ORIGINAL ARTICLE

Coproduction of xylose and biobutanol from corn stover via recycling of sulfuric acid pretreatment solution

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Abstract

Sulfuric acid was used in the pretreatment of corn stover to obtain xylose as a value-added by-product, and the pretreated corn stover (Pre-CS) was hydrolyzed to produce glucose for butanol fermentation. The aim of this work is to achieve high xylose accumulation and reduced wastewater by recycling the pretreatment solution. The pretreatment conditions were optimized as follows: 180 °C, 15 min, 1:7 solid–liquid ratio (w/w), 0.6% H_2SO_4 (w/w, first batch)/0.9% H_2SO_4 (w/w, second and third batches), in which pretreatment solution was recycled for three times. Under above conditions, pretreatment solution containing 56.3 g/L xylose and 4.5 g/L glucose was obtained. Pre-CS residue was further hydrolyzed by cellulase to achieve 35.7–39.9 g/L glucose. The condensed corn stover hydrolysate was subjected to simultaneous detoxification and sterilization using 1% (w/w) activated carbon and then applied in butanol fermentation. The highest butanol titer of 9.5 g/L was obtained in 72 h. The results provide a practical approach for coproducing xylose and biobutanol from corn stover.

Keywords Acid pretreatment · Recycle · Xylose · Butanol · Simultaneous detoxification and sterilization · *Clostridium saccharobutylicum*

Introduction

Increasing environmental pollution and fluctuating petroleum prices have stimulated great interest in biofuels production. In the USA, 58.87 billion liters of ethanol was produced from corn in 2016 [1, 2]. Growing demand for "environmental-friendly" biofuels has driven increased food prices, even food shortage in many countries. Fermentation of lignocellulosic biomass is an attractive and feasible route to biofuels that supplements the fossil fuels [1, 3]. It has been estimated that 442 billion liters of bioethanol per year can be produced from lignocellulosic biomass all over the world [4]. Butanol has attracted increasing attentions as a biofuel, due to its excellent properties such as high energy density, low volatile and explosive, and miscible with gasoline in any proportion [5].

Corn stover is an abundant lignocellulosic biomass [6–9], with a global annual production of 0.95 billion tons [10]. However, pretreatment of corn stover is required to release sugars that can be utilized by microorganisms to produce biofuels [5, 8, 11]. Corn stover is composed of 37% cellulose and 19.8% xylan [12]. Various pretreatment methods have been reported, such as alkaline [8, 13, 14], acid [3, 15, 16], acid and alkaline [17], ethanol [18], glycerol [19], hydrogen peroxide [20, 21], alkaline hydrogen peroxide [22, 23], ionic liquids [24, 25] and deep eutectic solvents [26, 27].

Acid treatment is a well-known method for processing lignocellulosic biomass [28], especially in xylose, xylitol [10] and furfural production [29–31]. Acid pretreatment could increase the accessibility of enzymes to the cellulosic fractions by solubilizing the hemicellulosic fraction of the biomass [32]. Dilute H_2SO_4 (0.5–2%) is usually used for pretreatment because of the strong corrosivity of concentrated H_2SO_4 [28, 33]. Acid pretreatment could produce aromatic and toxic compounds such as furfural, hydroxyl methyl furfural (HMF), acetic acid and formic acid, which are inhibitory to microbial fermentation [1, 3]. Acid-pretreated solutions are highly toxic and usually need to be

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detoxified before subjected to hydrolysis and fermentation [7]. Xylose is an important component in acid-pretreated solutions, whereas the utilization of xylose is often inconvenient due to its low concentration. Corn stover is known to contain less xylan than corn cob, which contains about 33% of xylan and is commonly used for xylose and xylitol production [10]. Since the dry mass content is generally less than 13%, the xylose concentration after hydrolysis of corn stover was reported to be 16.4–20.5 g/L [10, 34]. However, a significantly higher substrate (xylose) concentration of about 200 g/L is required for xylitol production [35, 36]. Vacuum evaporation concentration is undesirable, due to its high energy consuming and generation of inhibitors such as furfural and hydroxyl methyl furfural (HMF).

Herein, recycling of sulfuric acid pretreatment solution to accumulate xylose from corn stover was studied for the first time. Importantly, a high xylose concentration of 56.3 g/L was achieved as a by-product from recycled acid-pretreated solution. After pretreatment, the unwashed corn stover was subjected to hydrolyzation, and simultaneous detoxification and sterilization (SDS) fermentation, resulting in butanol titer of 9.5 g/L.

Materials and methods

Raw materials, strains and enzymes

Corn stover was kindly provided by Shandong Zesheng Bioengineering Technology. Corn stover was chopped and stored in a closed container at room temperature. Activated carbon was purchased from Sinopharm Chemical Reagent Co. Ltd.

Clostridium saccharobutylicum DSM 13864 was purchased from DSMZ and was manipulated as previously described [5, 7, 8, 11].

ACCELLERASE® 1500 cellulase was a generous gift from Genencor (Wuxi) Bio-Products Co.

Acid pretreatment of corn stover

One gram of untreated corn stover was filled into sealed stainless reactors with lining Teflon (200 mL) and soaked in 0–20% H_2SO_4 (w/w) at 170 °C or 180 °C for 0–1 h in an oil bath. After the pretreatment, the reactors were immersed in ice bath immediately to cool down. Addition of H_2SO_4 is based on the percent of liquid weight. Solid–liquid ratios (w/w) were 1:10, 1:8 and 1:7. Pretreated corn stover (Pre-CS) was filtered with a Buchner funnel. The pretreatment solution was collected and recycled for second batch of pretreatment. For unwashed group, filtered Pre-CS was soaked with water and adjusted pH to 4.8 with NaOH for the next enzymatic hydrolysis step. For washed group, filtered

Pre-CS was washed with tap water until pH reached 4.8–6.0, dried at 65 °C for 24 h in an oven and stocked in sealed plastic bags for further use.

Recycled pretreatment solution for pretreating corn stover

For recycling pretreatment solution, 1 g of untreated corn stover was soaked in first and second batches of pretreatment solution (solid–liquid ratios (w/w) of 1:10), and 0%, 0.2%, 0.4%, 0.6%, 0.8%, 0.9%, 1.0% and 1.2% (w/w) H_2SO_4 were added to the pretreatment solution for second and third batches of pretreatment, respectively.

To optimize the solid–liquid ratio, 1 g of untreated corn stover was soaked in 10 g, 8 g and 7 g of first and second batches of pretreatment solution, and then 0.9% (w/w) H_2SO_4 was added to the pretreatment solution for second and third batches of pretreatment. Others conditions were the same as in "Acid pretreatment of corn stover".

Enzymatic hydrolysis of pretreated corn stover

The washed Pre-CS and unwashed Pre-CS were soaked in citrate buffer (pH 4.8) in conical flasks with plugs. Then, 30 FPU/g (Pre-CS) cellulase was added to the mixture. Solid–liquid ratio was 1:10 to 1:7. The conical flasks were incubated in a water bath at 50 °C and 150 rpm for 48 h.

Enzymatic hydrolysates were condensed to 60–65 g/L of total reducing sugar for butanol fermentation using a rotary evaporator under vacuum at 60 °C.

Samples were taken at 24 h and 48 h and then subjected to centrifugation at 12,000 rpm for 10 min. The glucose concentration was determined using HPLC analysis as described in "Analytical methods".

Butanol fermentation

Butanol fermentation medium was composed of 55 g/L reducing sugar, 10 g/L corn steep liquor (CSL), 4 g/L $CaCO_3$, 2 g/L $(NH_4)_2SO_4$, 0.5 g/L K_2HPO_4 and 0.01 g/L $MnSO_4·H_2O$ [5]. *C. saccharobutylicum* DSM 13864 cultured at 37 °C for 16–18 h was used as seed culture for butanol fermentation at an inoculum size of 10% (v/v). The butanol fermentation was conducted as follows.

Detoxification with separate sterilization (*DSS*) [7] about 50 mL of undetoxified and activated carbon detoxified corn stover hydrolysates was adjusted to pH 6.5 with 2 mol/L NaOH and then transferred into 100-mL anaerobic bottles for sterilization, respectively. Mixture of CSL and mineral salts [$CaCO_3$, $(NH_4)_2SO_4$, K_2HPO_4, $MnSO_4·H_2O$] was adjusted to pH 6.5 and sterilized. The sterilized CSL and salts mixture was then transferred into sterilized detoxified FRs hydrolysate to prepare fermentation medium as

described above and then inoculated with 10% (v/v) seed culture for butanol fermentation.

Simultaneous detoxification and sterilization (SDS) [7] about 50 mL of un-detoxified corn stover hydrolysate was mixed with activated carbon, CSL and mineral salts [$CaCO_3$, $(NH_4)_2SO_4$, K_2HPO_4, $MnSO_4 \cdot H_2O$] and adjusted to pH 6.5 with 2 mol/L NaOH in a 100-mL anaerobic bottle for sterilization. Then, 10% (v/v) seed culture was inoculated for butanol fermentation.

Analytical methods

Corn stover hydrolysate was centrifuged (12,000 rpm, 5 min) and diluted for 10 or 20 folds. The total reducing sugar and monosaccharides in corn stover hydrolysate were measured as previous report [8].

Xylose, glucose and arabinose were analyzed by HPLC (Agilent 1260, Agilent Technologies Ltd., USA) using a refractive index detector (RID) and an Aminex HPX-87H column (300 mm × 7.8 mm, 9 μm; Bio-Rad, USA). The mobile phase was 5 mmol/L H_2SO_4 solution at a flow rate of 0.5 mL/min, and the column was operated at 55 °C.

Furfural was analyzed by HPLC using a UV detector at wavelength of 276 nm and a C18 column with 0.01% (v/v) H_2SO_4/CH_3OH (95/5, v/v) as the mobile phase at a flow rate of 1 mL/min [37].

The concentrations of butanol, acetone, and ethanol were determined by GC (6890 N; Agilent Technologies, Wilmington, DE, USA) according to previously described method equipped with the flame ionization detector (FID) and an capillary column PEG-20M (30 m × 0.32 mm × 0.5 μm, JK, China) using nitrogen as the carrier gas [11, 38].

Results and discussion

Pretreatment of corn stover using sulfuric acid

Increased temperature, acid dose and pretreatment time could promote the formation of inhibitory compounds during biomass pretreatment [28, 39]. However, lower pretreatment temperature often requires higher dose of acids. Germec and Turhan reported the pretreatment of rice straw using 5% (w/w) H_2SO_4 at 126.5 °C [3]. In Cao and coworkers' study, 0.5% (w/w) H_2SO_4 was applied in the pretreatment of poplar (solid loading 5% w/w) at 170 °C [40]. Pretreatment with higher acids concentration could result in higher ionic strength in hydrolysates, which is detrimental to further microbial fermentation [41]. Thus, 170 °C was chosen for H_2SO_4 pretreatment of corn stover.

Different doses of H_2SO_4 were tested in corn stover pretreatment at 170 °C (Fig. 1). Among xylan, dextran and araban, araban is regarded as the most stable polysaccharide
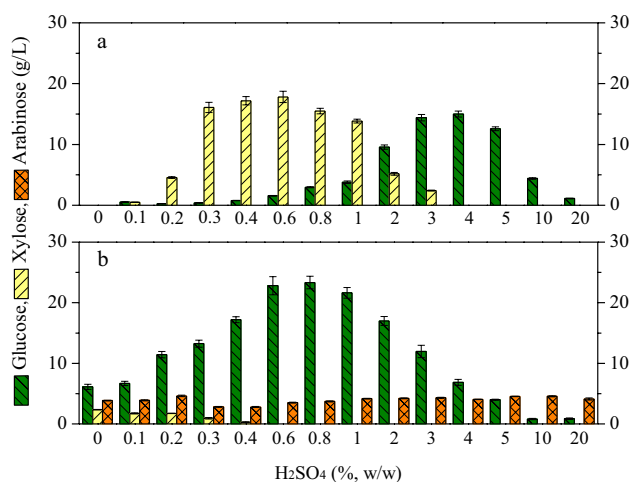

Fig. 1 Effect of H_2SO_4 concentration on corn stover pretreatment at 170 °C. **a** Sugar concentrations of pretreatment solution. **b** Sugar concentrations of enzymatic hydrolysates of H_2SO_4 Pre-CS. The solid–liquid ratio of pretreatment and enzymatic hydrolysis is 1:10, and pretreatment time is 1 h

under acidic conditions. At 10–20% (w/w) H_2SO_4, xylan and dextran were converted into furfural, 5-HMF, etc., [42]; araban, however, was hardly degraded. Under pretreatment conditions of 170 °C, 1 h and 10% (w/w) H_2SO_4, 0.8 g/L of glucose and 4.6 g/L of arabinose are detected in the enzymatic hydrolysate.

Using 0.6% (w/w) H_2SO_4, 17.2 g/L xylose and 22.8 g/L glucose were achieved in pretreatment solution and enzymatic hydrolysates, respectively. Although higher glucose (23.3 g/L) was reached at 0.8% (w/w) H_2SO_4 than that of 0.6% (w/w) H_2SO_4, lower xylose (15.5 g/L) was resulted at 0.8% (w/w) H_2SO_4 (Fig. 1). Thus, 0.6% (w/w) H_2SO_4 was used in further experiments.

Recycling of acid pretreatment solution

Because the price of xylose ($3500/ton) is significantly higher than that of glucose ($470/ton), it would be more practical to produce xylose (from hemicellulose) and glucose (from cellulose) separately in the pretreatment of lignocellulosic biomass. To achieve higher xylose and reduced waste liquid, the pretreatment solution was recycled in second batch of pretreatment (Fig. 2). Considering the reduced acidity after pretreatment [43], various amounts of sulfuric acid were added in the pretreatment solution. When 0.6% (w/w) of H_2SO_4 was added, the highest xylose concentration of 33.5 g/L was reached, along with 19.5 g/L glucose. At H_2SO_4 addition of 1.0% (w/w), the highest glucose level of 21.2 g/L was achieved, with reduced xylose of 29.2 g/L. Based on the above results, addition of 0.9% (w/w) H_2SO_4 was selected in second batch of pretreatment.
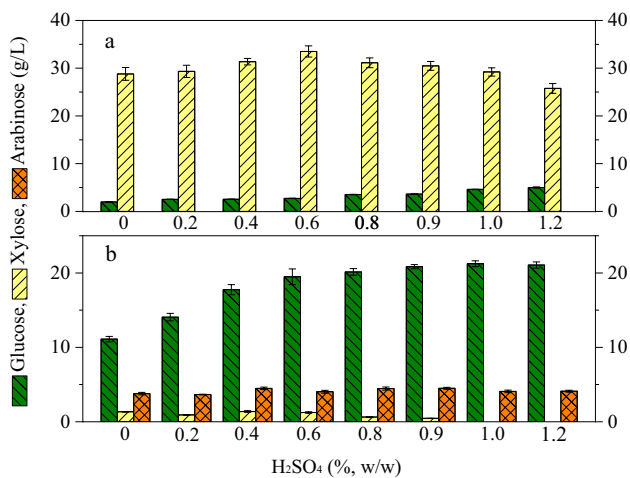
Fig. 2 Effect of H_2SO_4 addition in second batch of pretreatment at 170 °C. **a** Sugar concentrations of pretreatment solutions. **b** Sugar concentrations of enzymatic hydrolysates of H_2SO_4 Pre-CS. The solid–liquid ratio in pretreatment and enzymatic hydrolysis is 1:10, and pretreatment time is 1 h

To further evaluate the accumulation of xylose in third batch of pretreatment, different concentrations of H_2SO_4 were added in the pretreatment solution of second batch (Fig. 3). The results show that xylose concentrations in third batch were not significantly increased. When 0.9% (w/w) of H_2SO_4 was added, xylose concentration reached the maximum level of 32.9 g/L, which was slightly decreased compared with second batch (33.5 g/L xylose). It has been reported that xylose is unstable at higher temperatures and can easily transformed into furfural and other compounds [44–46]. The concentration of glucose (18.7 g/L) in the
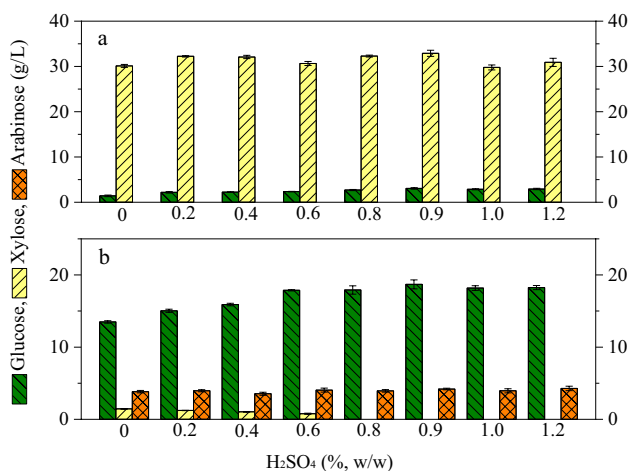

Fig. 3 Effect of H_2SO_4 addition in third batch of pretreatment at 170 °C. **a** Sugar concentration of pretreatment solutions. **b** Sugar concentrations of enzymatic hydrolysates of H_2SO_4 Pre-CS. The solid–liquid ratio in pretreatment and enzymatic hydrolysis is 1:10, and pretreatment time is 1 h

hydrolysates was also lower than that in second batch, suggesting a further strengthened buffer capacity and weakened acidity of third batch of pretreatment solution. Addition of 0.9% (w/w) H_2SO_4 was used in second and third batches of pretreatment in further experiments.

Process optimization of recycled acid pretreatment solution over three batches

Higher temperature and shorter reaction time could result in higher xylose titer and furfural yield [47]. Considering the severe degradation of xylose in third batch of pretreatment at 170 °C for 1 h, shortened pretreatment at higher temperature (180 °C) and solid–liquid ratio (1:7) was attempted to reduce the degradation of xylose.

As shown in Fig. 4a, xylose concentration did not increase linearly with the increase of solid–liquid ratio (1:10, 1:8 and 1:7) at pretreatment temperature of 170 °C. Although xylose concentrations increased in recycled batches at all solid–liquid ratios, only 40.5 g/L of xylose was obtained in third
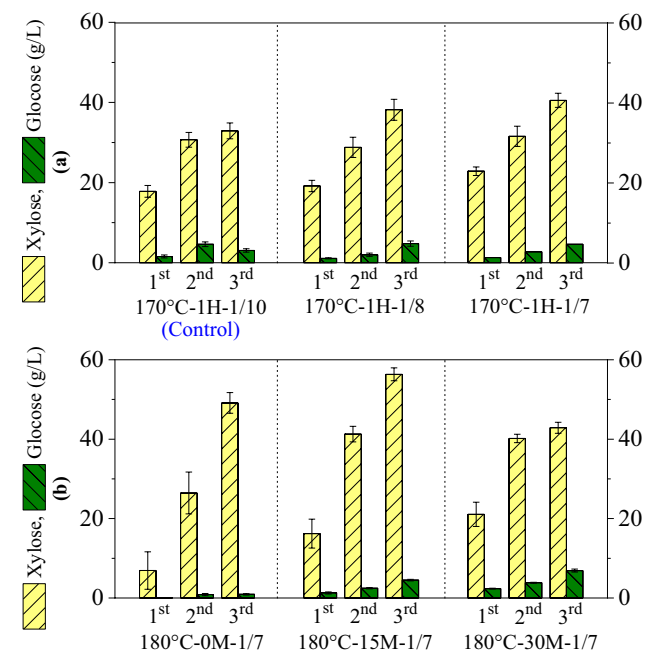

Fig. 4 Optimization of solid–liquid ratio, temperature and time for recycle of pretreatment solution. **a** Pretreatment at 170 °C for 1 h at various solid–liquid ratios; **b** pretreatment at 180 °C for 0 − 30 min at solid–liquid ratio of 1:7. 170 °C-1H-1/10: 170 °C pretreatment for 1 h, solid–liquid ratio of 1:10; 170 °C-1H-1/8: 170 °C pretreatment for 1 h, solid–liquid ratio of 1:8; 170 °C-1H-1/7: 170 °C pretreatment for 1 h, solid–liquid ratio of 1:7; 180 °C-0 M-1/7: 180 °C pretreatment for 0 min, solid–liquid ratio of 1:7; 180 °C-15 M-1/7: 180 °C pretreatment for 15 min, solid–liquid ratio of 1:7; 180 °C-30 M-1/7: 180 °C pretreatment for 30 min, solid–liquid ratio of 1:7. Addition of H_2SO_4: first: 0.6% H_2SO_4 (w/w); second: 0.9% H_2SO_4 (w/w); third: 0.9% H_2SO_4 (w/w)

batch under 170 °C-1H-1/7 (170 °C pretreatment for 1 h, solid–liquid ratio of 1:7).

To reduce the degradation of xylose, pretreatment at higher temperature and shorter time was attempted. As shown in Fig. 4b, the pretreatment condition was optimized to be 15 min and solid–liquid ratio 1:7 at 180 °C. In second batch, 41.3 g/L of xylose was accumulated, with only 2.5 g/L of glucose. In third batch, accumulation of xylose was further enhanced to 56.3 g/L, along with 4.5 g/L of glucose. For pretreatment at 170 °C, the highest xylose accumulation was merely 40 g/L, while glucose level reached 5 g/L (Fig. 4a). Consequently, higher temperature and shorter time could not only result in higher xylose, but also reduce the hydrolysis of cellulose. The recycled pretreatment conditions were optimized as follows: 180 °C, 15 min, 1:7 solid–liquid ratio (w/w), three batches of recycled pretreatment, 0.6% H_2SO_4 (w/w, first batch)/0.9% H_2SO_4 (w/w, second and third batches).

Enzymatic hydrolysis of acid-pretreated corn stover

To reduce the waste water generated in washing Pre-CS, unwashed Pre-CS was also subjected to enzymatic hydrolysis and further butanol fermentation. Washed Pre-CS and Unwashed Pre-CS in different batches were hydrolyzed, and the hydrolysates were analyzed (Table 1). Similar glucose concentrations of 39.96 g/L and 38.76 g/L were achieved from washed and unwashed Pre-CS in third batch, respectively. However, total reducing sugar of unwashed Pre-CS is 15.65 g/L higher than that of the washed Pre-CS in third batch, mainly due to the xylose accumulation in unwashed pretreatment solution. The highest xylose titer of 15.04 g/L was obtained from unwashed Pre-CS. This indicates that the pretreatment solution has little inhibitory effect on enzymatic hydrolysis. In further study, the potential application of hydrolysates of unwashed Pre-CS was evaluated in butanol fermentation.

Butanol fermentation utilizing SDS-detoxified hydrolysates

As shown in Fig. 5, butanol fermentation was carried out with enzymatic hydrolysates of acid Pre-CS washed (AW) and acid Pre-CS unwashed (AUW). The fermentation was performed following previously reported SDS fermentation [7]. Briefly, SDS is a process of detoxification and sterilization of the mixture containing hydrolysate, detoxification agents (resin or/and activated carbon), CSL and mineral salts in one pot at 121 °C for 20 min. SDS could not only reduce the toxicity of hydrolysates, but also remove toxic compounds generated during sterilization of hydrolysates (Maillard reaction). *Clostridium saccharobutylicum* DSM 13864 was used for biobutanol production, which has been successfully applied in the utilization of cane molasses, hydrolysates of rice straw and corn stover for producing biofuels [7, 8, 11, 26, 38]. When undetoxified medium was applied, cell growth was completely inhibited and no butanol was produced. The highest butanol titer of 9.5 g/L
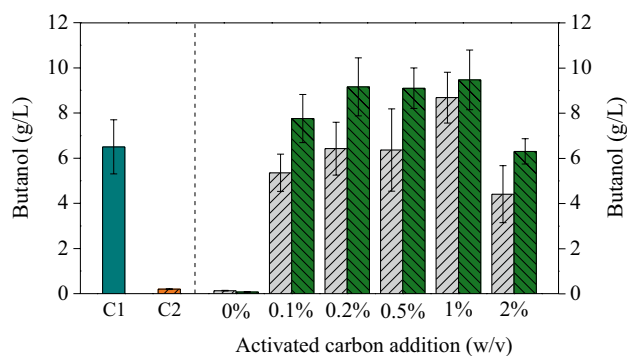

Fig. 5 Butanol fermentation using corn stover hydrolysates processed by simultaneous detoxification and sterilization (SDS). ▨: enzymatic hydrolysate of acid Pre-CS washed (AW); ▧: enzymatic hydrolysate of acid Pre-CS unwashed (AUW); C1 (▮): separately sterilized glucose as control 1; C2 (▨): corn stover hydrolysate processed by detoxification with separate sterilization (DSS) as control 2 [6]. SDS and DSS butanol fermentation were conducted in 100-mL anaerobic bottle at 37 °C for 72 h. Fermentation medium: 55 g/L reducing sugar, 10 g/L CSL, 4 g/L $CaCO_3$, 2 g/L $(NH_4)_2SO_4$, 0.5 g/L K_2HPO_4, 0.01 g/L $MnSO_4 \cdot H_2O$

Table 1 Enzymatic hydrolysis of Pre-CS in different batches

Reducing sugar	Washed pre-CS			Unwashed pre-CS		
	First batch	Second batch	Third batch	First batch	Second batch	Third batch
Xylose (g/L)	3.66 ± 0.18	0.98 ± 0.02	0.20 ± 0.05	6.64 ± 0.04	8.16 ± 0.05	15.04 ± 0.31
Glucose (g/L)	28.47 ± 0.30	37.96 ± 0.50	39.96 ± 0.97	27.55 ± 0.07	35.33 ± 1.26	38.76 ± 1.58
Arabinose (g/L)	3.09 ± 0.29	1.87 ± 0.14	2.20 ± 0.01	3.14 ± 0.09	3.53 ± 0.27	4.21 ± 0.39
Total sugar (g/L)	35.22 ± 0.76	40.81 ± 0.65	42.36 ± 1.03	37.33 ± 0.20	47.02 ± 1.56	58.01 ± 2.28

Corn stover was pretreated at 180 °C for 15 min at 1:7 solid–liquid ratio (w/w)

All data are an average of three parallel replicates and are represented as mean ± standard deviation

was obtained when 1% (w/w) activated carbon was added. Butanol titers of 9.5 g/L and 8.7 g/L were achieved at 72 h of fermentation from AUW and AW, respectively. When 2% of activated carbon was added, butanol concentration was significantly decreased, which could be attributed to the nonspecific adsorption of both toxins and nutrients in the medium by activated carbon [48–50]. In addition, higher butanol concentration was achieved with AUW than that of AW. It is speculated that trace amount of furfural remained in AUW might promote the cell growth and butanol production. Similar phenomenon was observed in previous study on butanol fermentation using furfural residues hydrolysates [7]. Low concentration of furfural (<0.1 g/L) did not inhibit the cell growth; on the contrary, it promoted the cell growth and butanol production of *C. saccharobutylicum* (data not shown). The same observations have also been reported with *Clostridium acetobutylicum* ATCC 824 [51] and *Clostridium beijerinckii* NCIMB 8052 [52]. Low concentration of furfural could induce the expression of aldo/keto reductase and short-chain dehydrogenase/reductase, which have been identified to be involved in furfural detoxification and tolerance. It is speculated that these enzymes could not only reduce furfural and HMF, but also promote the metabolic pathway of butanol production.

Using this approach, 82.7 ± 2.3 g of xylose and 61.0 ± 7.1 g of butanol could be produced from one kilogram of corn stover. As substitutes for sulfuric acid, nitric acid and phosphoric acid could potentially be applied in pretreatment. Nitrate and phosphate, formed upon being neutralized, could be used as nitrogen and phosphorus source for plants and microorganisms [53, 54] .

Conclusions

Sulfuric acid pretreatment of corn stover was optimized, and pretreatment solution containing 56.3 g/L xylose was obtained after recycling of pretreatment solution for 3 times. Pre-CS residue was enzymatically hydrolyzed to obtain 35.7–39.9 g/L glucose and 4.3–5.5 g/L arabinose. The concentrated hydrolysates were used for butanol fermentation following a SDS process. The highest butanol titer (9.5 g/L) was obtained at 72 h from hydrolysates of unwashed corn stover with 1% (w/w) activated carbon. This study provides a practical pretreatment approach for the utilization of corn stover for producing xylose and biobutanol.

Compliance with ethical standards

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