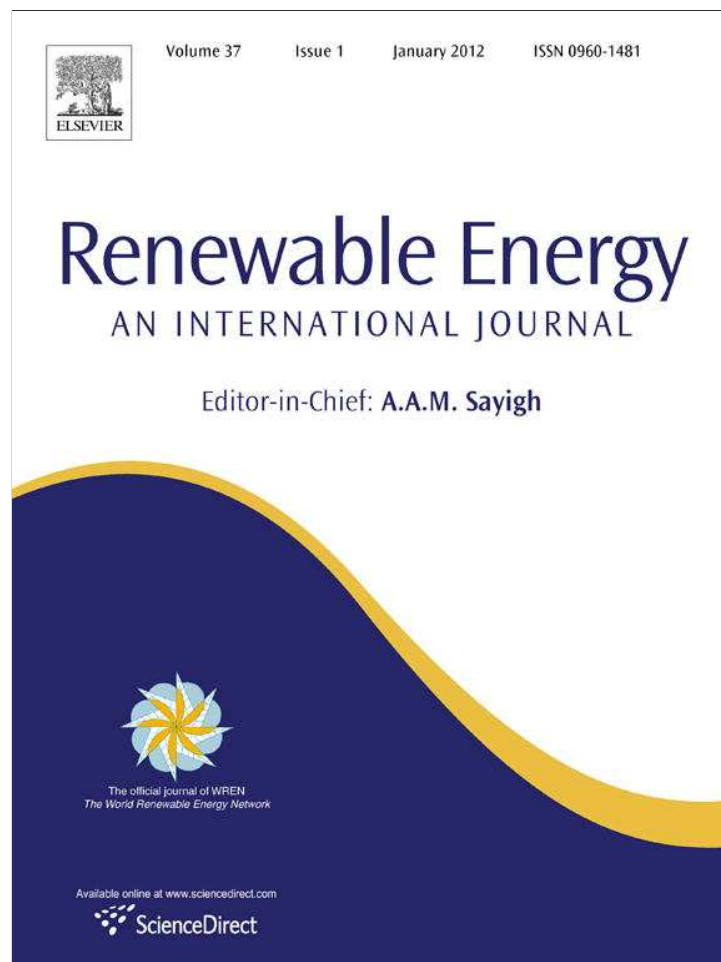


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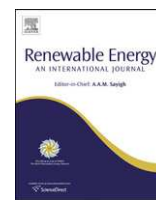
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Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse

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ABSTRACT

Production of bioethanol from lignocellulosic biomass is very challenging due to the heterogenous nature of the feedstock. An efficient pretreatment is necessary for maximizing the enzymatic hydrolysis efficiency and this in turn helps in reducing the total process economy. Conventional pretreatment using acid or alkali at high temperature and pressure is limited due to its high energy input. So there is a need for alternative heating techniques which not only reduce the energy input, but increases the total process efficiency. Microwave pretreatment may be a good alternative as it can reduce the pretreatment time at higher temperature. In the present study, a comparison of three types of microwave pretreatment such as microwave-acid, microwave-alkali and combined microwave-alkali-acid were tried using sugarcane bagasse as the lignocellulosic biomass. The enzymatic saccharification efficiency and lignin removal in each pretreatment method has been evaluated. Microwave treatment of sugarcane bagasse with 1% NaOH at 600 W for 4 min followed by enzymatic hydrolysis gave reducing sugar yield of 0.665 g/g dry biomass, while combined microwave-alkali-acid treatment with 1% NaOH followed by 1% sulfuric acid, the reducing sugar yield increased to 0.83 g/g dry biomass. Microwave-alkali treatment at 450 W for 5 min resulted almost 90% of lignin removal from the bagasse. The effect of pretreatment has been also evaluated by XRD, SEM and FTIR analysis. It was found that combined microwave-alkali-acid treatment for short duration enhanced the fermentable sugar yield.

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1. Introduction

Lignocellulosic biomass, the most abundant and lowest cost biomass in the world, can be used as alternative raw materials for production of fuel ethanol [1]. There occur several challenges in converting this feedstock into fuel ethanol mainly because of its heterogenous nature. The digestibility of cellulose present in lignocellulosic biomass is hindered by many physico-chemical, structural and compositional factors [2]. The cellulose is present as long chain polymers that are packed into micro fibrils. These micro fibrils are covered by hemicellulose and lignin. This cover of lignin and hemicellulose protects cellulose from enzymatic hydrolysis [3].

Sugarcane bagasse, the solid residue left after extraction of sugarcane juice, is one of the major lignocellulosic plant residues in many tropical countries [4]. However, like other lignocellulosic substrates, the use of bagasse as feedstock for biorefinery has been

limited because the chemical structure and high pentose fraction of bagasse makes it recalcitrant to enzymatic hydrolysis unless it is pretreated to a more accessible form. Pretreatment is one of the most expensive and least technologically mature steps in the process of converting biomass to fermentable sugars [5]. Costs are due to the use of steam and chemical products and the need for expensive corrosion resistant reactors [6]. Among different pretreatment methods, acid pretreatment is known to separate pentoses and hexoses; while alkali pretreatment is known to separate lignin from lignocellulosic biomass. Most of these conventional pretreatment methods produce compounds that might seriously inhibit the subsequent fermentation.

In order to hydrolyze lignocellulosic biomass with enzymes successfully, it is very important to use a suitable pretreatment, because crystallinity of cellulose, degree of polymerization, moisture content, available surface area, and lignin content are factors that hinder the attack of enzymes [7]. An effective pretreatment is characterized by several criteria. It should avoid the need for reducing the size of biomass particles, preserve the pentose (hemicellulose) fractions, limit formation of degradation products that inhibit growth of fermentative microorganism, minimizes

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energy demands and limit costs. Also, the pretreatment agent should have low cost and capable of recycling inexpensively [8].

Microwave is found to be alternative method for conventional heating [9] and it has been widely used in many areas because of its high heating efficiency and easy operation. Advantages of microwave-based technologies include reduction of process energy requirements, uniform and selective processing, and the ability to start and stop the process instantaneously [10,11]. Some studies have shown that microwave irradiation could change the ultrastructure of cellulose [12], degrade lignin and hemicellulose in lignocellulosic materials, and increase the enzymatic susceptibility of lignocellulosic materials [13]. It has been reported that microwave pretreatment in the presence of water could enhance the enzymatic hydrolysis of lignocellulosic materials [13,14]. Microwave pretreatment is generally carried out at elevated temperature (>160 °C) and, in essence, it is an acid-catalyzed autohydrolysis of lignocellulosic materials. Because acidity increases with increasing temperature during the microwave pretreatment, high temperature become essential [15]. Microwave irradiation could be easily combined with chemical reaction and can accelerate the chemical reaction rate [16]. Combination microwave treatment with either acid or alkali or combined acid/alkali might be an alternative for pretreatment of lignocellulosic materials.

The objective of the present study is to evaluate the microwave pretreatment efficiency of sugarcane bagasse. Different microwave treatment like, the microwave-acid (MA), microwave-alkali (MAL) and microwave-alkali followed by acid (MAA) were performed before enzymatic hydrolysis to find out the fermentable sugar production from sugarcane bagasse. The effect of microwave irradiation power and treatment time on the enzymatic hydrolysis and the optimization of the hydrolysis were also reported. The Scanning Electron Microscopic (SEM) analysis and X-ray diffraction (XRD) pattern of untreated and pretreated sugarcane bagasse were evaluated to find out the structural differences affected during microwave irradiation.

2. Materials and methods

2.1. 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 compositional analysis of native sugarcane bagasse was carried out by two stage acid hydrolysis protocol developed by National Renewable Energy Laboratory [17], and the result is shown in Table 1.

2.2. Microwave pretreatment

Microwave pretreatment was carried out using a domestic microwave oven (Samsung, CE2877 N, Korea) with an operating

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 C₅ sugars.

frequency of 2450 MHz and the power could be set at 100 W, 180 W, 300 W, 450 W, 600 W and 850 W. All experiments were carried out three times, and the given numbers are the mean values.

2.2.1. Microwave-alkali pretreatment

Microwave-alkali pretreatment was carried out using 1% NaOH as pretreatment reagent. 10% biomass was loaded to this alkaline solution in a stoppered flask and subjected to microwave pretreatment at varying power consumption 850 W, 600 W, 450 W, 300 W, 180 W and 100 W for different residence time varying from 1 min to 30 min. After pretreatment, biomass was thoroughly washed with water till pH 6.0 and dried in air. Pretreated liquor was analyzed for glucose, pentose, total reducing sugar and lignin. The dried solid residue was used for enzymatic hydrolysis and compositional analysis.

2.2.2. Microwave-acid pretreatment

For acid pretreatment, 1% H₂SO₄ was used. 10% solid loading was maintained and pretreatment was done as in the case of microwave-alkali. Pretreated biomass was washed with water to bring to pH 6.0. The air dried pretreated residue and pretreated liquor was used for further studies.

2.2.3. Microwave-alkali followed by acid pretreatment

Biomass was subjected to alkali pretreatment as mentioned above. Alkali pretreated biomass was washed with water and air dried. Dry biomass was once again subjected to acid pretreatment, as mentioned above.

2.3. Analytical methods

Reducing sugar estimation was done by Dinitrosalicylic acid method [18]. Pentose sugar estimation was carried out using Orcinol method [19]. The compositional analysis of pretreated sugarcane bagasse was carried out by two stage acid hydrolysis protocol developed by National Renewable Energy Laboratory [17]. Acid soluble and acid insoluble lignin was estimated as per the NREL protocol [20,21].

2.4. Enzymatic hydrolysis

The enzymatic saccharification of MA, MAL and MAA pretreated biomass was carried out using commercial cellulase from Zytex (Zytex India Private Limited, Mumbai, India). Two grams of pretreated feedstock was incubated with enzyme (30 Filter Paper Unit/g biomass) in stoppered conical flasks. 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

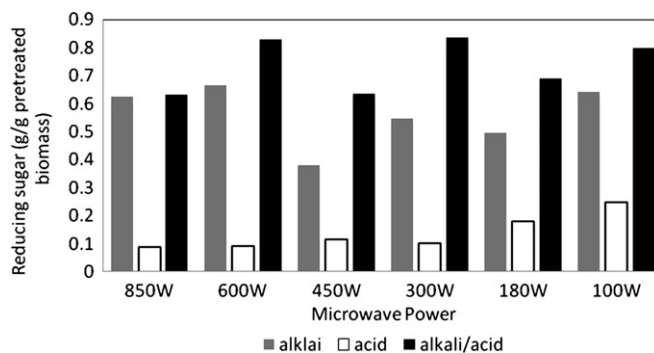


Fig. 1. Maximum reducing sugar yield for MA, MAL and MAA pretreatment at various microwave power.

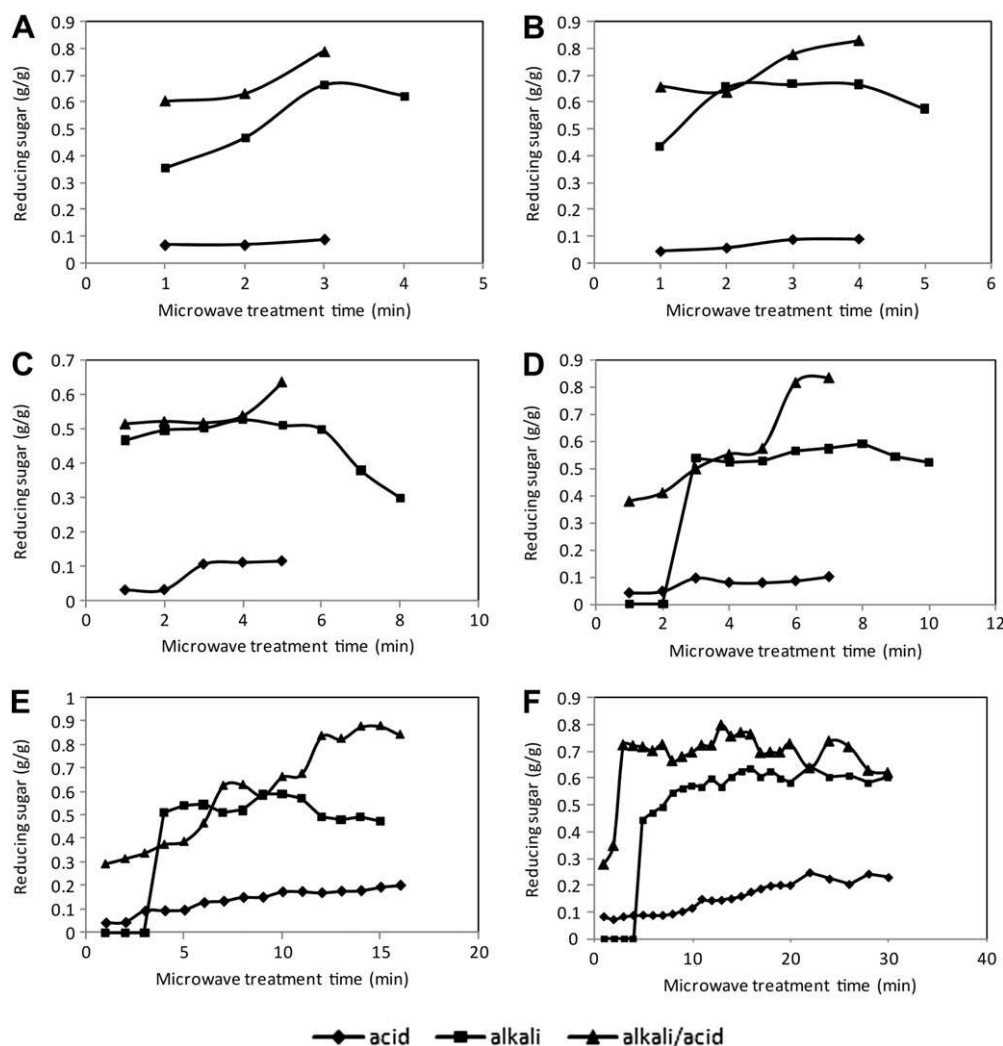


Fig. 2. Reducing sugar yield by microwave pretreatment at various microwave power and pretreatment time. A: 850 W; B: 600 W; C: 450 W; D: 300 W; E: 180 W; F: 100 W.

unhydrolyzed residue. The supernatant was used to estimate the reducing sugar analysis by 2, 5 dinitrosalicylic acid method [18,22,23]. The result expressed in g/g pretreated biomass, taken into consideration of material loss during each pretreatment stage.

2.5. Characterization of native and pretreated biomass

2.5.1. XRD analysis

Crystallinity of sugarcane bagasse before and after pretreatment was analyzed by XRD in a PANalytical (Netherlands), X-pert pro diffractometer set at 40 kV, 30 mA; radiation was Cu K α ($\lambda = 1.54 \text{ \AA}$), and grade range between 10 and 30 $^\circ$ with a step size of 0.03 $^\circ$. Crystallinity of cellulose was calculated according to the empirical method proposed by Segar et al. [24] for native cellulose

$$\text{CrI}(\%) = [(I_{002} - I_{18.0^\circ})/I_{002}] \times 100$$

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 $^\circ$, 2θ degrees.

The degree of crystallinity was calculated by Zhou et al. [25].

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

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.

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

Where $D_{(\text{hkl})}$ 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_0 is the full-width at half-maximum of the reflection hkl, and 2θ is the corresponding Bragg angle [26].

2.5.2. FTIR analysis

Fourier Transform Infrared 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 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.

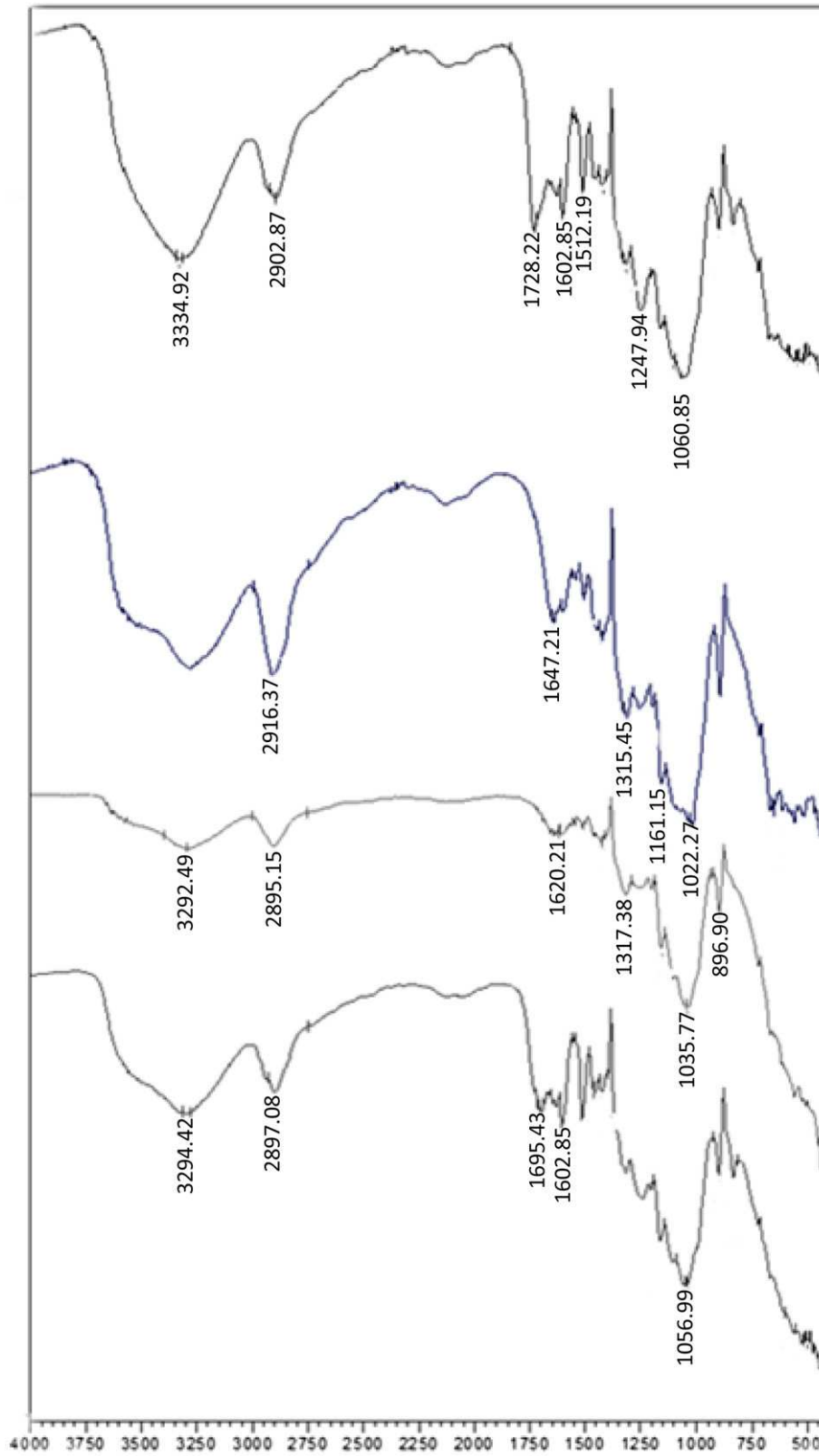


Fig. 3. FTIR spectra of native, MA, MAL and MAA treated sugarcane bagasse.

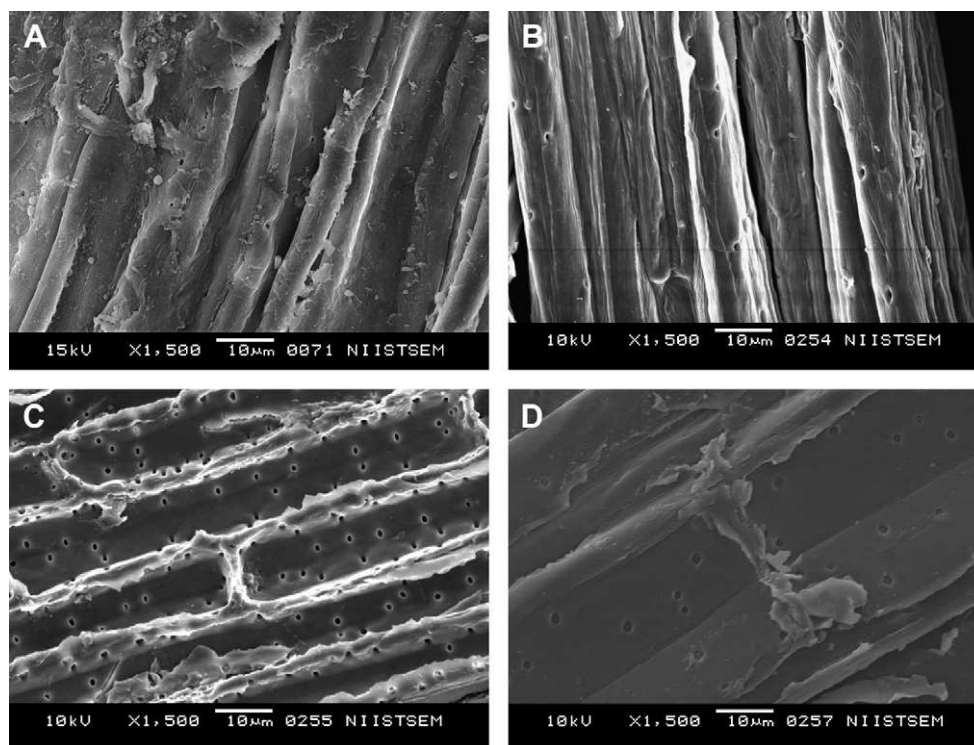


Fig. 4. Scanning electron microscopic images of MA, MAL and MAA pretreated sugarcane bagasse. A: Native sugarcane bagasse (without any pretreatment); B: MA pretreatment; C: MAL pretreatment; D: MAA pretreatment.

2.5.3. IR crystallinity index

The IR crystallinity index of cellulose was evaluated as the intensity ratio between IR absorptions at 1427 and 895 cm^{-1} which are assigned to CH_2 bending mode and deformation of anomeric CH respectively. To distinguish between cellulose I_α and I_β crystalline forms, the characteristic IR bands, 750 cm^{-1} for I_α and 710 cm^{-1} for I_β were analyzed [27].

2.5.4. SEM analysis

Physical changes in the native and formic acid pretreated sugarcane bagasse were observed by scanning electron microscopy (SEM). Images of the surfaces of the native and microwave pretreated sugarcane bagasse were taken at magnification 1500X using a JEOL JSM-5600 scanning electron microscope (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 10–15 kV.

3. Results

3.1. Effect microwave pretreatment on sugar yield

In the present study, we compared the structural changes and enzymatic hydrolysis efficiency of sugarcane bagasse with MA, MAL and MAA pretreatment. With 1% sulfuric acid pretreatment at 600 W for 4 min followed by enzymatic hydrolysis, the reducing sugar yield was 0.091 g/g pretreated biomass, while with 1% NaOH treatment at the same condition resulted 0.665 g/g pretreated biomass. But, when the 1% NaOH pretreated bagasse was treated with 1% sulfuric acid followed by enzymatic hydrolysis, the reducing sugar yield was increased from 0.665 g/g to 0.83 g/g pretreated bagasse. Maximum reducing sugar yield was obtained at 3 min treatment at 850 W, 4 min treatment at 600 W, 5 min at 450 W, 7 min at 300 W, 15 min at 180 W and 24 min at 100 W. Fig. 1

shows the maximum reducing sugar yield for MA, MAL and MAA pretreatment at various microwave power. For microwave-acid treatment, it was observed that by increasing the microwave power, there is a decrease in reducing sugar yield. Highest reducing sugar was recorded at 100 W microwave power. For MAL and MAA treatment, highest reducing sugar was yielded at 600 W power. It was found that by increasing the microwave power, the treatment time can be reduced. Since the exact temperature and pressure of pretreatment is not possible to directly measure in microwave oven, we expressed the pretreatment in terms of microwave power output that can be set on the instrument. Fig. 2 shows the effect of treatment time at various microwave irradiation power.

3.2. Characterization of microwave pretreated sugarcane bagasse

FTIR spectroscopy was used to investigate the changes of cellulose structures during microwave pretreatment. Fig. 3 shows the FTIR spectra of native sugarcane bagasse, sugarcane bagasse pretreated with MA, MAL and MAA. The most representative bands can be summarized as follows. The broad absorption at $3340\text{--}3412\text{ cm}^{-1}$ related to the stretching of H-bonded OH groups, and one at 2900 cm^{-1} to the C–H stretching [28,29]. The bands at 1431 cm^{-1} 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 cm^{-1} and 901 cm^{-1} arose from C–O–C stretching at the β -(1–4) 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 microwave pretreated sugarcane bagasse. This indicates that there were structural changes of cellulose after pretreatment. Major changes were broadening of band at $3200\text{--}3400\text{ cm}^{-1}$ which was

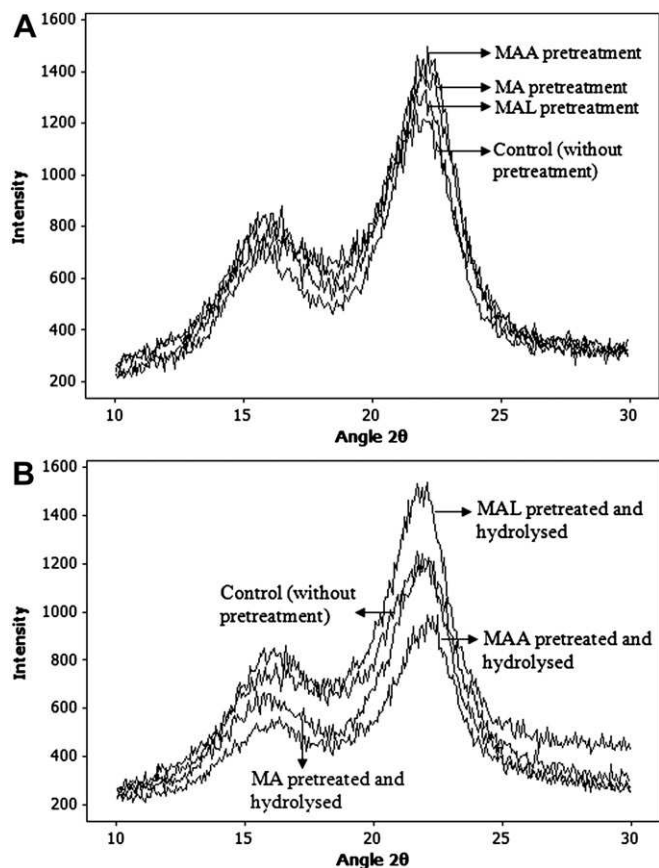


Fig. 5. X-ray diffraction pattern of microwave treated sugarcane bagasse. A: Different pretreated samples; B: Samples after enzymatic hydrolysis.

Table 2

Crystallinity index, crystalline size and crystallinity degree of microwave treated sugarcane bagasse.

Material	Crystallinity Index (%)	Crystalline size (nm)	Crystallinity degree (%)
Control (without pretreatment)	53.44	0.249	68.23
MA	58.79	0.116	70.82
MA followed by hydrolysis	62.26	0.202	72.60
MAL	65.29	0.173	74.23
MAL followed by hydrolysis	58.58	0.072	70.71
MAA	65.55	0.130	74.38
MAA followed by hydrolysis	59.05	0.149	70.95

Crystallinity index, crystalline size and crystallinity degree of microwave treated sugarcane bagasse.

of crystallinity and crystallite size of native as well as pretreated sugarcane bagasse is shown in Table 2. The crystallinity index of native sugarcane bagasse was less (53.44%) compared to other pretreated samples. MAA pretreatment gave the highest crystallinity index (65.55%). The crystalline size was found to be higher in native sugarcane bagasse than the pretreated ones. It is possible that the removal of lignin may be the reason for increased crystallinity index of microwave treated biomass. Compared to MA, MAL pretreated samples showed high crystallinity index as this indicate the removal of lignin by alkali. The crystallinity degree was more for pretreated biomass than that of untreated. This increase in degree of crystallinity indicates that the effect of microwave treatment on amorphous zone was more than crystalline zone. Degree of crystallinity for MAL pretreated sample (74.23%) and MAA pretreated samples (74.38) were the highest. In these two types of samples after enzymatic hydrolysis degree of crystallinity decreased. The data corresponding to the crystalline size also showed the effect of microwave treatment on the amorphous zone.

4. Discussion

Selection of suitable pretreatment reagent is an important factor that affects the efficiency of enzymatic hydrolysis. Generally alkali pretreatment removes lignin from the lignocellulosic biomass, leaving cellulose and hemicelluloses fraction in the solid residue. Acid pretreatment removes hemicelluloses that can be separated from the solid residue in soluble form. Each of these pretreatment methods has some limitations. Alkali pretreatment removes most of the lignin from biomass and the remaining solid residue on enzymatic hydrolysis results in the production of a mixture of C6 and C5 sugars, which require complex co-fermentation methods for complete sugar utilization. With acid pretreatment, the lignin present in the solid fraction inhibits enzymatic hydrolysis which in turn lowers the hydrolysis efficiency.

Microwave irradiation power and treatment time are two main factors that affect the microwave pretreatment. A series of experiment was carried out to investigate the effect of microwave irradiation power and treatment time on hydrolysis. At 850 W with 15% solid loading, charring of the samples occurred at 3 min of treatment for all the three methods of microwave treatment, while with 100 W power charring not occurred even at 30 min of treatment time. With 180 W, charring occurred at 16 min. When alkali treated sugarcane bagasse was treated with acid (2nd stage hydrolysis) at 850 W for 1–3 min, the highest reducing sugar yield was for 3 min (0.79 g/g). The highest reducing sugar yield with all these pretreatment was 0.879 g/g which occurred by combined treatment at 180 W for 15 min. With MA, maximum reducing sugar

associated with the O–H stretching of the hydrogen bonds [33]. The peak of $-\text{CH}_2$ stretching near 2900 cm^{-1} were easily distinguished from native as well as pretreated sugarcane bagasse. Bands at $1000\text{--}1200\text{ cm}^{-1}$ were related to structural features of cellulose and hemicelluloses. The enhancement of absorption peaks at $1000\text{--}1100\text{ cm}^{-1}$ after pretreatment indicate the increase in cellulose content in the solid residue [34]. The FTIR spectra showed the stretching of hydrogen bonds of pretreated sugarcane bagasse arose at higher number. This indicates that the structure of microwave pretreated sugarcane bagasse was looser than that of untreated ones. The peak of O–H stretching at 3300 cm^{-1} and the peak of $-\text{CH}_2$ stretching near 2900 cm^{-1} are the distinguished features of cellulose. It has been reported that microwave irradiation enhances the saponification of intermolecular ester bonds cross-linking xylan hemicelluloses and other components such as lignin and other hemicelluloses [35].

SEM observations of untreated and microwave pretreated sugarcane bagasse (Fig. 4) showed that the pretreatment induced physical changes in the biomass. The untreated sugarcane bagasse has smooth and continuous surface whereas the microwave pretreated sugarcane bagasse has a rough surface. MAL treated biomass showed a sieve like structure. 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 [36] and for rice straw pretreated with aqueous ammonia soaking pretreatment [37].

The X-ray diffraction profile of native and microwave pretreated sugarcane bagasse is shown in Fig. 5. The crystallinity index, degree

Table 3
Composition of solid residue after MAL pretreatment.

Microwave pretreatment power(W)/ treatment time (minutes)	Cellulose ^a (%)	Hemicellulose ^b (%)	Lignin (%)
850/2	46.7	26.4	7.8
600/3	66.6	26.5	4.9
600/4	66.5	26.3	3.9
450/4	65.3	26.5	0.6
450/5	50.3	26.4	1.8
300/7	57.3	26.8	3.9
180/8	52.2	26.5	2.9
100/12	56.4	18.0	5.3
100/17	49.3	10.9	7.4

^a Based on total glucan.

^b Based on total xylan and other C₅ sugars.

yield (0.249 g/g) was noted at 100 W for 22 min treatment time, while with MAL, maximum reducing sugar yield was at 600 W for 3 min. The study shows that for every microwave irradiation power, an optimal treatment time exists according to hydrolysis yield.

Efficiency of lignin removal by MAL pretreatment was examined by lignin estimation after pretreatment. It was found that microwave treatment at 450 W for 4 min removed about 96% lignin from sugarcane bagasse. When the bagasse was treated with 850 W for 2 min, 57% of lignin was removed, but at this high temperature, charring of the biomass occurred above 3 min. As the pretreatment temperature is lowered, time required for efficient lignin removal also increased. The compositional analysis of the solid residue shows that xylan fraction was almost constant for all the pretreatment condition except for microwave power of 100 W for residence time higher than 12 min. The cellulose percentage in the entire pretreated residue was increased. Residue with maximum cellulose content (66%) was obtained at the treatment condition of 600 W and 450 W power. At 450 W for 4 min residence time, maximum lignin removal was observed (96%) and the solid residue contained 65.3% cellulose and 26.5% hemicellulose. Table 3 shows the composition of solid residues at various pretreatment powers.

The crystallinity index of cellulose has been used for more than five decades to interpret changes in cellulose structure after various pretreatments [38]. It has thought to play an important role in enzymatic hydrolysis [39]. Cellulose structure is divided into two regions, an amorphous region that is easy for enzymes to digest and a crystalline region that is difficult to digest. This provides a ready explanation of observed cellulose digestion kinetics, where enzymes more rapidly digest the 'easy and presumed amorphous' material before more slowly digesting the more difficult crystalline cellulose.

The cellulose crystallinity was also investigated using different IR crystallinity ratios reported in the literature [40–42]. Three different IR ratios were calculated for different microwave pretreated samples (Table 4). These different peak height and peak area ratios were measured. The IR ratio A_{1370}/A_{670} was used by Ute et al. [40] to study the conversion of cellulose I to cellulose II during alkaline treatment. The value is constant for MA pretreated and untreated sugarcane bagasse (1.45), while for MAL and MAA, the

Table 4
IR crystallinity ratio of microwave treated sugarcane bagasse.

Sample	IR crystallinity ratio		
	A_{1370}/A_{670}	A_{1429}/A_{897}	A_{1372}/A_{2900}
Control	1.45	1.12	0.99
MA	1.45	1.19	0.98
MAL	1.13	1.04	1.03
MAA	1.27	1.09	1.00

values were decreased. This indicates the change in crystallinity after alkaline treatment and acid treatment has no effect. According to Åkerholm et al. [43], this IR ratio does not measure the cellulose crystallinity, but it indicates the cellulose I/cellulose II ratio. Hence the present study shows that by MAL and MAA pretreatment the cellulose II amount is increasing and MA treatment will not change the ratio of cellulose I to cellulose II. The IR ratios A_{1429}/A_{897} and A_{1372}/A_{2900} are ratios between different peak heights, 1429–897 and 1372–2900 cm^{-1} , respectively. The A_{1429}/A_{897} ratio for different microwave treated samples ranged from 1.19 to 1.04. This value for MA was highest (1.19) and for MAL it was 1.04. The IR ratio A_{1372}/A_{2900} was compared to be higher for MAA and MAL.

Microwave irradiation has been widely used in many areas because of its high heating efficiency and easy operation. Some studies have shown that microwave irradiation could change the ultrastructure of cellulose [12], degrade lignin and hemicellulose in lignocellulosic materials, and increase the enzymatic susceptibility of lignocellulosic materials [13]. Azuma et al. [13] and Ooshima et al. [14] reported that microwave pretreatment in the presence of water could enhance the enzymatic hydrolysis of lignocellulosic materials. Kitchaiya et al. [15] also reported that microwave pretreatment of rice straw in a glycerine medium with small amounts of water had similar results. Microwave irradiation could be easily combined with chemical reaction and, in some cases, accelerate the chemical reaction rate [16]. The study shows that microwave pretreatment of lignocellulosic biomass can reduce the pretreatment time. But, microwave pretreatment at high temperature may sometimes decompose the useful components. This will limit the application of microwave pretreatment to some extent.

5. Conclusions

Microwave pretreatment substantially improved the recovery of fermentable sugars from sugarcane bagasse. With microwave-alkali pretreatment an overall yield of 0.665 g/g dry biomass fermentable sugars were released. With microwave-acid, the reducing sugar yield was 0.249 g/g dry biomass at microwave power of 100 W with 30 min pretreatment time. Microwave-alkali followed by acid pretreatment gave an overall reducing sugar yield of 0.83 g/g dry biomass. The X-ray diffraction profile of native and microwave 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 showed the stretching of hydrogen bonds of pretreated sugarcane bagasse arose at higher number which indicates the structural changes during microwave treatment.

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