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Formic Acid as a Potential Pretreatment Agent for the Conversion of Sugarcane Bagasse to Bioethanol

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Abstract In recent years, growing attention has been focused on the use of lignocellulosic biomass as a feedstock for the production of ethanol, a possible renewable alternative to fossil fuels. Several pretreatment processes have been developed for decreasing the biomass recalcitrance, but only a few of them seem to be promising. In this study, effect of various organic solvents and organic acids on the pretreatment of sugarcane bagasse was studied. Among the different organic acids and organic solvents tested, formic acid was found to be effective. Optimization of process parameters for formic acid pretreatment was carried out. The structural changes before and after pretreatment was investigated by scanning electron microscopy, X-ray diffraction (XRD), and Fourier transform infrared (FTIR) analysis. The X-ray diffraction profile showed that the degree of crystallinity was more for pretreated biomass than that of untreated. The FTIR spectra shown at the stretching of hydrogen bonds of pretreated sugarcane bagasse arose at higher number. It also revealed that the cellulose content in the solid residue increased because the hemicelluloses fraction in raw materials was released by acid hydrolytic reaction.

Keywords Sugarcane bagasse · Bioethanol · Formic acid · Pretreatment · Lignocellulosic biomass

Introduction

Sugarcane bagasse, the fibrous waste product of the sugar refining industry, is currently used as a renewable resource in the manufacture of pulp and paper products and building materials. This agro residue has been persistently receiving attention as a raw material for production of ethanol because of its high cellulose (45–50%) and hemicellulose (23–30%) content. The statistics for agricultural crops in India show that 101.3 million metric tons of

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sugarcane bagasse is generated per year in India (1), out of which 6.4 million metric tons are available in surplus for biofuel production and the rest are consumed by free market industries like paper (2). Sugarcane bagasse has the most positive net energy balance of the cellulosic feed stocks discussed today (3).

Similar to other lignocellulosic biomass, sugarcane bagasse also require some types of pretreatment to remove the lignin seal so as to access cellulose by enzymes. Pretreatment is required to alter biomass structure as well as chemical composition so that hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yields.

There are several reports on pretreatment of sugarcane bagasse using acid (4); lime (5); gamma irradiation (6); wet oxidation (7); biological (8), organosolvent (9), ionic liquids (10); and combined process (11, 12). Few reports are available on organosolvent and organic acid pretreatment of lignocellulosic biomass. Organic acids like formic acid and acetic acid have been used for pulping of woody and herbaceous plants (13). Pretreatment of corn stover by acetic acid and/or lactic acid has been reported by Xu et al. (14). Pretreatment with formic acid (15, 16), phosphoric acid–acetone (17), bioorganosolvent (18), crude glycerol (19), and acetone (9) has been reported by several authors. Organic acid pretreatment involves mainly two steps—degradation of hemicelluloses and disruptions of lignin followed by solvations of the fragments. Organic acid solvent dissociate partial hydrogen ion to accelerate delignification and hydrolysis of hemicelluloses, and it also dissolves the lignin fragments (20). Organic acid pretreatment increase accessible surface area, solubilization of hemicelluloses, solubilization of lignin, and alteration of lignin structure (11). Organosolvent process yield dry lignin, an aqueous hemicelluloses stream and a pure cellulose fraction (21).

The aim of the present study was to identify the optimum conditions for formic acid pretreatment of sugarcane bagasse, which was analyzed by estimating reducing sugar yield after enzymatic hydrolysis of the pretreated biomass. The structural features of native as well as pretreated sugarcane bagasse were investigated by scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared (FTIR) analysis.

Materials and Methods

Feed Stock

Sugarcane bagasse used in this study was received from Godawari Sugar Mills, Maharashtra, India. It was dried and milled to a size less than 1 mm. The milled samples were stored at room temperature. The composition of feed stock has a major influence on pretreatment. The compositional analysis of native sugarcane bagasse was carried out by two-stage acid hydrolysis protocol developed by National Renewable Energy Laboratory, and the result is shown in Table 1.

Pretreatment

Pretreatment was carried out in a 250-mL Erlenmeyer flask with specified solid–liquid ratio. Two grams of biomass was taken and mixed with different organic solvents like glycerol, methanol, and acetone in a 1:1 ratio and organic acids like acetic and formic acid (30% v/v) in the presence and absence of catalysts (0.2% H_2SO_4). The pretreatment was

Table 1 Composition of native sugarcane bagasse.

Component	% w/w of sugarcane bagasse
Cellulose[a]	34
Hemicellulose[b]	27
Total lignin	18
Ash	4
Extractives	17

[a] Based on total glucan.

[b] Based on total xylan and other C_5 sugars.

carried out in an autoclave at 121 °C, 15-lb pressure for 60 min. After pretreatment, the sample was neutralized by washing with tap water and kept for drying at room temperature. The pretreatment efficiency was checked by measuring hydrolysis efficiency by reducing sugar estimation. After initial screening of various organic solvents and organic acids, the most effective reagent was taken for further optimization such as reagent concentration, effect of catalysts, catalyst concentration, biomass loading, particle size, incubation temperature, and reaction time.

Optimization of Process Parameters on Pretreatment

Effect of different catalysts like mineral acids, such as HCl, H_2SO_4, and H_3PO_4, organic acids (CH_3COOH), and alkali (NaOH, KOH, and Ca $(OH)_2$) on formic acid pretreatment were carried out. Different concentrations (0.2 - 0.8%) of catalysts were added along with formic acid during pretreatment to find the effect of catalyst concentration. Biomass 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 formic acid pretreatment of sugarcane bagasse was carried out with different particle sizes (>600, 600–1000, and <1000 μm). Effect of incubation temperature on formic acid 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, 60, and 90 min) to find out the optimum time.

Characterization of Native and Pretreated Biomass

XRD Analysis

Crystallinity of sugarcane bagasse before and after pretreatment can be analyzed by XRD in a PANalytical (the Netherlands), X-pert pro diffractometer set at 40 KV, 30 mA; radiation was Cu Kα (λ = 1.54 Å), and grade ranges between 10° and 30° with a step size of 0.03°. Crystallinity of cellulose was calculated according to the empirical method proposed by Segal et al. (22) for native cellulose.

$$\mathrm{CrI}(\%) = \left[\left(I_{002} - I_{18.0^\circ}\right)/_{I002}\right] \times 100$$

where CrI is the crystalline index, I_{002} is the maximum intensity of the (002) lattice diffraction, and $I_{18.0^\circ}$ is the intensity diffraction at 18.0°, 2θ degrees.

The degree of crystallinity was calculated by Zhou et al. (23)

$$\chi_c = F_c/(F_a + F_c) \times 100\%$$

where F_c and F_a are the area of crystalline and non-crystalline regions, respectively.

The crystallite size was calculated from the Scherrer equation, with the method based on the width of the diffraction patterns. The crystallite sizes were determined by using the diffraction pattern obtained from (002) of samples.

$$D_{(hkl)} = \frac{K\lambda}{\beta_0 \cos\theta}$$

where $D_{(hkl)}$ is the size of crystallite (nm), K is the Scherrer constant (0.94), λ is the X-ray wavelength (0.15418 nm for Cu). β_o is the full width at half maximum of the reflection hkl, and 2θ is the corresponding Bragg angle (24).

FTIR Analysis

FTIR spectroscopic analysis was carried out to detect changes in functional groups that may have been caused by the pretreatment. FTIR spectrum was recorded between 4000 and 400 cm^{-1} using a Shimadzu (Japan) spectrometer with detector at 4 cm^{-1} resolution and 25 scan per sample. Discs have been prepared by mixing 3 mg of dried sample with 300 mg of KBr (spectroscopic grade) in an agate mortar. The resulting mixture was successfully pressed at 10 MPa for 3 min.

SEM Analysis

Physical changes in the native and formic acid-pretreated sugarcane bagasse were observed by SEM. Images of the surfaces of the native and pretreated sugarcane bagasse were taken at magnification 1500× using a JEOL JSM-5600 SEM. 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 to 15 KV.

Enzymatic Hydrolysis

Enzymatic saccharification of the biomass was done by incubating 2 g of pretreated sample with commercial cellulase (Zytex India Private Limited, Mumbai, India) in stoppered conical flasks, and the 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, the samples were centrifuged to remove the unhydrolyzed residue. The supernatant was used to estimate the reducing sugar analysis by 2,5-dinitrosalicylic acid method (25–27).

Ethanol Fermentation

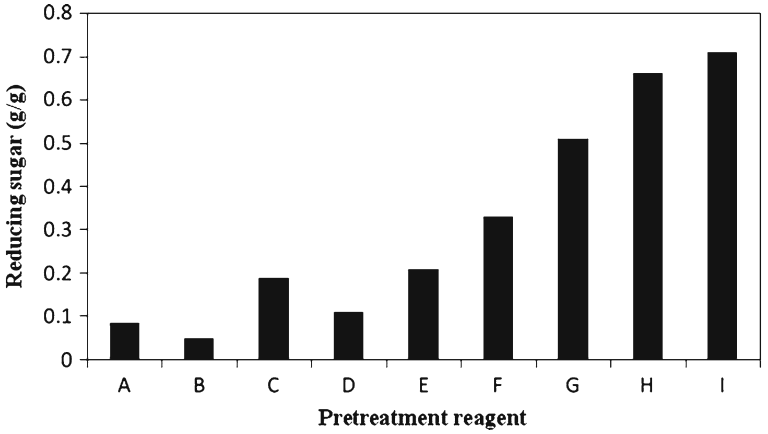
Fermentation was carried out using enzymatic hydrolysate of sugarcane bagasse. The hydrolysate was inoculated with seed culture of 12-h-old *Saccharomyces cerevisiae*. The samples were incubated at room temperature (28±2 °C) under static conditions. The supernatant was filtered using 0.4-μm filters, and the ethanol content was analyzed using gas chromatography.

Results and Discussion

Effect of Different Process Parameters on the Formic Acid Pretreatment of Sugarcane Bagasse

Among the chemicals, like glycerol, methanol, acetone, acetic acid, and formic acid, used for the pretreatment, formic acid was found to be more effective in terms of reducing sugar yield (Fig. 1). Acetic acid was also found to be effective, but the reducing sugar yield was low as compared to formic acid. Methanol, acetone, and glycerol were not found to be effective. Hence, formic acid was selected for further optimization. Pretreatment with different concentration of formic acid from 10% to 100 % (v/v) showed 60% (v/v) as optimum with reducing sugar yield of 0.74 g/g dry biomass. On increasing the concentration of formic acid beyond 60% (v/v), there was a decrease in the reducing sugar yield. This may be due to charring of the feed stock at high concentration of formic acid, which may inhibit the efficient enzymatic hydrolysis. Addition of catalyst during formic acid pretreatment increased the sugar yield. Pretreatment in the presence of 0.6% H_2SO_4 gave maximum reducing sugar (0.755 g/g) followed by 0.2% NaOH and KOH. The addition of acid catalysts permits the use of lower hydrolysis temperatures relative to the uncatalyzed systems (11). Optimization of catalyst concentration showed that the reducing sugar formation increased up to 0.6% concentration of catalyst beyond that there was a reduction in reducing sugar. Decrease in reducing sugar formation above 0.6% of catalyst may be due to generation of inhibitor compounds.

Effect of biomass loading on formic acid pretreatment showed that the reducing sugar concentration increased up to 15% (0.763 g/g) beyond that there is a reduction in the reducing sugar yield. The decrease in efficiency of pretreatment above 15% solid loading is



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

Fig. 1 Selection of pretreatment reagents. Glycerol/water (1:1) (**a**); acetone/water (1:1) (**b**); acetone/water (1:1) +0.2% H_2SO_4 (**c**); methanol/water (1:1) (**d**); methanol/water (1:1) +0.2% H_2SO_4 (**e**); acetic acid (30%) (**f**); acetic acid (30%) +0.2% H_2SO_4 (**g**); formic acid (30%) (**h**); formic acid (30%) + 0.2% H_2SO_4 (**i**)

due to decrease of accessible surface area for the pretreatment agent. Optimization of particle size on formic acid pretreatment of sugarcane bagasse showed that particle size less than 600 μm gave maximum reducing sugar (0.768 g/g). For smaller particle size, the accessible surface area is more when compared to mixed or large particle size. Thus, the pretreatment was found to be effective for smaller particle size sample.

With different pretreatment temperature tried, such as 80, 100, and 121 °C, maximum reducing sugar (0.764 g/g) was produced when the sugarcane bagasse was pretreated at 121 °C. Total reducing sugar produced was less than 50% when pretreatment was carried out at 80 °C (0.337 g/g) and 100 °C (0.347 g/g). The organosolvent process is effective only when the treatment is carried out at higher temperature because effective delignification takes place at this temperature (11). The result indicates that the hydrolysis of cellulose is strongly affected by temperature and a higher temperature can enhance the transformation ratio of cellulose. Studies on the effect of incubation time on formic acid pretreatment showed that maximum reducing sugar was produced after 90 min (0.791 g/g) of incubation. The reaction time plays a significant role in the formic acid pretreatment process.

From the study, it was found that the optimum conditions for formic acid pretreatment of sugarcane bagasse were 60% (v/v) concentration of formic acid, 0.6% concentration of H_2SO_4 as catalyst, 15% (w/w) solid loading, particle size >600 μm, incubation temperature 121 °C, and incubation time of 90 min (Table 2). The result emphasizes the capability of formic acid for pretreatment of sugarcane bagasse.

Characterization of Native and Pretreated Biomass

FTIR spectroscopy was used to investigate the changes of cellulose structures during formic acid pretreatment. Figure 2 shows the FTIR spectra of native sugarcane bagasse, sugarcane bagasse pretreated with formic acid, sugarcane bagasse pretreated with 0.6% H_2SO_4, and sugarcane bagasse pretreated with formic acid in the presence of 0.6% H_2SO_4 as catalyst. The most representative bands can be summarized as follows. The broad absorption at 3340 to 3412 cm^{-1} was related to the stretching of H-bonded OH groups, and one at 2900 cm^{-1} was related to the C–H stretching (28, 29). The bands at 1431 and 1316 cm^{-1} in the spectrum were assigned to the symmetric CH_2 bending and wagging (30); the C–H bending occurs at 1373 cm^{-1}, 1281 cm^{-1} (31). The absorption at 1201 cm^{-1} belonged to the C–O–H in-plane bending at C-6, and the bands at 1237 cm^{-1} was the bending of O–H (32). Two absorption bands at 1158 and 901 cm^{-1} arose from C–O–C stretching at the β-(1–4)

Table 2 Optimum conditions for formic acid pretreatment of sugarcane bagasse.

Parameters—optimum conditions	Reducing sugar (g/g)
Organosolvents (formic acid)	0.442
Formic acid concentration (60%)	0.735
Catalyst (H_2SO_4)	0.737
Catalyst concentration (0.6%)	0.755
Solid loading (15%)	0.763
Particle size (>600 μm)	0.768
Temperature (121 °C)	0.764
Incubation time (90 minutes)	0.791

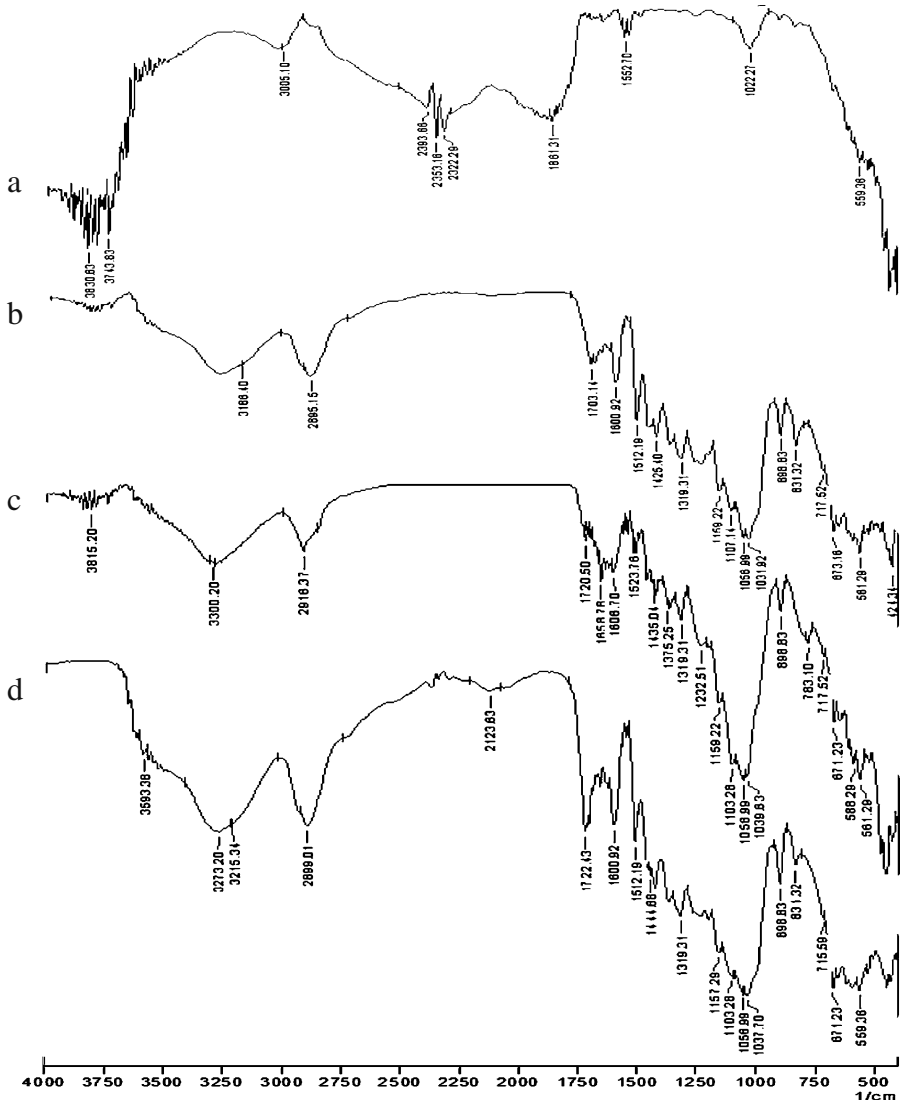


Fig. 2 FTIR spectra of native and pretreated sugarcane bagasse. Unpretreated (**a**), formic acid (60%) pretreated (**b**), 0.6% H_2SO_4 pretreated (**c**), formic acid (60%) in the presence of 0.6% H_2SO_4 (**d**)

glycosidic linkages (30). The peaks at 1061 cm^{-1} and 1033 cm^{-1} were indicative of C–O stretching at C-3, C–C stretching and C–O stretching at C-6.

The profile of the FTIR spectra was different for native and pretreated biomass. This indicates that there were structural changes of cellulose after pretreatment. Major changes were a broadening of band at 3200 to 3400 cm^{-1}, which was associated with the O–H stretching of the hydrogen bonds (33). The peak of $–CH_2$ stretching near 2900 cm^{-1} were easily distinguished from native as well as pretreated sugarcane bagasse. Bands at 1000 to 1200 cm^{-1} were related to structural features of cellulose and hemicelluloses. After pretreatment with formic acid, 0.6% H_2SO_4 and formic acid along with 0.6% H_2SO_4 as

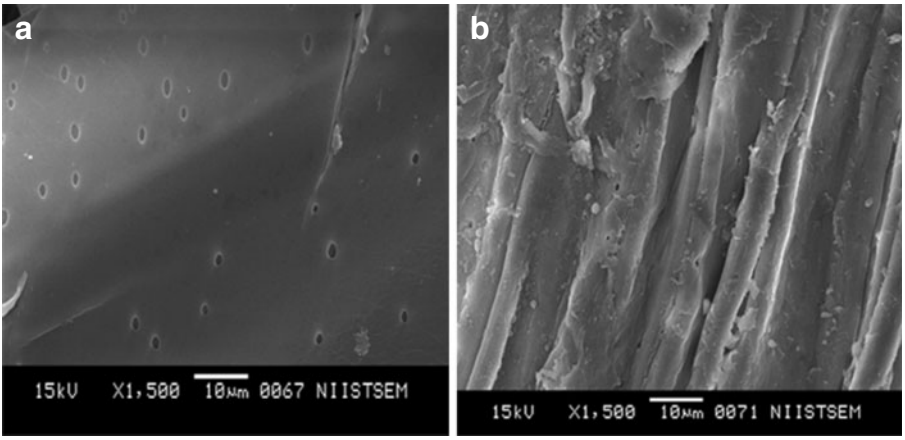


Fig. 3 Scanning electron micrographs of native and formic acid-pretreated sugarcane bagasse. **a** Native sugarcane bagasse. **b** Formic acid-pretreated sugarcane bagasse

catalyst, the absorption peaks of 1000 to 1100 were enhanced. This suggested that the cellulose content in the solid residue increased because the hemicelluloses fraction in raw materials was released by acid hydrolytic reaction (15). The FTIR spectra shown at the stretching of hydrogen bonds of pretreated sugarcane bagasse arose at higher number. This indicates that the structure of formic acid-pretreated sugarcane bagasse was looser than that of untreated ones. The peak of O–H stretching at 3500 cm^{-1} and the peak of $–CH_2$ stretching near 2900 cm^{-1} are the distinguished features of cellulose. This indicates that only intramolecular hydrogen bonds are formed when formic acid molecule penetrate into crystalline lattice of cellulose. Identical observations were earlier reported for formic acid pretreatment of bamboo (15).

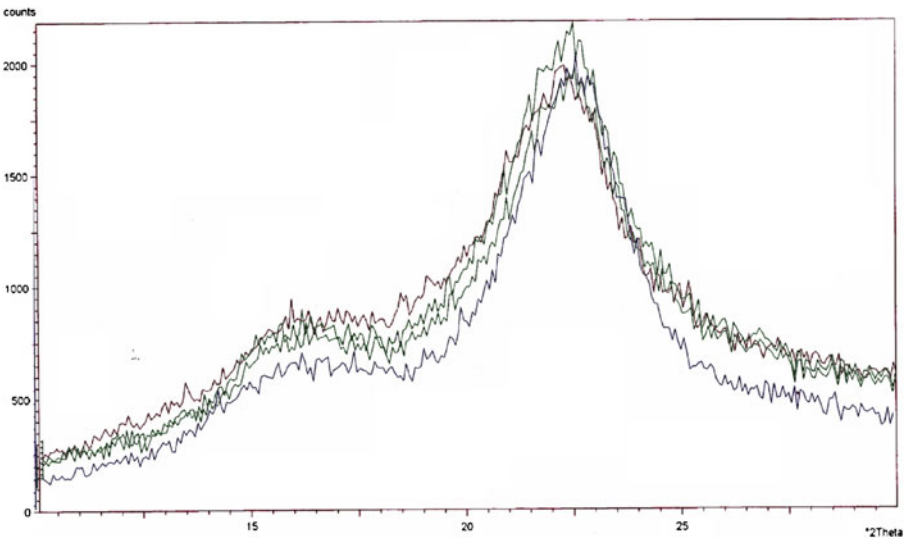


Fig. 4 X-ray diffraction pattern of native, formic acid (60%), 0.6% H_2SO_4 and formic acid (60%) pretreatment in the presence of 0.6% H_2SO_4 catalyst

Table 3 Crystallinity index, crystalline size, and degree of crystallinity of untreated and pretreated sugarcane bagasse.

Material	Crystallinity index (%)	Crystalline size (nm)	Crystalinity degree (%)
SB native	42.72	1.084	63.58
SB-FA	54.99	0.545	68.96
SB-H_2SO_4	63.16	0.726	73.08
SB-FA + H_2SO_4	67.83	0.440	75.66

SB Sugarcane bagasse
FA Formic acid

SEM observations of native and formic acid-pretreated sugarcane bagasse (Fig. 3) showed that the pretreatment induced physical changes in the biomass. The untreated sugarcane bagasses have smooth and continuous surface, whereas the formic acid-pretreated sugarcane bagasse have a rough surface. This indicates that pretreatment removed external fibers that 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 (34) and for rice straw pretreated with aqueous ammonia soaking pretreatment (35).

The X-ray diffraction profile of native and formic acid-pretreated sugarcane bagasse is shown in Fig. 4. The crystallinity index, degree of crystallinity, and crystallite size of native as well as pretreated sugarcane bagasse are shown in Table 3. The crystallinity index of native sugarcane bagasse was less (42.72%) compared to other pretreated samples. Pretreatment with formic acid in the presence of 0.6% H_2SO_4 as catalyst gave the highest crystallinity index (67.83%). The crystalline size was found to be higher in native sugarcane bagasse than the pretreated ones. However, the crystallinity index was found to be lower in native sugarcane bagasse. The change of cellulose crystallinity during organosolv pretreatment is not clear yet, but it has been found that the swelling of cellulose in organic solvent strongly depends on the species of organic solvents, solvent concentration, and temperature (36, 37). It is possible that the reason for the increase in the crystallinity index is the removal of lignin in samples with formic acid pretreatment. The crystallinity degree was more for pretreated biomass than that of untreated. This

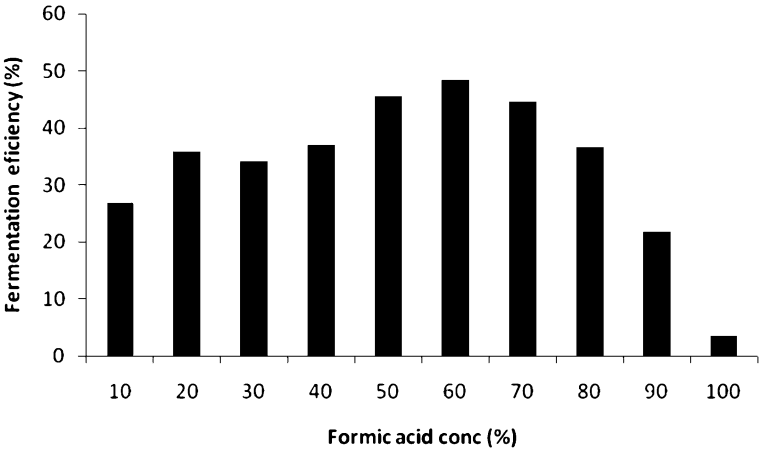


Fig. 5 Fermentation efficiency of formic acid-pretreated sugarcane bagasse

increase in degree of crystallinity indicates that the effect of formic acid on amorphous zone was more than crystalline zone. The data corresponding to the crystalline size also attested to the effect of acid solution on the amorphous zone. Formic acid is an effective delignification media in higher concentrations (80–100%) and has been widely used in paper-making industry. The study on degradation of hemicelluloses with 20% to 30% formic acid combined with acetic acid shows that a mixture of formic acid, acetic acid, and water in the ratio 30:60:10 gave the best result (38).

Ethanol Fermentation

Enzymatic hydrolysate was used as substrate for fermentation. The results of the fermentation of Formic acid-pretreated and enzymatically saccharified sugarcane bagasse are given in Fig. 5. The maximum ethanol concentration (18.45 g of ethanol) was obtained after 24 h using 60% formic acid-pretreated followed by enzymatic hydrolyzed sugarcane bagasse. The overall efficiency of the process was around 48%.

Conclusions

Formic acid pretreatment of sugarcane bagasse substantially improved the recovery of sugars with an overall yield of 0.791 g/g dry biomass. From the study, it was found that the optimum conditions for formic acid pretreatment of sugarcane bagasse were 60% (v/v) concentration of formic acid, 0.6% concentration of H_2SO_4 as catalyst, 15% (w/w) solid loading, particle size >600 μm, incubation temperature 121 °C and incubation time of 90 min. The X-ray diffraction profile of native and formic acid-pretreated sugarcane bagasse showed that the crystallinity index of native sugarcane bagasse was less compared to other pretreated samples. The crystalline size was found to be higher in native sugarcane bagasse than the pretreated ones. The FTIR spectra shown at the stretching of hydrogen bonds of pretreated sugarcane bagasse arose at higher number, which indicates that the structure of formic acid-pretreated sugarcane bagasse was looser than that of untreated. The present study showed that it could be possible to convert sugarcane bagasse to ethanol by formic acid treatment.

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