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A biorefining process: Sequential, combinational lignocellulose pretreatment procedure for improving biobutanol production from sugarcane bagasse

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HIGHLIGHTS

- A biorefining process: SCLPP was designed to pretreat lignocellulose.
- Improving biobutanol production from sugarcane bagasse.
- Enzymatic hydrolysis with simultaneous saccharification fermentation was effective.
- Developing SCLPP that increase biofuel from lignocellulose is feasible.

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ABSTRACT

Here, for the first time, we designed a sequential, combinatorial lignocellulose pretreatment procedure (SCLPP) for microbial biofuel fermentation to reduce generation of microbial growth inhibitors and furthermore increase sugar yields. We tested this pretreatment process using sugarcane bagasse as substrate and assessed the effectiveness by analysis of biobutanol production through microbial *clostridium beijerinckii* NCIMB 8052 conversion. Our results showed that there were no inhibitory effects when using the hydrolysates as fermentation substrate. Under the SSF scheme, we observed the highest concentrations of butanol (6.4 g/L) and total ABE (11.9 g/L), resulting in a higher ABE productivity, compared with the SHF method. These findings suggest that the SCLPP is a feasible method for improving ABE production, lowering microbial inhibitor generation, and ensuring success in the subsequent fermentation process. Therefore, our work demonstrated developing a tractable integrated process that facilitates to increase biofuel production from agricultural residues rich in lignocellulose is feasible.

1. Background

Several well-established pretreatment methods are available to convert cellulosic and hemicellulosic materials into sugars via hydrolysis. Microbial fermentation processes can convert these sugars into biofuels (Brown and Brown, 2013; Kumar et al., 2009; Yang and Wyman, 2008). Recently, advances in industrial biotechnology have provided additional opportunities for economical utilization of agro-industrial residues, such as sugarcane bagasse, a complex material and a major by-product of the sugarcane industry. Sugarcane is one of the most prevalent forms of agricultural waste and is very abundant each year in southern China. The composition of sugarcane bagasse is approximately 50% cellulose, 25% hemicellulose and 25% lignin. Therefore, using sugarcane bagasse to produce biobutanol is a promising approach for biofuel production. Butanol has a higher energy value and lower hygroscopicity than ethanol. However, the feasibility of producing biobutanol from sugarcane bagasse also depends on other factors such as pretreatment and detoxification (Jönsson et al., 2013). These processes are very important because conventional pretreatment approaches like acid hydrolysis often generate toxic

Abbreviations: SCLPP, sequential, combinational lignocellulose pretreatment procedure; LHW, liquid hot water pretreatment; HMF, hydroxymethylfurfural; HAN, HTR and HPJ, indicate the total hydrolysates were used as fermentation substrates of SHF; HANNY, HTRNY and HPJNY, indicate the total hydrolysates were used as fermentation substrates of SSF; AN ATCC 1015, *A. niger* ATCC 1015; TR ATCC 26921, *T. reesei* ATCC 26921; PJ ATCC 44750, *P. janthinellum* ATCC 44750; AR2, solid residue AN1 + solid residue AN2; TR2, solid residue TR1 + solid residue TR2; PR2, solid residue PJ1 + solid residue PJ2; AAR3, solid residue AAN; ATR3, solid residue ATR; APR3, solid residue APJ; EAR4, solid residue EAN; ETR4, solid residue ETR; EPR4, solid residue EPJ.

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substances (e.g., furfural and 5-hydroxymethylfurfural) that greatly inhibit the growth of bacteria.

Diverse approaches are available to pretreat crop residues rich in cellulose and hemicellulose including steam explosion (Li et al., 2013b) and pretreatments with dilute acids such as phosphoric acid (de Vasconcelos et al., 2013) and sulfuric acid. Acid hydrolysis, especially when using dilute sulfuric acid, is a common and effective pretreatment method because it is simple and inexpensive (Jung et al., 2013). Generally, the hemicellulose fraction of sugarcane bagasse can be hydrolyzed to monomeric sugars by dilute sulfuric acid. However, the microbial inhibitors produced during acid hydrolysis will inhibit microbial growth during fermentation, thereby suppress the fermentation efficiency (Bamufleh et al., 2013). For example, such inhibitory compounds had been found to significantly suppress cell growth and biobutanol production in *Clostridium beijerinckii* (Guo et al., 2013; Zhang and Ezeji, 2013). Furfural, hydroxymethyl furfural and phenolic compounds are the major inhibitors of biofuel fermentation, (Hayashi et al., 2003; Shimizu et al., 2005). Due to their toxicities, it is essential to remove inhibitory compounds from hydrolysates prior to biobutanol fermentation.

Unfortunately, detoxifying pretreated substrates is a complicated process. Although several detoxification approaches have been investigated previously, including lime treatment, evaporation, adsorption using ionic-exchange column chromatography or activated charcoal, and biological treatment (Jennings and Schell, 2011; Shen et al., 2013), most of which are either ineffective at toxin removal or cost prohibitive for industrial application due to high cost for waste disposal. At present, chemically degrading inhibitors is the most common detoxification technique because of its simplicity and low cost. However, the efficiency of toxin removal by chemical methods is dependent on the chemical structure similarity of the inhibitors, resulting in incomplete removal of toxic compounds. In addition, chemical detoxification leads to high salt ion concentrations in the fermentation liquids which can significantly inhibit microbial growth (Chen et al., 2008; Kim et al., 2009a) and result in a reduced biobutanol production.

Therefore, to reduce or avoid production of inhibitor compounds in the pretreatment stage, it is crucial to select appropriate pretreatment methods. To be effective, a pretreatment process must break down fiber with a high efficiency, produce a sufficient quantity of sugar, prevent the dissipation of sugar out of the desired fraction (i.e. pentosan fraction), and limit the extent to which the pretreated material inhibits microbial growth during fermentation. Some pretreatment methods can avoid or reduce the production of inhibitors such as microwave pretreatment (Tyagi and Lo, 2013), enzymatic hydrolysis (Ju et al., 2013), and liquid hot water pretreatment (Li et al., 2013a). In particular, biological pretreatment using microbiological degradation has shown excellent success in removing inhibitors from laccase-treated hydrolysates, enabling the substrates to be used for fermentation without generating inhibitory phenolic compounds (Krastanov et al., 2013). Finally, to be economically feasible, the pretreatment process should also aim to minimize energy demands and costs associated with construction materials, treatment of process residues, and reduction in feedstock size.

However, if only a single pretreatment method is used to help limit the production of inhibitors, the substrate will not be fully decomposed and the sugar production will not be very high. Therefore, to limit the generation of toxic substances as far as possible and completely break down substrate materials simultaneously, we believe combining various pretreatment methods is the optimal approach, instead of using just one or two. However, there are no reports integrating multiple (i.e., three or more) approaches to pretreat feedstock rich in lignocellulose.

In this study, using sugarcane bagasse as example feedstock, we designed a SCLPP involving a series of methods including microwave decomposition, enzyme hydrolysis, ammonia immersion, microbial decomposition, and liquid hot water pretreatment to limit the production of microbial inhibitors and obtain high sugar yields. Finally, we assessed the effectiveness of resulting hydrolysates to drive butanol production using two methods: separate hydrolysis and fermentation (SHF) and simultaneous saccharification fermentation (SSF).

2. Methods

2.1. Sugarcane bagasse

Sugarcane bagasse (stumps) for the pretreatment experiments was collected from a farmer in Yulin (Guangxi province, China). The sugarcane juice was removed with a squeezer (ET-ZZJ83, Guangzhao, Guangdong) and the remaining sugarcane bagasse was dried at 65 ± 2 °C for 2 days.

2.2. Experimental design and pretreatment procedure of the SCLPP

Experiments were conducted following the methodology illustrated in the pretreatment and fermentation process flow sheet (Fig. 1). The sugarcane bagasse was pretreated with five different methods of decomposition. The composition changes in and out processes were also presented in Fig. 1. Then the hydrolysis products were fermented by the *C. beijerinckii* strain NCIMB 8052. In addition, pure glucose and mixture sugars were also used to ferment.

2.2.1. Step 1: milling raw bagasse to powder

The original dried sugarcane bagasse with lengths of 5–10 cm was partially broken down using a pulverizer (AB03, Weifang city, China). The resulting material was screened to obtain particles with a maximum size of 830 μm (size 20 mesh) prior to pretreatment.

2.2.2. Step 2: liquid hot water pretreatment (LHW)

A 500 g sample of sugarcane bagasse powder was pretreated with liquid hot water pretreatment (LHW) as follows. The sugarcane bagasse was positioned at the bottom of a reactor (Autoclaves Sterilizer, SANYO*MLS-3750, Japan) and was completely immersed in 2 L of water at room temperature for 12 h to fully saturate the bagasse and facilitate the decomposition. Then, the feedstock was preheated using steam for 45 s before adding liquid water. For the rest of the procedure, the reaction temperature was maintained at 200 ± 3 °C for 1 h. After the pretreatment, the resulting hydrolysates were filtered and divided equally into six portions (Hydrolysate L1 through L6) for the fermentation experiments. The residual solids were dried at 65 °C and the solid loads were determined. Then the solids loads were used in the next pretreatment step.

2.2.3. Step 3: microwave pretreatment (MP)

The humidity of the remnants' solid fraction environment was adjusted to 40% for microwave pyrolysis. Prior to pretreatment, the sugarcane bagasse remnants were positioned into a three-necked, round-bottomed flask reactor. Nitrogen was infused to exclude all oxygen in the reaction flask, which was then kept overnight in a 4 °C refrigerator. Next day, the flask was placed in a microwave pyrolysis furnace (MCR-3, Gongyi Instrument Co. Ltd., Henan, China) at 120 °C for 7 min to allow decomposition. Then heating was stopped; the hot gas was allowed to completely escape the flask; the furnace was cooled to room temperature;