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ORIGINAL ARTICLE

Valorization of jute (*Corchorus* sp.) biomass for bioethanol production

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Abstract

The enormous availability of lignocellulosic biomass makes it a potential renewable feedstock for the continuous supply of second-generation bioethanol. The present study focuses on the exploitation of jute biomass left over after fiber extraction for bioethanol production by subjecting it to physical and chemical pretreatments (alkali, acid, ammonia fiber expansion, steam) followed by enzymatic saccharification and fermentation. The compositional analysis of jute biomass indicated that it contained cellulose (42.52 ± 5.54%), hemicellulose (12.24 ± 0.06%), lignin (31.58 ± 3.67%), and extractives (6.21 ± 0.42%). The biomass was subjected to different pretreatments and the structural and chemical changes along with crystallinity of cellulose were examined through scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction, respectively. Enzymatic saccharification of pretreated biomass revealed that among all the pretreatment methods, alkali (2% NaOH) treated substrate produced significantly higher amount of reducing sugar (19.51 g/L) compared with other pretreated biomass after 72 h of hydrolysis. The fermentation of the reducing sugars released during saccharification was carried out by a thermotolerant yeast *Saccharomyces cerevisiae* JRC6 resulting in 7.55 g/L of ethanol within 72 h of fermentation with 77.73% fermentation efficiency. Furthermore, lignin was aslo recovered from the spent liquor obtained after alkali pretreatment of the substrate and the remnant wash was analyzed by LC-MS for the presence of valuable platform chemicals. This study for the first time illustrates the potential of jute sticks as a feedstock for second-generation bioethanol.

Keywords Second-generation bioethanol · Jute · Pretreatment · Saccharification · Holocellulose · Reducing sugars · Lignin

1 Introduction

Currently, the use of fossil fuels, which are a non-renewable source of energy, is not recommended due to various concerns related to economy, ecology, and environment [1]. Therefore, switch from this non-renewable and unsustainable form of energy to sustainable and environmentally feasible sources of energy for our industrial economies and consumer societies has become the need of the hour [2]. In this regard, bioethanol, a renewable and sustainable liquid fuel, is expected to have a promising future in dealing with today's global energy crisis and the worsening environment quality. Bioethanol helps to reduce CO_2 emission up to 80% compared with the use of petrol, thus promoting a cleaner environment for the future [3].

In the future by 2030, there will be a drastic increase in global energy demand which will be almost 60% more than today, and of this 45% will be accounted by India and China together [4]. Therefore, lignocellulosic biomass may be a good option for second-generation bioethanol production to

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meet the world's energy demand. Generally, a large amount of biomass is generated from food crops (rice and wheat) and non-food crops (jute and cotton). Because of their abundance, vast availability, renewable character, and environmental sustainability, these agri-wastes can be a promising feedstock for second-generation bioethanol [5]. The biomass originating from food crops may find other applications in agricultural context as animal feed or may be recycled to the soil for maintaining fertility, but non-food crops are generally discarded or burnt because of their recalcitrant nature. Therefore, fiber crops may be explored for bioethanol production. Until now, among the main fiber crops of India i.e., cotton, jute, and mesta, only cotton [6] and mesta [7] have been explored for its bioethanol production potential. Reports are lacking on the use of jute stick for second-generation bioethanol production.

Jute (*Corchorus* sp.) is an important natural fiber crop in India next to cotton. Jute is a Kharif crop, sown from March to April on lowlands and in May to June on uplands. It is cultivated in tropical and subtropical regions and is a rain-fed crop with little need for fertilizers or pesticides. The Southeast Asian countries, like India, Bangladesh, Myanmar, China, and Thailand, and few South American countries are the largest producers of jute [8]. The lead jute producer states in India are Assam, Bihar, and West Bengal, among which West Bengal is the major cultivator of jute, contributing about three-fourth of the country's production [9]. It was estimated that the total cultivation area for jute was 0.73 million hectares with a production of 9.77 million bales of 180 kg each in the year 2018–2019 [10]. Jute fiber is extensively used for industrial and commercial applications for manufacturing gunny bags, hessian for carpet backing, decorative fabrics, etc., whereas the leftover part is twice the amount of jute fiber i.e., jute stick has very little commercial importance. Generally, 3–4 million tonnes of jute sticks remain unutilized annually after the extraction of jute fiber in India. In general, fiber crops require less production input per unit produce, are environmentally sustainable, and contain high cellulosic content coupled with low lignin which makes it fit for bioethanol production [7]. The major component of jute fiber is cellulose (58–63%) followed by hemicellulose (20–22%) and lignin (12–14%), whereas the jute stick has less cellulose (40.8%) and higher lignin (23.5%) as compared with the fiber [11]. To improve the overall effectiveness of bioethanol production, one strategy is the biorefinery model in which all components of biomass are fully used to produce a wide range of value-added products [12]. Lignin, making up to 10–25% of lignocellulosic biomass, is the second most abundant natural polymer [13]. Large amount of lignin is produced each year by the pulp and paper industry as byproducts of delignification and the biorefinery industry during the pretreatment process. This degraded lignin is then employed in low-value applications and energy production. Lignin continues to attract more attention because of its numerous potential applications [14]. The availability of high-quality lignin in large quantities would stimulate development in new lignin applications in the fields of fibers, biodegradable polymers, adhesives, and surface treatment (rust converter) [15]. Therefore, in this study, jute sticks remaining after the extraction of its fiber was explored, for bioethanol production by optimizing different pretreatment methods, saccharification, and fermentation process for achieving maximum saccharification and fermentation rate, respectively. For the complete valorization of the substrate, the spent liquor obtained after alkali pretreatment of the jute biomass was used for lignin recovery and the wash was further analyzed by LC-MS for any lignin monomers and oligomers, which can serve as potential high-value compounds in a biorefinery approach.

2 Materials and methods

2.1 Raw materials, chemicals, and microorganism used in the study

Jute biomass (leftover stalks after fiber extraction) was procured from ICAR-National Institute of Natural Fibre Engineering and Technology, Kolkata, India. The stalks were dried in the oven at 60 °C for 72 h, mechanically shredded to 0.5–1.0 cm, and stored in airtight containers at room temperature for further analysis. Commercial cellulase (Cat No. C2730, Sigma, USA) and beta-glucosidase enzyme (Cat No. C6105, Sigma, USA) used for saccharification were purchased from Sigma-Aldrich, USA. Stock solution of penicillin (10,000 U) and streptomycin (10 mg/mL in citrate buffer) was purchased from Himedia Labs, India. All other chemicals and solvents used were procured from Sisco Research Laboratory, Mumbai, India. Thermotolerant yeast *Saccharomyces cerevisiae* JRC6 (accession number KX668410) was collected from Division of Microbiology, IARI, New Delhi and was maintained on MGYP agar slants (malt extract 3.00 g L^{-1}, glucose 10.00 g L^{-1}, yeast extract 3.00 g L^{-1}, peptone 5.00 g L^{-1}, agar 18.00 g L^{-1}).

2.2 Proximate compositional analysis of jute biomass

The jute stalk samples were analyzed for different physico-chemical parameters (moisture and NPK content) as well as compositional analysis (cellulose, hemicellulose, and lignin). The moisture content was analyzed using an infrared moisture analyzer (Aczet, India). Nitrogen content was estimated by the Kjeldahl method, potassium by flame photometer, and phosphorus by spectrophotometric method [16]. The raw material was firstly characterized to determine cellulose, hemicellulose, and lignin content, using the standard laboratory analytical procedures for biomass analysis provided by the National Renewable Energy Laboratory, and the sugars formed were estimated as previously described [7, 17]. The percentage of acetyl group [18] and uronic acid [19] in jute biomass was also estimated to understand the complexity of biomass structure.

2.3 Removal of extractives from jute biomass

Extractives were removed from the biomass using ethanol:benzene (1:2). The biomass (10 g) was kept in a thimble in the Soxhlet apparatus and 250 mL of solvent was poured in it. It was then allowed to boil for 6–8 h followed by washing with 250 mL of ethanol for 4 h. The dewaxed jute biomass was weighed to quantify the amount of extractives gravimetrically and then subjected to various pretreatments after drying.

2.4 Pretreatment of the substrate and its compositional analysis

The shredded dewaxed biomass was subjected to various physical and chemical pretreatment methods. Steam pretreatment was carried out by autoclaving with a substrate loading of 20% in a flask at 121 °C for 20 min. For the ammonia fiber expansion (AFEX) method, the jute biomass was treated with liquid ammonia (NH_3) at the rate of 2 g/g of the substrate and autoclaved at 121 °C for 20 min, while in chemical method, the biomass was treated with four different concentrations of NaOH and H_2SO_4 (0.5%, 1%, 1.5%, 2% *v*/v) either with the substrate loading of 20% at room temperature for 1 h (cold treatment) or by autoclaving at 121 °C for 20 min (hot treatment). After the chemical pretreatments, the samples were filtered through a muslin cloth and washed with distilled water until the pH reached to neutral. The samples were then dried and analyzed for weight loss, cellulose, hemicellulose, and lignin content and for the percentage of acetyl groups and uronic acid as described earlier (Section 2.2).

2.5 Recovery of lignin

Black spent liquor obtained after dilute NaOH (2%) treatment was taken in a glass beaker and conc. HCl was slowly added (under constant stirring) to bring its pH to 2 [20]. The precipitated lignin was separated by centrifugation at 6800 rcf for 20 min and the precipitates were washed thrice with hot water (60 °C) to remove salts. Finally, the precipitates were dried at 60 °C until constant weight and analyzed by FTIR, while the wash remaining after the precipitation of lignin (acid precipitable, polyphenolic, polymeric lignin) was subjected to LC-MS analysis for the characterization of lignin monomers and oligomers present in it.

2.6 LC-MS analysis of wash from alkali pretreatment of jute biomass after lignin precipitation

For the identification of lignin monomers and oligomers released after alkali pretreatment of jute biomass, the wash of jute biomass was subjected to LC-MS analysis. The analysis was performed using Thermo Ultimate 3000 full MS scan type mass spectrometer coupled with Thermo Q Exactive mass spectrometer in the scan range of 100.0–1500.0 m/z, resolution of 140,000, and spray volume of 2.4 kV with capillary temperature of 275 °C in positive polarity mode. Separations were performed using Thermo Reverse phase C18 1.9u (100 * 2.1) column with column temperature of 40 °C. Isocratic elution was performed using buffer A (0.1% formic acid in water) and buffer B (0.1% formic acid in acetonitrile) delivered at a total flow rate of 400 uL/min. The analysis was carried out in the following gradient elution mode: Solvent B increased from 5 to 95% in 10 min and then kept constant (95% B) for 2 min and again returned to initial condition in 3 min. After the run was completed, the column was re-equilibrated in initial condition for 2 min. LC-MS data was processed using the Compound Discoverer 2.1 software.

2.7 Structural analysis of pretreated jute biomass

Raw and pretreated samples were examined under a scanning electron microscope (SEM), X-ray diffractometer (XRD), and Fourier transform infrared spectroscopy (FTIR) to study the morphological and compositional changes in the substrate after pretreatment as described previously [7].

2.8 Saccharification of pretreated jute biomass

Enzymatic saccharification of pretreated jute biomass was carried out using commercial cellulase and beta-glucosidase enzymes procured from Sigma-Aldrich, USA. The reaction was performed with substrate loading of 5% (*w*/*v*) and cellulase and beta-glucosidase enzyme loading of 25 FPU/g dried substrate and 10 μL/gds, respectively in 100 mL saccharification tubes containing 20 mL 0.05 M citrate buffer (pH 4.8). To prevent microbial contamination, 200 μL of the antibiotic solution of penicillin and streptomycin (100X) was also added into the tubes and the reaction mixtures were incubated at 50 °C, 150 rpm for 72 h. Aliquots were taken periodically at 24 h, 48 h, and 72 h from the tubes. The samples were filtered and centrifuged at 3000*g* for 10 min to remove unhydrolyzed residues. The reducing sugar content of the supernatant was determined using the 3,5-dinitrosalicylic acid method [21]. Results were expressed as mg reducing sugar per g dry biomass using the following equation:

$$\text{Reducing sugar yield (mg/g dry substrate)} = (a \times b)/x$$

where a is the concentration (mg/mL) of reducing sugars in the sample hydrolyzed, b is the total volume (mL) hydrolyzed, and x is the initial dry weight (g) of the pretreated jute biomass.

2.9 Bioethanol production from jute biomass

The submerged fermentation process for bioethanol production from jute biomass was carried out by using *Saccharomyces cerevisiae* JRC6 (accession number KX668410). After the saccharification, flasks were subsequently inoculated with 10% (*v*/v) inoculum of yeast grown in MGYP broth and incubated at 30 °C, under stationary conditions for 72 h. The samples were centrifuged at 10,000 rpm for 10 min. The supernatants were collected, filtered, and analyzed for residual sugar by DNSA method [21] and ethanol by HPLC as described previously [7]. The sugar released and the ethanol produced were expressed in g/L.

3 Results and discussion

3.1 Composition of dry jute stick biomass

The dried jute stick biomass contained cellulose (42.52 ± 5.54%), hemicelluloses (12.24 ± 0.06%), and lignin (31.58 ± 3.67%) as given in Table 1. The presence of 54% holocellulose in the jute stick makes it a potential source of monomeric fermentable sugars to be used for bioethanol production.

3.2 Effect of pretreatment on the composition of jute stick biomass

Pretreatment of biomass to remove the recalcitrant lignin is an extremely important step in the synthesis of biofuels from lignocellulosic biomasses. Solubilization and degradation of linked lignin in the polymeric cell wall of the biomass were observed due to various pretreatment methods. In the present study, the extent of lignin removal and an increase in cellulose content in response to different pretreatment methods are presented in Table 2. The highest amount of delignification (29.63%) was observed in hot 2% acid treatment. However, it is reported that monomeric pentoses are further degraded to byproducts such as furfural under acid treatment conditions which therefore put a negative impact on saccharification process [22]. Weak acids tend to remove lignin but result in poor hydrolysis of cellulose, whereas strong acid treatment occurs under relatively extreme corrosive conditions of high temperature and pH, which necessitate the use of expensive equipment. Ammonia fiber expansion (AFEX) treatment resulted in a higher amount of cellulose enrichment and reduced the crystallinity to increase enzyme accessibility for hydrolysis [23]. But, AFEX treatment is usually not a method of choice for pretreatment of biomass due to high ammonia cost and environmental concerns [24]. On the other hand, alkali pretreatment (2%) resulted in 23.46% delignification with considerable cellulose enrichment. Sodium hydroxide pretreatment of lignocellulosic biomass breaks the ester bonds cross-linking lignin and xylan, thus increasing the porosity of biomass [25]. Moreover, alkali pretreatment has the advantage of recovering lignin from the alkali wash generated during pretreatment of the biomass. Overall, the results revealed that all the pretreatments resulted in lignin removal in the range of 2.21–29.63% with considerable enrichment of cellulose. Overall, hemicellulose loss of 13.39% was recorded in the chemical pretreatments.

Table 1 Compositional analysis of raw jute stick

Composition (%)	Raw jute stick*
Cellulose	42.52 ± 5.54
Hemicellulose	12.24 ± 0.06
Lignin	31.58 ± 3.67
Ash	1.50 ± 0.09
Extractives	6.21 ± 0.42
Nitrogen	1.21 ± 0.005
Potassium	0.05 ± 0.008
Phosphorous	0.16 ± 0.007
Acetyl group	2.90 ± 0.18
Uronic acid	0.25 ± 0.14

* All components on dry weight basis

3.3 Determination of acetyl group in pretreated jute stick biomass

Lignin and presence of acetyl group linked with cell wall components inhibit enzymatic saccharification and acetate present in crude hydrolysates can forbid the growth and fermentation by yeast and other microorganisms [26]. Hemicellulosic acetylation in lignocellulosic biomass hinders the access and hydrolysis of hemicellulose by xylanolytic enzymes, thus impacting the hydrolysis of holocellulose into sugars [27]. The complexity of hemicellulose structure in the biomass will affect the saccharification process due to its cross-linking with cellulose, lignin, and cell wall proteins through a variety of covalent and non-covalent interactions [28]. Therefore, the acetyl group in the untreated and pretreated jute biomass was determined (Fig. 1) to understand the effect of pretreatment on the removal of these groups. The results revealed that raw jute biomass contains a higher percentage of the acetyl group (2.9%) in comparison with pretreated biomass. A major reduction of acetyl group was observed in NaOH-treated (0.19%) jute biomass, whereas in H_2SO_4-treated biomass, the percentage of acetyl group found was 0.41%. From the results, it can be concluded that alkali pretreatment resulted in maximum reduction of the acetyl group in jute biomass, compared with other pretreatment methods. In earlier studies, also the alkaline treatments tended to completely deacetylate the corn stover substrate [29]. In

Table 2 The compositional change in cellulose, hemicellulose, and lignin content in the pretreated jute stick biomass

Sample	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Total solid recovery (%)
Raw jute	42.52 ± 5.54	12.24 ± 0.06	31.58 ± 3.67	100.00 ± 0.00
Dewaxed jute	40.12 ± 0.23	10.96 ± 0.04	30.35 ± 1.52	62.10 ± 0.09
Steam	46.91 ± 0.14	11.78 ± 0.01	27.22 ± 1.17	97.60 ± 0.32
AFEX	51.18 ± 0.38	10.98 ± 0.20	23.51 ± 6.12	90.10 ± 0.97
Hot (121 °C) NaOH				
0.50%	41.62 ± 1.15	10.84 ± 0.01	28.33 ± 0.23	96.20 ± 0.34
1%	43.48 ± 0.62	10.91 ± 0.05	27.74 ± 1.17	89.90 ± 0.73
1.50%	44.79 ± 2.80	10.70 ± 0.23	25.89 ± 1.41	73.40 ± 1.24
2%	45.35 ± 2.97	10.60 ± 0.05	24.17 ± 0.94	76.71 ± 0.61
Cold (room temp.) NaOH				
0.50%	41.09 ± 0.11	10.87 ± 0.01	30.88 ± 1.64	96.16 ± 0.50
1%	43.58 ± 0.14	11.02 ± 0.21	30.27 ± 3.29	91.85 ± 0.86
1.50%	42.07 ± 0.40	11.33 ± 0.13	28.74 ± 0.70	91.07 ± 1.03
2%	43.77 ± 4.14	12.06 ± 0.18	27.18 ± 0.70	82.73 ± 1.75
Hot (121 °C) H_2SO_4				
0.50%	45.19 ± 0.07	11.88 ± 0.02	28.01 ± 2.35	89.26 ± 1.46
1%	49.50 ± 0.07	10.80 ± 0.07	25.74 ± 0.94	77.90 ± 0.95
1.50%	49.89 ± 0.09	10.75 ± 0.01	24.52 ± 1.64	73.46 ± 0.19
2%	49.96 ± 0.23	10.85 ± 0.19	22.22 ± 0.70	71.42 ± 1.93
Cold (room temp.) H_2SO_4				
0.50%	40.48 ± 0.07	10.91 ± 0.01	29.91 ± 2.12	97.93 ± 1.42
1%	43.52 ± 0.14	11.77 ± 0.01	28.56 ± 1.17	96.52 ± 0.46
1.50%	43.43 ± 0.07	11.86 ± 0.01	26.86 ± 0.94	94.60 ± 0.88
2%	43.72 ± 0.09	11.84 ± 0.01	26.10 ± 2.12	94.42 ± 0.52

another study, alkaline pretreatment of yellow poplar wood meal was reported to extract/remove the acetyl group and hence greatly enhanced the ethanol yield [30]. The pretreatment process also reduced the uronic acid content in the jute sticks (0.44%) as compared with raw jute sticks (5.05%). Removal of acetyl groups and uronic acid substitutions in hemicelluloses during alkali pretreatment also increases the accessibility of the carbohydrates to enzymatic hydrolysis [31]. It is reported that about 70% of xylan residues contain acetyl groups. It is thought that acetyl groups sterically hinder

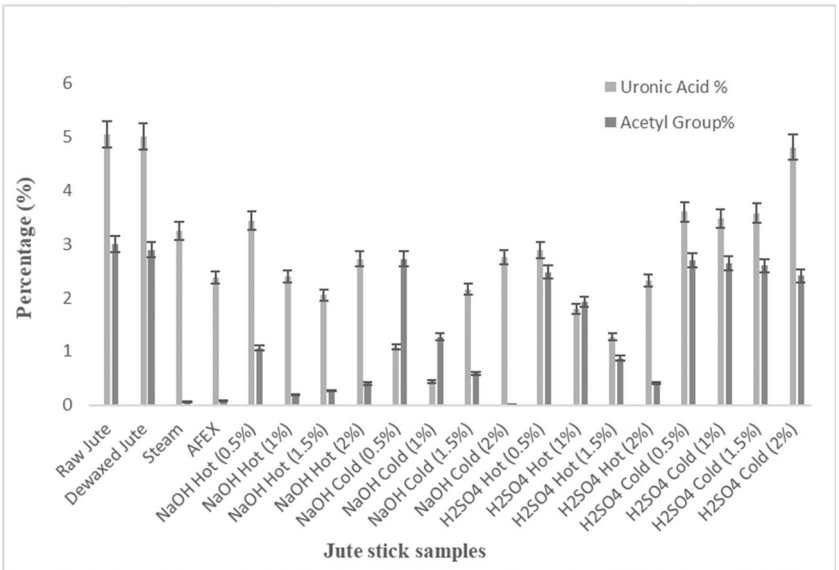


Fig. 1 Variation in acetyl group and uronic acid of untreated and treated jute stick biomass

xylanase activity. Deacetylation increased swellability [32] and enzymatic digestibility of poplar wood and wheat straw [33]. Removal of acetyl and uronic acid groups present on hemicelluloses enhances the accessibility of the enzyme that degrades hemicellulose [34]. It is reported that NaOH pretreatments not only removed lignin but also removed acetyl groups from hemicellulose [25] and reduced crystallinity [35].

3.4 Analysis of structural and chemical changes in jute biomass after chemical pretreatment

3.4.1 SEM analysis

SEM electron micrographs of raw and pretreated samples are presented in Fig. 2. SEM images revealed morphological differences between the native and the pretreated jute biomass samples. While the raw jute samples showed a rough, compact, and highly ordered structure, the pretreated samples showed a highly distorted structure. The cell wall of the pretreated biomass was degraded which indicates the breakdown of lignin and hemicellulose portion of the pretreated biomass. Similar results were observed in the study of palm tree trunk waste [36]. The pretreated biomass also exhibited numerous pores on the surface and on disturbed matrices which help in increasing the enzyme accessibility on the biomass and thus, increase the yield of reducing sugar after saccharification. Sharma and co-workers [37] also reported similar observations after the pretreatment of wheat straw and cotton stalk.

3.4.2 FTIR analysis

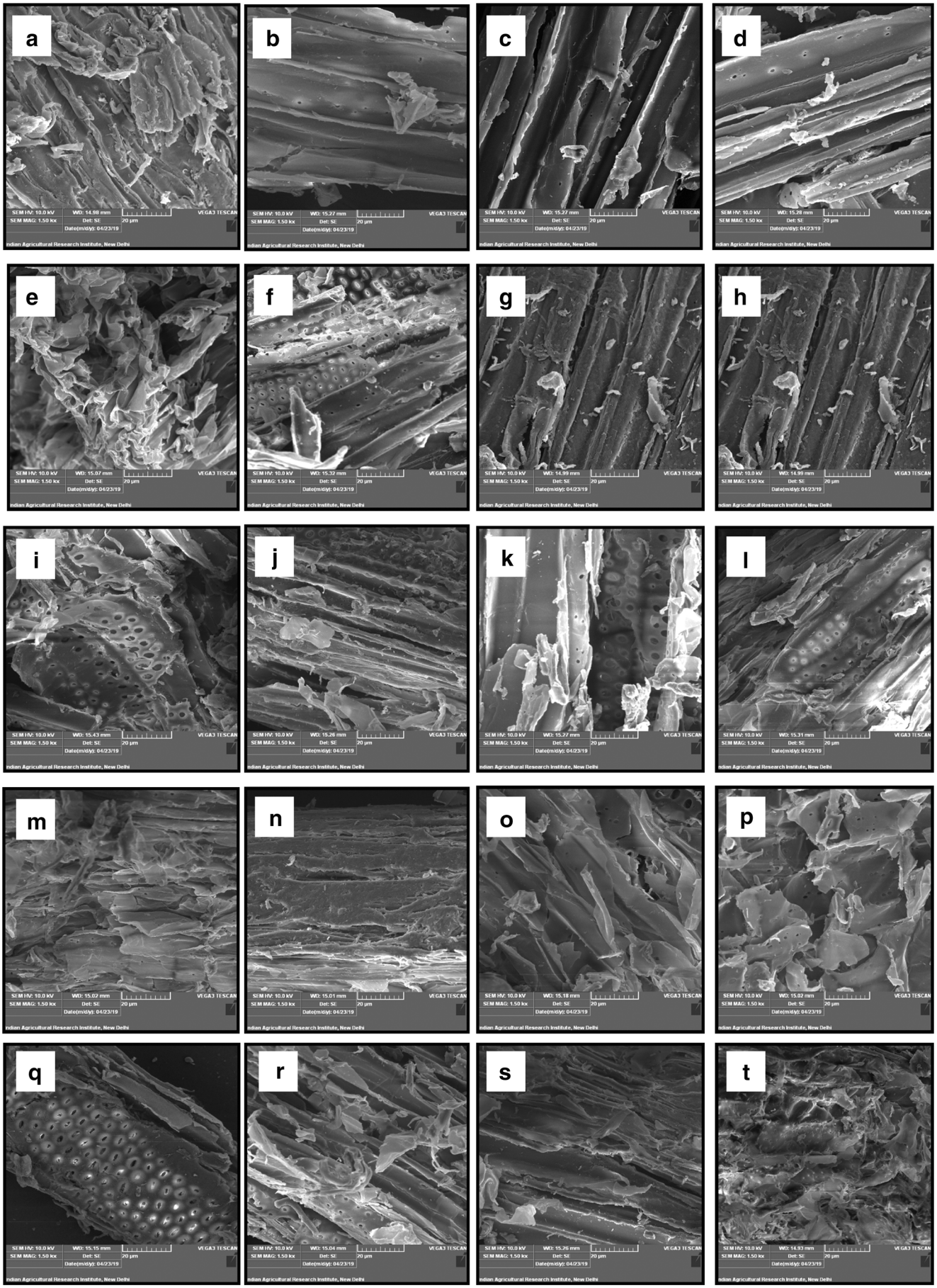
The study of functional groups present on raw and pretreated jute biomass was carried out by FTIR analysis (Fig. 3.) The jute stalk is composed mainly of cellulose, hemicelluloses, and lignin with functional groups like esters, alkanes, aromatic ketones, and alcohols present on them. The FTIR analysis revealed the loss of some peaks corresponding to these functional groups in the chemically pretreated biomass, indicating the removal of corresponding components from biomass after the pretreatment. The results showed that among all the pretreatments of the biomass used in the study, alkali (NaOH) treatment (2% hot) is the most effective treatment in terms of bond removal and cleavage of the functional groups of lignin, hemicelluloses, and acetyl groups. Crude lignin shows peaks at 836 and 1166 cm^{-1}, while syringyl (S) and guaiacyl (G) units are detected by vibrations of the aromatic skeleton at 1609, 1126, and 1330 cm^{-1} (S) and 1513, 1034, and 1265 cm^{-1} (G), respectively [38]. In our results, the reduction in the transmittance of the abovementioned peaks reveals that lignin was partially removed in all the pretreated jute biomass compared with the untreated biomass. A similar reduction in the transmittance of peaks at 1061 cm^{-1}, 1026 cm^{-1}, 1037 cm^{-1}, and 1054 cm^{-1} was observed in the case of pretreated mesta biomass [7]. The transmittance from 3344 to 2918 cm^{-1} indicates the presence of cellulose as the absorbance band in the region of 3344–3400 cm^{-1} and 2845–2920 cm^{-1} represents the hydrogen bond stretching bands of –OH groups and the –C-H groups of cellulose respectively [39]. In the pretreated jute biomass, reduction in peaks was observed at the transmittance from 3344 to 2918 cm^{-1} in comparison with the untreated jute biomass which shows a modification of cellulose structure thus increasing the affinity of cellulase enzyme for cellulose. Peak position at 2900 and 2860 cm^{-1} represents the C-H stretching of methyl and methylene group corresponding to aliphatic moieties in lignin and polysaccharide. Peak at 1726 cm^{-1} shows acetyl and uronic ester groups of hemicellulose or ester linkage of carboxylic group of the ferulic and p-coumaric acids of lignin/hemicellulose [40]. The reduction in the transmittance of these peaks in case of treated biomass in comparison with untreated biomass gives an indication of rupturing of the functional groups of lignin and cleavage of ester bond from hemicelluloses [41].

3.4.3 XRD analysis

The influence of pretreatment methods on cellulose crystallinity of jute biomass was calculated from XRD data and the results are summarized in Fig. 4. The crystallinity index (CrI) of biomass is the quantitative indication of its crystallinity which hinders the action of cellulase enzyme. The CrI of untreated jute biomass was found out to be 36.96%, while the CrI of NaOH (2%) and H_2SO_4 (2%) treated biomass was found out to be 23.61% and 18.42%, respectively. This decrease in CrI may be due the breakdown of inter and intra hydrogen bonding in the crystalline cellulose resulting in modified crystal structure [42].

3.5 LC-MS analysis

The compounds identified by LC-MS analysis of the alkaline (NaOH) wash (from which lignin has been precipitated) obtained after pretreatment of jute biomass are given in Supplementary Table 1. From the MS data, a total of 303 compounds were identified in the wash. Separation of the lignin component of biomass from the cellulose using pretreatment presents an opportunity to access various interesting products from the lignin fragments [43]. Lignin is a sizable renewable resource that provides a pool of important industrial chemicals [44]. Many high-value platform chemicals like vanillin, coniferyl alcohol, naphthol, ferulic acid, and catechol were detected in the spent wash (Supplementary Table 1). Mota et al. [45] have reviewed various extraction processes for the recovery of high-value monomeric chemicals from the disassociation of lignin in aqueous solutions via liquid-liquid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◀ **Fig. 2** SEM images of untreated and treated jute biomass. **a** Raw jute. **b** Dewaxed jute. **c** AFEX-treated jute. **d** Steam-treated jute. **e** H_2SO_4 hot (0.5%). **f** H_2SO_4 hot (1%). **g** H_2SO_4 hot (1.5%). **h** H_2SO_4 hot (2%). **i** H_2SO_4 cold (0.5%). **j** H_2SO_4 cold (1%). **k** H_2SO_4 cold (1.5%). **l** H_2SO_4 cold (2%). **m** NaOH hot (0.5%). **n** NaOH hot (1%). **o** NaOH hot (1.5%). **p** NaOH hot (2%). **q** NaOH cold (0.5%). **r** NaOH cold (1%). **s** NaOH cold (1.5%). **t** NaOH cold (2%). Hot pretreatment was carried out at 121 °C for 20 min by autoclaving at 15 lbs. pressure and cold pretreatment was carried out at room temperature (30 °C)

extraction, supercritical fluid extraction, distillation, crystallization, membrane separation, and adsorption. Further, very interestingly, the presence of 4-methylumbelliferyl acetate in the alkaline wash (Supplementary Table 1) indicates the removal of the acetyl group present in the cell wall that hinders enzymatic hydrolysis.

3.6 FTIR analysis of recovered lignin from the alkaline wash of pretreated jute biomass

The spent liquor obtained after NaOH pretreatment (2% *w*/*v*) resulted in lignin recovery of 51.67% from treated jute biomass. The recovered acid precipitable, polyphenolic, and polymeric lignin was subjected to FTIR analysis depicting the infrared (IR) spectra of the lignin to analyze the presence of functional groups (Supplementary Figure 1). The range of 500–1770 cm^{-1} is considered the lignin fingerprint region [46]. The bands around 2850–2922 cm^{-1} were attributed to the C-H stretching vibration of the aliphatic chain structure of lignin [47]. The absorption bands in the 3500–3400 cm^{-1} region reveal the presence of the OH functional group. The bands at 1715–1675 cm^{-1} are attributed to C=O stretching in conjugated p-substituted aryl ketones. Asymmetric deformation of C-H in CH_2 and CH_3 is observed at 1760 cm^{-1}. Bands occurring from 1650 to 1640 cm^{-1} represent the protein impurity and water associated with lignin. C=O functional group in coumaryl ether group or aldehyde group is indicated by the absorption at 1641 cm^{-1} in the spectra. Bands stretching from 1610 to 1500 cm^{-1} are characteristics of aromatic compounds (phenolic hydroxyl groups) and are attributed to aromatic skeleton vibrations where bands ranging from 1610 to 1595 cm^{-1} represent stretching of aromatic ring (S) and bands from 1515 to 1505 cm^{-1} attributed to C=C stretching of the aromatic ring (G) indicating presence of both syringyl and guaiacyl in lignin's chemical structure [15]. Peaks at band 1470 cm^{-1} indicate the presence of a methylene bridge [48]. IR spectra in the range of 1430–1422 cm^{-1} show asymmetric deformation of C-H in $–OCH_3$. The frequencies 1273 cm^{-1} and 1233 cm^{-1} indicate asymmetric stretching for phenolic C–C–OH. Also, the guaiacyl ring breathing, C-O stretch in lignin, C-O linkage in guaiacyl aromatic methoxyl groups, and syringyl ring breathing with C-O stretching absorption are at 1268 cm^{-1} and 1235–1230 cm^{-1}, respectively. Bond at 1166 cm^{-1} represents typical HGS lignin. These results confirmed the purity of lignin for further use in commercial products.

3.7 Enzymatic saccharification of chemically pretreated jute biomass

The total reducing sugar released after saccharification of pretreated jute biomass after different pretreatments is summarized in Table 3. From the results, it was observed that the maximum sugar yield was achieved from alkali-treated jute biomass i.e., 19.51 g/L compared with other pretreatments after 72 h. Therefore, it was concluded that the alkali pretreatment method is a better way to make the jute biomass more accessible to saccharifying enzymes by lowering the degree of

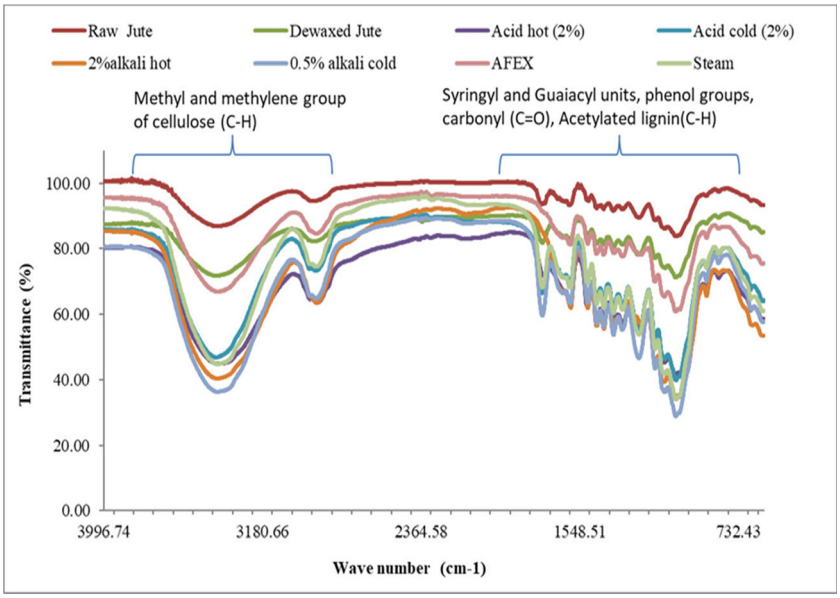

Fig. 3 FTIR pattern of untreated and treated jute biomass

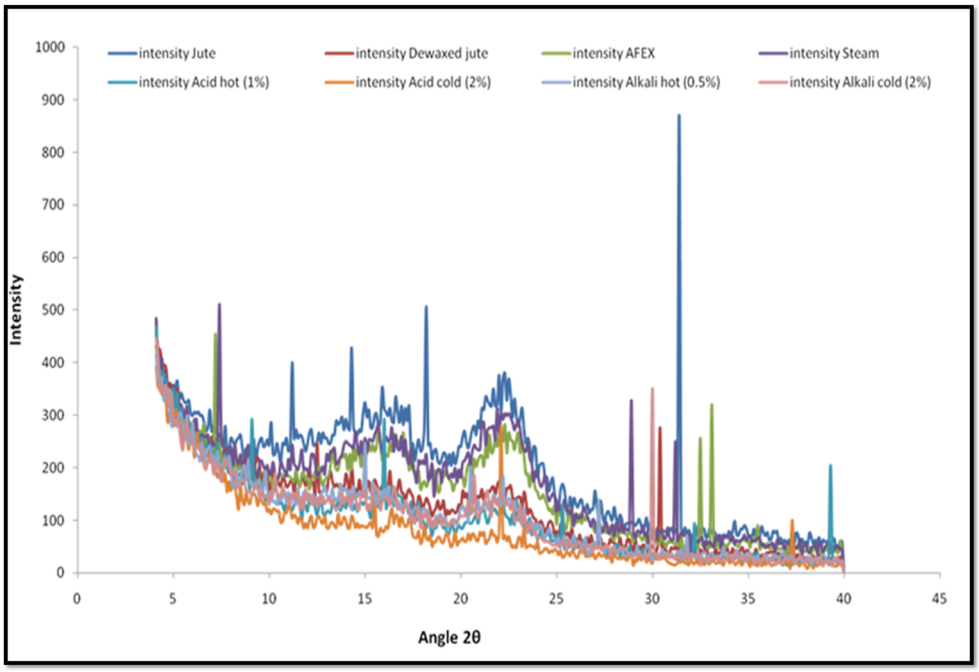

Fig. 4 XRD pattern of untreated and treated jute biomass

Table 3 Total reducing sugars (g/L) released at different intervals of time after enzymatic saccharification of pretreated jute biomass

Sample	Total reducing sugars released (g/L)		
	24 h	48 h	72 h
Raw	8.41 ± 0.86	9.46 ± 0.04	10.91 ± 0.08
Steam	6.49 ± 0.39	6.63 ± 0.23	6.93 ± 0.12
AFEX	10.13 ± 0.48	10.43 ± 0.20	10.90 ± 0.08
Hot (121 °C) NaOH			
0.50%	12.88 ± 0.44	14.40 ± 0.23	14.98 ± 0.46
1%	13.13 ± 0.25	15.30 ± 0.26	15.61 ± 0.42
1.50%	14.97 ± 0.45	16.14 ± 0.46	17.16 ± 0.78
2%	15.50 ± 0.80	17.77 ± 0.32	19.51 ± 0.56
Cold (room temp.) NaOH			
0.50%	9.33 ± 0.29	11.00 ± 0.58	12.02 ± 0.10
1%	9.85 ± 0.06	10.46 ± 0.11	12.55 ± 0.44
1.50%	12.61 ± 0.24	13.28 ± 0.15	14.12 ± 0.15
2%	13.44 ± 0.49	14.85 ± 0.50	15.76 ± 1.63
Hot (121 °C) H_2SO_4			
0.50%	8.83 ± 0.28	9.10 ± 0.16	9.48 ± 0.14
1%	6.50 ± 0.07	8.15 ± 0.21	8.42 ± 0.28
1.50%	6.47 ± 0.68	7.20 ± 0.21	7.52 ± 0.11
2%	6.85 ± 0.53	7.15 ± 0.08	7.38 ± 0.13
Cold (room temp.) H_2SO_4			
0.50%	8.93 ± 0.94	8.88 ± 0.47	9.08 ± 0.20
1%	7.91 ± 0.11	8.00 ± 0.09	8.32 ± 0.20
1.50%	7.21 ± 0.02	8.21 ± 0.13	8.59 ± 0.09
2%	6.10 ± 0.06	6.80 ± 0.12	7.05 ± 0.09

polymerization and crystallinity of the complex cellulose structure and hence disrupting the lignin. In lignocellulosic material, NaOH gives a better internal surface by swelling it leading to lignin degradation. The main effect of sodium hydroxide pretreatment on lignocellulosic biomass is delignification by breaking the ester bonds cross-linking lignin and xylan, thus, increasing the porosity of biomass [49]. Akhtar et al. [50] also reported that pretreatment with 2% NaOH increased the saccharification rates by 33.0, 25.5, and 35.5% for wheat straw, rice straw, and bagasse, respectively. Some studies have also reported that application of sodium hydroxide is a better treatment for other lignocellulosic resources, including hardwood, softwood, corn stover, cotton stalk, and wheat straw [51–54].

3.8 Ethanol production from alkali-pretreated jute biomass

The saccharified hydrolysate was used for ethanol production using *S. cerevisiae* JRC6 through submerged fermentation. The reducing sugar released after enzymatic saccharification of alkali-treated biomass was 19.51 g/L, accounting for 77.44% saccharification which is greater than the reducing sugar released by the saccharification of other pretreated jute biomass. When the alkali-treated saccharified hydrolysate was used for ethanol fermentation by *S. cerevisiae* JRC6, 7.55 g/L of ethanol was produced. The yeast strain has shown 77.73% fermentation efficiency during fermentation. This study proves the superiority of selected yeast strain as evident from ethanol yield reported for other crop residues (Table 4).

Table 4 Ethanol production potential of various crop residues

Biomass	Ethanol yield (g/L)	Reference
Sugarcane bagasse	4.50 g/L	[55]
Sri Lanka ecotype vetiver grass	1.14 g/L	[56]
Mesta	4.10 g/L	[7]
Jute sticks	7.55 g/L	Present study

4 Conclusion

This is the first report demonstrating utilization of jute biomass for bioethanol production along with the recovery of lignin as well as characterization of important platform chemicals in alkaline wash of the biomass. The potential of jute biomass as a source of fermentable sugar for bioethanol production was evaluated by subjecting the biomass to various pretreatments and estimating the sugar and ethanol yield during enzymatic saccharification and fermentation, respectively. SEM, FTIR, and XRD analysis confirmed the favorable structural and functional changes in the pretreated biomass. The maximum reducing sugar (19.51 g/L) released after the saccharification of alkali hot (2% NaOH) treated biomass and the subsequent fermentation of the hydrolysate with *S. cerevisiae* JRC6 produced 7.55 g/L of ethanol. Further, the extraction of lignin from the alkali wash of pretreated jute biomass followed by the analysis of the remaining wash for the presence of lignin monomers using LC-MS will help in efficient valorization of jute biomass in a biorefinery approach.

The work performed can help in future research wherein various extraction processes can be used for the recovery of monomeric chemicals obtained from the disassociation of lignin in the alkaline wash to be used as important high-value biochemicals and platform chemicals.

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References

1. Kamm B, Kamm M, Gruber PR, Kromus S (2006) Biorefinery systems–an overview. In: Kamm B, Gruber PR, Kamm M (eds) Biorefineries–industrial processes and products, status quo and future directions. Wiley-Verlag, Weinheim, pp 3–40
2. Mabee WE, Gregg DJ, Saddler JN (2005) Assessing the emerging biorefinery sector in Canada. Appl Biochem Biotechnol 123(1–3): 0765–0778. https://doi.org/10.1385/abab:123:1-3:0765
3. Lashinsky A, Schwartz ND (2006) How to beat the high cost of gasoline, Fortune 74, 82
4. Patil V, Tran K-Q, Giselrød HR (2008) Towards sustainable production of biofuels from microalgae. Int J Mol Sci 9(7):1188–1195. https://doi.org/10.3390/ijms9071188
5. Soo C-S, Yap W-S, Hon W-M, Phang L-Y (2015) Mini review: hydrogen and ethanol co-production from waste materials via microbial fermentation. World J Microbiol Biotechnol 31(10):1475–1488. https://doi.org/10.1007/s11274-015-1902-6
6. Wang M, Zhou D, Wang Y, Wei S, Yang W, Kuang M, Du S (2016) Bioethanol production from cotton stalk: a comparative study of various pretreatments. Fuel 184:527–532. https://doi.org/10.1016/j.fuel.2016.07.061
7. Lavanya A K, Sharma A, Choudhary S B, Sharma H K, Nain P K S, Singh S, Nain L (2019) Mesta (Hibiscus spp.)–a potential feedstock for bioethanol production. Energy Sources Part A 1–14. https://doi.org/10.1080/15567036.2019.1618980
8. Ghosh BK, Jethi A (2013) Growth and instability in world jute production: a disaggregated analysis. Int J Electron Commun Technol 4:191–195
9. Mondal D, Bandyopadhyay AK (2014) Adoption of jute production technology in West Bengal. Econ Aff 59:701–709
10. Directorate of Economics and Statistics (2018-2019) Department of Agriculture, Cooperation and Farmer's Welfare, Ministry of Agriculture and Farmer's Welfare, Govt of India https://eands.dacnet.nic.in/
11. National Jute Board (2010) Ministry of Textile, Govt. of India. https://www.jute.com/ [12] Ragauskas A J (2006) The Path Forward for Biofuels and Biomaterials. Science 311(5760): 484–489.https://doi.org/10.1126/science.1114736
12. Ragauskas AJ (2006) The Path Forward for Biofuels and Biomaterials. Science 311(5760):484–489. https://doi.org/10.1126/science.1114736
13. Hu L, Pan H, Zhou Y, Zhang M (2011) Methods to improve lignin's reactivity as a phenol substitute and as replacement for other phenolic compounds: a brief review. BioResources 6(3): 3515–3525
14. Min D, Waters Smith S, Chang H, Jameel H (2013) Influence of isolation condition on structure of milled wood lignin characterized by quantitative 13C nuclear magnetic resonance spectroscopy. BioResources 8(2):1790–1800. https://doi.org/10.15376/biores.8.2.1790-1800
15. Brosse N, Mohamad Ibrahim MN, Abdul Rahim A (2011) Biomass to bioethanol: initiatives of the future for lignin. ISRN Mater Sci 2011:1–10. https://doi.org/10.5402/2011/461482
16. Jackson ML (1973) Methods of chemical analysis. Prentice Hall of India Pvt, New Delhi
17. NREL (National Renewable Energy Laboratory), CO (2012). Determination of structural carbohydrates and lignin in biomass in the LAP (Laboratory Analytical Procedure)
18. Hestrin S (1949) The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine, and its analytical application. J Biol Chem 180(1):249–261
19. Blumenkrantz N, Asboe-Hansen G (1973) New method for quantitative determination of uronic acids. Anal Biochem 54(2):484–489. https://doi.org/10.1016/0003-2697(73)90377-1
20. Kaur A, Kuhad RC (2019) Valorization of rice straw for ethanol production and lignin recovery using combined acid-alkali pretreatment. BioEnergy Res 12(3):570–582. https://doi.org/10.1007/s12155-019-09988-3
21. Miller GL (1959) Use of dinitrosalycilic acid reagent for determination of reducing sugar. Anal Chem 31:426–428

22. Teymouri F, Laureano-Perez L, Alizadeh H, Dale BE (2005) Optimization of the ammonia fiber explosion (AFEX) treatment parameters for enzymatic hydrolysis of corn stover. Bioresour Technol 96(18):2014–2018. https://doi.org/10.1016/j.biortech.2005.01.016
23. Bajpai P (2016) Pretreatment of lignocellulosic biomass. Pretreatment of Lignocellulosic Biomass for Biofuel Production 2016: 17–70. https://doi.org/10.1007/978-981-10-0687-6_4
24. Lau MW, Gunawan C, Dale BE (2009) The impacts of pretreatment on the fermentability of pretreated lignocellulosic biomass: a comparative evaluation between ammonia fiber expansion and dilute acid pretreatment. Biotechnol Biofuels 2(1):30. https://doi.org/10.1186/1754-6834-2-30
25. Tarkow H, Feist W C (1969) A mechanism for improving the digestibility of lignocellulosic materials with dilute alkali and liquid ammonia. Cellulases and Their Applications 197–218. https://doi.org/10.1021/ba-1969-0095.ch012
26. Helle S, Cameron D, Lam J, White B, Duff S (2003) Effect of inhibitory compounds found in biomass hydrolysates on growth and xylose fermentation by a genetically engineered strain of *S. cerevisiae*. Enzym Microb Technol 33(6):786–792. https://doi.org/10.1016/s0141-0229(03)00214-x
27. Yuan Y, Teng Q, Zhong R, Haghighat M, Richardson EA, Ye ZH (2016) Mutations of arabidopsis TBL32 and TBL33 affect xylan acetylation and secondary wall deposition. PLoS One 11(1): e0146460. https://doi.org/10.1371/journal.pone.0146460
28. Sorek N, Yeats TH, Szemenyei H, Youngs H, Somerville CR (2014) The implications of lignocellulosic biomass chemical composition for the production of advanced biofuels. Bioscience 64(3): 192–201. https://doi.org/10.1093/biosci/bit037
29. Selig MJ, Adney WS, Himmel ME, Decker SR (2009) The impact of cell wall acetylation on corn stover hydrolysis by cellulolytic and xylanolytic enzymes. Cellulose 16(4):711–722. https://doi.org/10.1007/s10570-009-9322-0
30. Cho DH, Shin S-J, Bae Y, Park C, Kim YH (2010) Enhanced ethanol production from deacetylated yellow poplar acid hydrolysate by *Pichia stipitis*. Bioresour Technol 101(13):4947–4951. https://doi.org/10.1016/j.biortech.2009.11.014
31. Maurya DP, Singla A, Negi S (2015) An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol. 3. Biotech 5(5):597–609. https://doi.org/10.1007/s13205-015-0279-4
32. Chang VS, Holtzapple MT (2000) Fundamental factors affecting biomass enzymatic recativity. In: Finkelstein M, Davison BH (eds) Twenty-first symposium on biotechnology for fuels and chemicals. Springer
33. Grohmann K, Mitchell DJ, Himmel ME, Dale BE, Schroeder HA (1989) The role of ester groups in resistance of plant cell wall polysaccharides to enzymatic hydrolysis. Appl Biochem Biotechnol 20-21(1):45–61. https://doi.org/10.1007/bf02936472
34. Bouveng HO, Høeg H, Michelsen K, Nielsen GB, Nord H, Jart A (1961) Phenylisocyanate derivatives of carbohydrates. II. Location of the O-acetyl groups in birch xylan. Acta Chem Scand 15:96–100. https://doi.org/10.3891/acta.chem.scand.15-0096
35. Gharpuray MM, Lee Y-H, Fan LT (1983) Structural modification of lignocellulosics by pretreatments to enhance enzymatic hydrolysis. Biotechnol Bioeng 25(1):157–172. https://doi.org/10.1002/bit.260250113
36. Kusmiyati K, Anarki ST, Nugroho SW, Widiastutik R, Hadiyanto H (2019) Effect of dilute acid and alkaline pretreatments on enzymatic saccharfication of palm tree trunk waste for bioethanol production. Bull Chem React Eng Catal 14(3):705. https://doi.org/10.9767/bcrec.14.3.4256.705-714
37. Sharma S, Sharma V, Kuila A (2016) Cellulase production using natural medium and its application on enzymatic hydrolysis of thermo chemically pretreated biomass. 3 Biotech 6(2): https://doi.org/10.1007/s13205-016-0465-z
38. Zeng J, Singh D, Chen S (2011) Biological pretreatment of wheat straw by *Phanerochaetechrysosporium* supplemented with inorganic salts. Bioresour Technol 102(3):3206–3214. https://doi.org/10.1016/j.biortech.2010.11.008
39. Trache D, Hussin MH, Hui Chuin CT, Sabar S, Fazita MRN, Taiwo OFA, Haafiz MKM (2016) Microcrystalline cellulose: isolation, characterization and bio-composites application—a review. Int J Biol Macromol 93:789–804. https://doi.org/10.1016/j.ijbiomac.2016.09.056
40. Sun Y, Cheng J (2005) Dilute acid pretreatment of rye straw and Bermuda grass for ethanol production. Bioresour Technol 96(14): 1599–1606. https://doi.org/10.1016/j.biortech.2004.12.022
41. He Y, Pang Y, Liu Y, Li X, Wang K (2008) Physicochemical characterization of rice straw pretreated with sodium hydroxide in the solid state for enhancing biogas production. Energy Fuel 22(4): 2775–2781. https://doi.org/10.1021/ef8000967
42. Zhang S, Xu Y, Hanna MA (2011) Pretreatment of corn stover with twin-screw extrusion followed by enzymatic saccharification. Appl Biochem Biotechnol 166(2):458–469. https://doi.org/10.1007/s12010-011-9441-6
43. Hocking MB (1997) Vanillin: synthetic flavoring from spent sulfite liquor. J Chem Educ 74(9):1055. https://doi.org/10.1021/ed074p1055
44. Prothmann J, Spégel P, Sandahl M, Turner C (2018) Identification of lignin oligomers in Kraft lignin using ultra-high-performance liquid chromatography/high-resolution multiple-stage tandem mass spectrometry (UHPLC/HRMSn). Anal Bioanal Chem 410(29): 7803–7814. https://doi.org/10.1007/s00216-018-1400-4
45. Mota MIF, Rodrigues Pinto PC, Loureiro JM, Rodrigues AE (2015) Recovery of vanillin and syringaldehyde from lignin oxidation: a review of separation and purification processes. Sep Purif Rev 45(3):227–259. https://doi.org/10.1080/15422119.2015.1070178
46. Du S, Su X, Yang W, Wang Y, Kuang M, Ma L, Zhou D (2016) Enzymatic saccharification of high pressure assist-alkali pretreated cotton stalk and structural characterization. Carbohydr Polym 140: 279–286. https://doi.org/10.1016/j.carbpol.2015.12.056
47. Wang Q, Hu J, Shen F, Mei Z, Yang G, Zhang Y, Deng S (2016) Pretreating wheat straw by the concentrated phosphoric acid plus hydrogen peroxide (PHP): investigations on pretreatment conditions and structure changes. Bioresour Technol 199:245–257. https://doi.org/10.1016/j.biortech.2015.07.112
48. Khan MA, Ashraf SM, Malhotra VP (2004) Eucalyptus bark lignin substituted phenol formaldehyde adhesives: a study on optimization of reaction parameters and characterization. J Appl Polym Sci 92(6):3514–3523. https://doi.org/10.1002/app.20374
49. Kang KE, Jeong G-T, Park D-H (2011) Pretreatment of rapeseed straw by sodium hydroxide. Bioprocess Biosyst Eng 35(5):705–713. https://doi.org/10.1007/s00449-011-0650-8
50. Akhtar MS, Saleem M, Ruby G (2001) Enzymatic saccharification of lignocellulosic materials by the xylanase of *Bacillus subtilis*. J Biol Sci 1(5):398–400. https://doi.org/10.3923/jbs.2001.398.400
51. Balat M (2011) Production of bioethanol from lignocellulosic materials via the biochemical pathway: a review. Energy Convers Manag 52(2):858–875. https://doi.org/10.1016/j.enconman.2010.08.013
52. Silverstein RA, Chen Y, Sharma-Shivappa RR, Boyette MD, Osborne J (2007) A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. Bioresour Technol 98(16):3000–3011. https://doi.org/10.1016/j.biortech.2006.10.022
53. Millett MA, Baker AJ, Satter LD (1976) Physical and chemical pretreatments for enhancing cellulose saccharification. Biotechnol Bioeng Symp 6:125–153

54. Li Y, Ruan R, Chen PL, Liu Z, Pan X, Lin X, Yang T (2004) Enzymatic hydrolysis of corn stover pretreated by combined dilute alkaline treatment and homogenization. Trans ASAE 47(3):821–825. https://doi.org/10.13031/2013.16078
55. Khuong LD, Kondo R, De Leon R, Kim Anh T, Shimizu K, Kamei I (2014) Bioethanol production from alkaline-pretreated sugarcane bagasse by consolidated bioprocessing using Phlebia sp. MG-60. Int Biodeterioration Biodegradation 88:62–68. https://doi.org/10.1016/j.ibiod.2013.12.008
56. Wongwatanapaiboon J, Kangvansaichol K, Burapatana V, Inochanon R, Winayanuwattikun P, Yongvanich T, Chulalaksananukul W (2012) The potential of cellulosic ethanol production from grasses in Thailand. J Biomed Biotechnol 2012: 1–10. https://doi.org/10.1155/2012/303748