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Dual effect of soluble materials in pretreated lignocellulose on simultaneous saccharification and co-fermentation process for the bioethanol production

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HIGHLIGHTS

- Effect of different additions of soluble materials in biomass on SSCF was studied.
- Soluble materials inhibit enzymatic hydrolysis but stimulate fermentation.
- Soluble lignins at low concentrations stimulates xylose consumption in yeast.
- YP addition enhances ethanol yield in SSCF containing high inhibitor concentrations.

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ABSTRACT

In this study, wash liquors isolated from ethylenediamine and dry dilute acid pretreated corn stover were used to evaluate the effect of soluble materials in pretreated biomass on simultaneous saccharification and co-fermentation (SSCF) for ethanol production, respectively. Both of the wash liquors had different impacts on enzymatic hydrolysis and fermentation. Enzymatic conversions of glucan and xylan monotonically decreased as wash liquor concentration increased. Whereas, with low wash liquor concentrations, xylose consumption rate, cell viability and ethanol yield were maximally stimulated in fermentation without nutrient supplementary. Soluble lignins were found as the key composition which promoted sugars utilization and cell viability without nutrient supplementary. The dual effects of soluble materials on enzymatic hydrolysis and fermentation resulted in the reduction of ethanol yield as soluble materials increased in SSCF.

1. Introduction

Lignocellulosic biomass has long been recognized as a potential sustainable resource of mixed sugars used for fermentation to produce bioethanol (Himmel et al., 2007). The biorefinery from biomass to ethanol, which comprises of pretreatment, enzymatic hydrolysis, fermentation and distillation, needs stronger market competition by simplifying technical process and increasing ethanol yield and productivity (Cardona and Sánchez, 2007; Balan, 2014). Simultaneous saccharification and co-fermentation (SSCF) process, enabling the saccharification of the pretreated biomass to react simultaneously with the co-fermentation of hexose and pentose by microorganism, effectively increases ethanol yield and productivity in broth by reducing end-products inhibition and affording higher solid loading (Olofsson et al., 2010; Ojeda et al., 2011; Karagöz et al., 2012; Koppram et al., 2013; Liu and Chen, 2016). Increasing solid loading has significant effect on increasing ethanol concentration and decreasing distillation costs (Modenbach and Nokes, 2012).

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The increasing concentration of inhibitors at high solid loading is assumed as one of the limiting factors to get higher ethanol yield in SSCF process (Carrasco et al., 2011; Moreno et al., 2013; Zhu et al., 2015). Previous studies showed that soluble materials from various pretreated lignocelluloses significantly inhibit enzymatic hydrolysis, in which oligomeric sugars and lignin degradation products play the dominant role in inhibiting effects (Ximenes et al., 2011; Xue et al., 2015; Qin et al., 2016a,b). For fermentation, inhibitors in soluble materials, *i.e.* sugar degradation products (furfural and hydroxymethylfurfural (HMF)), organic acids and lignin degradation products, restrain yeast growth and ethanol productivity (Jönsson et al., 2013; Wang et al., 2015). Xylose utilizations of engineered yeast are reduced with the presence of biomass degradation products as previously reported (Jin et al., 2012a; Zhu et al., 2015). However, comprehensive influences of soluble materials derived from pretreatment on SSCF need to be evaluated yet.

Lignocellulose hydrolysates were generally co-fermented with the supplementation of inorganic salts and nitrogen sources (Karagöz et al., 2012; Erdei et al., 2013; Koppram et al., 2013), while several reports showed that various hydrolysates were able to be co-fermented well without nutrient supplementation (Van Eylen et al., 2011; Jin et al., 2012b; Zhu et al., 2014; Liu and Chen, 2016). This implies that there are some nutrients or accelerators for yeast in the lignocellulosic hydrolysates. It was speculated that amides produced from ammonolysis reaction during ammonia fiber expansion (AFEX) pretreatment might provide the nitrogen source to yeast (Chundawat et al., 2010). However, little work has been published on what enabling cell growth without nutrient supplementation in lignocellulosic hydrolysates.

In this study, we investigated the impacts of the wash liquors from pretreated biomass on SSCF. Wash liquors were separated from ethylenediamine (EDA) and dry dilute acid (DDA) pretreated corn stover (CS) to represent the soluble materials in alkaline and acidic pretreated biomass, respectively. The wash liquors were found playing different roles on enzymatic hydrolysis and fermentation. It was found that soluble lignins were important to facilitate xylose utilization in fermentation without nutrient supplementation.

2. Materials and methods

2.1. Materials

Corn stover (CS), harvested in suburb of Tianjin, China, was air-dried and milled. The particles between 20 and 80 mesh were collected for pretreatment. The moisture of the milled CS was 4–7%. Lignin (alkali), used as soluble lignin, was purchased from Sigma-Aldrich (Shanghai, CN). Cellulase Accellerase 1500 (89 mg protein/mL) and hemicellulase Multifect Xylanase (42 mg protein/mL) were gifted by Genencor (NY, US).

2.2. Pretreatment of corn stover and wash liquor preparation

Dry dilute acid (DDA) pretreatment reactor and process were described previously (He et al., 2014). Pretreatment conditions included: 50% (w/w) solid in 2.5% sulfuric acid, 175 °C, agitation speed 50 rpm and residence time 3 min. After pretreatment, no liquid fraction was left and the moisture content of the pretreated CS was 51%.

Ethylenediamine (EDA) pretreatment was carried out in a vacuum drying oven as described previously (Qin et al., 2015). EDA pretreatment conditions used for this study included: EDA to biomass loading 1.0 mL/g dry CS, temperature 150 °C, residence time 20 min and drying time 60 min. The moisture content of the pretreated CS is 2%.

After pretreatment, 95 and 48 mL distilled water was added to 20 g (wet weight) EDA and DDA pretreated solid, respectively (the slurries equivalent to 6% glucan loading). The wash liquors containing soluble materials were then squeezed from the slurries. The pH of wash liquors was adjusted to 5.5 by adding concentrated sulfuric acid or concentrated sodium hydroxide. The obtained wash liquors contained 6% glucan loading equivalent soluble materials.

The solids were further washed twice with 10 mL distilled water per g solid (dry weight). The final pretreated CS was dried at room temperature until the moisture was less than 10%, and then used for composition analysis, enzymatic hydrolysis and SSCF.

2.3. Analytical methods

The compositions of biomass were determined following the Laboratory Analytical Procedure of the National Renewable Energy Laboratory (Sluiter et al., 2008). Glucose and xylose concentrations were analyzed using HPLC with an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) at 65 °C, using 5 mM H_2SO_4 as eluent, at a flow rate of 0.6 mL/min. Soluble lignins concentrations were estimated by UV spectrophotometer at 210 nm. Compositions of raw CS and pretreated CS are shown in Table 1. Sugars in wash liquors and ethanol in fermentation broth were also determined by HPX-87H column. The compositions of wash liquors were shown in Table 2.

2.4. Enzymatic hydrolysis with different wash liquors additions

Enzymatic hydrolysis of pretreated CS using commercial enzymes was conducted at 6% (w/w) glucan loadings in a 100 mL flask with 20 mL total mixture at pH 4.8, 50 °C, and 200 rpm. Sodium azide (0.2%, w/w) was used to inhibit microbial contamination. Cellulase Accellerase 1500 and hemicellulase Multifect Xylanase were added with 20 and 10 mg protein/g glucan, respectively. 0, 40 and 80% (v/v) wash liquors were added. Control groups without pretreated CS were carried out to determine the sugar released from wash liquors.

2.5. Microorganism and seed culture preparation

Genetically engineered xylose-fermenting strain *Saccharomyces cerevisiae* SyBE005 was used in this study (Zha et al., 2013). Seed culture was prepared by inoculating a single colony from a YPX-Agar plate (per liter: 10 g yeast extract, 20 g peptone, 20 g xylose, and 20 g agar) into a tube containing 5 mL YPX medium (per liter: 10 g yeast extract, 20 g peptone and 20 g xylose) at 30 °C, 250 rpm for 24 h; and then 500 μL culture was inoculated into 100 mL YPX medium in a 250 mL flask and incubated for 18 ~ 24 h at 30 °C and 250 rpm (Zhu et al., 2014). The final OD_{600} was around 5 (equal to 2.5 g dry cell/L). The cells were harvested for fermentation inoculation by centrifugation at 4000 rpm for 5 min.

2.6. Fermentation with different wash liquors additions

Fermentation was conducted in a 100 mL flask with 20 mL total mixture at pH 5.5, 34 °C and 150 rpm. 0, 50 and 100% (v/v) wash liquors were added. 40 g/L glucose and 20 g/L xylose were added into the media. 10 g/L yeast extract and 20 g/L peptone were also added in specific trials. 50 mg/L Ampicillin was used to prevent bacterial contamination. Inoculum size was 2.5 g dry cell/L. The flasks were sealed by a rubber stopper with a syringe needle inside in order to release the carbon dioxide produced during fermenta-