production.

1. Introduction

Over the past two hundred years, a rapidly developing human society has relied on non-renewable fossil resources (e.g. coal, petroleum, natural gas). The exhaustion of these resources and growing environmental problems are driving scientific interest towards renewable feedstocks. In this sense, lignocellulosic biomass is a promising candidate because it is an abundant and carbon-neutral energy resource (Somerville et al., 2010). The monosaccharides obtained from the hydrolysis of cellulose and hemicellulose can be converted to bio-based liquid fuels (e.g. ethanol) or other high-value chemicals (e.g. succinic acid) (Sheldon, 2014). However, the recalcitrant structure of lignocellulosic biomass impedes enzymatic hydrolysis of the polysaccharide (Himmel et al., 2007). Therefore, development of efficient and economical pretreatment technology will play a pivotal role in improving the economic feasibility and sustainability of biofuel production from lignocellulosic biomass.

Pretreatment techniques overcome the recalcitrant structure by disrupting cell wall physical barriers, reducing cellulose crystallinity, and removing the lignin, so that hydrolytic enzymes can access the biomass macrostructure. Various physical, chemical, and biological methods have been applied, either alone or in combination, for lignocellulose pretreatment. Several recent review articles provide a general overview of the field (Mosier et al., 2005; Hendriks and Zeeman, 2009; Alvira et al., 2010). Chemical pretreatments, usually using acid or alkali as the catalyst and water as the solvent, have been most widely studied. However, acid catalysts typically produce inhibitors such as carboxylic acids, furans, and phenolic compounds, which can inhibit microbial growth and fermentation, resulting in poor productivity of biofuels (Taherzadeh and Keikhosro, 2007). Furthermore, equipment corrosion problems and acid recovery are important drawbacks of concentrated acid pretreatments. Alkali pretreatment of lignocellulose uses alkaline catalysts such as NaOH, $Ca(OH)_2$ (lime), or ammonia, to break the ester bonds between lignin, hemicellulose, and cellulose, and increase the solubility of lignin. Alkali pretreatment usually causes swelling of the biomass, increasing its internal

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surface. Furthermore, alkali pretreatment could suppress the dissolution of cellulose and hemicellulose, which is often observed with acid or hydrothermal pretreatment processes (Alvira et al., 2010). Increased enzyme accessibility to cellulose and improved saccharification, especially for agricultural residues, are reported with alkali pretreatment (Zhu et al., 2010; Karp et al., 2014; Singh et al., 2015). However, alkali pretreatment still suffers from a long processing times and high consumption of alkali.

Organosolv pretreatment is an alternative strategy compared with acid or alkali processing. Organic solvents, including methanol, ethanol, butanol, acetone, tetrahydrofuran, glycerol, ethylene glycol, and propylene glycol, have been used for lignocellulose pretreatment (Zhang et al., 2016). The presence of organic solvent reduces the viscosity of the pretreatment medium, improves penetration into the biomass, and facilitates a more efficient removal of lignin. Organosolv pretreatment was found to be effective at improving the digestibility of cellulose (Perez-Cantu et al., 2013). The organosolv process is more expensive than other pretreatment processes owing to the additional cost of organic solvents. Organosolv pretreatment can be also performed with a catalyst. Mineral acids such as hydrochloric, sulfuric, and phosphoric acids have been used in organosolv pretreatment, and were shown to enhance the delignification process and enzymatic digestibility of cellulose (Caspeta et al., 2014). However, organosolv pretreatment is always performed at high temperatures (usually higher than 150 °C) when using acid as a catalyst. Under these sever conditions, acid catalysts might cause the dissolution of all hemicellulose and a limited content cellulose and can contribute to corrosion. Several fermentation inhibitors (levulinic acid, furfural, and 5-hydroxylmethylfurfural) can be generated from the degradation of pentose and hexose sugars. Furthermore, the use of alcohols such as ethanol and methanol generates high pressure at elevated temperatures (Rezayati-Charani et al., 2006). From an economic perspective, the cost of the equipment is greatly increased.

As a typical herbaceous biomass, cornstalks has been widely used as a feedstock for biofuel production. Compared with woody biomass, cornstalk has a higher ash content, but lower lignin content. Therefore, an organosolv processing was selected for the efficient recovery of lignin, as most of the ash remains in the solid phase during pretreatment. To avoid the degradation of cellulose and hemicellulose and generation of fermentation inhibitors, an alkali catalyst was used in the system. The aim of this study was to combine the aforementioned advantages, using an alkali-catalyzed organosolv (ACOS) pretreatment on cornstalks. The pretreated cornstalks was enzyme hydrolyzed with a cellulase and xylanase, prior to acetone-butanol-ethanol (ABE) fermentation, and the produced lignin was also characterized.

2. Methods

2.1. Materials

Cornstalks was provided by a local factory in Lianyungang, Jiangsu, China, was milled and screened to 30–50 mesh, and dried at 105 °C to constant weight. The chemical composition of the raw cornstalk (on a dry weight basis) was 36.3% cellulose, 25.9% hemicellulose, 17.4% lignin, 3.9% ash, and 16.5% unknown components. Cellulase (245 FPU/ml), was obtained from Tianguan Co. (Nanyang, China). Xylanase (439 IU/g, from *Thermomyces lanuginosus*) was purchased from Sigma (St Louis, MO, USA).

2.2. Alkali-catalyzed organosolv pretreatment

The pretreatment processes were carried out in a high-pressure reactor equipped with a mechanical agitator and temperature controller by Yanzheng Co. in China. The reactor was made of 316 stainless steel with the total volume of 250 mL. Reactions were performed using 10.0 g cornstalk (dry basis) with aqueous ethanol (0–100%, 100 mL), and NaOH as a catalyst (2–8% w/w dry cornstalks). The pretreatment was conducted at different temperatures (70–150 °C) and for different reaction times (30–150 min). After completion, the reactor was cooled using a water bath, and the obtained solid and liquid fractions were separated by filtration. The solids were washed with water (2 × 25 mL) and dried at 105 °C to constant weight; then the amount of solid remaining was calculated and sampled for composition analysis. Ethanol was recovered under vacuum, and lignin was then obtained by precipitating in concentrated sulfuric acid precipitation. Finally, the pretreated cornstalk was used for enzymatic hydrolysis.

2.3. Enzymatic hydrolysis of biomass

Enzymatic hydrolysis of raw cornstalks and pretreated solids was performed at 50 °C/150 rpm in 50 mM sodium citrate buffer (pH 4.8). All experiments were performed in 20-mL volumes at a solid consistency of 5%. Cellulase and xylanase were added at 15 FPU and 10 U per gram of substrate, respectively. Periodically, aliquot samples of the hydrolysate were taken for monosaccharide analysis by HPLC.

2.4. Butanol fermentation

Clostridium beijerinckii NCIMB 4110 (a Cbei_4110-inactivated mutant strain), from our team, was grown anaerobically for 12–14 h in yeast extract-peptone-starch (YPS) medium (1.0% soluble starch, 0.5% peptone, 0.3% yeast extract, 0.2% ammonium acetate, 0.3% NaCl, 0.3% $MgSO_4 \cdot 7H_2O$, 0.1% KH_2PO_4, 0.1% K_2HPO_4, and 0.01% $FeSO_4 \cdot 7H_2O$) at 37 °C (Liu et al., 2016). The culture was transferred to 10 vols of ABE production medium [containing P2 stock solutions (buffer solution, mineral solution, and vitamin solution) with 30 g/L of glucose or cornstalks hydrolysates (30 g/L total sugar) as a carbon source] for fermentation. The cornstalk hydrolysate medium was adjusted to pH 6.5, and sterilized at 115 °C for 20 min. Cultured *C. beijerinckii* cells were inoculated to a 10-fold excess at fermentation medium (v/v). Periodically, samples were collected for analysis of sugar consumption and ABE production (Liu et al., 2016).

2.5. Analytical methods

2.5.1. Composition analysis of biomass

Composition of raw cornstalk and pretreated solid was analyzed by acid hydrolysis, according to National Renewable Energy Laboratory (NREL) procedures (Sluiter et al., 2008). Glucose, xylose, and arabinose levels were analyzed by HPLC (Agilent 1200 series; Hewlett-Packard, Palo Alto, CA, USA) with a refractive index detector, using an Aminex HPX-87H ion exclusion column (300 × 7.8 mm; Bio-Rad Laboratories, Hercules, CA, USA), with 5.0 mM H_2SO_4 used as the mobile phase (0.6 mL/min) at 55 °C. The acid-insoluble lignin content was determined by gravimetric analysis of the calcined acid insoluble residue at 575 °C for 24 h. The acid-soluble lignin content was determined by UV spectroscopy, measuring the absorbance at 205 nm. The ash content of cornstalk was measured by gravimetric analysis of the calcined biomass at 575 °C for 24 h.

2.5.2. Analysis of fermentation products

Acetone, ethanol, and butanol were analyzed using gas chromatography (7890A, Agilent, Wilmington, DE, USA) equipped with a flame ionization detector (FID) and an Agilent HP-INNOWAX column (0.25 mm × 60 m). The oven was programmed to heat from 70 to 190 °C at a rate of 20 °C/min, with an initial holding time of

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