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Research paper

Efficient sugar production from sugarcane bagasse by microwave assisted acid and alkali pretreatment

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ABSTRACT

Sugarcane bagasse represents one of the best potential feedstocks for the production of second generation bioethanol. The most efficient method to produce fermentable sugars is by enzymatic hydrolysis, assisted by thermochemical pretreatments. Previous research was focused on conventional heating pretreatment and the pretreated biomass residue characteristics. In this work, microwave energy is applied to facilitate sodium hydroxide (NaOH) and sulphuric acid (H_2SO_4) pretreatments on sugarcane bagasse and the efficiency of sugar production was evaluated on the soluble sugars released during pretreatment. The results show that microwave assisted pretreatment was more efficient than conventional heating pretreatment and it gave rise to 4 times higher reducing sugar release by using 5.7 times less pretreatment time. It is highlighted that enrichment of xylose and glucose can be tuned by changing pretreatment media ($NaOH/H_2SO_4$) and holding time. SEM study shows significant delignification effect of NaOH pretreatment, suggesting a possible improved enzymatic hydrolysis process. However, severe acid conditions should be avoided (long holding time or high acid concentration) under microwave heating conditions. It led to biomass carbonization, reducing sugar production and forming 'humins'. Overall, in comparison with conventional pretreatment, microwave assisted pretreatment removed significant amount of hemicellulose and lignin and led to high amount of sugar production during pretreatment process, suggesting microwave heating pretreatment is an effective and efficient pretreatment method.

1. Introduction

In recent years, there has been increasing interest in the use of agricultural waste, as feedstock for second generation biofuels production. Sugarcane is the major bioenergy crop in Brazil, where it has been successfully used for the production of bioethanol [1]. The development of new technologies for lignocellulosic ethanol production from sugarcane bagasse is of special interest, since it would increase the efficiency of ethanol production without expanding the agricultural areas, avoiding the current conflict produced by change in land use to meet growing energy demands [1].

Lignocellulosic biomass is a recalcitrant material, composed of cellulose, hemicellulose and lignin, organised in a network of polymers that evolved to develop recalcitrance against enzyme hydrolysis produced by microorganism in nature [2]. To release the sugars locked in biomass in industrial processes, various pretreatments have been proposed and trialled at laboratory and pilot scale. Among them are steam explosion [3], ammonia fibre explosion [4], hot water [5], supercritical CO_2 [6], biological [4] and acid or alkaline pretreatments [7,8] and others. An effective pretreatment must meet the following requirements: 1. Improve sugar production or the ability to subsequently form sugars by hydrolysis; 2. Avoid degradation or carbohydrate loss; 3. Avoid by-product formation, such as inhibitory chemicals to the subsequent hydrolysis and fermentation processes; 4. Be cost effective [4]. Pretreatments may alter the structure of cellulosic biomass to make it more accessible for enzymes, as well as decrease the degree of polymerization and cellulose crystallinity. Additionally, it can selectively remove hemicellulose and lignin from the lignocellulosic matrix [3,9]. Compared to the other pretreatment methodologies listed above. Acid and alkaline pretreatments are considered effective, which explains their extensive use in most cases during biomass pretreatment [2,9–17]. For instance, Marasabessy et al. reported that, with 30 min 0.9% (w/v) H_2SO_4 pretreatment at 178 °C before enzymatic hydrolysis, 100% of all pentoses present in *Atropha curcas* fruit hull were released (71% yield and 29% degradation to furfural) after 24 h enzymatic hydrolysis. Meanwhile, 83% of the hexoses (78% yield and 5% degradation to 5-hydroxymethylfurfural) is achieved [18]. Zhu et al. studied microwave assisted dilute NaOH (1%) pretreatment of wheat straw, and the weight loss of hemicellulose and lignin after pretreatment is 76–84.4% and 81–86% respectively [17].

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There is a growing interest in using microwaves as a tool to bring about various chemical processes, both in synthetic chemistry and in the treatment of lignocellulosic biomass [19,20]. Microwaves are electromagnetic waves, whose frequencies lie between 300 MHz and 300 GHz by general definition. In the case of conventional heating, heat is transferred by means of convection, conduction, and radiation from the surfaces to the inner areas of the materials. Thus, while in conventional heating, the energy transfer is the result of thermal gradients, microwave energy is transferred directly to the material being heated by electromagnetic radiation [21]. Under the influence of the incident radiation, a substance possessing dipole moments and sufficient mobility will align itself to the electromagnetic field by rotation. Further, the rotation will give rise to frictions between neighbouring molecules and increase the temperature. Therefore, microwave heating is more direct, rapid and uniform than conventional heating [21]. Significant research has been carried out in the field of microwave assisted pretreatment, with different feedstock including sugarcane bagasse [2], switchgrass [22], wheat straw [17], and so on. Hu and Wen found that the total sugar amount (glucose and xylose) from pretreatment and hydrolysis of switchgrass was 58.5% of the maximum sugars in the material [22]. Nikolic et al. studied microwave pretreatment for corn starch and the results showed that the glucose concentration in pretreatment liquor was increased by 8.48% compared to untreated control sample and the percentage of theoretical ethanol yield was 92.27% after 44 h of the simultaneous saccharification and fermentation (SSF) [23]. Lu et al. reported that the glucose yield of rape straw from enzymatic hydrolysis was enhanced by 56.2% (11.5% for raw rape straw) after microwave pretreatment [24]. Chen et al. studied the effect of microwave assisted sulphuric acid pretreatment for sugarcane bagasse, and revealed that at 190 ℃ the fragmentation of particles become very pronounced and almost all hemicellulose was removed and the crystalline structure of cellulose disappeared [25]. These studies show that microwave combined with thermochemical processes could be a promising pretreatment method.

As it is well known, conventional heating systems have the disadvantage of being time consuming and also lead to significant sugar degradation [26–30]. Microwave heating, on the contrary, is faster, and is gaining growing attention, being applied for pyrolytic and hydrolytic activation [19,20,31,32]. Most of the current microwave assistance studies were concentrated on pyrolytic activation of lignocellulose material, but hydrolytic activation has been less studied. Besides, previous research concentrated on sugar production from enzymatic hydrolysis of biomass after pretreatment. In our study, rapid microwave heating is used to enhance the pretreatment process and improve the sugar production. Only a few results have been reported on sugar production during the pretreatment process under microwave conditions. In the present work, sugarcane bagasse is processed under a range of microwave assisted acid (H_2SO_4) and alkaline (NaOH) conditions. The sugar production and the chemical changes produced during pretreatments were evaluated in order to explain the unique microwave effects on biomass composition and structure, while informing the optimisation of sugar production. The current study is very useful for the comprehension of the microwave effect on the sugar production enhancement from lignocellulosic material during pretreatment and the unique microwave effects on biomass composition and structure.

2. Material and method

2.1. Raw material

Sugarcane bagasse was provided by the Cosan Mill (Ibaté, SP, Brazil). The raw material previously washed and roughly ground was dried in a convection oven at 60 °C for 24 h and further ground by knife milling into small pieces (625 μm × 188 μm avg.). The biomass compositions of raw sugarcane bagasse are cellulose (25 ± 2.7%), hemicellulose (48 ± 2.3%), lignin (31 ± 1.2%) and ash (0.83 ± 0.03%).

2.2. Microwave pretreatment and conventional thermochemical pretreatment method

Microwave acid digestion vessel (Vessel 4781, Parr Instruments) was used as a reactor and a domestic microwave oven (SAMSUNG Model CM1629A; Dimensions: W 464 × D 557 × H 368 mm; power output: 1600 W) was used as a microwave source. A total weight of 0.2 g of sugarcane bagasse was immersed in 8 ml 0.2 M or 0.4 M H_2SO_4 or NaOH. The pretreatment was carried out at 320 W for various holding time (3–10 min), resulting in more heat being transferred to the sample as time increased. The internal temperature of the pressure vessel was estimated by using an indirect method and found it to be 170 °C ± 5 °C when the microwave heating time was 3–5 min [33]. Pretreatment temperature was not measured. The biomass solid residue resulting from the pretreatments was separated from the liquor by centrifugation, and then rinsed with ethanol (3 × 10 ml), and dried in oven at 50 °C for 24 h. Both the liquor and the solid residues were analysed.

Conventional thermo-chemical pretreatment is conducted in the acid digestion vessel (Vessel 4745, Parr Instruments) under 120 °C for 40 min. Same biomass loading, sample separation, washing and drying procedures are performed in order carry out following analysis.

2.3. Chemical analysis of the liquors from pretreatment media

The liquor resulting from the acid and the alkaline pretreatments were neutralized to pH 7 by 1 M HCl and 1 M NaOH solutions, respectively. Then the analyses of monosaccharides were carried out by using a Dionex High Performance Anion Exchange Chromatography (ICS-3000PC, Thermal scientific, USA) equipped with electrochemical detector [34].

2.4. Lignin quantification

Lignin was quantified as follows: 3.5 mg of biomass (untreated or pretreated) was dissolved in acetyl bromide solution (25% v/v acetyl bromide/glacial acetic acid), then 1 ml of NaOH 2 M and 175 μl hydroxylamine hydrochloride of 0.5 M were added in a 5 ml volumetric flask. The solution was taken to 5 ml with acetic acid and diluted 10 times. The absorbance was read at 280 nm and the percentage of lignin calculated using the following formula [35]:

$$\mathrm{ABSL\%} = \{\mathrm{abs}/(\mathrm{coeff} \times \mathrm{pathlength})\} \times \{(\mathrm{total\ volume} \times 100\%)/\mathrm{biomass\ weight}\}$$

Coefficient = 17.75; Path length = 1 cm; Total volume = 5 ml; Biomass = 3.5 mg.

2.5. Chemical analysis of solid residues

In order to investigate the effect of microwave on biomass during pretreatment, the chemical composition of solid residues before and after the pretreatments was analysed by Fourier transform infrared spectrometry (FT-IR) and by ^{13}C nuclear magnetic resonance (NMR).

Attenuated total reflection–Fourier transform infrared spectroscopy was conducted by using a Bruker Optics Vertex system (VERTEX 70, Bruker) with built-in diamond-germanium ATR single reflection crystal. Untreated and pretreated samples were pressed firmly against the diamond surface using a screw loaded anvil. Sample spectra were obtained under 64 scans between 600 cm^{-1} to 2000 cm^{-1} with a spectra resolution of 4 cm^{-1}. Air is used as background for untreated and pretreated sugarcane bagasse.

Solid state ^{13}C experiments were carried out in a Varian VNMRS 400 Spectrometer at ^{13}C frequency of 100.562 MHz.

2.6. Morphological analysis

In order to study biomass surface change after pretreatment, morphological characteristics of the raw sugarcane bagasse and the pretreated biomass solid residue were analysed using a high resolution environmental scanning electron microscope, equipped with a field emission gun (FESEM) (FEI, Quanta 650, USA). Prior to analysis, the samples were coated with Au in a SCD 050 sputter coater (Oerlikon-Balzers, Balzers, Lichtenstein). Both equipment were available at the National Laboratory of Nanotechnology (LNNano) located in Campinas-SP/Brazil. Images were obtained under vacuum, using a 5 kV accelerating voltage and a secondary electron detector. A large number of images was obtained on different areas of the samples (at least 20 images per sample) to assure the reproducibility of the results.

2.7. Hemicellulose quantification

4 mg biomass was hydrolysed by adding 0.5 ml 2 M Trifluroacetic acid (TFA). The vials were flushed with dry argon, then heated at 100 °C for 4 h. Then vials were cooled at room temperature, and TFA is completely removed by evaporator with fume extraction overnight. Biomass was washed two times by adding 2 × 500 μl Propan-2-ol. After evaporate Propan-2-ol, the biomass sample was resuspend in 200 μl ultra purified water. After centrifuge, the supernatant was transferred into a new tube and diluted 20 times to measure the monosaccharides in hemicellulose on DionexICS-3000PC [36].

3. Results and discussion

3.1. Removal of sugars from bagasse during microwave pretreatment

3.1.1. The effect of NaOH

Hemicellulose is a polysaccharide that comprises xyloglucans, xylans, mannans and glucomannans. The most significant biological role of hemicellulose is that they strengthen the cell wall by interaction with cellulose and, in some walls, with lignin [37]. It acts as an amorphous matrix material, holding the stiff cellulose fibrils in place [38]. Alike to cellulose, it can be degraded into monosaccharides to produce fermentable sugars. Therefore, it is important to decompose both hemicellulose to enhance sugar production during pretreatment process.

Alkali can facilitate dissociation of entire wall polymers by breaking hydrogen and covalent bonds, and lignin can be removed effectively [3]. Fig. 1(A) and (B) show the release of sugars from sugarcane bagasse after alkaline pretreatments, using NaOH concentrations of 0.2 M and 0.4 M. The results show that, in both NaOH concentrations, the total amount of sugars released increases with the holding time up to 7 min, and subsequently decreases. 0.4 M NaOH results in a higher sugar release than 0.2 M NaOH. The reducing sugar amount is up to 1.51 μmol/mg biomass and 1.90 μmol/mg biomass respectively by using 0.2 M or 0.4 M NaOH for 7 min. Meanwhile, remarkable xylose yields from available carbohydrate (21%–22%) are achieved, suggesting the decomposition of hemicellulose. Longer holding times (10 min) led to a lower sugar production in the pretreatment liquor, resulting from sugar degradation [39]. The major components of hemicellulose in this material are glucuronoarabinoxylans and the xylose, arabinose, and glucose being the predominant monosaccharides in the liquor [27]. The proportion of each monosaccharide does not change significantly across the conditions assayed, except for the pretreatment with 0.4 M NaOH during 7 min, where the proportion of glucose increases significantly (Fig. 1(B)). This increase in glucose might represent a partial degradation of the cellulosic fraction.

3.1.2. The effect of H_2SO_4

H_2SO_4 is commonly used in pretreatments [2,7,8]. Acid is able to remove hemicellulose by breaking strong chemical bonds to yield soluble mono- and oligosaccharides at high temperatures [40,41]. Gomez et al. studied conventional heating pretreatment of maize stover. Their results showed that substantial quantities of sugars were released from the biomass by using NaOH or H_2SO_4, particularly from hemicellulose fraction [42]. Fig. 1(C) shows that high sugar yields is obtained from bagasse by using both concentrations of H_2SO_4 for 7 min. With reaction conditions of 0.4 M H_2SO_4 for 10 min, the amount of sugars present in the liquor dropped to 0.105 μmol/mg of biomass.

Holding time has a significant impact on sugar constitutions. The monosaccharide compositions in the pretreatment liquors of acid treated samples during 3 min do not differ largely from the alkali treated samples, compare Fig. 1(A)–(C). However, with the increasing holding time, the sugar composition of pretreatment media shows a drastic change. When holding time is increased to 7 min, glucose is the predominant product when 0.2 M and 0.4 M H_2SO_4 are applied, of which the yield is up to 64% from available carbohydrate. The results here are similar to our previous studies with *Miscanthus*, where we showed that the combination of microwave and H_2SO_4 can selectively produce glucose from *Miscanthus* [43]. Xylose yield is reduced when longer holding time with acid presence, because it is further degraded into other chemicals such as furfural, formaldehyde, formic acid, crotonaldehyde, lactic acid, acetaldehyde, dihydroxyacetone [44]. Unlike alkali conditions, acid pretreatments combined with microwave show a progressive hydrolysis of the cellulosic fraction with increasing holding times. More severe conditions (i.e. 10 min holding time) contribute to further degradation of produced sugars, therefore should be avoided.

In order to compare, conventional thermo-chemical pretreatment was conducted at the condition of 120 °C for 40 min, in order to obtain an effective pretreatment. The total sugar amount is 0.63 μmol/mg biomass and 0.85 μmol/mg biomass by using NaOH or H_2SO_4 re-

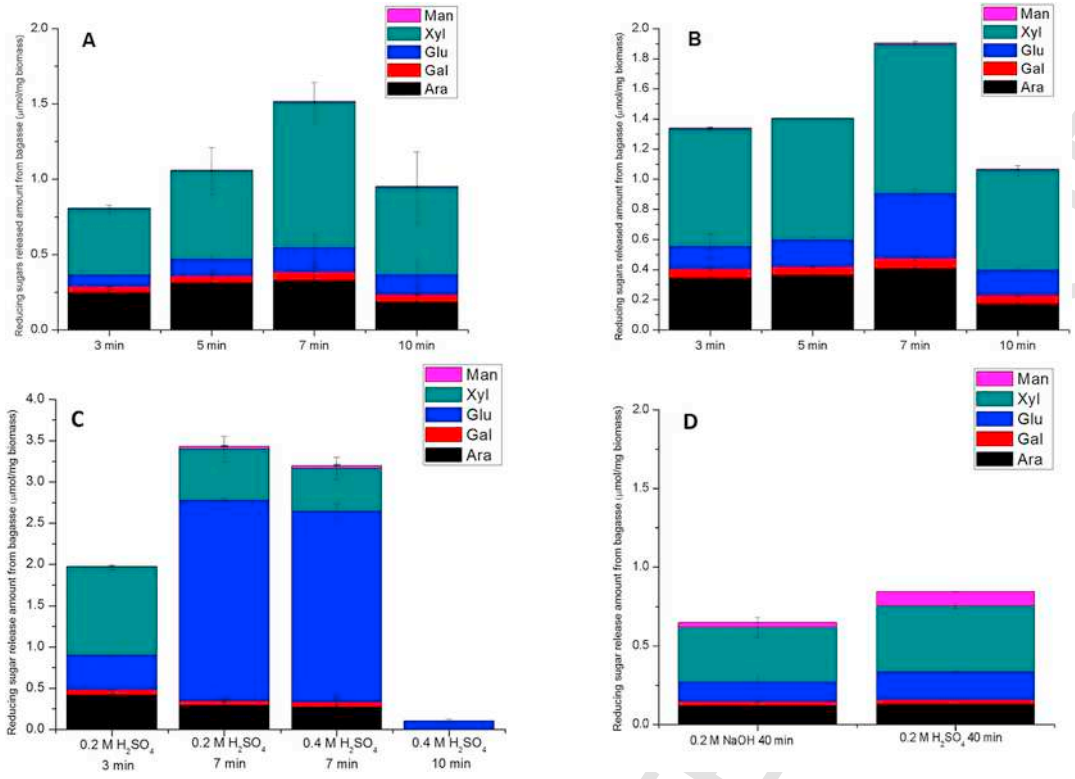

Fig. 1. Sugar analysis of liquid fraction. (A) monosaccharides release of 0.2 M NaOH pretreatment (microwave power of 320 W); (B) monosaccharides release of 0.4 M NaOH pretreatment (microwave power of 320 W); (C) monosaccharides release of H_2SO_4 pretreatment (microwave power of 320 W); (D) monosaccharides release by using conventional heating (120 °C, 40 min).

spectively (see Fig. 1(D)). Xylose is the major sugar component, indicating that hemicellulose is broken down without cellulose degradation. With 0.2 M H_2SO_4 as pretreatment media, the total sugar removal from sugarcane bagasse undergoing microwave pretreatment is about 4 times higher than that of conventional heating pretreatment. At the same time, a significant amount of glucose is obtained by using microwave assisted pretreatment, suggesting an effective depolymerisation of crystalline cellulose. More importantly, holding time of microwave assisted pretreatment is 5.7 times less. The reason of such efficient sugar production is that microwave heating leads to much higher pretreatment temperature (170 ± 5 °C) than conventional heating in a short period of time (3 min). It is worth mentioning that the pretreatment temperature could be even higher when microwave heating time is longer (7 or 10 min), however this led to increased sugar degradation.

3.2. Lignin content

Lignin has been proposed as one of the main factors behind the biomass recalcitrance. Lignin concentration and distribution in the cell wall is closely related to cell wall recalcitrance and to the low yields of enzymatic hydrolysis for ethanol production. It has a phenolic and hydrophobic character and forms a branched network around cellulose, thus hindering the accessibility of cellulases [45,46]. Therefore, pretreatment is required to remove lignin from lignocellulosic material and contribute to an energy-efficient biomass destruction process. It is known that alkaline and oxidative treatments, such as alkaline peroxide and lime and oxygen, have been extensively applied to remove lignin [47–49]. The lignin percentage in raw bagasse is 31.7%. This content is decreases by using different microwave assisted pretreatments (Table 1). Lignin percentage in the solid fraction is 27% after 0.2 M H_2SO_4 is applied, regardless of the increase in holding time. By using 0.4 M H_2SO_4 the lignin percentage decreased to 23%–24% when holding time is 7 min or 10 min. Increasing severity of H_2SO_4 pretreatment removes more lignin. Lignin percentages are approximately half or less after NaOH pretreatments in comparison to untreated bagasse. Furthermore, more lignin is removed when the holding time is increased from 3 min to 7 min using NaOH. In comparison with H_2SO_4 pretreatments, NaOH pretreatments lead to lower lignin amounts in the solid fraction (Table 1), which is agreement with results previously published [9,17,48].

Lignin percentage of conventional heating pretreated sugarcane bagasse is also measured in order to compare, which is similar to the ones undergoing microwave pretreatment. Therefore, microwave as-

Table 1
Lignin content in un/pretreated sugarcane bagasse material.

Pretreatment conditions	Lignin percentage (%)
Raw bagasse	31.71 ± 0.46
0.2 M H_2SO_4 3 min	27.56 ± 1.76
0.2 M H_2SO_4 7 min	27.12 ± 1.82
0.4 M H_2SO_4 7 min	24.14 ± 1.19
0.4 M H_2SO_4 10 min	23.14 ± 1.30
0.2 M NaOH 3 min	17.70 ± 1.24
0.2 M NaOH 7 min	13.32 ± 1.20
0.4 M NaOH 3 min	17.70 ± 1.14
0.4 M NaOH 7 min	12.07 ± 0.80
Con. 0.2 M NaOH, 40 min	16.06 ± 0.68
Con. 0.2 M H_2SO_4, 40 min	27.50 ± 0.50

Con. stands for conventional heating pretreatment; each condition is repeated three times and standard deviation is obtained.

sistance has little influence on lignin removal with the appearance of NaOH or H_2SO_4. This result is expected, because chemical structure of lignin is less polar than polysaccharides, which means they are less interactive with microwaves.

3.3. NMR analysis for H_2SO_4 pretreated sugar cane bagasse

Solid state ^{13}C NMR was carried out in order to have a better understanding of the biomass changes after microwave assisted pretreatment. Detailed information of chemical composition of sugarcane bagasse was obtained by ^{13}C solid-state NMR. Fig. 3 shows the NMR spectrum for the untreated and acid treated bagasse samples.

The signal at 22 ppm (attributed to the CH_3 groups of the acetylated hemicellulose alcohols) disappeared in the spectra of acid pretreated bagasse during 3 and 10 min (Fig. 2), indicating that hemicellulose is completely deacetylated after acid pretreatment. Liquid fraction analysis above also indicated that the hemicellulose is substantially hydrolysed under these conditions. The 20 and 30 ppm signals could be attributed to small amount of fatty acids/lipids. Peak at 84 ppm is contributed by C_4 carbon of amorphous cellulose and hemicellulose and $-OC_\beta H_2$ carbon of lignin. The peak at 89 ppm is related to C_4 carbon of crystalline cellulose. It can be noticed that after H_2SO_4 pretreatment, the relative intensities of the 84 ppm and 89 ppm signals are altered, indicating that the remaining sugar cane bagasse becomes more crystalline [50]. The crystallinity in 10 min pretreated bagasse is higher than that of 3 min pretreated one, presumably due to the selective removal of amorphous material. Two signals at 116 ppm and 147 ppm, respectively, appeared in samples from 10 min pretreatment, and these could be contributed by 'humins'. It is a condensation reaction product from sugars and furfuraldehydes derived from C_5 and C_6 sugars under acid catalysis condition [28]. The peak at 130 ppm is attributed to aromatic carbon, suggesting the high aromatic character of carbon can be found when bagasse is treated with 0.4 M H_2SO_4 for 10 min [51].

3.4. FT-IR absorption spectra and analysis

Chemical changes in the surface of samples were qualitatively analysed by ATR-FTIR spectroscopy. Cellulose is a homopolysaccharide composed of β-D-glucopyranose unit linked together by (1–>4)-glycosidic bonds [2]. The solid fraction of bagasse after 7 min

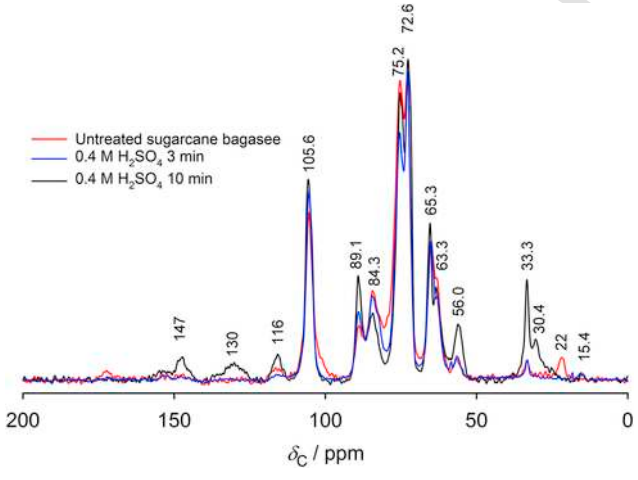

Fig. 2. NMR spectra of untreated sugar cane and bagasse treated with 0. 4 M H_2SO_4 for 3 min or 10 min at a microwave power of 320 W.

of H_2SO_4 or NaOH pretreatment was analysed by FT-IR. Fig. 3(A) and (B) show sharp bands at 897 cm^{-1}, 1033 cm^{-1}, 1104 cm^{-1}, and 1160 cm^{-1} that can be associated with cellulose [25,52]. The pronounced peaks at 1033 cm^{-1} relate to C-O stretching at C-6 [52]. Fig. 3(A) shows that peaks at 1033 cm^{-1} and 1104 cm^{-1} become more pronounced, indicating that hemicellulose is removed and these characteristic peaks of cellulose are enhanced after H_2SO_4 pretreatment [25]. These signals are stronger after 0.2 M H_2SO_4 treatment than of the corresponding ones in the 0.4 M H_2SO_4 treatment, due to the degradation of cellulose under higher concentration acid.

Lignin has absorbance at around 1422 cm^{-1}, 1511 cm^{-1} and 1600 cm^{-1} [25]. These lignin absorption fingerprints in the raw bagasse and H_2SO_4 pretreated bagasse are observed (Fig. 3(A). The absorption at 1424 cm^{-1} is proposed to be related to the methyl group presenting in lignin [53]. The absorption at 1513 cm^{-1} is related to aromatic stretches [45]. Fig. 4(A) shows that the peak at 1241 cm^{-1} (acetyl C-O stretching of hemicellulose) is absent after acid pretreatment, implying that hemicellulose is effectively deacetylated [54]. This suggests an effective removal of hemicelluloses in these conditions. The peak at 1731 cm^{-1} represents the complex linkages between hemicellulose and lignin, such as ester-linked acetyl, feruloyl and p-coumaroyl groups [55]. After acid pretreatment, there is no absorbance at this position, indicating that these linkages were also broken, despite the fact that lignin is hardly influenced. As it mentioned before, due to the nature of microwave heating, polar groups are largely affected by microwaves, while less polar parts (lignin) is less interactive with microwaves.

Infrared spectra of NaOH pretreated bagasse is also studied (see Fig. 3(B)). In contrast to the spectra of acid treated samples, the main changes are seen in peaks corresponding to lignin. Peaks at 1513 cm^{-1}, and 1604 cm^{-1} have almost disappeared, indicating the removal of lignin. The peak at 1424 cm^{-1} remains present because some $-OCH_3$ groups still remain after NaOH pretreatment. Various phenolates can be released during treatment, such as p-coumaric acid, ferulic acid, vanillin, syringic acid and p-hydroxybenzoic acid, among which syringic acid has two $-OCH_3$ groups [10]. It will be difficult for a base to remove the second one of these, as the first removal of the $-OCH_3$ group will create a negative charge on the ring. There is no absorbance at 1730 cm^{-1} after the alkali pretreatment, indicating that the linkages between hemicellulose and lignin were broken. After the 0.4 M NaOH pretreatment, a peak appears around 1104 cm^{-1} indicating that cellulose is more exposed after pretreatment. The peak at 1241 cm^{-1} completely disappears, due to the removal of acetyl groups on hemicellulose. In summary, both H_2SO_4 and NaOH can efficiently degrade hemicellulose and broke linkages between lignin and hemicellulose. However, NaOH can effectively remove lignin. In order to confirm that hemicellulose is effectively removed, we measured the hemicellulose percentage after NaOH or H_2SO_4 pretreatments when holding time is 7 min. As can be seen from Table 2, hemicellulose is effectively removed, and H_2SO_4 removed more hemicellulose than NaOH.

3.5. Morphological analysis

Scanning electron microscope was used to study the morphologic characteristics of raw and pre-treated *Miscanthus*. Untreated bagasse has a relatively flat and clean surface, as shown in Fig. 4(a–c), with conducting fibre packed in bundles (Fig. 4(a)).

Fig. 5 shows the biomass surface characteristics under the condition of 0.2 M NaOH microwave pretreatment. As can be seen, the surface coating of biomass is removed and the fibre bundles which

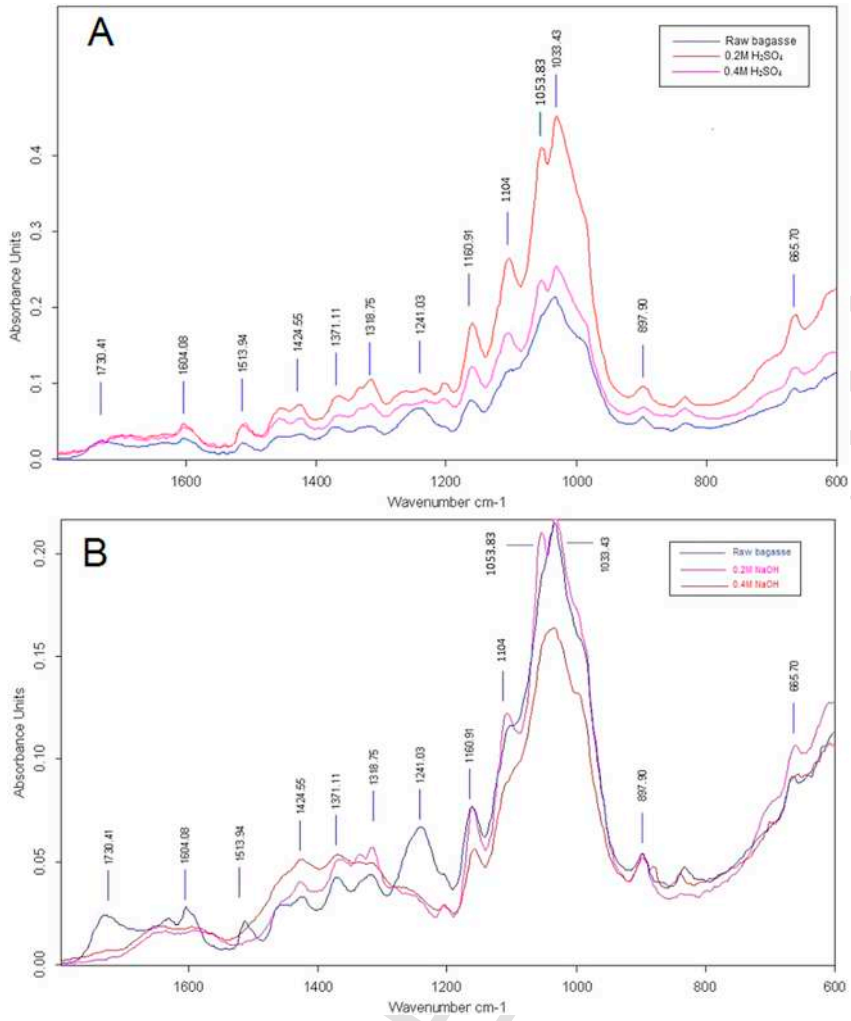

Fig. 3. FT-IR spectrum of sugarcane bagasse pretreated for 7 min at a microwave power of 320 W. (A) H_2SO_4 pretreated biomass; (B) NaOH pretreated biomass.

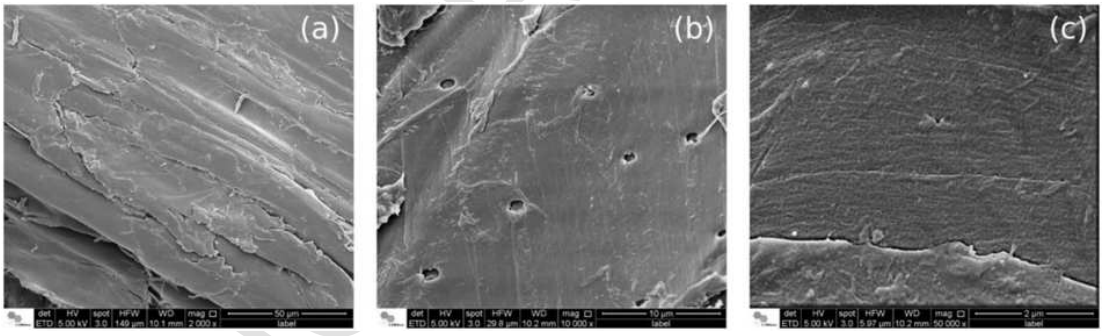

Fig. 4. Surface images of the untreated sugarcane bagasse obtained by FESEM. (a) general view of a fibre surface, bar scale: 50 μm; (b) flat surface of a fibre showing pits, bar scale: 10 μm and (c) amplification of the surface, bar scale: 2 μm.

Table 2
Hemicellulose percentage of un/pretreated biomass when holding time is 7 min.

	Untreated biomass	0.2 M NaOH	0.4 M NaOH	0.2 M H_2SO_4	0.4 M H_2SO_4
Hemicellulose percentage (%)	48 ± 2.30	5.42 ± 0.23	5.41 ± 0.05	1.39 ± 0.13	1.92 ± 0.04

Each condition is repeated three times and standard deviation is obtained.

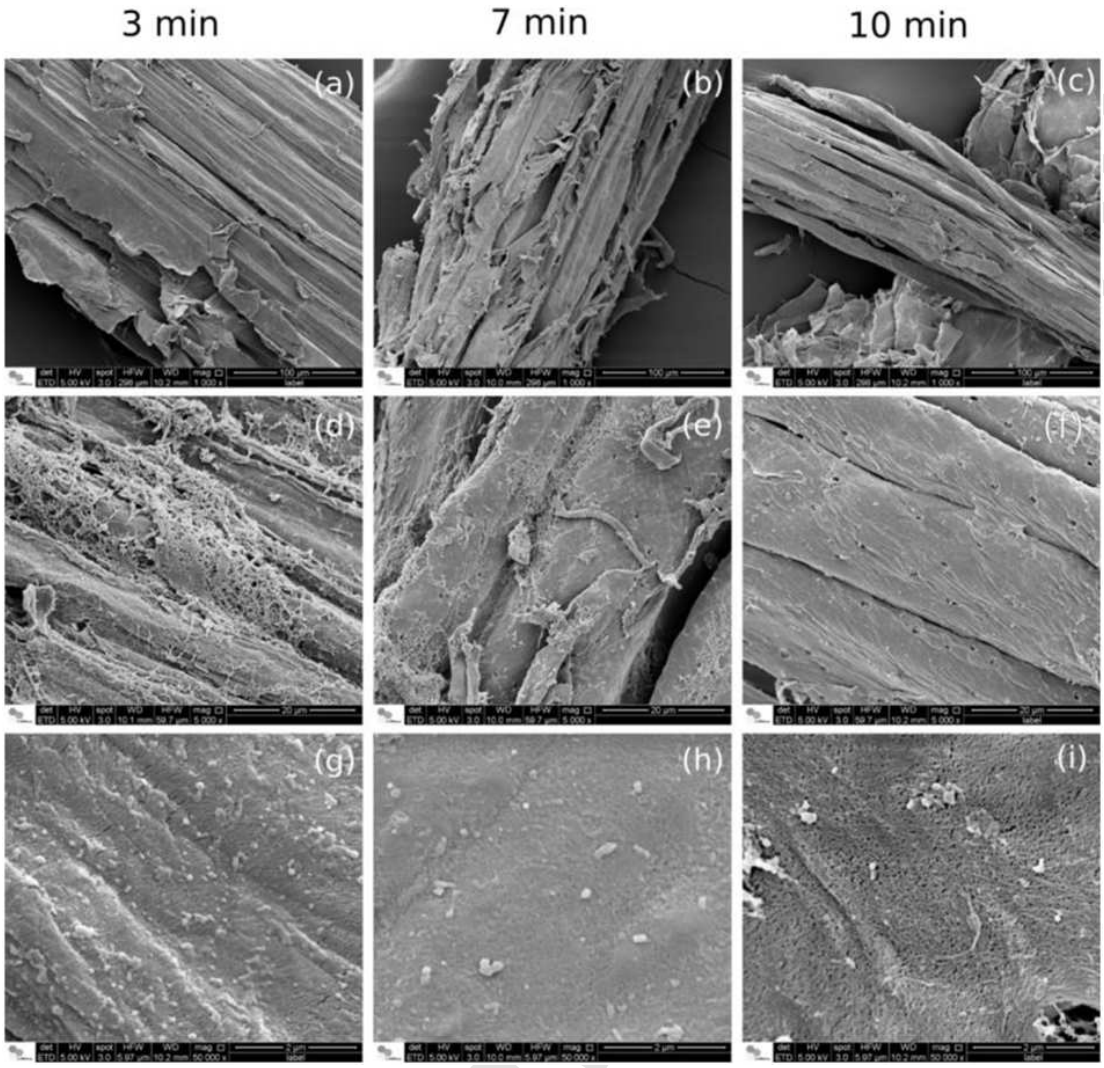

Fig. 5. Surface images obtained by FESEM on sugarcane bagasse treated with NaOH 0.2 M, under a 320 W microwave power with variable holding times: (a, d, g) 3 min; (b, e, h) 7 min, (c, f, i) 10 min. Three different magnifications with scale bars between 100 μm and 2 μm are shown for each treatment condition.

were tightly packed in the raw bagasse start to dismantle under the NaOH action (Fig. 5). These changes can be attributed to lignin removal from the interstices between the fibres of the bundle and is in agreement with the decrease in lignin content measured in the bagasse samples. The separations of the bundles into independent fibres increases with the holding time interestingly, deposits of material can be observed on the fibre surface, particularly after shorter holding times. These are probably lignin aggregates, formed by lignin extraction from the inner regions of the cell wall, followed by condensation due to pH conditions and re-deposition on the surface. Lignin re-deposition has been observed in other lignocellulosic samples treated under alkaline conditions [27,56,57]. These surfaces also show distinct cellulose microfibres pretreated by voids and holes on the surface, which are particularly visible on samples treated under a 10 min holding time (Fig. 5(G–I)). The presence of voids between the cellulose microfibers are an important feature resulting from microwave action, due to the removal of other components of the cell wall, leaving the cellulose network exposed. When the NaOH concentration is increased from 0.2 to 0.4 M, similar but more drastic effects are observed (Supplementary Fig. 1). Delignification effect of NaOH results in the formation of voids and holes on biomass surface, which can increase the cellulose accessibility during the enzymatic saccharification process. Therefore, it is a very important characteristic to be noted.

Fig. 6 show the changes on the surface of sugarcane bagasse treated with 0.2 M H_2SO_4, when holding times is between 3 and 7 min. Samples undergoing short holding times (3 min) at both H_2SO_4 concentrations show a very similar morphology to the raw bagasse, indicating that the acid treatment produces hardly any morphological change under these conditions (Fig. 6(a) and (d) and Supplementary Fig. 2(a,d)). Longer holding time (10 min), on the other hand, shows a large degree of aggregation between structures (Fig. 6(c) and (f)). Macroscopically, the samples become black and under SEM present spherical particles of carbonized samples (Fig. 6(f) and Supplementary Fig. 2(c,f)). This carbonization material could be 'humins', which could explain very low amount of sugar found in the pretreatment liquor (see Fig. 1(C)). This result is consistent with the NMR results for 'humins' formation under acid pretreatment (see Section 3.3). Acid treatments do not seem to remove interstitial material from the cell wall, and increasing the holding time causes degradation instead of fractionation of the components. It is worth mentioning that samples treated with acid do not present the

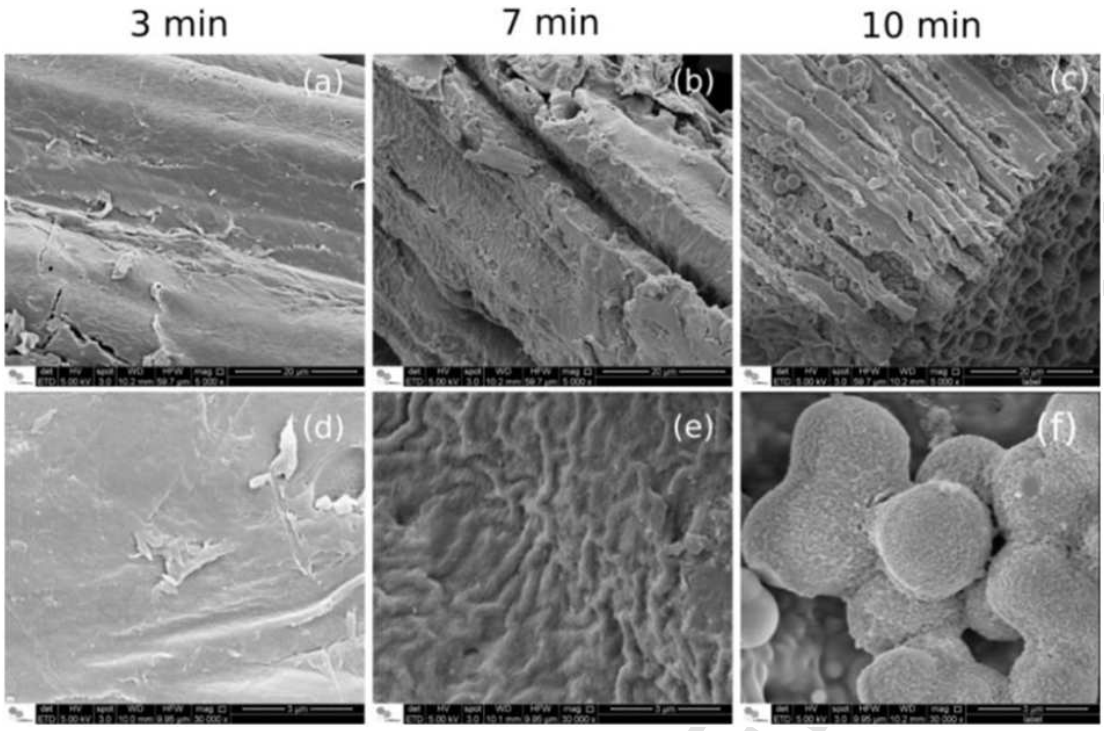

Fig. 6. Surface images obtained by FESEM on sugarcane bagasse treated with H_2SO_4 0.2 M, under a 320 W microwave power and variable holding times: (a, d) 3 min; (b, e) 7 min, (c, f) 10 min. Three different magnifications with scale bars between 20 μm and 3 μm are shown for each treatment condition.

lignin domains in the form of droplets or networks characteristic of alkali treatments.

In comparison with microwave assisted pretreatment, conventional pretreatments bring little change to the biomass surface, as all the biomass samples present flat and smooth surface (see Supplementary Fig. 3).

4. Conclusion

As we know, conventional pretreatment is time consuming and suffers from sugar degradation disadvantages. In this work, sugarcane bagasse was pretreated by NaOH and H_2SO_4 under microwave conditions. The results showed that a very effective and efficient sugar removal process was achieved by using microwave assisted pretreatment. Firstly it is highlight that changing pretreatment media and holding time, the major sugar components can be tuned. NaOH pretreatment effectively extracted hemicellulose and remove lignin, resulting in a xylose-rich pretreatment liquor. Maximum sugar yield (86%) in the pretreatment media was achieved by using 0.2 M H_2SO_4 for 7 min, producing enrichment in glucose of 64%. Secondly, microwave assisted pretreatment is faster than conventional heating pretreatment, giving rise to 4 times higher reducing sugar release than conventional heating pretreatment by using 5.7 times less pretreatment time. Thirdly, from morphological studies by SEM, we observed that NaOH had a significant impact on biomass surface. Biomass bundles are dismantled, and the inner structure of the cell wall is opened showing voids that are absent in the untreated bagasse, which potentially can lead to an improved enzymatic hydrolysis process of pretreated biomass. It is worth mentioning that microwave assisted acid condition should be controlled at a moderate condition, because sever acid condition leads to the formation of 'humins', which reduces the amount of sugar release in the pretreatment medium. Overall, our results shows microwave assisted pretreatment for lignocellulosic material provides a significant production of fermentable sugars during pretreatment procedure and industrial scale up process need further study. It is an efficient method to assist the thermo-chemical conversion for biomass, and shows promising potential in the process of 2nd energy generation biofuel production.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biombioe.2016.06.017.

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