



Evaluation of an integrated process to fully utilize bamboo biomass during the production of bioethanol



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HIGHLIGHTS

- Sequential autohydrolysis-alkaline extraction efficiently fractionated bamboo.
- Autohydrolysis improved the accessibility of silica and lignin to NaOH.
- Silica, lignin and sugars were recovered from the two pre-extraction liquors.
- Both recovered sugars and two-stage treated solids were used to produce ethanol.

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ABSTRACT

Aiming for the complete utilization of bamboo biomass, an integrated process which combines ethanol production with the recovery of silica and lignin was proposed. To reduce chemical charge required for the fractionation of silica and lignin from bamboo and improve the digestibility of the obtained substrate, a sequentially two-stage pretreatment process of autohydrolysis and alkaline extraction was carried out. From the view of enhancing enzymatic hydrolysis and recovery of silica and lignin, a two-stage treatment of autohydrolysis at 180 °C for 90 min followed by alkaline extraction at 100 °C with 6% NaOH (based on pretreated chips) for 120 min was optimized. About 93.7% of original silica and 75.7% of original lignin could be recovered from bamboo. Enzymatic hydrolysis and fermentation of carbohydrates showed that an overall sugar yield of 88.6% of original sugar content and an ethanol recovery of 0.467 g/g sugar were achieved based on the proposed scheme.

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1. Introduction

Lignocellulosic biomass, a renewable resource, is regarded as one of the most attractive raw materials for numerous applications such as for the production of bioethanol and biochemicals (Hamelinck et al., 2005; Naik et al., 2010). Among the lignocellulosic feedstocks, bamboo, a carbohydrate-rich natural lignocellulosic material, is considered to be a promising raw material due to its high abundance, quickly growing (3–5 years) and similar chemical composition to wood (Gratani et al., 2008; Scurlock et al., 2000). Moreover, bamboo requires relative low chemicals and nutrients during growing and can be cultivated on marginal land for bio-based products (García-Aparicio et al., 2011; Littlewood et al., 2013). However, bamboo contains a much higher level of silica content compared to wood. The high silica level

creates various challenges in the biorefinery processes (Talukder et al., 2017; Veen et al., 2007). For example, in the production of bioethanol from bamboo, the silica stays in the pretreated solids interfering the enzymatic hydrolysis by the interaction with cellulase (Talukder et al., 2017). Thus, to improve the efficiency of bioethanol production from bamboo, it is necessary to eliminate the silica associated challenges. The ideal approach to resolve the silica problems could be that completely separate silica from bamboo prior to enzymatic hydrolysis.

In our earlier studies (Yuan et al., 2016, 2017), it was observed that most of silica in bamboo could be extracted by low temperature alkaline pre-extraction with sodium hydroxide (NaOH) and recovered as high purity amorphous silica particles by lowering liquor pH with carbon dioxide (CO₂). The recovered silica can also be used as the sustainable feedstock for the production of nanosilica particles, catalysts, composite fillers and pharmaceuticals (Morpurgo et al., 2010; Zhang et al., 2013). However, relatively high NaOH charge and long reaction time were normally required for the one-stage pre-extraction of silica from original bamboo

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chips due to the consumption of alkali by extractives, acetyl and uronic acid groups and the limited accessibility of silica to NaOH. Additionally, low temperature alkaline pretreatment of original bamboo chips did not show significant effect on the removal of lignin (Yuan et al., 2016), which is also a critical parameter during bioethanol production from lignocellulosic materials (Chandra et al., 2015; Klein-Marcuschamer et al., 2012; Sun and Cheng, 2002). Within this context, a pre-condition stage that could decrease chemical charge (alkali), reduce reaction time and increase lignin removal during alkaline extraction of silica is of great importance in processing bamboo for the production of ethanol.

Autohydrolysis, typically performed by treating biomass at 140–180 °C with either water or steam, is regarded to be an essential and environmental friendly unit operation and has been commercially applied in the production of dissolving pulp and biofuels from lignocellulosic biomasses. During autohydrolysis, a portion of the hemicelluloses is hydrolyzed and removed from biomasses (Trajano and Wyman, 2013). The breaking of glycosidic linkages between monomeric sugars, the major bonds found in hemicelluloses, is generally utilized as the basis hydrolysis mechanism. Moreover, reactions of deacetylation, ash neutralization and lignin removal also take place during autohydrolysis of bamboo (Kapu et al., 2016). Therefore, autohydrolysis has the potential to remove alkaline consumption components (extractives, acetyl groups) and increase the accessibility of silica and lignin to alkali, thereby reducing the alkali charge during alkaline extraction of autohydrolyzed bamboo chips.

In this study, a two-step pretreatment of bamboo was explored from the view of improving the extraction of silica and lignin as well as enhancing the hydrolysis sugar yield. Moreover, dissolved silica and lignin in the pretreatment liquor were recovered as sustainable sources for various bio-based products (Pye, 2008; Zhang et al., 2013). After separating silica and lignin, dissolved hemicellulosic and cellulosic sugars in liquors were also recovered by nanofiltration and added back into the two-stage pretreated insoluble solids for enzymatic hydrolysis to achieve a good overall sugar yield. The obtained sugar hydrolysate was used for the production of ethanol by a metabolically engineered *Saccharomyces cerevisiae* strain.

2. Materials and methods

2.1. Organization of experimental work

Fig. 1 shows the proposed scheme to produce ethanol and recovery of high purity silica and lignin from bamboo. Commercial bamboo chips were initially pretreated with hot water (autohydrolysis) to remove hemicelluloses and increase the accessibility of lignin and silica to chemicals. The pretreated bamboo chips were subsequently subjected to alkaline extraction for the removal of silica and lignin. The dissolved materials, including silica, lignin and carbohydrates (hemicellulose and cellulose) in the two pre-extraction liquors (autohydrolysis liquor and alkaline extraction liquor), were recovered from the process. Silica and lignin were stored separately while the recovered carbohydrates was added back into the two-stage treated cellulosic substrate for enzymatic hydrolysis and ethanol production.

2.2. Materials

Bamboo chips prepared from 3 to 7 year old trees were provided by the Lee & Man Paper Manufacturing Ltd. China. The obtained commercial bamboo chips with a chip size of 1–2 cm in width and 3–4 cm in length were washed with deionized water

for 10 min at a liquid-to-wood ratio of 20 L/kg using a laboratory mixer. The washed chips were air dried for approximately 24 h and collected as the raw material from pretreatment. The moisture content of the chips was about 22%. Compositional analysis of the raw bamboo chips shows that it contains 48.4% cellulose, 21.8% hemicelluloses (20.3% xylan, 0.7% galactan, 0.8% arabinan, mannan undetected), 25.1% lignin (sum of acid-soluble and insoluble lignin), and 1.12% silica on a dry basis. All chemicals were reagent grade and purchased from Fisher Scientific, Canada. All experiments were performed at least in triplicate.

2.3. Autohydrolysis of bamboo chips

A number of autohydrolysis runs were conducted at two temperatures (160 and 180 °C) for 15–150 min. For an autohydrolysis run, 100 g oven dried (o.d.) bamboo chips and 500 mL deionized water were mixed and placed in a 1 L stainless steel reactor. The temperature ramp-up time was kept constant at 25 min. Then, the reactor was heated at the target temperature and routinely rotated at 50 rpm throughout the reaction process. Upon completion of a run, the reactor was rapidly cooled in an ice-water bath and auto-hydrolyzed chips were recovered through filtration. Chips were washed with excess deionized water to remove all soluble substances and stored at –20 °C for further analysis and utilization. The autohydrolysis liquor (AL) was collected and stored at –20 °C before further utilization.

2.4. Alkaline extraction of autohydrolyzed bamboo chips

The pretreated solid fractions obtained from autohydrolysis was then subjected to alkaline treatment using 2–6% NaOH charge (based on o.d. mass of treated bamboo chips) at 100 °C for 30–180 min in the same reactor used for autohydrolysis. The liquid to wood ratio was fixed at 4 L/kg. For an alkaline extraction run, 60 g o.d. treated bamboo biomass and the calculated volume of deionized water and NaOH solution (stock concentration of 50 g/L) were mixed and placed in a reactor. After alkaline post-treatment, the resulting slurry was collected and the water-soluble fraction was separated from the solid fraction with filtration. The solid fraction was thoroughly washed with deionized water and stored at –20 °C for analysis and enzymatic hydrolysis. The alkaline extraction liquor (AEL) was collected and stored at –20 °C until further used.

2.5. Separation of silica, lignin and sugars from the liquor

The silica dissolved in the AEL was separated by reducing the pH of the liquor to pH 8 by bubbling carbon dioxide (CO₂) at 60 °C (Yuan et al., 2017). After the silica separation, the retained AEL was mixed with autohydrolysis liquor (AL) in a conical flask with a laboratory mixer at 150 rpm for 30 min. The dissolved lignin in the liquor mixture was isolated by reducing the pH of the liquor to pH 2.5 with 72% sulfuric acid (H₂SO₄). The lignin precipitate was collected through filtration. After the separation of silica and lignin, the residual liquor was concentrated and filtered using a cellulose-acetate-based nanofiltration membrane (GE Osmosis) with a high pressure stirred cell (Sterlitech HP4750, USA) at pressure of 500 Psi for 150 min to recover dissolved carbohydrates.

The silica and lignin precipitates were air-dried overnight and vacuum dried at 45 °C for 48 h to obtain constant weight for further analysis and experimentation. The recovered carbohydrates from the liquor was subjected to an overliming detoxification procedure to remove any inhibitory compounds affecting enzymatic hydrolysis and ethanol fermentation following Martinez et al. (2000). Briefly, Ca(OH)₂ solution was added to the concentrated carbohydrates liquor until pH 10. Then, the liquor was agitated

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