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Ethanol production from bamboo using mild alkaline pre-extraction followed by alkaline hydrogen peroxide pretreatment

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ABSTRACT

A sequential two-stage pretreatment process comprising alkaline pre-extraction and alkaline hydrogen peroxide pretreatment (AHP) was investigated to convert bamboo carbohydrates into bioethanol. The results showed that mild alkaline pre-extraction using 8% (w/w) sodium hydroxide (NaOH) at 100 °C for 180 min followed by AHP pretreatment with 4% (w/w) hydrogen peroxide (H_2O_2) was sufficient to generate a substrate that could be efficiently digested with low enzyme loadings. Moreover, alkali pre-extraction enabled the use of lower H_2O_2 charges in AHP treatment. Two-stage pretreatment followed by enzymatic hydrolysis with only 9 FPU/g cellulose led to the recovery of 87% of the original sugars in the raw feedstock. The use of the pentose-hexose fermenting *Saccharomyces cerevisiae* SR8u strain enabled the utilization of 95.7% sugars in the hydrolysate to reach 4.6% w/v ethanol titer. The overall process also enabled the recovery of 62.9% lignin and 93.8% silica at high levels of purity.

1. Introduction

Due to diminishing fossil resources, sustainable and clean renewable energy and bio-based chemicals from lignocellulosic feedstocks are considered as promising alternatives to petroleum-based products (Qing et al., 2017; Tuck et al., 2012). Bamboo is a promising species for use as a feedstock in a biorefinery for the production of pulp, bioethanol, and other chemicals due to several reasons. For example, it not only grows fast but also has a chemical composition similar to that of woody species. Additionally, it can grow on marginal land (Littlewood et al., 2013; Yuan et al., 2017a,b). However, bamboo contains a much higher level of silica compared to wood (Ding et al., 2008; Torelli and Čufar, 1995). One of the poorly explored problems is that silica interferes with the enzymatic hydrolysis of carbohydrates due to its interaction with cellulase during the production of ethanol (Le et al., 2015; Talukder et al., 2017; Veen et al., 2007). In addition, in the case of lignocellulosic ethanol, the lignin content of biomass is also a critical parameter which greatly affects the efficiency of enzymatic hydrolysis (Chandra et al., 2015; Sun et al., 2011; Talebnia et al., 2010). In this context, pre-extraction of silica and lignin during the pretreatment stage is of great importance for the production of bioethanol from bamboo.

Alkaline hydrogen peroxide (AHP) treatment, traditionally employed in the papermaking industry as a bleaching method, is a chemical pretreatment technology that has been used in the production of bioethanol from lignocellulosic feedstocks such as wood and grasses (Alvarez-Vasco and Zhang, 2017; Banerjee et al., 2012; Song et al., 2016). It has been shown that AHP pretreatment could significantly improve the digestibility of biomass because it not only selectively removes lignin but also deconstructs the cell walls (Banerjee et al., 2011; Bhalla et al., 2016; Liu et al., 2014; Wyman, 2013). In addition, AHP pretreatment is normally carried out at pH 11.5–12.6 using sodium hydroxide (NaOH) (Song et al., 2016). Our previous work demonstrated that NaOH can remove silica from bamboo by converting insoluble silica particles into soluble sodium silicates, thus alleviating silica associated challenges during bamboo processing. However, in a single-step AHP pretreatment of biomass a relatively high charge of hydrogen peroxide (H_2O_2) (about 10% on biomass) is generally required (Alvarez-Vasco and Zhang, 2017). This is probably due to the consumption of H_2O_2 by alkali-solubilized aromatics and the decomposition of H_2O_2 by transition metals (iron, copper, manganese) in the biomass (Alvarez-Vasco and Zhang, 2017; Hage and Lienke, 2006; Lin and Gurol, 1998; Liu et al., 2014). The high H_2O_2 consumption increases the processing cost, which is not favorable for the production of bioethanol from bamboo or other lignocellulosic feedstocks (Li et al., 2013). In this context, a pre-condition stage that could reduce hydrogen peroxide charge and improve delignification during AHP treatment while maintaining the overall sugar yields from enzymatic hydrolysis

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should be identified.

Our previous studies showed that mild alkaline (≤100 °C) pre-extraction of bamboo chips with sodium hydroxide (NaOH) selectively removed all silica, a portion of ash (silica not included), and some hemicellulose while maintaining cellulose content (Yuan et al., 2016, 2017a,b). In addition, the dissolved silica and hemicelluloses in the pre-extraction liquor could also be recovered as by-products for various applications (Yuan et al., 2017a,b). For example, the recovered silica particles could be used as a sustainable feedstock for treating waste water and producing pharmaceuticals, catalysts, and composite fillers (Mor et al., 2017; Morpurgo et al., 2010; Zhang et al., 2013). Recovered hemicelluloses could be used for the production of bio-polymeric films and ethanol (Yuan et al., 2017a,b). Moreover, alkaline pre-extraction also increases the accessibility of the treated substrate to chemicals or enzymes used in subsequent process steps thereby potentially reducing chemical or enzyme charge (Huang et al., 2010). Thus, the implementation of NaOH pre-extraction prior to AHP treatment is a viable option to reduce the charges of H_2O_2 and NaOH during the production of bioethanol from bamboo chips.

The literature on two-stage pretreatment methods to improve overall sugar recovery from lignocellulosics and reduce inhibitory compounds generation is substantial (Liu et al., 2014; Qing et al., 2017; Yuan and Wen, 2017). However, the application of alkaline pre-extraction followed by AHP delignification to improve enzymatic saccharification of bamboo biomass has not yet been reported. In the present work, a mild alkaline pre-extraction was conducted to partially remove hemicelluloses and silica, and swell cellulosic fibres. Thereafter, AHP treatment was conducted on alkali-pretreated bamboo chips to solubilize lignin, further extract silica, and provide a cellulosic substrate that could be readily hydrolyzed using low enzyme loadings. The obtained sugar hydrolysate was fermented by a metabolically engineered *Saccharomyces cerevisiae* strain to produce ethanol. The objectives of this study were to a) reduce the H_2O_2 charge of AHP treatment, b) pre-extract silica and lignin to generate a substrate that can be easily hydrolyzed using cellulolytic enzymes, and c) generate multiple products from bamboo. Our results demonstrated the technical feasibility of using a two-stage pretreatment to accomplish these objectives.

2. Materials and methods

2.1. Raw materials and chemicals

Commercial bamboo chips prepared from three to seven years old trees were provided by Lee & Man Paper Manufacturing Ltd. China. The commercial chips were rechipped to 3–5 mm in width and 15–25 mm in length and washed with deionized water at a liquid-to-wood ratio of 20 L/kg using a laboratory mixer to remove impurities such as soil and sand. The washed chips were air dried for approximately 24 h and stored at 4 °C until used for subsequent experiments. The moisture content of the bamboo chips was about 22%. Compositional analysis of the raw bamboo chips showed that it contained 48.4% cellulose, 21.8% hemicelluloses (20.3% xylan, 0.7% galactan, and 0.8% arabinan. There was no detectable amount of mannan), 25.1% lignin (sum of acid-soluble and insoluble lignin), 0.99% ash, and 1.12% silica on dry weight basis. Hereafter, the ash content, unless specified in the text, represents the non-silica ash. All chemicals were reagent grade and purchased from Sigma-Aldrich (Beijing, China). All experiments were performed at least in triplicate.

2.2. Alkaline pre-extraction and AHP pretreatment

NaOH pre-extraction of bamboo chips was carried out at 100 °C using a rotating reactor system (Greenwood Instruments, USA). Bamboo chips were treated with 4–10% NaOH charge (based on oven dried mass of bamboo chips) for 30–180 min. Reactors were continuously rotated at 50 rpm during the reaction and the liquid-to-wood ratio was kept constant at 10 L/kg. For each alkaline pre-extraction run, 100 g (oven dried: o.d.) bamboo chips and the calculated volume of deionized water and NaOH solution (stock concentration of 100 g/L) were mixed and placed in a digester. The temperature ramp-up time was fixed at 20 min. After alkaline pre-extraction, the pretreated bamboo chips were collected through filtration and washed with excess deionized water (about 4 L) to remove all soluble materials. In the laboratory experiments, the substrate was extensively washed to carefully measure the composition of alkali pre-extracted solid substrate and track the changes that occur during the subsequent AHP treatment. The recovered insoluble solids were stored at 4 °C until used for subsequent experiments.

Before subjecting the alkali-extracted bamboo chips to the alkaline hydrogen peroxide (AHP) treatment, the moisture content of the wet sample was determined. AHP pretreatment runs were carried out in 250-mL Erlenmeyer flasks in a water bath with orbital shaking at 150 rpm. During AHP pretreatment of alkali-extracted chips, the loading of H_2O_2 on pretreated bamboo chips (o.d.) ranged from 0% to 6% (grams of peroxide per gram of o.d. bamboo biomass). AHP treatment was conducted at 75 °C for 180 min and the liquid-to-wood ratio was kept constant at 10 L/kg. Time zero was taken to be the time at which the reactor reached the target temperature. The pH of the reaction was periodically adjusted to pH 11.5 using 6 mol/L NaOH. The volume of NaOH (6 mol/L) used to adjust the pH to 11.5 ranged from 0.5 to 1.5 mL (depending on H_2O_2 charge). Upon completion of a run, the solid fraction (water insoluble solids) and the liquid fraction (water soluble materials) were separated and collected by filtration. The solid fraction was thoroughly washed with deionized water and stored at 4 °C until used for further experiments and analyses. Fig. 1 illustrates the experimental process flow. The processed liquors obtained from the two pretreatment stages were mixed and subjected to silica and lignin recovery. Then, silica and lignin free liquor was concentrated by vacuum evaporation and added back to the treated solids for enzymatic hydrolysis. All experiments were carried out at least in triplicate.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis was performed using the cellulolytic enzyme mixture Cellic CTec2 (Novozymes investments Co. Ltd, Bagsværd, Denmark) which had an activity of 113 FPU/mL and a protein content of 137.6 mg/mL (determined by the bicinchoninic acid assay) and a commercial β-glucosidase preparation (Novozym 188, Novozymes A/S Bagsværd, Denmark) of 160 CBU/mL activity and 120 mg/mL protein content. In the enzyme mixture, the protein mass ratio of Cellic CTec2 to β-glucosidase was 5:1. A series of enzyme loadings ranging from 5.5 to 18 FPU/g cellulose were tested. Enzymatic hydrolysis was carried out in a shaking incubator (HNY-111C, Zhengzhou, China) at 5% solids consistency, 150 rpm, and 50 °C for up to 96 h with 100 mM sodium citrate buffer at pH 5. Sodium azide (2.5 mg/L) was added to prevent microbial growth. Approximately 2 mL of the liquid hydrolysate were sampled periodically for the analysis of sugar concentration using a HPLC (high performance liquid chromatography) system following NREL methods (Sluiter et al., 2012).

After enzymatic hydrolysis, the hydrolysate was centrifuged at 12000 rpm for 40 min to separate the liquid hydrolysate from the residual solids. The obtained liquid bamboo hydrolysate was concentrated to a sugar concentration of 100 g/L with a rotary evaporator and stored at −20 °C for ethanol fermentation. All experiments were carried out at least in triplicate.

2.4. Fermentation

Saccharomyces cerevisiae SR8u strain engineered for xylose fermentation (in addition to C6-fermentation) was used in this study (Zhang et al., 2017). The microorganism was cultured in medium containing glucose (20 g/L), yeast extract (10 g/L), and peptone (20 g/L) at 30 °C