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ORIGINAL PAPER

Organosolvent pretreatment and enzymatic hydrolysis of rice straw for the production of bioethanol

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Abstract The present study investigates the operational conditions for organosolvent pretreatment and hydrolysis of rice straw. Among the different organic acids and organic solvents tested, acetone was found to be most effective based on the fermentable sugar yield. Optimization of process parameters for acetone pretreatment were carried out. The structural changes before and after pretreatment were investigated by scanning electron microscopy, X-ray diffraction and Fourier transform infrared (FTIR) analysis. The X-ray diffraction profile showed that the degree of crystallinity was higher for acetone pretreated biomass than that of the native. FTIR spectrum also exhibited significant difference between the native and pretreated samples. Under optimum pretreatment conditions 0.458 g of reducing sugar was produced per gram of pretreated biomass with a fermentation efficiency of 39%. Optimization of process parameters for hydrolysis such as biomass loading, enzyme loading, surfactant concentration and incubation time was done using Box–Benhken design. The results indicate that acetone pretreated rice straw can be used as a good feed stock for bioethanol production.

Keywords Rice straw · Bioethanol · Acetone · Pretreatment · Lignocellulosic biomass · Response surface methodology

Introduction

Driven by increasing environmental concerns, high oil prices, and the uncertainty of petroleum reserves, the production of bioethanol from lignocellulose is receiving increasing attention. The conversion of lignocellulosic biomass to ethanol has many technical and economical challenges that delayed its commercialization. To make the biomass conversion economical, it is necessary to lower the cost of the pretreatment and enzymatic hydrolysis. Pretreatment is essential to make biomass digestible by altering biomass structural features (Zhu et al. 2010). Structural modifications of lignocelluloses are highly dependent on the type of pretreatment employed and have a great effect on enzymatic hydrolysis (Kumar and Wyman 2009).

Rice straw is an attractive lignocellulosic material for bioethanol production since it is one of the most abundant renewable resources. It has several characteristics that make it a potential feedstock for fuel ethanol production. It has high cellulose and hemicellulose content that can be readily hydrolysed into fermentable sugars (Binod et al. 2010).

For the conversion of lignocellulosic biomass into fermentable sugars, it is necessary to have a pretreatment step to break the lignin and to expose cellulose and hemicelluloses for enzymatic saccharification. There are several reports on pretreatment of rice straw using electron beam irradiation (Jin and Chen 2006), microwave (Zhu et al. 2005), alkali (Zhang and Cai 2008), ammonia (Kim and Lee 2007), acid (Sumphanwanich et al. 2008), wet oxidation (Wei and Cheng 1985), organosolvent (Jamshid et al. 2005; Pan et al. 2005; Pan et al. 2006), biological using white—rot fungi (Taniguchi et al. 2005), organic acids (Jahan et al. 2006), ionic liquids (Nguyen et al. 2010) and a combined process (Kun et al. 2009).

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Few reports are available on organosolvent pretreatment of lignocellulosic biomass. Pretreatment of hybrid poplar (Zhang et al. 2007), switch grass (Moxley et al. 2008) and rape seed (Li et al. 2009) with phosphoric acid–acetone were reported in the literature. Others include bioorganic solvent pretreatment of beech wood (Itoh et al. 2003); crude glycerol pretreatment of wheat straw (Sun and Chen 2008); acetone pretreatment of sugarcane bagasse (Araque et al. 2008); and formic acid pretreatment of sugarcane bagasse (Sindhu et al. 2010). Organosolvent process yield dry lignin, an aqueous hemicelluloses stream and pure cellulose fraction (Duff and Murray 1996).

The aim of the present study was to optimize process parameters for acetone pretreatment of rice straw and to study the effect of various parameters for hydrolysis of acetone pretreated rice straw. The structural features of native as well as pretreated rice straw were investigated by scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared (FTIR) analysis.

Materials and methods

Feed stock

Rice straw received from Hyderabad, Andhra Pradesh, India was used in this study. The raw materials were air dried and milled to a size less than 1 mm. The dried materials were stored at room temperature until further use. Their dry weight content was measured at 105°C for 24 h. The compositional analysis of native rice straw was carried out by two stage acid hydrolysis protocol developed by National Renewable Energy Laboratory (NREL) and the result is shown in Table 1.

Pretreatment

Two gram of biomass was taken in a 250 ml stoppered conical flasks and mixed with different organic solvents like glycerol, methanol and acetone with water in 1:1 ratio and organic acids like acetic acid (30% v/v) and formic acid (30% v/v) in presence and absence of catalysts (0.2% H_2SO_4). The pretreatment was carried out in an autoclave at 121°C, 15 lb pressure for 60 min. Neutralization of pretreated sample was carried out by washing with tap water and dried at room temperature. The pretreatment efficiency was checked by measuring hydrolysis efficiency by reducing sugar estimation. After primary screening of various organic solvents and organic acids, the most effective pretreatment agent was chosen for further optimization of process conditions.

Table 1 Composition of native rice straw

Component	% w/w of rice straw
Cellulose[a]	34.12 ± 3.5
Hemicellulose[b]	28.45 ± 3.2
Total lignin	18.12 ± 3.02
Ash	7.45 ± 0.318
Extractives	11.93 ± 0.113

[a] Based on total glucan

[b] Based on total xylan and other C_5 sugars

Optimization of process parameters on pretreatment

The various process parameters involved in the pretreatment was optimized in a step wise manner, i.e. selecting one parameter at a time approach. Effects of different catalysts like mineral acids, organic acids and alkali on acetone pretreatment were carried out. Different concentrations (0.2–0.8%) of H_2SO_4 were added along with acetone (60% v/v) during pretreatment to find the effect of catalyst concentration. Solid loading during pretreatment was optimized by adjusting various solid–liquid ratios such as 5, 10, 15, 20, 25 and 30% (w/w). Effect of particle size on acetone pretreatment of rice straw was carried out with different particle sizes (>600, 600–1,000 and <1,000 μ). Effect of incubation temperature on acetone pretreatment was carried out by keeping the flasks in autoclave at different temperatures such as 80, 100 and 121°C. Pretreatment was performed at different time points (30–120 min) to find out the optimum time.

Characterization of native and pretreated biomass

XRD analysis

The crystallinity of native and pretreated samples was measured by X-ray diffraction using a PANalytical (Netherlands), X-pert pro diffractometer set at 40 kV, 30 mA; radiation was Cu Kα ($\lambda = 1.54$ Å). The samples were scanned in a 2θ range from 10 to 30° and a step size of 0.03° was used for the analysis. The crystallinity index of each sample was expressed using the following equation (Kim and Holtzapple 2006).

$$\text{CrI } (\%) = \left[(I_{002} - I_{am})/_{I002}\right] \times 100$$

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

FTIR analysis

Fourier Transform Infrared (FTIR) spectroscopic analysis was carried out to detect changes in functional groups that may have been caused by the pretreatment (Sindhu et al. 2010).

SEM analysis

Scanning electron micrographs (SEM) were taken at magnification 1,500× for both native and acetone pretreated rice straw (particle size >600 μm) using a JEOL JSM-5600 scanning electron microscope (Sindhu et al. 2010).

Enzymatic hydrolysis

Enzymatic hydrolysis of the acetone pretreated rice straw was carried out in 150 ml stoppered conical flasks by incubating 2 g of biomass with commercial cellulase (Zytex India Private Limited, Mumbai, India). The enzyme loading was 30 FPU/g of pretreated dried biomass 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 72 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; Aswathy et al. 2010).

Optimization of enzymatic saccharification by Box–Benhken design

The process parameters such as biomass loading, enzyme loading, surfactant concentration and incubation time that affects the enzymatic hydrolysis of acetone pretreated rice straw were optimized using a response surface methodology. A Box and Behnken (1960) design with four variables at three levels and a total of 27 runs were used for the study. The three levels of each variable were coded as −1, 0 and +1, which corresponded to the lower, middle and higher values, respectively. For individual parameters these were, biomass loading (7.5, 11.25, 15% w/w), enzyme concentration (20, 50, 80 FPU/g substrate), Tween 80 concentration (0.05, 0.125, 0.2% w/w), and incubation time (24, 42, 60 h). The software Minitab 15 (Minitab Inc, USA) was used for experimental design, data analysis and quadratic model building. The response surface graphs were obtained using the software to understand the effect of variables individually and in combination, and to determine their optimum levels. The experimental setup of Box–Benhken Design is shown in Table 2.

Ethanol fermentation

After enzymatic hydrolysis, solids and liquids were separated by centrifugation and the liquid portion (designated as hydrolyzate) was used for fermentation. Ethanol fermentation was carried out in 250 ml stoppered conical flasks containing 150 ml hydrolysate with reducing sugar concentration of 34.7 mg/ml. The flasks were inoculated with seed culture (2% v/v) of 12 h old *Saccharomyces cerevisiae* and incubated at room temperature (28 ± 2°C) under static conditions. After 72 h of incubation the entire fermented broth was centrifuged at 10,000 rpm for 5 min at 4°C. The supernatant was filtered using 0.4 μm filters and the ethanol content was analyzed using gas chromatography set at an oven temperature 150°C, injector temperature 175°C and detector temperature 250°C. Nitrogen with flow rate 30 ml per min was used as carrier gas. The ethanol concentrations of the fermented samples were calculated based on the calibration curve drawn with known concentrations as external standard. The analyses were done in triplicate and the mean values were presented.

Results

Effect of different process parameters on the acetone pretreatment of rice straw

The result presented in Fig. 1 indicates that among the different reagent (with and without catalyst) tested, acetone with 0.2% H_2SO_4 as catalyst was found to be more effective followed by methanol (with 0.2% H_2SO_4) in terms of reducing sugar yield. Formic acid, acetic acid and glycerol were not effective. Hence acetone along with H_2SO_4 as catalyst was selected as pretreatment agent for further studies. The optimization of different concentration of acetone from 20 to 100% (v/v) in presence of 0.2% H_2SO_4 as catalyst showed that 60% (v/v) was optimum with a reducing sugar yield of 0.347 g/g dry biomass. Further increase from 60% in acetone concentration leads to slight decrease in reducing sugar formation. Acetone loading above 80% leads to low reducing sugar yield. Control experiments were carried out by pretreatment with water, 0.2% H_2SO_4 and acetone alone. Pretreatment with water, acetone and 0.2% H_2SO_4 yielded 0.02, 0.029 and 0.045 g/g of reducing sugar, respectively. The results indicate that pretreatment was effective when acetone was pretreated in the presence of 0.2% H_2SO_4 as catalyst.

Among the catalysts used for acetone pretreatment like mineral acids (HCl, H_2SO_4 and H_3PO_4), organic acids (CH_3COOH and formic acid) and alkali (NaOH, KOH and Ca $(OH)_2$), H_2SO_4 was found to be effective in terms of reducing sugar yield (0.347 g/g) followed by KOH (0.296 g/g), NaOH (0.294 g/g) and HCl (0.284 g/g). The optimization of concentration of H_2SO_4 as catalyst (0.2–0.8%, v/v) showed that 0.6% was found to be good for better reducing sugar yield (0.405 g/g).

Table 2 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	0.285
2	15	50	0.125	60	0.295
3	7.5	50	0.2	42	0.215
4	7.5	50	0.125	24	0.519
5	15	20	0.125	42	0.45
6	11.25	50	0.125	42	0.619
7	11.25	50	0.2	60	0.655
8	11.25	50	0.2	24	0.291
9	7.5	20	0.125	42	0.394
10	7.5	50	0.05	42	0.452
11	11.25	80	0.05	42	0.61
12	15	80	0.125	42	0.555
13	11.25	20	0.05	42	0.48
14	11.25	50	0.125	42	0.391
15	7.5	50	0.125	60	0.599
16	11.25	50	0.125	42	0.522
17	11.25	80	0.125	24	0.501
18	15	50	0.2	42	0.399
19	15	50	0.05	42	0.439
20	11.25	80	0.2	42	0.537
21	11.25	50	0.05	24	0.545
22	11.25	80	0.125	60	0.536
23	11.25	20	0.125	60	0.594
24	11.25	50	0.05	60	0.439
25	11.25	20	0.125	24	0.253
26	11.25	20	0.2	42	0.195
27	7.5	80	0.125	42	0.189

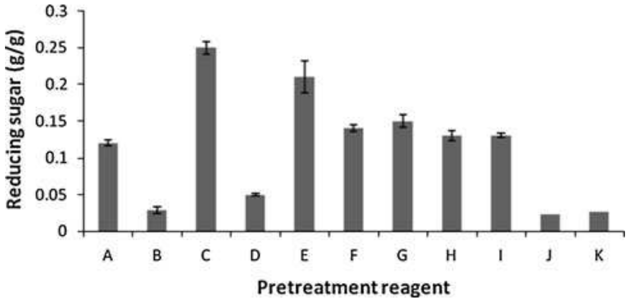


Fig. 1 Selection of pretreatment reagents. *A* Glycerol: water (1:1). *B* Acetone: water (1:1). *C* Acetone: water (1:1) + 0.2% H_2SO_4. *D* Methanol: water (1:1). *E* Methanol: water (1:1) + 0.2% H_2SO_4. *F* Acetic acid (30%). *G* Acetic acid (30%) + 0.2% H_2SO_4. *H* Formic acid (30%). *I* Formic acid (30%) + 0.2% H_2SO_4. *J* Water. *K* 0.2% H_2SO_4

The solid loading for pretreatment was carried out from 5 to 30%, w/w and 15% was found to be optimum with a reducing sugar yield of 0.433 g/g. Further increase in biomass loading decreased the total sugar yield. Optimization of particle size on acetone pretreatment of rice straw showed that particle size less than 600 μ gave maximum reducing sugar (0.436 g/g).

Effect of temperature on acetone pretreatment showed that maximum reducing sugar yield (0.431 g/g) was achieved when the rice straw was pretreated at 121°C. Total reducing sugar produced was less than 57% when carried out at 100°C (0.246 g/g). Effect of incubation time on acetone pretreatment showed that maximum reducing sugar was produced at 90 min (0.458 g/g). The pretreatment beyond 90 min showed reduction in sugar yield and at 120 min the reducing sugar yield was 0.440 g/g.

Characterization of native and pretreated biomass

FTIR spectroscopy was used to detect changes in cellulose structure during pretreatment. There is difference in the FTIR spectra of native and pretreated sample. Figure 2 shows the FTIR spectra of native rice straw, rice straw pretreated with acetone, rice straw pretreated with 0.6% H_2SO_4 and rice straw pretreated with acetone in presence of 0.6% H_2SO_4 as catalyst. The most representative bands can be summarized as follows.

The band absorption in the 3,250 cm^{-1} region was related to stretching of H-bonded OH groups and the two absorption bands 1,100 and 900 cm^{-1} arose from C–O–C stretching at the β-1,4-glycosidic linkages (Cao and Tan 2004; Hinterstoisser et al. 2001). The peak of CH_2 stretching near 2,850 cm^{-1} region is distinguished feature of cellulose (Sun et al. 2007). During acetone pretreatment, the rice straw absorbs some materials containing –OH (H_2SO_4 added as catalyst) and this results in a peak at 1,600 cm^{-1}. The bands at 1,350 cm^{-1} region corresponds to symmetric CH_2 bending and wagging (Cao and Tan 2004). A small peak at 670 cm^{-1} region corresponds to the out of plane bending of C–O–H (Liu et al. 2006). Compared to control sample the peak at 1,700 cm^{-1} region is stronger, which arose due to intra molecular hydrogen bond formation (Sun et al. 2007). The peak at 1,030 cm^{-1} corresponds to C–O-C–O-C bonds in cellulose.

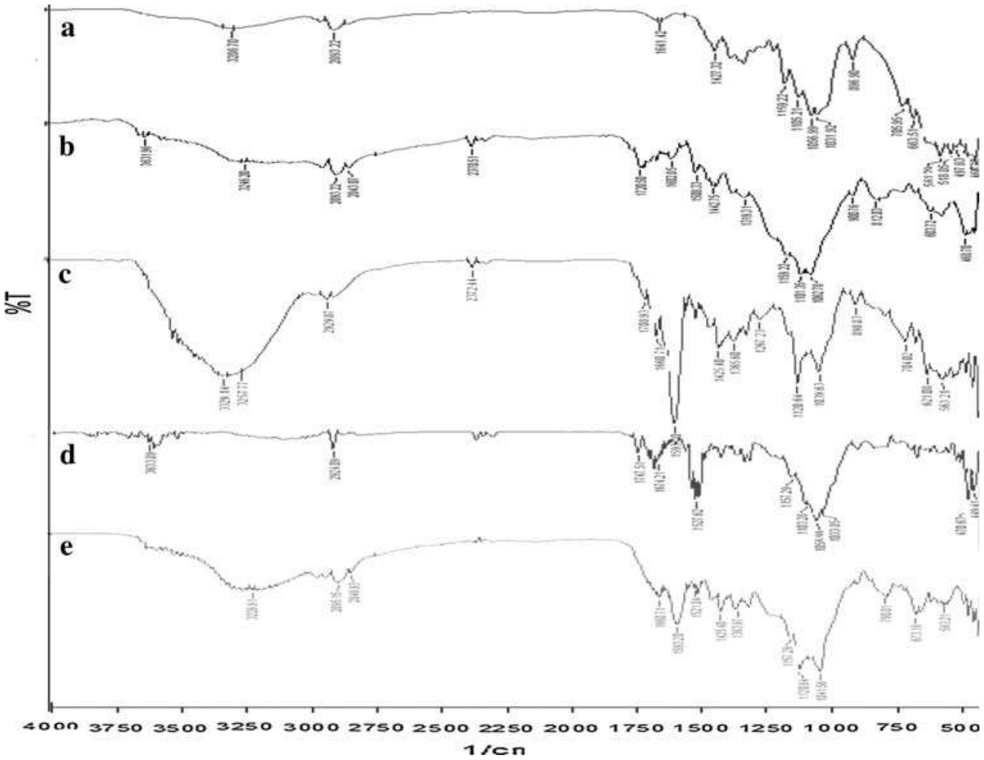
The FTIR spectra were different for native and pretreated biomass. The intensity of intermolecular hydrogen bonds is responsible for the shift of –OH vibration in the FTIR spectra. The peak shifts to a higher wave number if the intensity of intermolecular hydrogen bond is weak (Oh et al. 2005).

SEM observations of native and acetone pretreated rice straw were taken at 1,500× magnification. Figure 3 showed that the pretreatment induced physical changes in the biomass. The native rice straw have smooth and uniform surface. The acetone pretreated rice straw has a rough surface. This indicates that pretreatment removed external fibers which in turn increase surface area so that cellulose becomes more accessible to enzymes. Similar structural changes were earlier reported for rice straw pretreated with electron beam irradiation (Bak et al. 2009) and for rice straw pretreated with aqueous ammonia soaking pretreatment (Ko et al. 2009). Rice straw pretreated by acetone in presence of 0.6% H_2SO_4 as catalyst became rough and porous with a number of visible fibrous structures were formed.

Crystallinity index was calculated according to the results of XRD analysis. The X-ray diffraction profile of native and acetone pretreated rice straw is shown in Fig. 4. The crystallinity index of native rice straw was less (49.03%) compared to other pretreated samples. Pretreatment with acetone in presence of 0.6% H_2SO_4 as catalyst gave the highest crystallinity index (58.59%). If the pretreatment is effective the amorphous components like hemicelluloses and lignin were removed and the biomass become more crystalline. In the present study the pretreated sample gave the highest crystallinity index, indicating that the pretreatment is effective.

Cellulose crystallinity has been considered as an important factor in determining the hydrolysis rate (Alvira et al. 2010). Mansfield et al. (1999) reported that pretreatment increases the crystallinity of the cellulose fraction. This may be due to the removal or reduction of more easily available amorphous cellulose after pretreatment.

Fig. 2 FTIR spectra of native and pretreated rice straw **a** microcrystalline cellulose, **b** unpretreated, **c** acetone (60%) pretreated, **d** 0.6% H_2SO_4 pretreated, **e** acetone (60%) in presence of 0.6% H_2SO_4



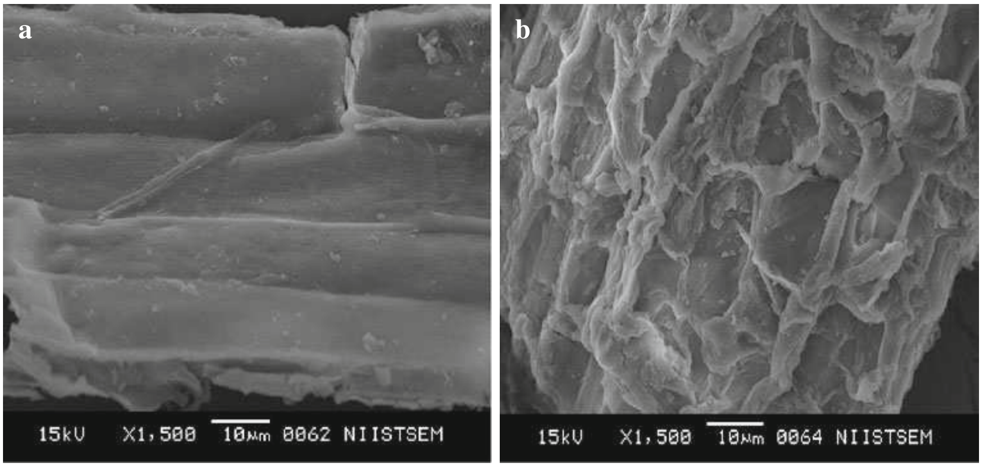


Fig. 3 Scanning electron micrographs of native and acetone pretreated rice straw **a** native rice straw, **b** acetone pretreated rice straw

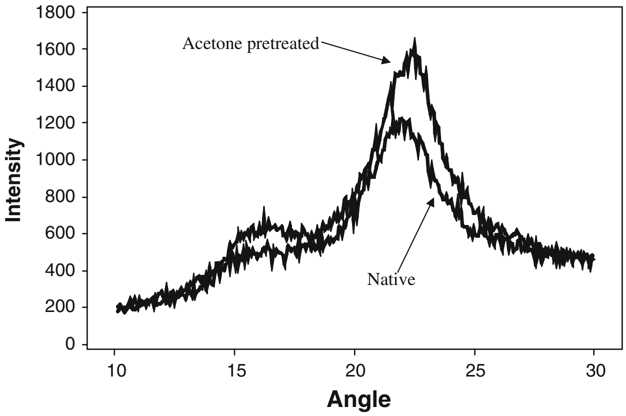


Fig. 4 X-ray diffraction pattern of native and acetone (60%) pretreated rice straw

A contrary observation was reported by Kumar and Wyman (2009). High pH pretreatments have been shown to have less effect and even reduced biomass crystallinity.

Optimization of enzymatic saccharification by Box–Benhken design

Response surface optimization is more advantageous than the traditional single parameter optimization in that it saves time, space and raw material. There were a total of 27 runs for optimizing the four individual parameters in the current Box–Behnken design. The data were analyzed by multiple regression analysis using the Minitab software and the following polynomial equation was derived to represent reducing sugar yield as a function of the variables tested.

$$\begin{aligned}Y = {} & 0.20 + 0.10X_1 - 6.76X_2 - 6.10X_3 + 0.01X_4 \\ & - 0.01X_1^2 - 3.12X_2^2 - 5.81X_3^2 - 2.44X_4^2 \\ & + 0.01X_1X_2 + 0.18X_1X_3 - 2.59X_1X_4 + 0.02X_2X_3 \\ & - 1.42X_2X_4 + 0.09X_3X_4\end{aligned}$$

where Y is the predicted reducing sugar yield and X_1, X_2, X_3 and X_4 are the coded values for biomass loading, enzyme loading, surfactant concentration and incubation time, respectively.

The experimental data were statistically analyzed using the Fischer's statistical test for analysis of variance (ANOVA) and the results are shown in Table 3. The ANOVA of the quadratic Regression model indicated that the model was moderately significant, as the *F* value for the model was 1.4. The Prob > *F* value of the model was 0.05, which also confirmed that the model was significant.

Surface plot describing combined effect between pair of factors on hydrolysis of acetone pretreated rice straw were given in Figs. 5, 6, 7, 8, and 9, keeping other two variables

Table 3 Analysis of variance (ANOVA) for the response surface model

Source	Sum of squares	Degree of freedom	Mean square	*F* value	Prob > *F*
Model	0.23	10	0.02	1.40	0.051
Linear	0.12	4	0.13	1.62	0.218
Interaction	0.12	6	0.02	1.25	0.334
Residual error	0.27	16	0.17		
Lack of fit	0.03	2	0.17	1.31	0.515
Pure error	0.03	2	0.01		
Total	0.50	26			

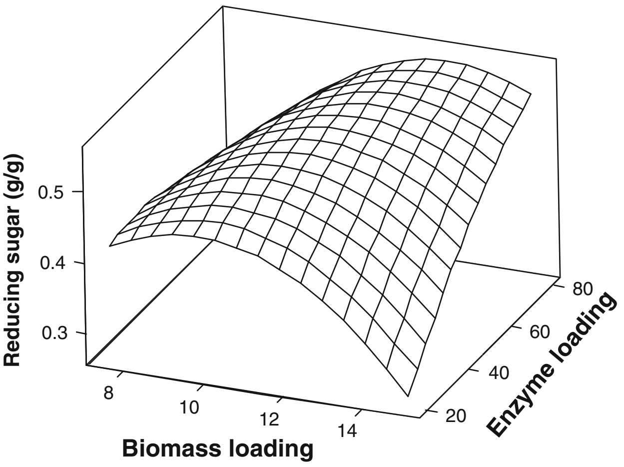


Fig. 5 Effect of biomass loading and enzyme loading on reducing sugar yield

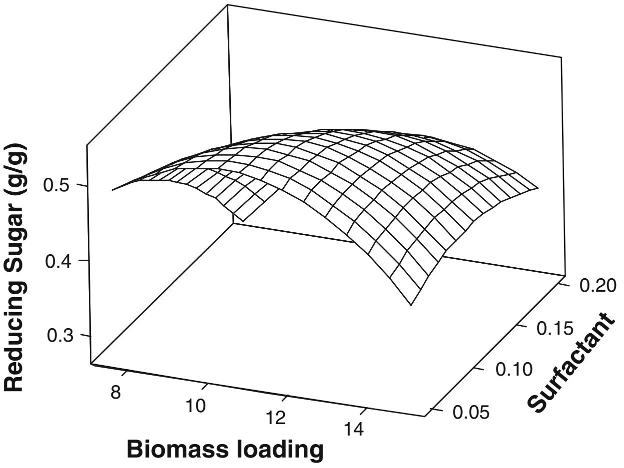


Fig. 6 Effect of biomass loading and surfactant concentration on reducing sugar yield

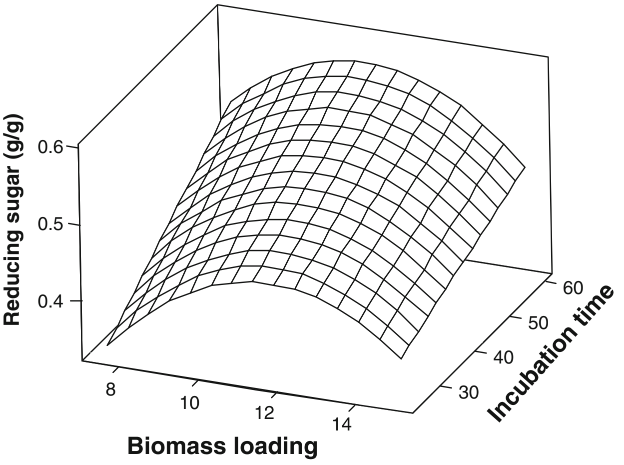


Fig. 7 Effect of biomass loading and incubation time on reducing sugar yield

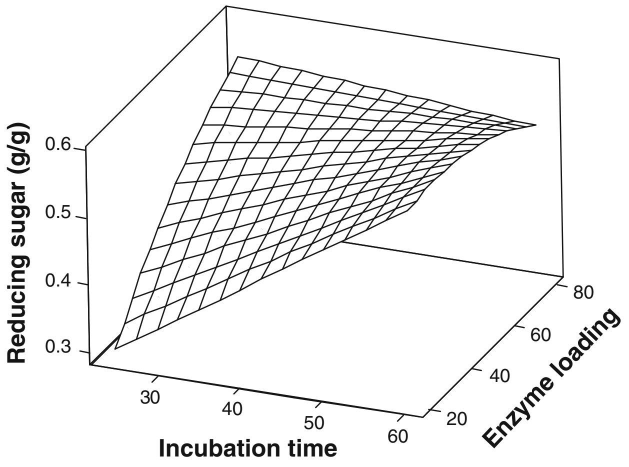


Fig. 8 Effect of incubation time and enzyme loading on reducing sugar yield

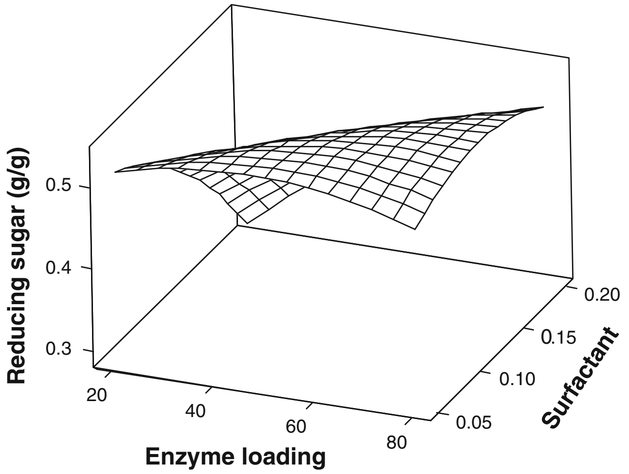


Fig. 9 Effect of enzyme loading and surfactant concentration on reducing sugar yield

constant at their middle level. Figure 5 indicates reducing sugar yield as a function of enzyme loading and biomass loading. It was observed that the average level of biomass loading (11.25%) and at the average level of enzyme loading (60 FPU) the reducing sugar yield was high. Figure 6 indicates reducing sugar yield as a function of surfactant concentration and biomass loading. The middle level of biomass loading (11.25%) and low level of surfactant concentration (0.05%) shows the maximum reducing sugar yield. The interaction effect of incubation time and biomass loading was shown in Fig. 7. This surface plot explains that at middle value of biomass loading (11.25%) and long duration of incubation time (60 h) gives maximum reducing sugar. The effect of incubation time and enzyme loading on reducing sugar yield was given in Fig. 8. Increased effect on reducing sugar yield was

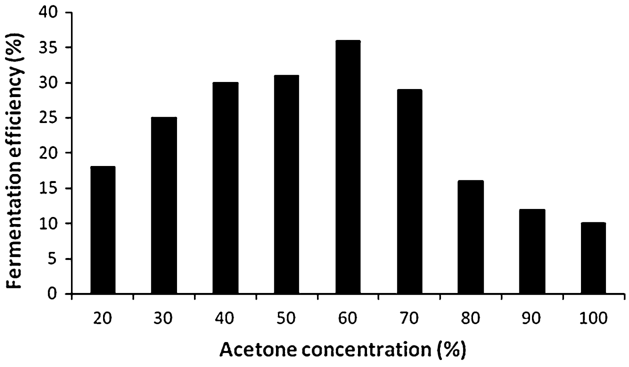


Fig. 10 Fermentation efficiency of acetone pretreated rice straw

observed at high level of incubation time (60 h) and a linear increase in reducing sugar yield was observed from low to high enzyme concentration. Figure 9 explains the interaction between surfactant concentration and enzyme loading with the reducing sugar yield. Increased effect on reducing sugar yield was observed at low level of surfactant concentration and a linear increase in reducing sugar yield from low to high level of enzyme concentration.

Ethanol fermentation

The results of the fermentation of acetone pretreated and enzymatically saccharified rice straw are given in Fig. 10. The maximum ethanol concentration (0.954 g of ethanol) was obtained after 72 h using 60% acetone pretreatment followed by enzymatically hydrolysed rice straw. The overall efficiency of the process was around 39%. The calculation of efficiency is based on the theoretical ethanol yield from glucose. By definition it is the percentage conversion of reducing sugar (considered as glucose) initially present in the fermentation medium to ethanol.

Discussion

Among the various pretreatment methods as described in introduction, none of them can be adopted as a universal process for recovering cellulose from all types of lignocellulosic biomass. Based on the composition of biomass as well as the product of interest, it is necessary to choose a pretreatment process. The most common pretreatment methods in commercial use are steam explosion and dilute acid hydrolysis. The main drawbacks of these processes are the formation of inhibitors which affect the subsequent fermentation. A comparison of various pretreatment methods for lignocellulosic biomass shows that carbonic acid, alkaline extraction and organosolv pretreatment are the best process in terms of higher sugar yield and less formation of inhibitors (Gnansounou 2009).

Literature survey shows that organosolvent pretreatment is less exploited in the lignocellulosic biomass to ethanol process. The major drawbacks in this process are the high cost of the solvents and additional revenue needed for solvent recycling which otherwise inhibit the fermenting organisms. The organosolv pretreatment process covers a broad range of organic solvents such as methanol, ethanol, acetic acid, peracetic acid, acetone, and so on (Zhao et al. 2009). Pretreatment with low boiling point alcohol such as methanol and ethanol seems to be the most promising organosolv process due to the lower chemical costs and easy recovery of solvents, but the utilization of ethanol for the production of ethanol is absurd. Acetone pretreatment is similar to low boiling point alcohol pretreatment, but the cost of acetone is higher than those of methanol and ethanol. Acetone is the most efficient ketone for delignification (Pan and Sano 1999). Cellulose containing materials, such as wood, could be rapidly converted to sugars by an acetone–water mixture. An acid catalysed organosolv saccharification process was developed by Paszner et al. (1985), by which wood was hydrolyzed to fermentable sugars and lignin. In 2007, Zhang et al. reported a new process for fractionation of lignocellulosic biomass in concentrated phosphoric acid-acetone mixture. Li et al. (2009) reported phosphoric acid–acetone pretreatment of rape seed, bermuda grass and reed for bioethanol production. In this method phosphoric acid removed hemicelluloses and partial lignin, and no inhibitors were generated in this process. In the present study, acetone pretreatment at 121°C in presence of 0.6% H_2SO_4 improved the sugar yield after enzymatic hydrolysis. Identical observation was earlier recorded by Sindhu et al. (2010) for formic acid pretreatment of sugarcane bagasse using H_2SO_4 as catalyst. Effectiveness of organosolvent treatment depends on the treatment temperature. By the addition of catalysts, the treatment temperature can be reduced (Duff and Murray 1996). At high temperature, the organic acids generated during pretreatment acts as catalyst (Sun and Cheng 2002). Acetone extracts most of the organic components and produces pure cellulose, which facilitates easy enzymatic hydrolysis (Li et al. 2009).

Solid loading is an important parameter in biomass pretreatment since it affects the overall energy balance and capital costs for fermentation and downstream recovery (Eggerman and Elander 2005). Hence maintaining high solid concentration during pretreatment improves the total sugar recovery. But higher solid loading beyond a certain level has an unfavourable effect. Higher solid–liquid ratio may impart less accessibility of biomass by the pretreatment reagent which in turn results in incomplete pretreatment. In the present study 15% of solid loading was found to be best as the reducing sugar yield decreased beyond this value. Studies with solid loading of 5 and 10% gave almost the same sugar yield as that of 15% loading (0.433 g/g). The decrease in reducing sugar yield above 15% of solid loading may be due to the decreased accessibility of the biomass by acetone in presence of H_2SO_4 as catalyst which might cause the incomplete removal of lignin. Presence of lignin in pretreated biomass irreversibly binds with cellulase during enzymatic hydrolysis resulting less sugar release (Binod et al. in press). As reported by Kootstra et al. (2009) and Noureddini and Byun (2010) in their studies on the pretreatment of wheat straw and distiller's grains, respectively, high solid loading can also promote the formation of inhibitory compounds, such as furfural, which will inhibit subsequent cellulose hydrolysis. Most of the reported literatures show that a solid loading of 10–15% is good for biomass pretreatment (Li et al. 2009; Itoh et al. 2003; Sindhu et al. 2010; Satyanagalakshmi et al. 2011).

Another important parameter in pretreatment of lignocellulosic materials is particle size. As the size of the particle decrease, the accessible surface area for reagent to act increases which in turn improves the efficiency. Milling and sieving is the easiest way to decrease the particle size. In the present work, rice straw was milled and sieved to attain the particle size in the range of <600, 600–1,000 and >1,000 μm among which smallest particle size gave the best result. This may be due to increased exposure of biomass materials by the action of reagent which ultimately increases accessibility of cellulose by enzymes in the subsequent enzymatic hydrolysis stage.

Complete removal of lignin from lignocellulosic material during pretreatment is a necessary prerequisite for successful enzymatic hydrolysis of cellulosic fraction. Organic solvent pretreatment breaks the internal lignin and hemicellulose bonds and separate the lignin and hemicellulose fraction. Temperature plays a major role in this reaction. The hard woods and herbaceous materials are readily delignified in acid-catalysed systems, where as soft woods require higher temperature. The present study shows that at lower temperature (80 and 100°C) the sugar yield was substantially low where as at 121°C, 0.431 g/g reducing sugar yield was observed. The results indicate that the temperature had a significant effect on pretreatment. Identical observations were earlier reported for acid pretreatment of water hyacinth by Satyanagalakshmi et al. (2011) and formic acid pretreatment of sugarcane bagasse by Sindhu et al. (2010), when pretreatment carried out at three different temperatures 121°C was found to be optimum. During acid pretreatment increase in temperature is reported to increase delignification (Silverstein et al. 2007).

Residence time for pretreatment is also crucial for sugar generation. For acid pretreatment, it has been reported that, increase residence time at higher temperature causes the degradation of sugars. In the present study maximum reducing sugar was produced at 90 min (0.458 g/g) and beyond that there was a drastic decrease. Similar observation was earlier reported for formic acid pretreatment of sugarcane bagasse (Sindhu et al. 2010).

The second-order polynomial regression equation obtained from the experimental data can be used to predict the hydrolysis rate at any enzyme loading, substrate concentration, surfactant concentration, and hydrolysis time within the range of the experimental design. The optimum conditions of enzymatic hydrolysis of acetone pretreated rice straw were biomass loading (11.25% w/w), enzyme loading (60 FPU), surfactant concentration (0.05% w/w) and incubation time (60 h) with reducing sugar yield of 0.655 g/g. Xu et al. (2007) performed enzymatic hydrolysis of ammonia liquor pretreated soybean straw using an enzyme loading of 50 FPU/g substrate and obtained a maximum hydrolysis yield of 51.22% at 5% (w/v) substrate concentration for 36 h. Sun and Chen (2008) observed that an enzyme loading of 44 FPU/g substrate was necessary to have a hydrolysis yield of 92% from enzymatic digestion of wheat straw pretreated by atmospheric glycerol autocatalysis at 2% (w/w) substrate concentration after 48 h. Mussato et al. (2008) concluded that 45 FPU/g substrate was an enzyme loading enough to achieve complete conversion of cellulose from brewer's spent grain pretreated by a two-step chemical pretreatment process using dilute acid and alkali at 2% (w/v) substrate concentration for 96 h. Several such studies have evaluated the influence of the cellulase loading in enzymatic hydrolysis. Chen et al. (2007) demonstrated the requirement of higher cellulase concentrations to corncob cellulose hydrolysis, verifying an increase in hydrolysis yield from 50 to 150 FPU/g substrate, although above 100 FPU/g substrate the raise was weaker. Tengborg et al. (2001) studied the hydrolysis of whole slurry of steam-pretreated softwood with a cellulases loading until 120 FPU/g cellulose do not achieve a complete hydrolysis even after 120 h. Lu et al. (2007) reached to saturation with an increase in enzyme concentration from 20 to 25 FPU/g with an optimum for cellulase concentration of 22 FPU/g substrate. These studies show that different biomass and different conditions of pretreatment and enzymatic hydrolysis reaction may lead to very different results. In our study it was observed a rising model response with the increase of cellulase concentration from 21 to 60 FPU/g dry biomass.

Acetone pretreatment of rice straw substantially improved the recovery of sugars with an overall yield of 0.458 g/g dry biomass. One of the most important advantage of using acetone as a pretreatment agent is that it will not generate any inhibitors and the acetone can be recycled. Several authors have reported that pretreatment using organosolv process will not generate or produce minimal level of inhibitors which will not affect subsequent fermentation. If pretreatment is carried out at higher temperatures, there will be a chance for formation of inhibitors. Therefore, the process temperature should preferably be limited (Huijgen et al. 2008). Fermentation experiments using separate hydrolysis and fermentation as well as simultaneous saccharification and fermentation indicates that there is no significant generation of inhibitors during the organosolv pulping (Pan et al. 2005; Weil et al. 2002).

The results indicate that the optimum conditions for acetone pretreatment of rice straw were 60% (v/v) concentration of acetone, 0.6% concentration of H_2SO_4 as catalyst, 15% (w/w) solid loading, particle size >600 μ, incubation temperature 121°C and incubation time of 90 min. The structural changes had a significant influence on the capacity of cellulase to adsorb and diffuse along the cellulose surface. The X-ray diffraction profile of native and acetone pretreated rice straw showed that the crystallinity index of native rice straw was less when compared to other pretreated samples. The optimum conditions of enzymatic hydrolysis of acetone pretreated rice straw were biomass loading (11.25% w/w), enzyme loading (60 FPU), surfactant concentration (0.05% w/w) and incubation time (60 h). The maximum reducing sugar yield at optimum conditions was 0.655 g/g. From the results it can conclude that acetone pretreatment followed by enzymatic saccharification is an effective method for the production of fermentable sugars which further can be utilized for bioethanol production.

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