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Optimization of alkali concentration in the pretreatment of sugarcane bagasse for ethanol production

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Abstract

This study was aimed for the investigation of the effect of pretreatment procedure of alkaline, based on the chemical arrangement, surface morphology, structural composition and enzymatic assimilation of sugarcane bagasse for sugars and ethanol production. Alkali pretreatment (0 to 8% w/v of NaOH) assists to reduce the lignin portion (from 19.57±0.03% to 9.91±0.02%) and increase the cellulose content of the treated SB (from 34.66±0.05% to 63.58±0.05%) simultaneously. The optimal conditions for alkali pretreatment were 8% NaOH charge at 100^{o}C for 90 min. Enzymatic digestibility of alkali treated SB was significantly improved and hydrolysis yield reached to 89.59% glucose and 61.23% xylose at an prime level using *Trichoderma viridae*. Further hydrolysate of 8% (w/v) alkali treated SB sample was fermented by *Saccharomyces cerevisiae* to convert sugar into ethanol and yield was 16.81±0.32% in 24 h. Alkali pretreatment was found to be a treatment of choice for cellulose hydrolysis in SB and subsequent sugar acquired for the production of ethanol during fermentation.

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Introduction

Due to a possible source of renewable energy and a reduction in Green house gases (GHG) emissions, recently emphasize on exclusive products using lignocellulosic materials has increased. (Den *et al.* 2018; Janker-Obermeier *et al.* 2012). In comparison to first generation biofuel, second generation biofuel made from lignocellulosic sources has more energetic, economic, and environmental benefits (Hirani *et al.* 2018). Mostly three biological polymers- cellulose, lignin and hemicellulose comprise the lignocellulosic biomass in which supramolecular associtions of cellulose and its combination with lignin and hemicellulose provide physical and chemical barriers in plant tissue. In general the purposes of pretreatment are (1) enlargement of the available surface area and breakdown the cellulose crystal (2) partial depolymerization of cellulose, (3) solubolize lignin and/or hemicellulose (4) modification of enzyme accessibility to improve digestibility of cellulose (Zhao *et al.* 2012). The process design that has a clear impact on the cellulose, hemicellulose and lignin fragments should be consider solemnly while selecting the pretreatment techniques and constrains (Alvira *et al.* 2010).

Agricultural remains in Bangladesh could be the potential source of bioethanol production. Sugarcane bagasse is loosely bonded anatomical metric,

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composed of vasculer bundles surrounded by non-fibrous parenchymatic cell (Jahan *et al.* 2009). Usually it is available as agricultural residue and byproduct from sugar mill industries. In the year of 2015-16, there was 2,95,162 metric ton sugarcane bagasse was obtained from sugar mills of Bangladesh (http://www.bsfic.gov.bd).

To enrich cellulose content in biomass for enzymatic saccarification, current research has focused on various pretreatment processes. Currently, scientists have gone through a lot of studies on different pretreatment techniques to remove the compact and rigid composition by open up the cellulosic structure (Alvira *et al.* 2010). Pretreatment technologies are commonly abandoned to reduce the structural barriers and boost cellulose availability based on not only chemicals such as acid, alkali, oxidant, etc. but also several treatment settings. Alkali treatment is one of the most widespread and economical methods used for surface modification of lignocellulosic biomass. In the field of biorefinary, alkali pretreatment is intensively employed to develop the cellulosic materials both mechanically as well as chemically and such properties include tensile strength, dyeability, stability of dimension and reactivity (Wu *et al.* 2011). In alkali pretreatment biomass is treated under moderate reaction conditions ensuring inexpensiveness, inflated recycling possibilities of water and chemical agents (McIntosh and Vancov, 2010). Usually alkali treatment is the most effective pretreatment for agricultural residues, herbaceous crops and hardwood containing low lignin content in the compariosn of softwood containing high lignin content (Agbor *et al.* 2011; Canilha *et al.* 2012).

This research aims is to investigate the alkaline pretreatment repercussions based on the chemical structure, surface morphology, structure and enzymatic digestion of sugarcane bagasse for sugars and ethanol production.

Materials and methods

Sugarcane bagasse (SB) was taken from street juice vendor. Warm tap water was used for washing purpose to eliminate the residual free sugars once again. This washing and pressing step was then repeated for three times. After that drying was done in an oven at 65°C for 16 h. Crushing of dried bagasse was done successively using a locally made crusher and sieved (Retsch, D-42759, HAAN, Germany) to have the particle size of 20-40 mesh on average. Before usage, the 20-40 mesh bagasse was kept at room temperature in an airtight plastic container (Sujan *et al.* 2018).

Fig. 1. 20-40 mesh size SB

Moisture and Ash content

Moisture content of raw materials was measured using the ASTM D 4442-07 procedure. In a glass crucible, 2 grams of pretreatment sample containing 20-40 mesh were dried in oven at 105±2°C. The moisture content was expressed in percent wet basis and weight measurements were taken every 3 h. A muffle furnace was used for burning the Oven-dried samples at 575±25°C for ash content determination following the ASTM Standard E 1755-01.

Volatile matter

The volatile matter analysis was carried out in accordance with ASTM Standard D 271-48. Four grams of raw materials were heated in a furnace at 950±20°C for seven minutes. The weight loss, excluding the weight of moisture pushed off at 105°C, is then used to calculate volatile matter.

Fixed carbon

The difference between 100 and the total of volatile matter, moisture, and ash content was used to compute the fixed carbon percentage.

Chemical analysis

The technical association of the pulp and paper industry (TAPPI) method detects **α**-cellulose (T 203 cm-99), pentosan (T 223 cm-01), klason lignin (T211 om-83), and acid soluble lignin (T UM 250) on a dry basis.

Ultimate analysis

Ultimate analysis of samples was done by following the procedure ASTM Standard D 5291-02. Organic elemental analyzer (Flash 2000, Thermo Scientific, USA) was used with a specific condition (Reactor temp. 900°C, He: 250 kPa, O_2: 250 kPa, TCD).

Low concentration alkali pretreatment

Different alkali concentration (0%, 2%, 4%, 6% and 8%), time (60, 90 and 120 min) and temperature (80, 100 and 120°C) were applied on raw material to check alkali effect on SB shown in Table 3. A portion of the ground bagasse 20-40 mesh (10g) was taken into plastic zipper bag (temperature 80°C, figure 2a and 2b) and stainless-steel reactor (temperature 100°C and 120°C, figure 2c and 2d). Different alkali concentration was applied during the pretreatment process (alkali to SB ratio ranging between 1:12). The mixture was heated at a particular temperature such as in a water bath (80°C) and oil bath (100°C and 120°C) for a desired length of time. In the course of pretreatment process the sample was manually mixed 2-3 times to attain proper alkali treatment. The treated SB was placed into a polyester bag to remove excess alkali water by pressing it. After that it was vigorously washed with tap water (repeated for five times) to remove the remaining alkali. Finally the SB was dried in an oven at 65°C for 72 h and stored in a close container at room temperature for further experiment.

2a 2b 2c 2d

Fig. 2. a) 20-40 mesh size SB and alkali were taking into plastic zipper bag; b) samples were pretreated into water bath at a temperature below 100°C; c) 20-40 mesh size SB and alkali were taking into stainless-steel reactor; d) sample pretreated into oil bath at a temperature above 100°C

Regression model

Regression model has been developed for prediction of *α*-cellulose, pentosan and lignin in SB. The general form of the model is:

$$y=\alpha+\alpha_1 x_1+\alpha_2 x_2+.....+\alpha_n x_n+\varepsilon.....(1)$$

where α is the constant term. α_i are the coefficient of variables x_i. ε is the random error term which is minimized with Simple Least Squares Regression (SLSR).

Regression coefficients of the independent variables namely alkali concentration, temperature and time are estimated by SLSR method for developing regression model to predict α-cellulose, pentosan and lignin.

Efficiencies of these models are expressed by coefficient of multiple determination (R^2) and Adjusted R^2.

2.9 Separate hydrolysis and fermentation (SHF)

The Hydrolysis experiment took place in 100 ml conical flux 10ml enzyme solution with 200 mg (2% dry wt.) in citrate buffer (0.05 M, pH 5.0) at 50°C for 48 h. In this case *Trichoderma viride* was used for hydrolysis. Hydrolysate was then heated for 15 min in a boiling water bath and centrifugation was done to remove solid particles. The supernatant was used for analysis of released sugars as described by Jamal *et al.* (2011).

During fermentation process according to Firoz *et al.* (2012), 100 ml media was prepared and 0.5 g of commercial yeast *Saccharomyces cerevisiae* was used as inoculum which showed good performance to converts sugar into bioethanol. This inoculated media mixture was poured in a suitable glassware and was kept in a shaking incubator for 48 h. 10 ml of this medium was then added into the flask and it was properly covered with aluminum foil. Then it was placed in the incubator at 30°C for 24 h, 48 h, 72 h and 96 h for fermentation of sugars to bio-ethanol according to Sujan *et al.* (2018). Samples from hydrolysis and fermentation were performed by HPLC.

High performance liquid chromatography (HPLC)

In characterize part, concentration of Sugars and ethanol were determined by HPLC (Ultimate 3000, Thermo Scientific, USA) method using Hyper Rez XP carbohydrate H^+ 8 µm column (100×7.7 mm) equipped with a Refractive Index (Shodex RI-101) detector. The mobile phase was degassed with deionized water with a flow rate of 0.7 ml/min and column temperature was maintained at 70°C.

It is possible to measure the total sugar concentration in the hydrolysis liquid fraction by comparing its peak area detected by HPLC with peak area of 1% standard sugar which consists of two sugars namely glucose and xylose (Sujan *et al.* 2018). The same column which is specialized for fermentation broth analysis is used for ethanol detection. The kinetic parameters of ethanol fermentation were determined as follows (Islam *et al.* 2019):

$$\text{Ethanol concentration (Ec)\%} = \frac{\text{Ethanol produced (g)}}{\text{Volume of reaction mixture (L)}}$$

Scanning electron microscopy (SEM) analysis

In this research, SEM (ZEISS EVO 18 SEM) was used to detect the change of pretreated bagasse fibers. SEM images were taken of different pretreated bagasse samples with acceleration voltage of 2.0 KV.

Results and discussion

Proximate analysis

Proximate analysis of SB sample (20-40 mesh) are presented in Table I. Primarily this analysis usually evaluate the fuel characteristics of raw materials. According to Sun *et al.,* 2009, higher moisture and ashcontent in samples lessen the heating value.

Table I. Proximate analysis of sugarcane bagasse sample

Name of sample	Moisture (%)	Ash (%)	Volatile matter (%)	Fixed carbon (%)
SB	9.44±0.14	1.75±0.06	4.99±0.11	83.82±0.17

Crystallinity measurement

X-ray diffraction (XRD) was used to determine the crystalline structure of the SB samples using a diffractometer (GBC XRD) and filtered copper K radiation (λ = 0.1542 nm) by a monochromator at 35.50 kV voltage and 28 mA current, with a speed of about 2°/min and scanning in the range of 10 - 80ºC. The crystallinity index (CrI) was obtained from the ratio between the intensity of the 002 peak (I_{002}, 2θ = 22.5) and the minimum dip (I_{am}, 2 θ = 18.5) according to the following equation (Roberta *et al.,* 2012):

$$\text{CrI (\%)} = [(I_{002} - I_{am})/I_{002}] \times 100 \quad \text{..... (2)}$$

where I_{002} is the highest peak intensity of plane 002 and I_{am} is related to the amorphous structure.

In present study, the average crystallite sizes were determined from the Scherrer equation by using the diffraction pattern obtained from the 002 (*hkl*) lattice planes of cellulose samples

$$D_{(hkl)} = [(K\lambda / B_{(hkl)} \cos 2\theta] \quad \text{..... (3)}$$

Where $D_{(hkl)}$ (Crystallite size), K (Scherrer constant, 0.84), λ (X-ray wavelength, 0.154nm), $B_{(hkl)}$(Full width half maximum of the measured *hkl* reflection), and 2θ (Corresponding Bragg angle).

Ultimate analysis

Ultimate analysis denotes the elemental configuration of SB such as carbon, hydrogen, oxygen, nitrogen and sulfur which are shown in Table II. This examination helps to measure the percentage of carbon and hydrogen content in biomass that is responsible to determine the amount of air is required for complete combustion, composition of combustion gases and heat is generated by it (Poddar *et al.* 2014).

Chemical properties

Raw SB contained 34.66±12% α-cellulose, 22.43±08% pentosan and 19.57±06% klason lignin in which 1.75±04% acid soluble lignin (dry basis) was detected by technical association of the pulp and paper industry (TAPPI) method. The chemical composition of SB was determined by acid hydrolysis and it was calculatedby HPLC method as 45.35% glucose and 30.64% xylose (Sujan *et al.,* 2018).

α-cellulose yield

Bagasse is mainly composed of cellulose, hemicellulose and lignin. Besides these there are some extractives such as ash, wax, gum, pectin etc. During alkali pretreatment usually most of the extractives are removed with the increasing of alkali concentration. Pretreatment of SB with different alkali concentration based on raw material (0%, 2%, 4%, 6% and 8%), time (60, 90 and 120 min) and temperature (80, 100 and

Table II. Ultimate analysis of Sugarcane bagasse (20-40 mesh)

Name of sample	Carbon (%)	Oxygen (%)	Hydrogen (%)	Nitrogen (%)	Sulfur (%)
SB	44.89	49.6	5.51	0	0

120°C) are shown in Table III. Consequences of each independent experiment varying with alkali concentration, temperature and time on the α-cellulose yield were analyzed using MATLAB software. Apparently, it was observed that α-cellulose yield was considerably increased with changes of alkali concentration ranging from 0% to 8% (34.66% to 63.58%). But no noticeable variation was observed in temperature-time alteration during pretreatment of SB. Based on cellulose percentage obtained in treated bagasse, the optimum conditions for pretreatment reaction were selected as alkali concentration 8%, time 90 min and temperature 100°C. Although cellulose content in treated SB was slowly increased with the increase of alkali concentration 12% attime 90 min and temperature 100°C but as a consequence of huge chemical consumption, recovery problem, chance of losses cellulose and hemicellulose, alkali concentrations

Table III. Chemical pretreatment of SB with different concentration of NaOH, temperature and time for the yield of α-cellulose, pentosan and lignin yield

Alkali (NaOH) solution (%)	Temperature (°C)	Time (min)	α-cellulose yield (%)	Pentosan yield (%)	Lignin yield (%)
0%	80	60	34.66±0.05	22.43±0.02	19.57±0.03
0%	80	90	34.76±0.02	22.56±0.01	19.42±0.01
0%	100	60	34.88±0.01	22.68±0.03	19.39±0.01
0%	100	120	34.90±0.01	22.75±0.02	19.25±0.06
0%	120	90	34.98±0.02	22.88±0.02	19.16±0.01
2%	80	60	37.02±0.10	23.20±0.01	17.77±0.09
2%	80	90	37.32±0.08	23.35±0.03	17.23±0.05
2%	100	60	38.13±0.03	23.60±0.04	17.14±0.01
2%	100	90	38.99±0.05	23.84±0.03	17.05±0.02
2%	120	120	38.46±0.08	23.25±0.02	16.73±0.06
4%	80	90	44.98±0.13	23.96±0.05	14.96±0.08
4%	80	120	45.46±0.09	24.26±0.08	14.85±0.01
4%	100	60	47.43±0.11	24.53±0.04	14.66±0.05
4%	100	90	48.05±0.06	24.87±0.05	14.50±0.01
4%	120	60	47.86±0.09	24.95±0.02	14.41±0.06
6%	80	60	53.45±0.11	25.09±0.03	12.86±0.08
6%	80	90	53.95±0.08	25.21±0.02	12.58±0.06
6%	100	90	55.79±0.05	25.86±0.03	12.29±0.02
6%	100	120	54.35±0.03	25.89±0.01	12.11±0.02
6%	120	60	54.88±0.04	25.99±0.02	12.01±0.02
8%	80	60	59.24±0.14	26.15±0.04	10.79±0.10
8%	80	120	59.95±0.02	26.32±0.02	10.69±0.01
8%	100	60	61.67±0.11	26.56±0.03	10.17±0.08
8%	**100**	**90**	**63.58±0.05**	**26.75±0.02**	**09.01±0.02**
8%	120	90	62.59±0.04	26.86±0.02	09.30±0.04
12%	100	90	66.14±0.06	27.46±0.04	06.22±0.05

for pretreatment above 8% was not considered as ideal concentration.

Effect of alkali concentration (AC), cooking time and temperature (temp) charge on α-cellulose yield as well as their statistical significance on the basis of F-test number are presented in regression equations (4). As shown in equations, cooking time at the maximum temperature had no significant effect on α-cellulose yield followed by alkali concentration charge. Effect of temperature on α-cellulose yield was less in employed cooking conditions.

For α-cellulose yield:

α-cellulose yield = 82.05-0.151×temp-1.56×time-0.69×AC (R^2=0.89, adjusted R^2=0.87) ..... (4)

For Pentosan:

Pentosan = 13.58+0.086×temp+0.006×time+0.445×AC (R^2=0.67, adjusted R^2=0.63) ..... (5)

For Lignin:

Lignin = 27.662-0.041×temp-0.001×time-01.242×AC (R^2=0.93, adjusted R^2=0.92) ..... (6)

For predicting α-cellulose, percentage of pentosan and lignin, the most influential factor was alkali concentration and then cooking temperature for α-cellulose yield, which exhibited an almost linear dependence on both operational variables. The coefficient of determinations is good for α-cellulose yield and lignin percentage which hovers around 90 percent, although the figure is moderate more than 60 percent for pentosan. All these three models are significant ($p<0.05$) at 5% level of significance.

In order to perceive the impact of alkali concentration and temperature on these three parameters, three-dimensional (3D) response surface plots were created by plotting the response (α-cellulose yield) pentosan and lignin on the Z-axis versus the most influential one independent variable alkali concentration and temperature as shown in Fig. 3 (a), (b) and (c).

XRD analysis

In 19th century the cellulose crystalline structure has been discovered and later it was verified by X-ray crystallography (Meyer and Misch, 1937; Wilkie, 1961). The crystallinity index (CrI) of non-woody biomass, such as grasses and agricultural residues, varies depending on the type of biomass. Generally, non-woody biomass has a lower crystallinity index than woody biomass, typically ranging

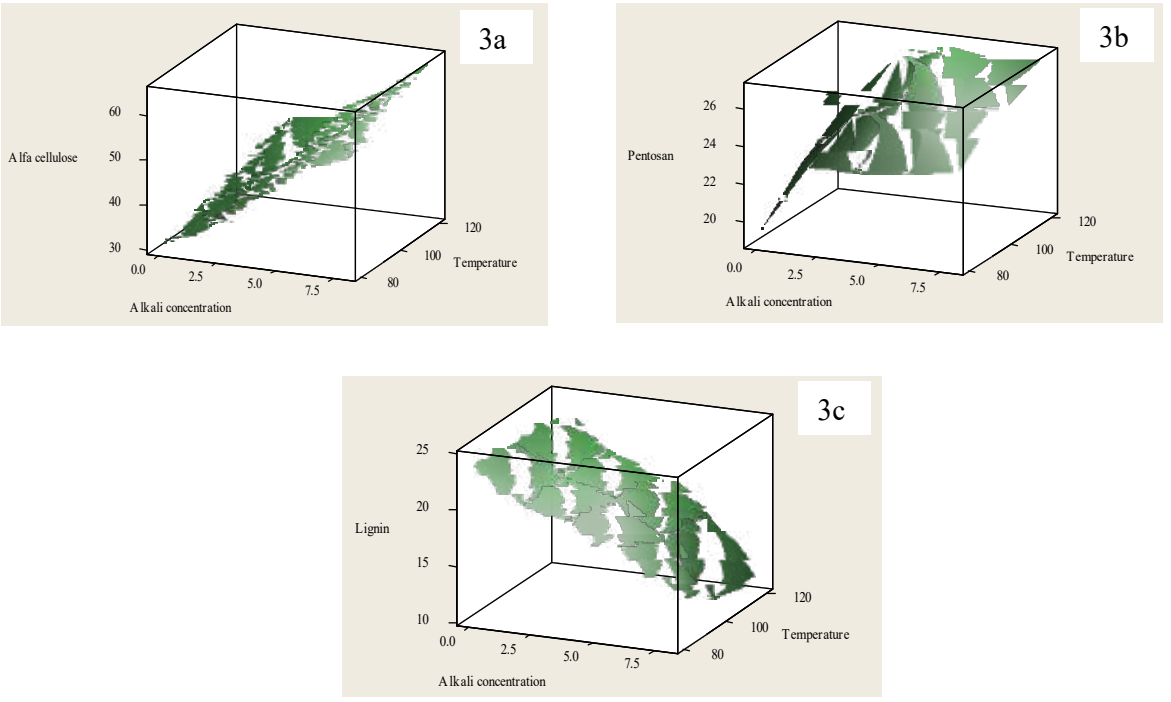


Fig. 3. Surface plot of (a) α-cellulose, (b) Pentosan and (c) Lignin against alkali concentration and temperature

from 20-40%. The crystallinity index of non-woody biomass affects its digestibility and energy production potential.Recently, researchers are being paid more attention on cellulose index because of its potential use in bioenergy production. Since then several different models of cellulose index have been proposed. The most popular two-phase cellulose model describes cellulose chains as containing both crystalline (ordered) and amorphous (less ordered) region (Park *et al.,* 2010).

Alkali treatment of bagasse has been found to increase the crystallinity index of the bagasse. Alkali treatment breaks down hemicellulose, making the cellulose molecules more ordered and crystalline, resulting in a higher crystallinity index. Alkali treatment also increases the digestibility of the bagasse, making it easier to break down and therefore more suitable for bioenergy production. X-ray diffraction spectra of the SB and alkali treated SB were presented in Figure 4. It was observed that the intensity of 101 and 002 peaks were gradually increased. The relative amount of crystalline cellulose (CrI) in the total solid were calculated based on the equation (2) and obtained 18.68%, 24.80%, 27.23%, 32.69%, 36.45% and 39.19% for raw SB, 0%, 2%, 4%, 6% and 8% alkali treated SB samples respectively. The CrI of alkali treated SB samples (not cellulose crystallinity) were intensely influenced by the composition of the samples. In case of lignocellulosic biomass examples, cellulose CrI measured the relative amount of crystalline cellulose in the total solid. Therefore, amorphous part of lignin and hemicellulose in biomass specimens were partially removed with the delignification process as a result the proportion of α-cellulose was increased and hence CrI would be increased gradually.

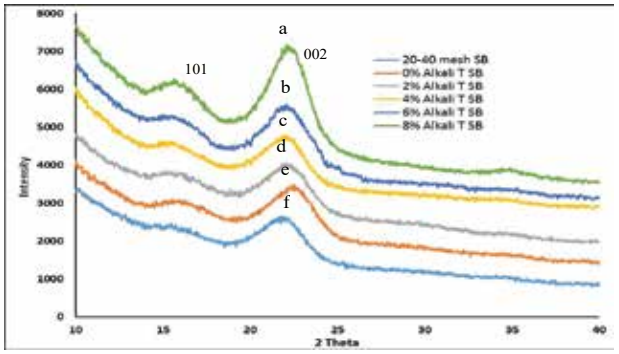


Fig. 4. XRD spectra of a) 20-40 mesh size SB; b) 0% alkali treated sample; c) 2% alkali treated sample; d) 4% alkali treated sample; e) 6% alkali treated sample; f) 8% alkali treated sample

This interpretation could be proved by the fact that alkali-treated SB had higher CrI than raw SB (Zhao *et al.* 2010) and the portion of cellulose in the treated SB was also increased gradually.

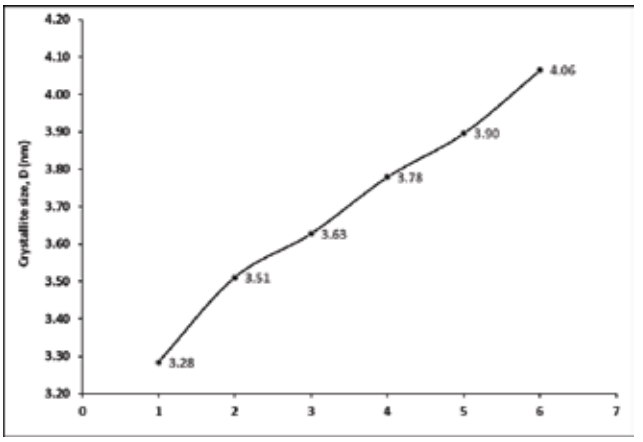


Fig. 5. Average size of crystalline for raw SB, 0, 2, 4, 6 and 8% alkali treated sample

According to the equation 3, the average sizes of crystallite obtained were 3.28, 3.51, 3.63, 3.78, 3.90 and 4.06 nm for raw SB, 0%, 2%, 4%, 6% and 8% alkali treated SB samples respectively. The experimental data revealed that during delignification, the size of crystallite was increased.

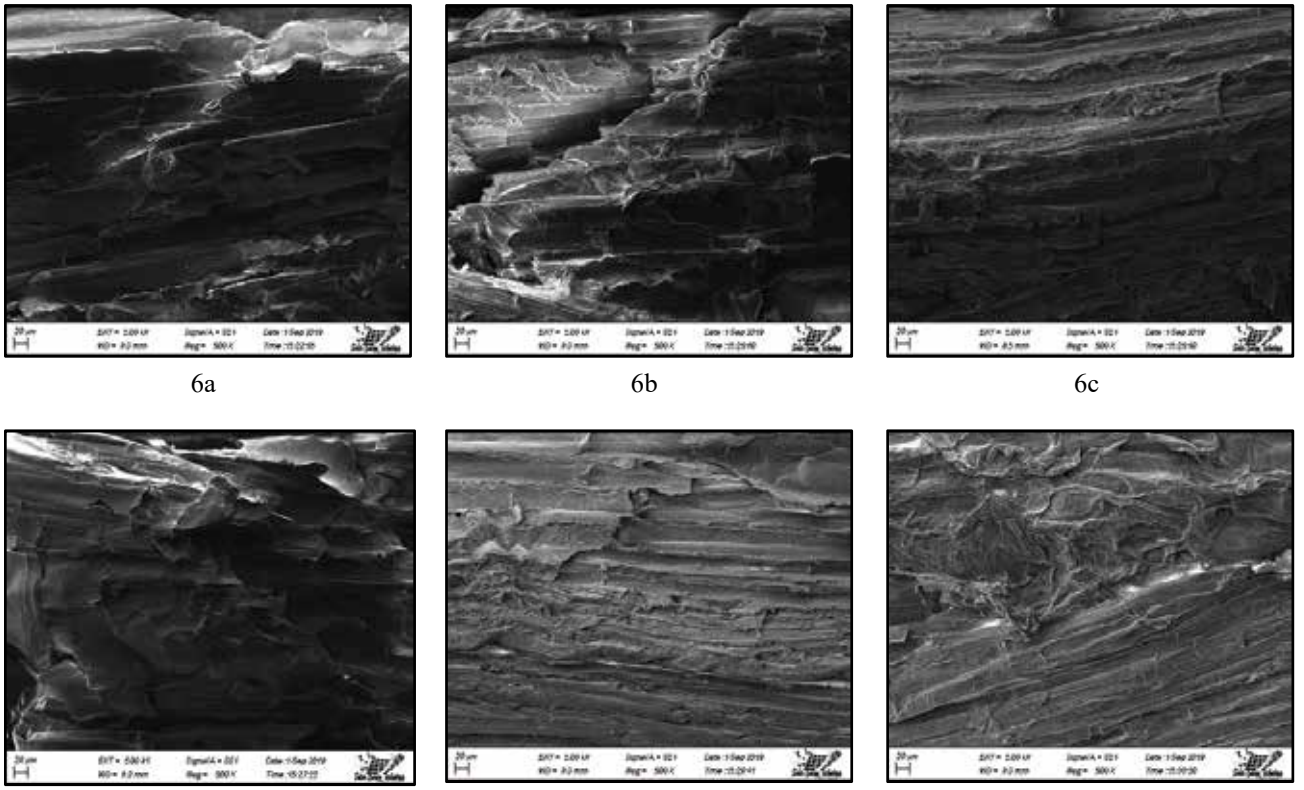
SEM analysis

SEM is one of the most commanding tools widely used to investigate the lignocellulosic biomass surface (Amiri and Karimi, 2015). SEM is usually employed for surface characterization, morphology and inspection of microstructure. In respect of biomass example through SEM images we can compare the untreated and pretreated models which may lead to different insight into the biomass (Karimi and Taherzadeh, 2016).

SEM provides two-dimensional images of raw SB and alkali treated SB, which were taken and compared to the outcome of NaOH treatment. All testers were coated with carbon tape and magnification of 500x was used. Figure 4 shows the wall of raw SB (Figure 6a) was intact where the alkali treated SB (Figures 6c-6f), the cell wall was ruptured or splitted and hence packing of the fibers were partially loosened (Firoz *et al.* 2012).

Sugar and ethanol yield

Enzymatic digestibility is the ability of enzymes to break down molecules into smaller molecules, such as glucose. It is used to measure the efficiency of enzyme-catalyzed reactions and the digestibility of cellulose. The enzymatic digestibility of a compound is affected by factors such as the compound's crystallinity index, the type of enzyme used, and the temperature



6a 6b 6c

6d 6e 6f

Fig. 6(a-f). SEM images a) 20-40 mesh size SB; b) 0% alkali treated sample; c) 2% alkali treated sample; d) 4% alkali treated sample; e) 6% alkali treated sample; f) 8% alkali treated sample

and pH of the reaction. In this study the enzymatic digestibility of the alkali pretreated SB was improved by increasing pretreatment conditions. Typically the pretreatment settings are selected by considering various factors such as feedstock characteristics, pretreatment chemical cost, energy consumption and recovery efficiency (Wu *et al.* 2011).

α-cellulose obtained by 8% alkali treated of SB was used for hydrolysis reaction and the reaction was carried out at an optimum condition set by Sujan *et al.* (2018). The most effective enzymatic hydrolysis was taken place with *Trichoderma viride*at 48 h and the theoretical yield of sugars i.e. glucose and xylose were obtained 89.59% and 61.23%, respectively.

Through fermentation process generated sugars were used to check ethanol production. Yeast *Saccharomyces cerevisiae* presented worthy performance to convert C_6 sugar into ethanol when it was incubated at 30°C for 24 h and 16.81±0.32% ethanol yield was detected.

Conclusion

α-cellulose yield was optimized by varying alkali concentration, temperature and cooking time. The most vital influencing factor for α-cellulose yield was alkali concentration and after that temperature performed a little bit. The optimum α-cellulose yield (63.58±0.05%) was obtained at 8% alkali concentration, 100°C and 90 minutes. In hydrolysis step of

Table IV. The percentage of ethanol yield with different time of fermentation at 30°C

Time (h)	24 h	48 h	72 h	96 h
Ec (%)	16.81±0.32	13.25±0.16	12.12±0.20	10.41±0.23

Ec: Ethanol concentration

SB, *Trichoderma viridae*was used to convert 8% alkali treated SB into sugars and it was attained 89.59% glucose and 61.23% xylose which gave higher yield in compare with our previous study such as ball milled and mesh size varied SB sample (Sujan *et al.* 2018). At fermentation step *Saccharomyces cerevisiae* was used to convert hydrolysate of 8% alkali treated SB sample and ethanol yield was obtained 16.81±0.32% at 24 h. Compositional analysis, imaging and crystallinity are three methods were performed. SEM images can give different clues about SB including morphology, surface disruption and creation of highly accessible surface area iii) CrI (18.68% to 39.19%) and crystal size (3.28 nm to 4.06 nm) of SB are increased with different alkali treatment (0%-8%). In this case it was observed that crystallinity, crystal size, accessible surface area, porosity, particle size, lignin and hemicellulose content and enzyme adsorption/desorption were acted as the most impressive factors for digestibility of sugarcane bagasse.

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