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Study of Various Pretreatments on Waste Jute Caddies for the Production of Ethanol

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***Abstract*— Lignocellulosic biomass is most abundant renewable resource suitable for a continuous supply of bioethanol. Waste Jute caddies (WJC) from Jute mills can be a potential feedstock for the production of ethanol. In the present study, the effect of various pretreatments on WJC was compared by glucose and ethanol yields. The WJC was pretreated using physical and chemical pretreatments viz. steam, sodium hydroxide, sulfuric acid, Triton x-100. The cellulose fractions of pretreated WJC were allowed to undergo saccharification using cellulase enzymes. S. cerevisiae was used in the anaerobic fermentation of glucose and effect of supplementation with benzoic acid on ethanol yield was also investigated. The quantitation of glucose and ethanol was performed by High performance liquid chromatography (HPLC). The changes in chemical nature of biomass due to pretreatment were determined using XRD and FTIR. The highest yield ethanol was observed after fermentation with sodium hydroxide pretreated WJC. Based on fermentation studies conducted on WJC, the ethanol yield was enhanced up to 8% by supplementation of benzoic acid.**

***Keywords* — Bioethanol, Waste Jute Caddies, Lignocellulose, Pretreatment**

I. INTRODUCTION

The rise in carbon emissions besides energy security has strengthened the interest in alternative, non-petroleum based sources of fuels. Biomass is the major suitable and renewable primary energy resource that can provide alternative transportation fuels such as bioethanol [1]. Jute caddies which are the waste after processing jute can be used as raw material for production of ethanol. Jute caddies are produced in large amount in Bangladesh, India, and China etc. Jute is a kharif crop, sown in March to April on lowlands and in May to June on uplands. Jute is a thirsty plant and requires sufficient rainfall over the period of growth. According to FAO statistics, India produced 1.9 M tonnes of jute in the year of 2016 and became world's largest producer for the year [2]. The crude oil imports of India are raising due to the demand in transportation sector [3]. Further the policies on non-grain based ethanol blending in petrol, environmental protection laws have increased focus on bioenergy research. Jute with high cellulose content is considered to be suitable for glucose and ethanol production [5]. The common step in the production of ethanol is pretreatment followed by saccharification and fermentation. The cellulose, hemicellulose and lignin polymers exhibit recalcitrance to hydrolytic enzymes during saccharification. The extent of hydrolysis or sugar formation depends on the efficiency of pretreatment. Acid pretreatment, a major chemical treatment can be divided into dilute acid and concentrated acid pretreatment. The objective of acid pretreatment is to partially or completely hydrolyze hemicellulose, break down the lignin structure and disrupt the cellulose crystallinity for further enzymatic digestion to release fermentable sugars. Generally, concentrated acid (H2SO4 and HCl) pretreatment is considered to be too corrosive and hazardous to operate. So, dilute acid pretreatments has been studied under different residencies in time are many researchers. Various agro-industrial residues, including corn cobs, corn stover, whey straw, whey bran, sugarcane bagasse, and cassava bagasse, have been studied. Physical pretreatment, especially mechanical pretreatment, employs machinery chipping, grinding, or milling to reduce the size of biomass and the cellulose crystallinity improving easy acid/enzyme access. Depending on the requirements, biomass can first be sent through a chipping machine to obtain particles at sizes of 10-30 mm; and if fine powder is preferred, the biomass can be further sent for grinding or milling to reduce the size to 0.2-2 mm. In general, the smaller the particle size, the easier for the microorganism or enzyme to digest. Steam explosion has been applied to and is recognized as one of the most effective pretreatment methods for lignocellulosic materials, particularly agricultural residues and hardwood. Advantages of steam explosion mainly include reducing the biomass size, effective removal of lignin and hemicellulose without dilution of the resulting sugars and lower energy cost compared to mechanical milling. In the present work, waste jute caddies were employed as raw material for the production of glucose and ethanol. Various pretreatments like acid, alkaline, steam and surfactant based pretreatments were compared with untreated biomass. The effect of efficient pretreatment was investigated in terms of cellulose crystallinity using XRD analysis of materials before and after pretreatment. In addition, fermentation of hydrolysate was carried out using Saccharomyces cerevisiae in the presence and absence of benzoic acid supplementation and corresponding ethanol yields were compared. At each stage,

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the comparison was made with untreated biomass. The changes in chemical groups were compared using FTIR.

II. MATERIALS AND METHODS

A. Waste jute caddies, enzymes, organism and chemicals

WJC was procured from Vinayaka group, India. The sample was stored in vacuum desiccator until use. The moisture content of WJC was determined using oven and found to be 7.4% according to National renewable energy laboratory (NREL) protocol [23]. The composition of biomass compositions was determined by NREL procedure [22]. The basic principle is hydrolysis of polymers to monomers and determination of these monomers subsequently using HPLC. According to this procedure, a 300 mg of WJC sample was hydrolyzed with 3 ml of 72% (w/w) H2SO4 kept in 30 °C water bath for 60 min with random agitation. The mixture was diluted using 4% H2SO4 and autoclaved at 15 psi, and temperature of 121°C for 60 min. The resultant hydrolysate was vacuum filtered and analyzed for glucose, arabinose, xylose, other sugars and acetate concentrations by using HPLC as described below. The soluble lignin (Acid soluble) was quantified using spectrophotometer at absorbance of 240 nm. The solid remained after vacuum filtration was subjected to drying at 105 °C for one day, subsequently heated in muffle furnace up to 575 °C for one day. The acid insoluble lignin (AIL) weight was used as ash content value. The composition of WJC in terms of component weights are shown in Table 1. The saccharification enzyme celluclast B4 was gifted by Brenntag (India). HPLC carbohydrates and sulfuric acid were procured from Sigma-Aldrich (India). HPLC grade ethanol was obtained from Merck (India). The fermentation organism, S. cerevisiae MTCC 170 for ethanol production was obtained from the MTCC, Chandigarh, India. The yeast strain S. cerevisiae was cultured using a YPD broth (yeast extract 10 g/L, peptone 20 g/L, and glucose 20 g/L) with pH 6.5 and incubated at 30 °C and 140 rpm for 20 h. Then agar slants were prepared by 2% agar in above YPD broth and stored in refrigerator at 4 °C until use.

Table 1 waste jute caddies (WJC) composition

Constituent	Waste caddies Jute
Cellulose	59.5
Hemicellulose	21.9
Lignin	12.5
Others	6.1

B. Analysis of carbohydrate monomers, acetate and ethanol by HPLC

All the non-corrosive liquid samples were filtered through 0.2 µm syringe filters (Diameter: 1.5cm,) with nylon membrane and all the corrosive liquid samples containing acid or base were filtered through syringe filter of same dia. having PTFE membrane. A sample volume of 20 µl was injected in HPLC having Refractive index detector (Shimadzu LC20-A, Japan), degassing unit and Hi-plex H column (7.7 × 300 mm, 8 µm) by Agilent, India. Sulphuric acid (5m mol l-1) was used as mobile phase with a flow rate of 0.7ml/min. The column temperature was maintained at 60 °C. The amounts of various compounds were determined using standard concentrations of carbohydrates, acetate and ethanol.

C. WJC pretreatment

A 5% (v/v) biomass loading was employed for all pretreatments. Dilute acid pretreatment was carried out using 1% (v/v) H2SO4, Alkaline pretreatment was conducted with 2% NaOH, and Surfactant based pretreatment was conducted with 0.5% triton x-100. The pretreatment flasks were kept in the in an orbital shaker at 200 rpm at temperature of 60 °C for 24 h. After this the flasks were sterilized using autoclave at 121°C and 15 psi for 15 min along with a control. Steam pretreatment was carried out using autoclave at 121°C and 15 psi for 60 min. Then, pretreated flasks were removed and allowed to cool at room temperature before further operation.

D. Saccharification of pretreated WJC

Hydrolysis of pretreated solid was carried out with 2% biomass i.e., 1g in 50ml citrate buffer at 45 °C and 140 rpm for 72 h in incubator (Reico, India). A hydrolysis mixture of 50mL contains 1g of biomass (pretreated), 200 µL cellulase enzyme (filtered through syringe filter), 0.1 M citrate buffer of pH 4.8 and 1 mg of NaZ (antibacterial). After hydrolysis, the liquid obtained was filtered through cheesecloth and analyzed for the presence of glucose using HPLC.

E.Fermentation of hydrolysate

The inoculum was prepared using agar slants of yeast in 100 ml YPD broth as described earlier at pH 6.5 and incubated at 30°C, 140 rpm for 20 h under aerobic conditions. The inoculum volume was determined in order to get fermentation hydrolysate OD (optical density) of 0.5 at 600 nm. The inoculum was then centrifuged at 4000 rpm for 5 minutes and 0.5 ml of pre-sterilized water was added aseptically to pellet. Fermentations under anaerobic conditions were performed with and without addition of benzoic acid (0.5g/L) by using 9.5 ml hydrolyzate in a glass tube both for pretreated and control WJC biomass samples. In addition, these samples were supplemented with 2% peptone and adjusted to pH 6.5. These tubes were autoclaved at temperature of 121 °C and pressure of 15 psi for 15 min. Then, these tubes were inoculated with 0.5ml of prepared inoculum. The glass tubes were kept in orbital shaker at 30 °C, 130 rpm for 72 h under anaerobic environment. The

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samples were analyzed for the presence of glucose and ethanol using HPLC by collecting after 72 h.

F. XRD and FTIR Analysis of solid samples

The crystallinity of cellulose in raw materials, pretreated samples were analyzed using X-ray diffractometer (X-Pert, Netherlands) using Cu K-alpha (k = 0.154 nm); The operating current, voltage were kept at 20mA and 30kV, respectively. The 2θ is kept in the range of 5° to 30° at a scan speed of 10° per min. The crystallinity in the biomass sample was defined as crystallinity index (CrI) and calculated using the following formula [24].

$$\mathrm{CrI}\ (\%) = \left[\left(I_{002} - I_{am} \right) / I_{002} \right] 100 \quad (1)$$

where CrI, crystallinity index, I002, maximum intensity of the 002 peak at 2θ = 22.53°, and Iam, intensity at 2θ =18.48°. The chemical groups present in untreated and pretreated biomass samples was examined using FTIR spectrometer (PerkinElmer) in the range of 400-4000 cm-1. The Potassium bromide (KBr) method was used by mixing 1 mg of biomass with 200mg KBr powder and pressed as 13mm pellets using a hydraulic press for few seconds.

III. RESULTS AND DISCUSSION

A. Effect of various pretreatments

Acid pretreatment is considered as one of the most important technique that aims for high sugar yield from lignocellulosic biomass. Alkaline pretreatment of LC digests the lignin and makes cellulose and hemicellulose available for enzymatic degradation. Alkali treatment of lignocellulose disrupts the cell wall by dissolving hemicelluloses, lignin and silica, by hydrolyzing uronic and acetic esters and by swelling cellulose and because of swelling the crystallinity of cellulose decreases. The triton X-100 pretreatment increases the accessibility of enzymes to carbohydrate polymers on the basis of surfactant action.

B. Effect of NaOH pretreatment on WJC characteristics

The decrystallization of pretreated biomass was widely studied using XRD technique. The crystallinity of biomass was considered as significant for enzyme hydrolysis by many groups of researchers [30]. In the present study, peak height method was used to determine crystallinity index given by Segal and coworkers [24]. This is an empirical formula which allows rapid determination of crystallinity of cellulosic materials after various pretreatments. The CrI of cellulose was determined using I002 (18.48°) peak and the maximum height I002 peak (22.53°) obtained from the XRD spectra. The XRD spectra obtained for NaOH pretreatment along with untreated WJC is shown in Fig. 1.

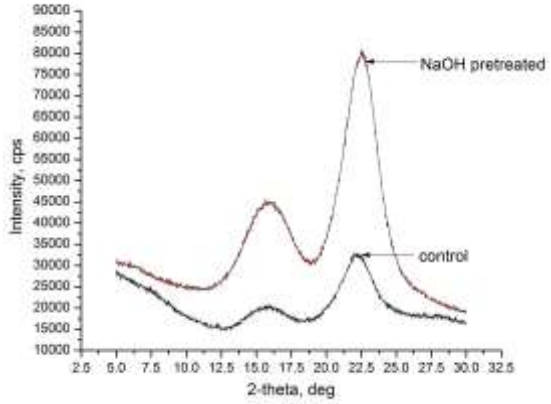

Fig. 1 X-ray spectrum of waste jute caddies (WJC) pretreated with sodium hydroxide in red line and control in black line.

The structure of cellulose can be changed by various pretreatments by breaking H bonds of intra- and interchain chains of microfibrils present in cellulose polymer [31]. Thus we investigated the effect of pretreatment using cellulose crystallinity. Sodium hydroxide pretreatment resulted in increased H bond intensity (Fig. 1). Further, the H bonds leads to the binding of water increasing enzyme interactions during hydrolysis and thus, producing sugars [32]. The crystallinity index of untreated and pretreated WJC are 30% and 45%.

The changes in chemical groups of the untreated and pretreated WJC was also examined using FTIR spectroscopy. The dotted line indicate WJC pretreated with NaOH and solid line indicate control FTIR specra and shown in Fig. 2. The peak at 3418 cm-1 was mainly associated with hydroxyl groups in cellulose which was unaltered in both the samples. It implies that crystalline cellulose was mildly disrupted due to the action of NaOH. The peak at 2918 cm-1 related to C-H stretching in methylene of cellulose. This peak was slightly increased in WJC. The peaks at 1610 cm-1 and 1517 cm-1 are related to C=C bonds vibrations of lignin subunits of S and G respectively. The bond stretchings of peak at 1610 cm-1 for pretreated WJC was increased and coalesced with band near 1650 cm-1 which indicates degradation of lignin subunit. The peak at 1517 cm-1 were also dimnished in WJC after pretreatment. The peaks at 1377 cm-1 were assigned to CH bendings of celluloses and hemiculloses. The variation is only slightly observed in pretreated WJC which implies that cellulose was not affected much due to pretreatment at respective conditions. Moreover, the peaks at 1200-1000 cm-1 were designated to of cellulose and hemicelluloses also suggests that these components are affected by pretreatment.

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The peak at 910 cm-1 is corresponds to beta-1,4 glycosidic linkage of cellulose indicating the dissociations of subunits links after pretreatment of WJC.

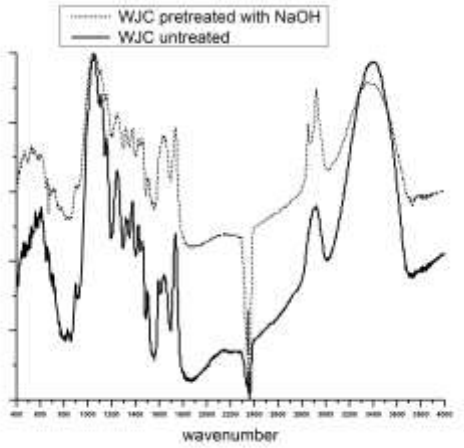

Fig. 3 Fourier transform infrared spectra of waste jute caddies (WJC) pretreated with sodium hydroxide along with untreated WJC.

C. Saccharification and fermentation

The hydrolysis of cellulose depends on the pretreatmet severity. The cellulase enzymes are very specific for cellulose. The effect of the pretreatment directly influence the glucose yields in saccharification. The untreated samples are also compared with pretreated samples in terms of glucose yields. The saccharification yields of obtained after 3 days for various pretreatments along with untreated controls WJC are shown in Table 2. It was observed that the saccharification of untreated sample was less compared with pretread samples. Thus, it suggests that pretreatment increases available surface area for enzymatic saccharification. The enzymatic digestion of NaOH treated WJC showed a glucose yield of 434 mg/g of biomass compared to untreated which is only 2744 mg/g of biomass. The results significantly highlighted the various pretreatments viz., acid, base, surfactant, and steam affects the enzymatic hydrolysis.

Pretreatment Methods	Sugar concentration (mg/gm of biomass)	Ethanol concentration (mg/gm of biomass)
Triton X100	358	166
H2SO4	301	138
NaOH	434	199
Steam	284	131
Untreated	274	126

The enzyme hydrolysates from various pretreatments and untreated samples were fermented for ethanol production using S.cerevisiae under anaerobic conditions without benzoic acid and supplemented with benzoic acid. The pathway in yeast will break glucose to ethanol and water releasing CO2 under anaerobic conditions. But, under aerobic conditions ethanol is not produced. It was also proved that the yields of ethanol were less in aerobic conditions even after supplementing with benzoic acid like reducing agents [19]. So, complete anaerobic conditions were adapted in this study. S. cerevisiae is preferred organism widely used for ethanol fermentation because of its resistance to high ethanol concentrations and inhibitory compounds. It can metabolize hexoses like glucose, galactose and mannose to ethanol [20]. Thus, this organism was used to study its potential to produce ethanol from various saccharified WJC samples. The ethanol yields obtained from saccharified WJC samples pretreated with NaOH is 166 mg/g of biomass when compared to untreated sample which is only 126 mg/g of biomass. This represents an increase of 31.7% when compared with untreated WJC sample. The yield of ethanol was further enhanced with supplementation of benzoic acid by 8% with NaOH pretreated biomass. It constitutes a final ethanol yield of 172 mg/g of biomass. The yields of sugars and ethanol can also be improved using xylanase enzyme in saccharification and xylose-fermenting yeasts. However, their usage affects process economics and should be evaluated. The reducing agent, cysteine hydrochloride showed a positive effect on ethanol production under anaerobic condition in both types of biomass. Moreover, cysteine hydrochloride is less toxic and the reducing agent is commonly used to grow anaerobic bacteria and fungi [35, 36]. Our results also showed that low levels of cysteine hydrochloride (0.5 g/L) could increase ethanol yield on supplementation under anaerobic conditions.

IV. CONCLUSION

The various pretreatments were compared in terms of physical and chemical analysis. The XRD showed an increase in crystallinity of 15% after NaOH pretreatment. The enzymatic digestion of NaOH treated WJC showed a glucose yield of 434 mg/g of biomass compared to untreated which is only 2744 mg/g of biomass. The ethanol yields obtained from saccharified WJC samples pretreated with NaOH is 166 mg/g of biomass when compared to untreated sample which is only 126 mg/g of biomass. This represents an increase of 31.7% when compared with untreated WJC sample. The yield of ethanol was further enhanced with supplementation of benzoic acid by 8% with NaOH pretreated biomass.