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Influence of dilute acid and alkali pretreatment on reducing sugar production from corncobs by crude enzymatic method: A comparative study

Rama Raju Baadhe [a], Ravichandra Potumarthi [b,*], Naveen Kumar Mekala [a]

[a] Department of Biotechnology, National Institute of Technology, Warangal 506004, India
[b] Department of Chemical Engineering, Monash University, Clayton, Victoria 3800, Australia

HIGHLIGHTS

- Attempted to identify optimum solid to liquid ratio for higher reducing sugars.
- Solid to liquid ratio of 0.05 released 415.12 mg/mL of sugars by dilute acid.
- 0.25 M acid improved the glucan percentage achieved highest xylan solubilization.
- Produced 398.95 mg/mL of reducing sugars by enzymatic hydrolysis.

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ABSTRACT

In the present study, two commonly used catalysts in chemical pretreatment, sulfuric acid and sodium hydroxide, were tested to evaluate the effect of solid-to-liquid ratio on pretreatment and enzymatic hydrolysis. Solid to liquid ratio (S/L) was influential on sugars released with an increase in the S/L ratio between 0.03 and 0.2. Enzymatic digestibility of 0.25 M H_2SO_4 pretreated corncobs were released more sugars (415.12 mg/mL); whereas, corncobs pretreated with NaOH released 350.12 mg/mL of reducing sugars at S/L 0.05. Further, in comparison with NaOH pretreated corncobs, acid treated material substantially increased the accessibility and digestibility of cellulose during crude enzymatic hydrolysis (28.96 FPU) and released 398.95 mg/mL reducing sugars.

1. Introduction

Enormous amounts of renewable carbon is produced in the ecosystem and about 77 × 10^9 tons, fixed via photosynthesis leads to the production of 1 × 10^{11} tons of biomass annually (Bozell and Patel, 2006). This abundant and economical biomass composed of complex lignocellulosic materials, which are well-known sources for diverse complex molecules: arabinoxylan, cellulose and lignin, which can be converted biotechnologically into valuable products like reducing sugars, fuel, chemicals and wide variety of enzymes (Torrea et al., 2008; Miura et al., 2004; Kahar et al., 2010; Potumarthi et al., 2012, 2013a,b; Mekala et al., 2013). Moreover advancements in these conversion technologies offers an outstanding and potential benefits to the national and global interests through: (1) improved strategic security, (2) decreased trade deficits, (3) healthier rural economies, (4) improved environmental quality, (5) technology exports, and (6) a sustainable energy resource supply (Angenent et al., 2004; Kamm and Kamm, 2004; Mekala et al., 2008, 2013; Potumarthi et al., 2013b).

Corncob is one of the most copious byproducts of the agricultural industry in India while cultivating maize or corn. Area wise it is the third largest crop in India and contributing 2% corn (maize) production globally. India produces 22.5 million metric tons of corn annually (Annual Report, 2012–13), which contributes approximately 6.75 million tons (Potumarthi et al., 2012) of corncobs annually. Easy availability, high cellulose (32.3–45.6%), low lignin (6.7–13.9%) content and high energy densities (4960–5210 MJ/kg) made corncobs as one of the finest feedstock for the production of a variety of compounds (Potumarthi et al., 2012). Advances in triumphant pretreatment methods to break the complex structure, subsequently to increase the vulnerability of cellulose to enzyme attack are major challenge for process commercialization. Various pretreatments methods have been

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* Corresponding author.
E-mail address: pravichandra@gmail.com (R. Potumarthi).

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developed to facilitate enzymatic hydrolysis of various types of lignocellulosic materials (Wang et al., 2011; Chen et al., 2009; Ken-lin et al., 2011; Erdei et al., 2010; Pedersen et al., 2011; Michael et al., 2009). In recent times, much attention is gaining on corncobs as a viable feed stock for the production of reducing sugars, and some efforts are done on the pretreatment of corncobs (Foster et al., 2007; Zakpaa et al., 2009; Potumarthi et al., 2012, 2013a). For effective biomass pre-treatment and hydrolysis of agricultural wastes, methods such as dilute acid, lime, microbial, ionic liquid, ammonia fiber expansion, oxalic acid, aqueous ammonia, individually and in combinations are well studied (Cheng et al., 2010; Potumarthi et al., 2013a). Pretreatment step is critical in the entire sugar production or bioethanol production process as it involves high cost of operation. To make this step and whole process economically viable, process should be at low energy and water consumption, low investment costs, inexpensive and easily recoverable reagents and applicability to various types of raw material are required (Vallejos et al., 2012). Now, lots of efforts are underway to determine the type of pretreatment more efficient for lignocellulosic materials, but which method is suitable is not yet clear. However, irrespective of pretreatment technology, for reducing the energy costs of the process, a low solid–liquid ratio (S/L) and mild temperatures in the pretreatment are important, thus economizing both steam and electricity (Vallejos et al., 2012). Although various studies were carried out on S/L ratio, limited studies have been done on the corncob with combination of acid and alkali treatment (Vallejos et al., 2012; Tao et al., 2013).

In the present study chemical pretreatment processes of corncob carried out by commonly used dilute H_2SO_4 and NaOH with varied concentrations. During pretreatment step, solid to liquid ratio varied to understand the effect on pretreatment and the concentration of sugar released. Further pretreated corncobs subjected to crude enzymatic hydrolysis to produce reducing sugars. Work is carried out to study the effect of catalyst concentration on concentration of sugars release along with changes in solid to liquid ratio, during the pretreatment and enzymatic hydrolysis.

2. Methods

2.1. Raw material and compositional analysis

Corncobs (pH 3.0–5.0) obtained from local farms (Warangal, India) with 10–20% moisture content. In ordered to neutralize and to remove toxic and interference compounds, corncobs submerged in fresh water for 24 h and then washed with distilled. Subsequently sun dried and pre milled and screened to get 8–10 mm size of the granules and average composition was determined according to method given by Sluiter et al. (2008). All the samples either used in the experiments or stored at room temperature. All chemicals, media and reagents used were of analytical grade and purchased from either from Merck, Sigma or Himedia.

2.2. Acid and alkali pretreatment

To evaluate solid to liquid ratio (S/L) impact on chemical pretreatment, 10 g of corncob mixed with varied volumes (50–300 ml) of 0.05 M sulfuric acid and 0.05 M NaOH. Further, corncobs treated with varied concentrations of H_2SO_4 (0.05 M, 0.1 M, 0.15 M, 0.2 M, 0.25 M, 0.3 M and 0.5 M) and NaOH (0.05 M, 0.1 M, 0.25 M, and 1 M) with best S/L (w/v) ratio. All these experiments were carried out in Erlenmeyer flasks (Borosil) and kept at 121 °C for 20 min in an autoclave. Subsequently residual solid biomass separated, neutralized either used in sacchariffication or dried at 60 °C and stored for further experiments.

2.3. Microbial culture

Lyophilized slant culture of *Phanerochaete chrysosporium NCIM 1106* were procured from NCL Pune, India and maintained on czapekdox media at 30 °C. Sporulated slants were obtained after 5 days of growth and used immediately or stored at 4 °C. Seed inoculum with a conidial suspension was prepared by adding 5 ml sterile distilled water supplemented with 0.1% (w/v) Tween – 80 into agar slants and used for production of crude cellulase enzyme (Mekala et al., 2008).

2.4. Enzyme production

Mandel's enriched medium containing (g/l) Urea, 0.3; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2.0; $CaCl_2{\cdot}2H_2O$, 0.4; $MgSO_4{\cdot}7H_2O$, 3.0; Peptone, 1.0; Tween 80, 0.2; $FeSO_4{\cdot}7H_2O$, 0.005; $MnSO_4{\cdot}7H_2O$, 0.0016; $ZnSO_4{\cdot}7H_2O$, 0.0014; $CoCl_2{\cdot}6H_2O$, 0.002; cellulose, 10.0 for the production of cellulase enzyme was prepared in four 250 ml conical flasks. The pH of the medium was adjusted to 4.5–5.0 and autoclaved at 121 °C, 15 lb pressure for 20 min. Following sterilization flasks were cooled to room temperature and inoculated with 1 ml of seed inoculum and kept in a shaking incubator (Daihan lab tech) maintained at 28 ± 2 °C, 150 rpm. After 96 h, the fungal biomass was separated from the solution by filtration. The filtrate obtained is centrifuged at 10,000 rpm at 4 °C for 10 min; supernatant contained crude enzyme immediately used for enzymatic hydrolysis or stored at 4 °C.

2.5. Crude enzymatic hydrolysis

Solid residues obtained from the H_2SO_4 and NaOH pretreatment were further subjected to crude enzyme obtained in the above method. 2 g of pretreated corncob sample from different methods, subjected to crude enzyme (28.96 FPU) with 10 ml sodium acetate buffer (pH 4.8) at 50 °C using a shaking incubator (Daihan lab tech) at 100 rpm. Penicillin (40 mg/L) was used to prevent microbial contamination; hydrolysates were sampled periodically for sugars analysis. Simultaneously enzymatic hydrolysis of untreated corncob sample was performed and used as a control.

2.6. Analytical methods

The filter paper assay was performed to know the enzyme activity, according to the standard procedures recommended by the International Union of Pure and Applied Chemistry (Ghose, 1987). One FPU is defined as the concentration of enzyme that releases 1 μM of glucose equivalents from Whatman No. 1 filter paper per min. The reducing sugar was estimated by using the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). Further quantification of glucose, xylose and arabinose in the soluble fraction was determined by HPLC (Shimadzu). HPLC equipped with an Aminex HPX-87H column (Bio-Rad, 300 × 7.8 mm) and refractive index detector (RID). 5 mM H_2SO_4 used as the mobile phase at 0.3 ml/min for 55 min under isocratic mode. All the analyses were carried out in triplicate.

3. Results and discussion

3.1. Chemical composition of corncob

The initial composition of the corncob samples used as a raw material as the average of six replicate analysis on wt.% are cellulose (glucan), 45.1; xylan, 30.2; araban, 3.80; acetyl groups, 3.08; klason lignin, 17.2; ash, 1.3 and ethanol extractives, 3.1.

3.2. Influence of solid to liquid ratio on the reducing sugar production

In our earlier experiments, 0.2 S/L ratio released 435.6 mg of sugars/10 g of corncobs when 0.5 M H_2SO_4 used as a catalyst (Potumarthi et al., 2012). To know the effect of solid to liquid (S/L) on corncob pretreatment, in the present study, S/L ratio employed with dilute H_2SO_4 (0.05 M) and NaOH (0.05 M) at different ratios (0.2–0.03) shown in Fig. 1. It is observed that at 0.05 S/L ratio maximum sugars released was 350.12 mg/10 g of corncobs with dilute acid (0.05 M). Decrement in the S/L ratio increased the release of reducing sugars up to S/L (0.03) beyond this there was a slight decrease in the sugar production. Consequently, S/L (0.05) with 10 g of corncob and 200 ml of catalyst was used in further experiments.

3.3. Acid and alkali pretreatment

With best possible S/L (1/20) (Fig. 1) ratio improved the amount of sugars with different concentration of acid and alkali Fig. 2A and B). During the sulfuric acid (0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.5 M) pretreatment maximum amount of sugars released with 0.25 M sulfuric acid compared with dilute 0.05 M acid. There is only a gradual increase in the amount of sugars released from 0.05 M to 0.5 M, but there is an extreme change with decrement in the amount sugars between 0.25 M and 0.5 M sulfuric acid. Since lift in concentration of acid released more sugars along with the inhibitors like furfural and hydroxyl methyl furfural. The glucan part of the solid residue was improved and xylan was decreased after treatment with 0.25 M acid (Table 1) further increase in the acid concentration not improved the glucan part. It was proved that the acid attack on the polysaccharides, particularly hemicelluloses easily hydrolyzed than cellulose and lignin. Further acid treatment is not adequate to hydrolyze the crystalline cellulose which remains as insoluble solid. Consequently, the cellulose and lignin fractions almost unaltered in the solid phase. Corncob contains up to 30% xylan portion which can readily be hydrolyzed into xylose by dilute acids. After the removal of xylan from corncob, cellulose residue is accessible for cellulases and converted into glucose, and further converted to ethanol (Cheng et al., 2010; Chen et al., 2007).

NaOH pretreatment resulted in the swelling of the corncob particles subsequently there is a possibility to remove of the lignin and the hemi cellulose. Fibers were depolymerized due to the separation of hydrogen bonds of cellulose. The swelling of the substrate particles was due to the saponification of inter molecular ester bonds (Potumarthi et al., 2013b). NaOH treatment released a low amount of sugars compared to H_2SO_4 (Parveen et al., 2009; Potumarthi et al., 2012) pretreatment and increase in concentration of NaOH decreased the sugar liberation. Fig. 2 shows sugars released during pretreatment of corncobs with different concentrations of NaOH. 0.05 M NaOH, produced more sugars and which were higher than that of the amount of sugars released with other NaOH (0.1 M, 0.25 M, 1 M) concentrations. In contrast to other concentrations, 1 M NaOH released a low amount of sugars. Which was also confirmed with compositional analysis of solid residue (Table 1). It was observed that the digestibility of NaOH-treated biomass increased with the decrease of lignin (⩽20%) content. However, minor effect of dilute NaOH pretreatment was found in softwoods with lignin content ⩾26% (Zheng et al., 2009). More swelling was observed in 1 M NaOH treated corncob samples,

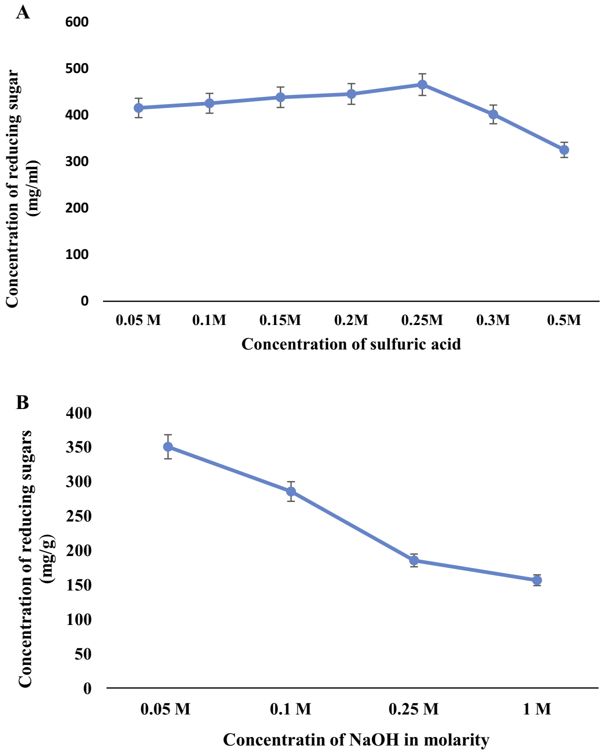

Fig. 2. Effect of sulfuric acid (A) and NaOH (B) concentrations on the reducing sugars at S/L 1:20. The data are displayed as the mean ± 1 SD, and are derived from three independent experiments.

Table 1
Chemical composition of solid residues from hydrothermal treatments (results expressed as weight percent, oven dry weight basis).

Concentration of catalyst (M)	Solid residue yield (%)	Cellulose (%)	Xylan (%)	Araban (%)	Acetyl groups (%)	Klason lignin (%)
Acid (H_2SO_4)						
0.05	98.2	45.16	28.27	2.84	2.98	–
0.1	92.9	47.54	23.56	1.95	2.45	18.14
0.15	80.5	49.25	15.35	0.596	1.10	21.83
0.2	71.9	53.58	10.25	0.565	0.838	23
0.25	62.9	59.75	3.56	0.0324	0.257	25.89
0.3	68.2	58.50	5.89	0.0428	0.624	24.9
0.5	70.3	52.78	7.51	0.453	0.409	25.14
Alkali (NaOH)						
0.05	85.23	55.18	8.89	0.468	0.584	28.55
0.1	89.35	53.25	12.85	0.986	0.982	24.52
0.25	92.35	49.35	18.36	1.95	1.89	20.58
1	95.65	46.25	22.35	2.98	2.05	19.55

% weight percentage.

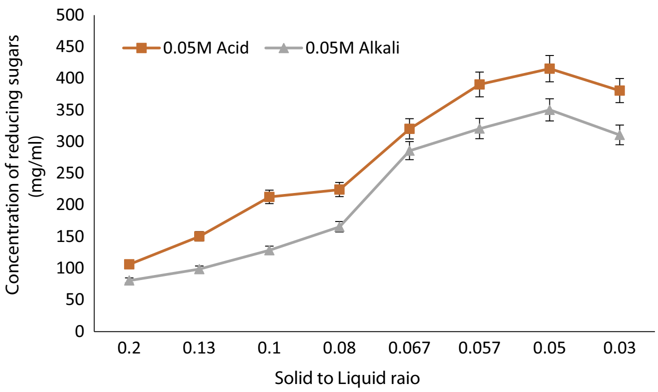

Fig. 1. Effect of solid to liquid ratio S/L (w/v) ratio on the liberation of the reducing sugars. The data are displayed as the mean ± 1 SD, and are derived from three independent experiments.

compared with other concentrations (0.05 M, 0.1 M. 025 M), which had an impact on enzymatic hydrolysis.

3.4. Enzymatic hydrolysis

Cellulase produced from this process utilized for crude enzymatic hydrolysis, where highest cellulase activity was observed (28.96) IU during the 96 h of fungal growth. A further increase in the production time decreased the enzyme activities revealing the optimum production of active enzyme during 96 h. The rate of hydrolysis using crude enzyme depends on the initial activity of the enzyme i.e., more the enzyme activity results in increased yield of sugars. Fig. 3A shows the pattern of sugar yield for different concentrations of H_2SO_4 for every 12 h. It was observed that there is an improvement in the sugar production at 36 h and has drastically decreased at the end of 48 h. Corncobs pretreated at 0.05 M concentration of H_2SO_4 resulted in sugar yield of 129.76 mg/mL at 24 h, which was decreased gradually to 58.15 mg/mL at the end of 72 h. Since during pretreatment step xylan removal and glucan increased as H_2SO_4 charge increased (Table 1). So highest sugars released during EH of 0.25 M acid treated corncob at 36 h (398.5 mg/mL) which has good agreement with highest weight percent of cellulose after 0.25 M H_2SO_4 pretreatment.

In contrast to above results the pattern of sugar yield by different concentrations of NaOH was shown in Fig. 3B that there was an increase in the concentration of sugars released during 36 h and this gradually decreased at the end of 72 h. Corncobs pretreated with 1 M NaOH released more amount of sugars (320.75 mg/mL) at 36 h than 0.05 M NaOH which yielded 145.7 mg/mL. There is a gradual decrease in the sugars production except 1 M NaOH treated corncob sample, which had a rapid transformation after 48 h. Decline in the sugar production may be due to the end product inhibition by the glucose (Xiao et al., 2004). Moreover during the growth of cellulase producing fungal strains in batch culture, accumulation of glucose in the media exerts catabolite repression to block the synthesis of cellulase (Potumarthi et al., 2013a). NaOH treated corncobs released less sugars in contrast to H_2SO_4, since H_2SO_4 treatment induces swelling of the biomass, which leads to increase in internal surface area and decrease in crystalline nature by unravelling the structural linkages between lignin and xylan, consequently converts them into soluble form effectively than NaOH (Table 1). Then, disruption of lignin structure takes place, which leads to an increase in corncobs porosity by removing the cross links. In addition, exclusion of acetyl and uronic acid components of hemicelluloses, increases accessibility of enzyme to hemicellulose and cellulose surface in the enzymatic hydrolysis (Huber et al., 2006; Mosier et al., 2005).

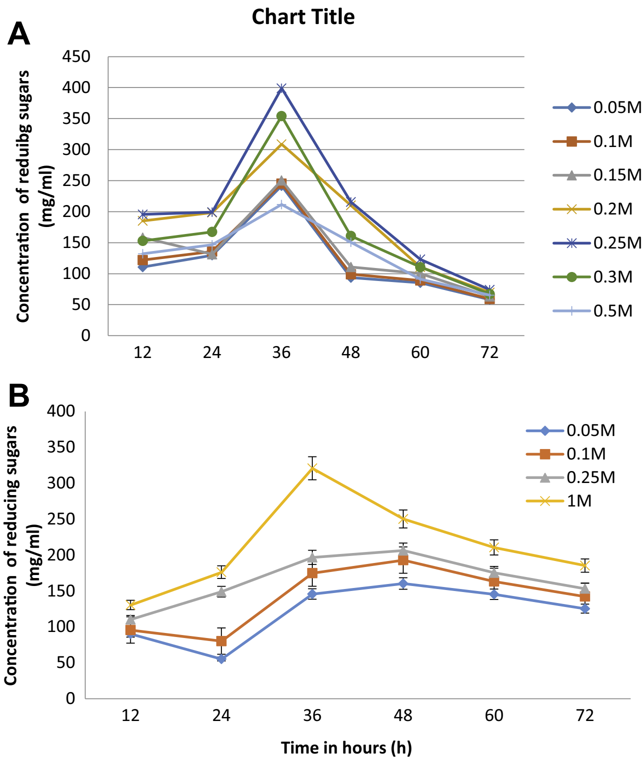

Fig. 3. Enzymatic hydrolysis of (A) H_2SO_4 and (B) NaOH pretreated corncobs with S/L 1:20. The data are displayed as the mean ± 1 SD, and are derived from three independent experiments.

4. Conclusions

Increasing fuel crisis and environmental concerns, advert biomass as a potential alternative. Nevertheless pretreatment of biomass is great challenge, still considerable efforts enduring for commercializing the pretreatment technology. During pretreatment, ratio of solid (biomass)/ to catalyst (acid/alkali) plays an important role; a connection between solid to liquid ratio and concentration of released sugars was observed along with increased enzymatic digestibility of the pretreated corncobs. Solid to liquid ratio (1:20) significantly detached the xylan part and improved the enzymatic hydrolysis for reducing sugar production. Compared with H_2SO_4 pretreatment, NaOH pretreatment of corncob substantially produced low reducing sugars. Moreover, enzymatic hydrolysate from H_2SO_4-pretreated corncobs contained higher concentrations of fermentable sugars which is more suitable for the subsequent fermentation process for value added chemicals production. Future efforts on optimization of S/L ratio improves the sugar production from corncobs.

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