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Dilute acid pretreatment and enzymatic saccharification of sugarcane tops for bioethanol production

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ABSTRACT

The aim of this work was to study the feasibility of using sugarcane tops as feedstock for the production of bioethanol. The process involved the pretreatment using acid followed by enzymatic saccharification using cellulases and the process was optimized for various parameters such as biomass loading, enzyme loading, surfactant concentration and incubation time using Box–Behnken design. Under optimum hydrolysis conditions, 0.685 g/g of reducing sugar was produced per gram of pretreated biomass. The fermentation of the hydrolyzate using *Saccharomyces cerevisae* produced 11.365 g/L of bioethanol with an efficiency of about 50%. This is the first report on utilization of sugarcane tops for bioethanol production.

1. Introduction

The importance of production of bioethanol from lignocellulosic feed stock has gained importance in the last few years due to various factors such as increased costs of petroleum fuel, benefits in environmental pollution control, energy security, etc. (Sukumaran and Pandey, 2009). Lignocellulosic biomass such as agricultural crop residues provides a low cost feed stock for the production of biofuels. The bioconversion of lignocellulosic biomass to fuel offers potential economical and environmental advantage over traditional fossil based fuels. Among the different conversion technologies from lignocellulosic biomass to ethanol, pretreatment followed by enzymatic hydrolysis and fermentation is claimed to be more beneficial (Sonderegger et al., 2004). Pretreatment of cellulosic biomass in a cost effective manner is a major challenge of cellulose to ethanol technology research and development. Agricultural crop residues such as rice straw, wheat straw, sugarcane bagasse, sugarcane tops, cotton stalk, soft bamboo, bamboo processing wastes are considered as abundantly available feed stocks for bioethanol production globally, and particularly in tropical countries such as India (Pandey et al., 2009).

Sugarcane is cultivated over a large area with a production of more than 199.1 million metric tons (MMT) in India (Sukumaran et al., 2010). This results in the generation of large amount of post harvest residues, including sugarcane tops (SCT), which could be an abundant, inexpensive and readily available source of lignocellulosic biomass. Little effort has been made to utilize SCT as a substrate for bioethanol production. According to Pandey et al. (2009), SCT is the most surplus residue (79.4 MMT) available in India (among various lignocellulosic residues) and is usually burnt in the field itself as it does not find any suitable application. It can be used as animal fodder for a few days before the leaves start rotting. Usually for every 1 MT of sugarcane produced, 0.25–0.30 MT of sugarcane tops (leaves plus top portion of plant which is cut away) is produced (Pandey et al., 2009).

The first step in the process of making bioethanol from lignocellulosic biomass is the pretreatment of the feedstock which is required to alter biomass structure as well as chemical composition, so that hydrolysis of carbohydrate to monomeric sugars can be achieved more rapidly with higher yields. The efficiency of hydrolysis of pretreated substrate depends on several process parameters such as enzyme loading, biomass loading, incubation time and surfactant concentration (Qi et al., 2009). There will be interaction between these factors and optimization of enzymatic hydrolysis plays an important role in hydrolysis improvement. Basically, this optimization consists of performing statistically designed experiments, estimating the regression coefficient in a mathematical model, predicting the response and checking the adequacy of the model. A mathematical model enables simple manipulation of variables to be accomplished, in order to determine how the process behaves in different situations. A model

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generally incorporates a number of parameters that are used to describe the desired process. Mathematical modeling of enzymatic degradation of cellulose is highly challenging as it is necessary to balance complex biological processes with many variables (Binod et al., 2011). Although, there are some reports on the use of post harvest sugarcane residues for bioethanol production (Braunbeck et al., 1999; Krishnan et al., 2005; Dawson and Boopathy, 2007), no concentrated effort has been made to use SCT for bioethanol production. Thus, the aim of this work was to develop a process for the pretreatment of SCT chemically, hydrolyze the pretreated material enzymatically and to ferment the hydrolyzate using the yeast.

2. Methods

2.1. Feed stock

Sugarcane tops (SCT) was obtained from the Godavari Biorefineries, Mumbai, Maharashtra, India. The dried feed stock was milled in a knife mill. The compositional analysis of native and dilute acid pretreated SCT were carried out by two stage dilute acid hydrolysis method following NREL protocol (Ruiz and Ehrman, 1994).

2.2. Pretreatment

2.2.1. Primary screening

Primary screening for the pretreatment was carried out to select the preferred acid. The milled SCT was mixed with HCl and H_2SO_4 (2%, w/w) so as to obtain 10% solid loading. The mixed material was then heated at 121 °C for 60 min and cooled. The samples were further neutralized with 1 N NaOH, washed with tap water and dried at room temperature (30 °C).

2.2.2. Optimization of process parameters for pretreatment

After primary screening, the effective acid was used for further optimization of process parameters, which included the influence of acid concentration, biomass loading, particle size of SCT and incubation time in a one parameter at a time mode. Different concentrations of H_2SO_4 (1–5%, w/w) were added to find the effect of acid concentration on pretreatment efficiency. Biomass loading during pretreatment was optimized by adjusting various solid liquid ratios (5%, 10%, 15%, 20%, 25% and 30%, w/w). Effect of particle size on pretreatment was optimized by using SCT of different particle size (mixed (dried, knife milled, unsorted SCT), <600 μ, 600–1000 μ and >1000 μ, respectively). Finally, the pretreatment was carried out for different time duration (30, 60 and 90 min) to find the optimum time.

2.3. Characterization of native and acid pretreated sugarcane tops

2.3.1. FTIR analysis

Fourier Transform Infrared spectra were studied using a Schimadzu spectrometer (Japan). For this, 3.0 mg of native as well as pretreated SCT were dispersed in 300 mg of spectroscopic grade KBr and subsequently pressed into disks at 10 MPa for 3 min. The spectra were obtained with an average of 25 scans and a resolution of 4 cm^{-1} in the range of 4000–400 cm^{-1}.

2.3.2. SEM analysis

The structural differences in the lignocellulosic morphology of SCT before and after the acid pretreatment were taken by JEOL JSM-5600 scanning electron microscope (Sindhu et al., 2010). Images of native and acid pretreated SCT were taken at a magnification of 500×. The specimens to be coated were mounted on a conductive tape and coated with gold palladium using a JEOL–JFC-1200 fine coater and observed using a voltage of 10–15 kV.

2.3.3. XRD analysis

Crystallinity of native and dilute acid pretreated SCT were characterized by XRD analysis performed with a PANalytical (Netherlands), X-pert pro diffractometer with a step size of 0.03° using a Cu–Ka radiation X ray (λ = 1.54 Å) at a voltage of 40 kV and current, 30 mA. The crystallinity of cellulose was calculated according to the empirical method of Segal et al. (1959) for native cellulose.

$$\mathrm{CrI}(\%) = [(I_{002} - I_{\mathrm{am}})/I_{002}] \times 100$$

where I_{002} is the intensity of 002 peak at $2\theta = 22.4°$ and I_{am} is the intensity of the background scatter at $2\theta = 18.0°$.

2.4. Enzymatic hydrolysis

Enzymatic saccharification of the acid pretreated SCT was carried out in 150 ml stoppered conical flasks by incubating 7% w/w of biomass with commercial cellulase (Zytex India Private Limited, Mumbai, India). The enzyme loading was 60 FPU/g of pretreated dried biomass, 0.1% w/w Tween-80 was used as surfactant, 200 μl of 1× antibiotic solution (Penicillin Streptomycin cock tail) and the total reaction volume was made up to 30 ml with 0.1 M citrate buffer (pH 4.8). The samples were incubated at 50 °C for 48 h in a shaking water bath (120 rpm). After incubation, samples were centrifuged to remove the unhydrolyzed residue. The hydrolyzate was used for reducing sugar analysis by 2,5-dinitrosalicylic acid method (Miller, 1959).

2.5. Optimization of enzymatic saccharification by Box–Benhken design

A Box–Behnken design (Box and Behnken, 1960) was employed to determine the effects of independent variables on the response and factor interactions with different combination of variables. This included three level four factorial designs to investigate and validate the process parameters affecting the yield of reducing sugars from the enzymatic saccharification of SCT. Four independent variables – biomass loading, enzyme loading, surfactant concentration and incubation time were studied at three levels −1, 0 and +1, which corresponded to the low, medium and high values, respectively. The variable input parameters were biomass loading (7.5–15%, w/w), enzyme loading (20–80 FPU/g pretreated substrate), surfactant concentration (0.05–0.2%, w/w) and incubation time (24–60 h) and the reducing sugar yield was the output factor. The results were analyzed by software Minitab 15 (Minitab Inc., USA). Both the linear and quadratic effects and the possible interactions of the four variables were calculated.

2.6. Ethanol fermentation

The enzymatic hydrolyzate was centrifuged (10,000×g at 4 °C for 10 min) to remove the solids. Ethanol fermentation was carried out in 250 ml stoppered conical flasks containing 100 ml hydrolyzate with reducing sugar concentration of 5.5%. The hydrolyzate was inoculated with seed culture (2% v/v) of *Saccharomyces cerevisiae* (18 h old culture) and incubated at 28 ± 2 °C for 72 h. The sample was centrifuged at 10,000×g at 4 °C. The supernatant was filtered using 0.4 μm PES membrane filters (Pall, USA) and the filtrate was analyzed by gas chromatography. Ethanol was detected using an FID detector kept at 250 °C. Other conditions of operation were – Nitrogen as mobile phase (30 ml/min), Column temperature −150 °C, injector temperature −175 °C and injection volume 1 μl. The concentrations of ethanol were calculated based on elution time

and peak areas of known concentration of ethanol. The analysis was done in triplicate and the mean values were presented.

3. Results and discussion

3.1. Effect of different process parameters on dilute acid pretreatment of sugarcane tops

Among the two mineral acids used for pretreatment, H_2SO_4 gave higher reducing sugar (0.437 g/g) than HCl (0.224 g/g) (data not shown). Untreated SCT yielded 0.11 g/g of reducing sugar after enzymatic saccharification. The results indicated that H_2SO_4 pretreatment improves the hydrolysis efficiency fourfold when compared to native SCT. Identical observations were earlier reported for H_2SO_4 pretreatment of water hyacinth by Satyanagalakshmi et al. (2011) and sugarcane bagasse pretreatment by Martin et al. (2007). Dilute acid prehydrolysis resulted in 2.7–3.7-fold increase for enzymatic convertibility of water hyacinth and sugarcane bagasse. Contrary observations were reported for peanut shells, cassava stalks and rice hulls were H_2SO_4 pretreatment were not efficient for improving the enzymatic hydrolysis (Martin et al., 2007). Optimization of acid concentration (1–5%, w/w) showed that 3% w/w was most suitable as it resulted in maximum reducing sugar (0.611 g/g). Acid pretreatment solubilizes the hemicelluloses fraction of the lignocellulosic biomass, making cellulose better accessible to the enzymes (Binod et al., 2010; Jeong et al., 2010). Similar results were reported for dilute acid pretreatment of wheat straw by Saha et al. (2005).

Studies on the effect of biomass loading on acid pretreatment of SCT showed that biomass loading from 10% to 25% w/w gave almost same reducing sugar yield, although the concentration of the reducing sugar increased marginally with increase in biomass loading and maximum was at 25% w/w (0.645 g/g); beyond that loading, there was reduction in reducing sugar yield. The decrease in efficiency of pretreatment above 25% w/w biomass loading could be due to decrease in the accessibility of the pretreatment agent. Increased solid loading have several advantages; it decreases the process cost by lowering the reactor size and energy requirements during the pretreatment and produces a more concentrated product stream (Kootstra et al., 2009). A high solid loading (45% w/w) was reported for acid pretreatment of corn stover using steam digester reactor (Weiss et al., 2009). In dilute acid pretreatment, solid loading could usually vary from 5% to 15% dry lignocellulosic biomass as reported by Kim et al. (2008).

Optimization of particle size showed that the mixed particle size SCT (dried and knife milled, unsorted SCT was used as such for pretreatment) was most suitable as it gave maximum reducing sugar yield (0.649 g/g). This was followed by particle size >1000 μm (0.57 g/g), 600–1000 μm (0.519 g/g) and <600 μm (0.49 g/g). Particle size of the feedstock plays an important role in determining the process efficiency. Sindhu et al. (2010) reported that pretreatment was effective with smaller particle size feedstock. One of the major advantages of utilization of mixed particle size feedstock is that there will be no wastage of milled lignocellulosic biomass.

Studies on the effect of incubation time on H_2SO_4 pretreatment of SCT showed that maximum reducing sugars (0.652 g/g) were produced after 60 min. Beyond 60 min, there was a reduction in reducing sugar yield (0.552 g/g).

The compositional analysis of native and H_2SO_4 treated SCT for cellulose, hemicellulose and lignin content were carried out. There was 30% loss after pretreatment. The cellulose content of the pretreated SCT increased to 46.5% from 29.9% of native SCT. Hemicelluloses content decreased to 6.16% from 18.9%.

From these results, it could be concluded that the optimum conditions for acid pretreatment of SCT were: 3% w/w H_2SO_4, 15% w/w solid loading, mixed particle size and incubation time of 60 min.

3.2. Optimization of enzymatic saccharification of pretreated SCT by Box–Behnken design

The objective of the experimental design was to optimize the hydrolysis condition for maximum reducing sugar yield. Since step wise optimization by single parameter at a time cannot examine all the possible combinations of independent variables, statistical experimental design tools for optimization are important. Experimental design and experimental reducing sugar yields are presented in Table 1. Polynomial equation for the model used was as below:

$$\begin{aligned}\text{Sugar yield}(g/g) = {} & 0.527 + 0.106X_1 + 0.046X_2 + 0.063X_3 \\ & - 0.043X_4 + 0.065X_1^2 - 0.091X_2^2 + 0.001X_3^2 \\ & - 0.011X_4^2 - 0.005X_1X_2 + 0.037X_1X_3 \\ & + 0.022X_1X_4 - 0.037X_2X_3 - 0.054X_2X_4 \\ & - 0.015X_3X_4\end{aligned}$$

where X_1, X_2, X_3 and X_4 are biomass loading, enzyme loading, surfactant concentration and incubation time respectively.

Response surface curves were plotted to find out the interaction of variables and to determine the optimum level of each variable for maximum response. Surface plot showing the interaction between a pair of factors on hydrolysis of acid pretreated SCT are given in Figs. 1–6.

The effects of biomass loading and incubation time on the hydrolysis yield of SCT, when the other two factors are at their center points are shown in Fig. 1. At low levels of biomass loading (7.5%) and incubation time, the reducing sugar yield was low. Significant improvement in the hydrolysis yield could be obtained by increasing biomass loading. When the biomass loading was set at middle level (11.25%) the sugar yield reached a maximum value of 0.6 g/g treated biomass and further increase in biomass loading does not increase the sugar level. For enzymatic reaction, a fixed substrate concentration is required to reach the adsorption saturation of enzymes and further increase in substrate concentration results in a constant rate of product formation. Biomass loading is considered to be one of the major factors affecting the conversion rate of enzymatic hydrolysis of cellulose (Qi et al., 2009). High substrate concentration results in low hydrolysis yield due to product inhibition, enzymatic inactivation, and a decrease in the reactivity of cellulosic substrate with proceeding of hydrolysis process (Gregg and Saddler, 1996).

Fig. 2 shows reducing sugar yield as a function of enzyme loading and incubation time. It was observed that at high level of incubation time (60 h) and middle level of enzyme loading (60 FPU), the reducing sugars yield was high. At low levels of enzyme loading, reducing sugar yield was low and with increase in enzyme loading, there was a significant increase in reducing sugar yield till certain level and further increase in the enzyme loading resulted in a decreased sugar yield. This may be due to the feed-back inhibition of produced glucose.

Fig. 3 explains the interaction between concentration of the surfactant and incubation time on reducing sugar yield. It was observed that at low level of surfactant concentration and low level of incubation time, the reducing sugars yield was low. Incubation time is an important factor affecting the enzymatic hydrolysis. It was observed that the reducing sugar yield increased with increase in incubation time. At high surfactant concentration, there was only a slight increase in reducing sugar yield. This surface plot explained that at the middle level of surfactant concentration

Table 1
Reducing sugar yields for individual runs of the RSM design.

Run	Biomass loading (% w/w)	Enzyme loading (FPU/g)	Surfactant (% w/w)	Incubation time (h)	Reducing sugar (g/g)
1	15	50	0.125	24	0.388
2	15	50	0.125	60	0.399
3	7.5	50	0.2	42	0.159
4	7.5	50	0.125	24	0.428
5	15	20	0.125	42	0.356
6	11.25	50	0.125	42	0.519
7	11.25	50	0.2	60	0.685
8	11.25	50	0.2	24	0.190
9	7.5	20	0.125	42	0.099
10	7.5	50	0.05	42	0.340
11	11.25	80	0.05	42	0.509
12	15	80	0.125	42	0.443
13	11.25	20	0.05	42	0.378
14	11.25	50	0.125	42	0.479
15	7.5	50	0.125	60	0.281
16	11.25	50	0.125	42	0.498
17	11.25	80	0.125	24	0.412
18	15	50	0.2	42	0.405
19	15	50	0.05	42	0.290
20	11.25	80	0.2	42	0.329
21	11.25	50	0.05	24	0.426
22	11.25	80	0.125	60	0.435
23	11.25	20	0.125	60	0.426
24	11.25	50	0.05	60	0.482
25	11.25	20	0.125	24	0.320
26	11.25	20	0.2	42	0.198
27	7.5	80	0.125	42	0.089

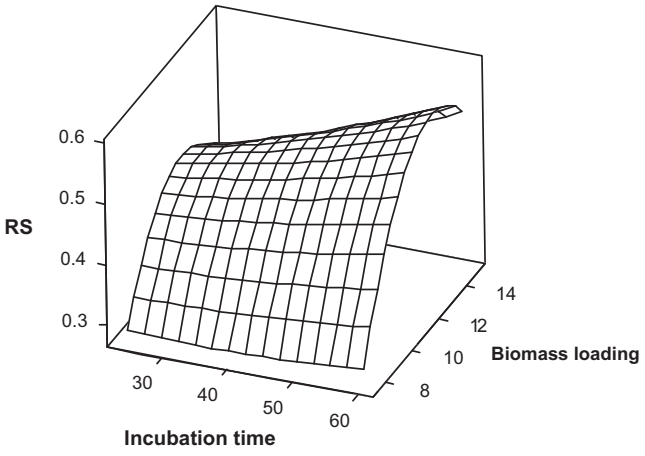

Fig. 1. Effect of biomass loading and incubation time on reducing sugar yield.

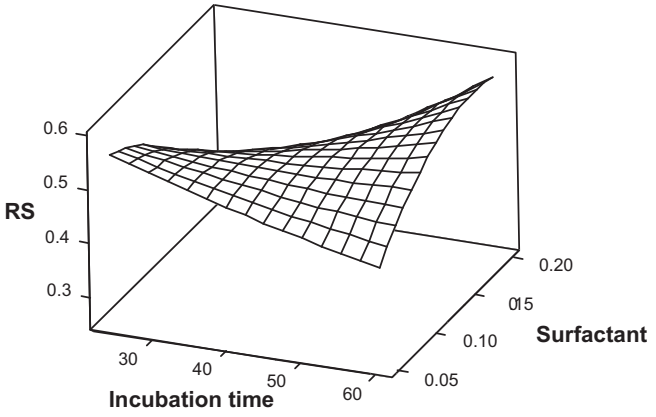

Fig. 3. Effect of surfactant concentration and incubation time on reducing sugar yield.

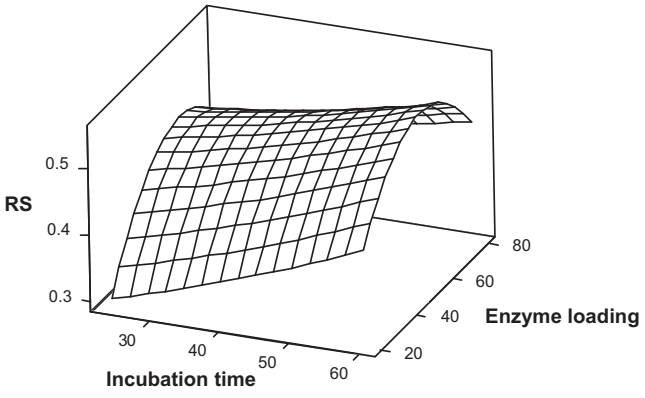

Fig. 2. Effect of enzyme loading and incubation time on reducing sugar yield.

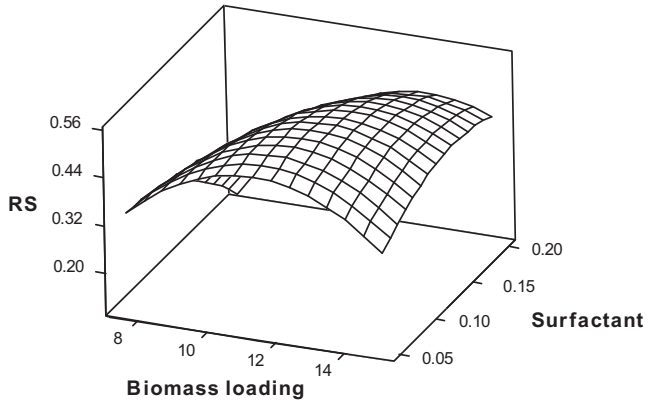

Fig. 4. Effect of biomass loading and surfactant concentration on reducing sugar yield.

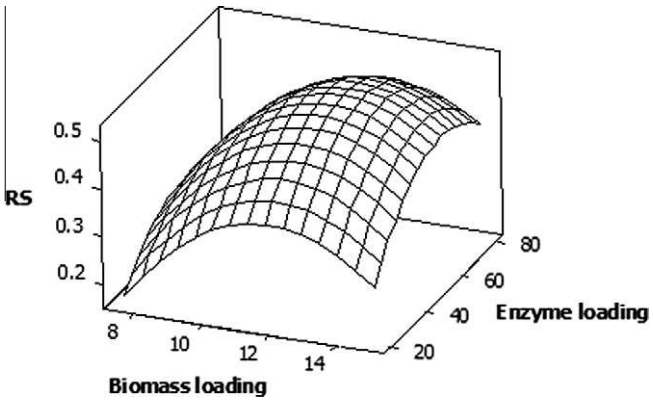

Fig. 5. Effect of biomass loading and enzyme loading on reducing sugar yield.

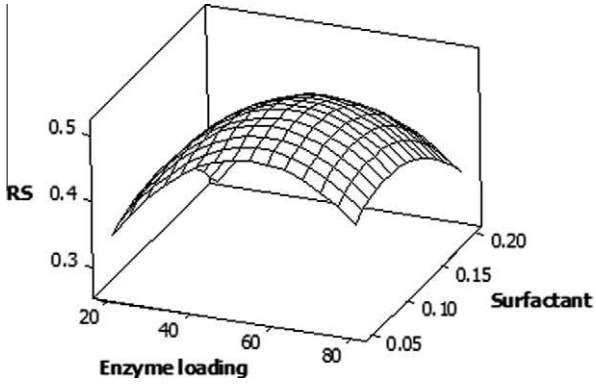

Fig. 6. Effect of enzyme loading and surfactant concentration on reducing sugar yield.

(0.125%) and high level of incubation time (60 h), the reducing sugar yield was high.

The interaction effects of surfactant concentration and biomass loading on the reducing sugar yield are shown in Fig. 4. Biomass loading is an important factor affecting the enzymatic hydrolysis. It was observed that at low level of biomass loading, increase in surfactant concentration has no effect on the yield of reducing sugars. The reducing sugar yield increased slowly with an increase in substrate concentration from 7.5% to 11.25%; above 11.25% there was a decrease in the yield of the reducing sugars. This surface plot explains that the middle level of biomass loading (11.25%) and middle level of surfactant concentration (0.125%) shows maximum reducing sugar yield. At high solid loading and surfactant concentration, there was a reduction in the reducing sugar yield. Different kinds of surfactants have different effects on the stability and activity of enzymes. When the net charge on the enzyme molecule is opposite to that on the surfactant layer, the enzyme molecule interacts with the surfactant layer remarkably and at high surfactant concentration there is a decrease in enzyme activity due to the formation of reverse micelles (Chen et al., 2006).

The effects of biomass loading and enzyme loading on the hydrolysis of SCT are shown in Fig. 5. At low levels of enzyme loading and low levels of biomass loading, the reducing sugar yield was low. Middle level of biomass loading (11.25%) and middle level of enzyme loading (60 FPU) showed maximum reducing sugar yield. At high biomass loading, the amount of available free water becomes less, which in turn decreases the hydrolysis efficiency. High biomass loading is associated with difficulties in mixing as well as end-product inhibition.

The interaction effects of enzyme loading and surfactant concentration on reducing sugar yield of sugarcane tops are shown in Fig. 6. At low enzyme loading, the reducing sugar yield was low. A significant increase in the yield of reducing sugar was obtained by increasing the enzyme loading to certain extent. At low level of enzyme loading, increasing the surfactant loading had no effect on reducing sugar yield. This surface plot explained that the middle level of enzyme loading (60 FPU) and middle level of surfactant concentration (0.125%) gave maximum reducing sugars. Similar observations were earlier reported for enzymatic hydrolysis of wheat straw pretreated with alkaline peroxide (Qi et al., 2009).

Table 2
Analysis of variance (ANOVA) for the response surface model.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	14	0.360,708	0.360,708	0.025,765	2.52	0.058
Linear	4	0.123,620	0.123,620	0.030,905	3.02	0.061
Square	4	0.156,688	0.156,688	0.039,172	3.83	0.031
Interaction	6	0.080,400	0.080,400	0.013,400	1.31	0.324
Residual error	12	0.122,734	0.122,734	0.010,228		
Lack-of-fit	10	0.121,933	0.121,933	0.012,193	30.46	0.032
Pure error	2	0.000,801	0.000,801	0.000,400		
Total	26	0.483,442				

Table 3
Model predicted value for reducing sugar yield at optimum condition.

Biomass loading (% w/w)	Enzyme concentration (FPU)	Surfactant concentration (%)	Incubation time (h)	Reducing sugar yield (g/g)	
				Predicted	Experimental
11.25	50	0.2	60	0.665	0.685
15	50	0.2	42	0.415	0.405
11.25	50	0.125	42	0.475	0.498

p-value was used as a tool to check the significance of each of the coefficients. Smaller the *p*-value, more significant is the correlation with the corresponding coefficient. The regression coefficient for reducing sugar yield was found to be best with biomass loading (*p*-value 0.036). The details are shown in Table 2.

The polynomial regression equation obtained from the experimental data was used to predict the hydrolysis rate at different enzyme loading, biomass loading, surfactant concentration, and incubation time within the range of the experimental design. In order to validate the model, three confirmation experiments were performed within the range of levels defined previously. Correlation analysis was performed on the actual responses and predicted values for each solution and the correlation coefficient was found to be 0.994, hence the empirical models developed were reasonably accurate. The details are shown in Table 3.

3.3. Characterization of native and pretreated biomass

FTIR spectra of native and acid pretreated SCT indicated structural changes in the biomass on acid pretreatment are shown in Supplementary Fig. S1. Bands at 1000 to 1200 cm^{-1} were related to structural features of cellulose and hemicelluloses (Langkilde and Svantesson, 1995). After pretreatment with H_2SO_4, the absorption peaks at 1122 cm^{-1} were enhanced. Acid pretreatment removes hemicelluloses fraction from the raw material which in turn increases the cellulose content. Band widening at 1316 cm^{-1} was related to CH_2–wagging vibrations in the cellulose and hemicelluloses (Liying and Hongzhang, 2006). Similar result was earlier reported by Sun et al. (2008) for formic acid pretreated bamboo. Band widening at 3302 cm^{-1} corresponds to stretching of H- bonded OH groups. Intensity of the band at 1045 cm^{-1} in the FTIR spectrum originated from typical absorbance of xylan decreased in the acid pretreated SCT, indicating that hemicelluloses

were removed in the pretreatment process. Similar observations have been reported by Qi et al. (2009) for wheat straw pretreated with alkaline peroxide.

Scanning electron micrographs of native and acid pretreated sugarcane tops are shown in Supplementary Fig. S2. The native sample displayed a regular, compact and smooth surface, indicating a highly ordered surface structure, while the acid pretreated sample displayed a rough surface. The pretreatment exposed some internal areas in the biomass compared with the images for untreated samples. Identical observations were earlier reported by Narayanaswamy et al. (2011) for corn stover and switch grass pretreated with supercritical carbon dioxide. Qi et al. (2009) also reported similar findings for Chinese white poplar fiber pretreated with ultra sound and for wheat straw pretreated with alkaline peroxide. The SEM images show how the native structure is opened during the pretreatment, which enhances the surface area for enzymatic hydrolysis (Cybulska et al., 2010).

Cellulose crystallinity plays an important role in enzymatic hydrolysis (Fan et al., 1980). XRD profile of native and pretreated SCT as studied here is presented in Supplementary Fig. S3. The crystallinity index of the native SCT was 37.74% which changed to 43.33% after the acid pretreatment. Increase in crystallinity index of acid pretreated samples has been reported by Converse et al. (1988) for microcrystalline cellulose, Sindhu et al. (2010) for sugarcane bagasse and Satyanagalakshmi et al. (2011) for water hyacinth. An efficient pretreatment method removes amorphous components such as hemicelluloses and lignin, which in turn increase the crystallinity of the pretreated sample. In the present investigation, pretreatment increased the crystallinity indicating that the pretreatment process was effective. However, contrary observations were reported by Hsu et al. (2010) for lignocellulosic biomass pretreated with dilute acids. Park et al. (2010) reported that cellulose accessibility was affected by crystallinity and also by other parameters such as lignin/hemicelluloses content and their distribution, porosity and particle size.

3.4. Ethanol fermentation

Fermentation of the hydrolyzate obtained after enzymatic saccharification of pretreated SCT with the yeast yielded 11.365 g/L ethanol. The initial sugar level for fermentation was 5.5% and 1.44% ethanol was recovered from the fermentation broth. Thus, the efficiency of the process was about 50% based on the theoretical ethanol yield from glucose. This could be due to the presence of inhibitory substances present in the hydrolyzate, which affected the growth and fermentation by the yeast culture. Several studies on yeast fermentation show that presence of furfural and hydroxyl methyl furfural severely affect the ethanol yield by inhibiting central enzymes, such as hexokinase, phosphofructokinase and triosphosphate dehydrogenase, involved in glycolysis (Palmqvist et al., 1999). In the present study, the hydrolyzate used for the fermentation was without any detoxification. Pretreatment of lignocellulosic biomass may produce degradation products with an inhibitory effect on the fermentation process. Major types of inhibitors are sugar and lignin degradation products. At lower temperature (less than 180 °C) lignin as well as sugar degradation is negligible. Pan et al. (2005) reported that pretreatment at lower temperature, results in very less inhibitor formation (in some cases no inhibitors at all) which was under the tolerance level of subsequent fermenting organisms.

4. Conclusions

From the results, it could be concluded that SCT could be a potential feedstock for bioethanol production. Acid pretreatment of the SCT resulted in residue with decreased hemicelluloses and lignin and the cellulose content was enhanced which in turn helped in efficient enzymatic hydrolysis and improved the reducing sugar yield. The Box–Behnken Design showed biomass loading as the most important factor influencing the sugar yield. The results offered an alternative avenue for SCT utilization.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2011.09.066.

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