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Co-fermentation of hemicellulose and starch from barley straw and grain for efficient pentoses utilization in acetone–butanol–ethanol production

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HIGHLIGHTS

- Two co-fermentations of hemicellulose and starch-based biomass were studied.
- Lower concentration of H_2SO_4 resulted in good ABE fermentability in process I.
- Process II facilitated pentoses utilization during the fermentation.
- Process II could relieve the effect of inhibitors on fermentation.
- Process II is attractive for ABE production from hemicellulosic biomass.

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ABSTRACT

This study aims to efficiently use hemicellulose-based biomass for ABE (acetone–butanol–ethanol) production by co-fermentation with starch-based biomass. Two processes were investigated: (I) co-fermentation of sugars derived from hemicellulose and starch in a mixture of barley straw and grain that was pretreated with dilute acid; (II) co-fermentation of straw hemicellulosic hydrolysate and gelatinized grain slurry in which the straw was pretreated with dilute acid. The two processes produced 11.3 and 13.5 g/L ABE that contains 7.4 and 7.8 g/L butanol, respectively. In process I, pretreatment with 1.0% H_2SO_4 resulted in better ABE fermentability than with 1.5% H_2SO_4, but only 19% of pentoses were consumed. In process II, 95% of pentoses were utilized even in the hemicellulosic hydrolysate pretreated with more severe condition (1.5% H_2SO_4). The results suggest that process II is more favorable for hemicellulosic biomass utilization, and it is also attractive for sustainable biofuel production due to great biomass availability.

1. Introduction

Biofuels offer the potential to mitigate the situation of global warming, environmental pollution and diminishing oil resources (Bessou et al., 2011). Inexpensive and efficient production methods from renewable resources are critical for biofuels production. The traditional biofuels production from starch based feedstocks that potentially compete with food supply is causing food versus fuel debate (Jang et al., 2012). Lignocellulosic feedstocks, such as agricultural and forest residues, industrial and municipal wastes, and dedicated energy crops hold tremendous potential for large-scale biofuel production (Banerjee et al., 2010). However, one of the major challenges for commercial production is that hemicellulose currently represents the largest polysaccharide fraction wasted in most cellulosic ethanol pilot and demonstration plants due to their heterogeneous polymeric nature and low fermentability by the most common industrial microbial strains (Gírio et al., 2012).

As an alternative liquid fuel, butanol can be produced by ABE fermentation using variety of substrates including monosaccharides (hexoses and pentoses) and polysaccharides (starch) (Madihah et al., 2001; Ezeji and Blaschek, 2008). Pentoses (derived from hemicelluloses, e.g. D-xylose and L-arabinose) that represent about 20–35% of lignocellulosic biomass have great potential as a butanol fermentation substrate (Sun and Liu, 2012). Dilute acid

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pretreatment is commonly applied to solubilize hemicellulose and increase the accessibility of the cellulose in lignocelluloses (Hendriks and Zeeman, 2009). However, during the pretreatment, certain chemicals, such as 5-hydroxymethylfurfural (HMF), furfural, weak organic acids and phenolic compounds that are produced have been shown to inhibit enzymatic hydrolysis and Clostridium fermentation (Cantarella et al., 2004; Ezeji et al., 2007a). ABE fermentation requires the availability of excess fermentable sugars for both the onset and maintenance of ABE production (Ezeji and Blaschek, 2008). Fermentation of hemicellulose-derived sugars directly needs the concentration process due to the low concentration of pentoses for butanol production. It will also increase the concentration of the inhibitory compounds. From an economical point of view, removal of inhibitors from hemicellulosic hydrolysates may not be cost effective.

One of hypotheses in this study is that co-fermentation of fermentable sugars derived from hemicelluloses and starch could increase sugar level in the fermentation media and achieve a higher ABE concentration. In a commercial biorefinery, for both economic and environmental reasons, mixed feedstocks from a variety of sources must be utilized. These are likely to include starch based materials such as corn, barley, sorghum, potato and sweet potato. The conversion of both starch and lignocellulose in these materials simultaneously to fermentable sugars could leave out the biomass separation step, and potentially reduce the production costs. According to previous studies, dilute sulfuric acid pretreatment is highly efficient in hydrolysis of hemicellulose and starch to its monomeric units, rendering the cellulose more available in the mixture of starch and lignocellulosic materials (Agbor et al., 2011; Yang et al., 2013).

Another hypothesis is that co-fermentation of hemicellulosic hydrolysate and gelatinized starch slurry could increase the sugar level for fermentation by adjusting the starch content, and also dilute the concentration of the inhibitors in hemicellulosic hydrolysate. Thus, it could relieve the inhibitory effects on fermentation of hemicellulosic hydrolysates and increase the ABE production. As described in previous studies, mixing wheat grain hydrolysate with wheat straw hydrolysate would be beneficial for both first generation and second generation ethanol production, and biohydrogen production in a biorefinery (Erdei et al., 2010; Panagiotopoulos et al., 2013).

Forage barley, a starch and lignocellulose-based feedstock, has been regarded as a good supplement to corn biofuel production before the commercialization of lignocellulosic biofuel (Nghiem et al., 2010; Kim et al., 2011). It has a very short growing season in regions with mild winters, allowing harvest early enough for double cropping with soybeans without conflicts with fuel-versus-food issues (Nghiem et al., 2010). This study used forage barley straw and grain as substrates, and aims to evaluate two processes: (I) co-fermentation of sugars derived from hemicellulose and starch in barley straw and grain; (II) co-fermentation of straw hemicellulosic hydrolysate and gelatinized grain slurry. The effects on pentoses utilization, ABE concentration and yields of varying the proportions of hemicellulose and starch based sugars were investigated.

2. Methods

2.1. Raw materials

The barley grain and straw were collected separately in 2011 from the field in North Karelia, Finland. Barley straw and grain were air-dried at 60 °C for seven days and milled using a cutting mill to pieces of 0.25 mm with a moisture content of 8%. The mixture of barley straw and grain contained 40% of milled straw and 60% of milled grain in dry weight. Barley grain contained 55.2% (w/w) starch, 6.2% glucan, 11.6% xylan and 3.1% arabinan. The straw had 38.1% (w/w) glucan, 26.9% xylan and 2.6% arabinan (Yang et al., 2013).

2.2. Substrates processing

In control batch fermentation, milled grain (6% dry matter loading, w/v) was used as substrate, which was heat treated with water at 121 °C, 1.1 bar for 60 min for starch gelatinization. In process I, a mixture of grain and straw (10% dry matter loading, w/v) that contains 6% grain and 4% straw was heat treated with dilute acid (1.0% and 1.5% sulfuric acid, w/v) at 121 °C, 1.1 bar for 60 min in a 250 mL triangle glass flask. After pretreatment, the slurry was cooled to room temperature, and the pH of pretreated slurry was adjusted to 5–6 with solid NaOH. The slurry was then pressed through filter paper to separate the pretreated hydrolysates from solid residual fractions. The pretreated hydrolysates were used for ABE fermentation. In further experiments, the mixtures with two different proportions of grain and straw were tested for hydrolysis with 1.0% sulfuric acid and fermentation. The two proportions of grain and straw were (1) 4% grain and 6% straw, and (2) 2% grain and 8% straw. The pretreated hydrolysates from the two mixtures were supplemented with 10 and 15 g/L respectively to keep a suitable sugar concentration for ABE fermentation.

In process II, the straw (7% dry matter loading, w/v) was heat treated with dilute acid (1.0% and 1.5% sulfuric acid, w/v) at 121 °C, 1.1 bar for 60 min in a 250 mL triangle glass flask. After pretreatment, the slurry was cooled to room temperature, and the pH of the pretreated slurry was adjusted to 5–6 with solid NaOH. The slurry was then pressed through filter paper to separate pretreated hydrolysates from solid residual fractions. The grain was gelatinized at 121 °C for 60 min, and mixed with straw pretreated hydrolysates in a ratio of 1:1 (v/v). The mixtures contained 6% (w/v) grain were used for ABE fermentation. In further experiments, two gelatinized grain slurry with lower amount grain were mixed with straw hemicellulosic hydrolysates (treated with 1.5% sulfuric acid). The final grain content was 4% and 2% (w/v), respectively. For keeping a suitable fermentation sugar level, 10 and 20 g/L xylose was supplemented into the two mixtures, respectively. The fermentations in process I and II were all compared with the fermentation of straw hemicellulosic hydrolysate (treated with 1.0 sulfuric acid) supplemented with 30 g/L xylose.

2.3. Microorganism and culture conditions

Freeze-stored bacteria *Clostridium acetobutylicum* DSM 1731 (DSMZ, Braunschweig, Germany) were activated in RCM media (Hirsch and Grinsted, 1954) for 14–16 h. Then 1 mL of active growing cells was inoculated into 50 mL of sterilized pre-fermentation P2 media prepared in a 125 mL screw-capped bottle. The pre-fermentation P2 media contained glucose 30 g/L and yeast extract 1 g/L. Before inoculation, each of the filter-sterilized stock solutions (buffer: KH_2PO_4, 50 g/L; K_2HPO_4, 50 g/L; ammonium acetate, 220 g/L; mineral: $MgSO_4{\cdot}7H_2O$, 20 g/L; $MnSO_4{\cdot}H_2O$, 1 g/L; $FeSO_4{\cdot}7H_2O$, 1 g/L; NaCl, 1 g/L; and vitamin: para-aminobenzoic acid, 0.1 g/L; thiamin, 0.1 g/L; biotin, 0.001 g/L) was added into the P2 media. The culture was allowed to grow for approximately 16 h at 37 °C before inoculation into the ABE production media. All experiments were conducted in duplicate.

2.4. ABE fermentation

The hydrolysates derived from the two processes were used as ABE production media. The fermentation of pure sugar media was also conducted for studying the xylose utilization by *C. acetobutylicum* DSM 1731. The pure sugar media contained xylose or