Comparative study of various pre-treatment techniques for saccharifications of water hyacinth (eichhornia crassipes) cellulose

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Abstract
Alkali, acid and hydrogen peroxide pretreatments were compared for their effect on lignin removal from water hyacinth, among them alkali pretreatment gave good results as compared to others. The highest sugar content in alkali pretreated samples was observed in water hyacinth treated with 5% NaOH solution at 20 psi pressure for 20 minute is 97.50%. Whereas the acid pretreatment had less effect on conversion of lignocellulose in water hyacinth to sugar is 80.6% at the same pressure and time. For hydrogen peroxide pretreatment it is around 67.00%. In this study, we have demonstrated the suitability of physical pretreatment under high pressure and temperature for production of fermentable sugar. Morphology inspection had also provided evidences of structure disruption that led to higher sugar recovery from water hyacinth in alkali pretreatment process than acid and hydrogen peroxide pretreatment process under the same conditions.

Key words*: Physical pretreatment, Water hyacinth, Reducing sugar, Morphology*

I. INTRODUCTION

Water hyacinth (Eichhornia crassipes) is first growing aquatic plants which are potential sources for producing a usable grade of ethanol or biogas for energy production. Potential plants use as sustainable energy sources include trees, certain grasses, weeds, crop such as corn, sugarcane and aquatics such as water hyacinth, algae etc.. The harvested frequency for water hyacinth tends to the order of days whereas the frequency of the trees and crops are in the order of months and year. Water hyacinth is considered as an alternative raw material for the production of fuel ethanol because of its availability in large quantities at low cost all over the world [1]. Water hyacinth grows rapidly and produces almost two tons of biomass per acre of land and population doubles every 10 to 15 days [2].

Studies in our laboratory indicated that water hyacinth collected from different ponds from Sonarpur, South 24 parganas district, West Bengal, contains 20.5 % cellulose, 40.1% hemicelluloses and 13.9% lignin. Water hyacinth is thus a lignocellulosic biomass and for better utilization it can be used as a good source for production of reducing sugars, which can be farther used for the production of ethanol, organic acid and other chemicals [3].

One of the major difficulties for production of bioethanol from lignocellulosic materials is low solubilisation of cellulose and hemicellulose during the hydrolysis process to produce sugar. In a lignocellulosic material the cellulose, hemicellulose and lignin are linked and formed strong structures which are difficult to be processed in raw condition [4].Thus the pretreatment is required in order to render those compositions for farther exploitations leading to improve hydrolysis process.

The present studies aims at investigating chemical pretreatment of water hyacinth using acid, alkali and hydrogen peroxide at different pressure, temperature and time. Emphasis was directed toward investigating the appropriate conditions leading to good delignification with the least loss of hemicelluloses and cellulose contents.

II. MATERIAL AND METHODS

Substrate

Fresh water hyacinth plant with long steam was collected from natural pond. The water hyacinth was thoroughly washed several times with water to remove adhering dirt. Then it chopped in a small piece (1-2 cm) and finally dried in a hot air oven at 80 ^{0}C with air circulation for 8 hours. The dried material was stored at room temperature until used.

Screening of best pre-treatment for the estimation of cellulose, hemicellulose and lignin:

A. Alkali pretreatment:

The solid substrate 5% (w/v) was pretreated in an autoclave with NaOH at concentration of 4% (w/v) as soaking agent for residence time 20 minitue at 121 ^{0}C temperature and 15 psi pressure. After cooling, the contents were filtered with two layers of cheese cloth and residues were washed several times with water to neutralize the pH followed by final rinse in distilled water, after that the residue was air dried by spreading on a tray at 80 ^{0}C for 8 hour for subsequent analysis.

B. Acid pretreatment:

The substrate 5% (w/v) was pretreated in autoclave with H_2SO_4 at concentration of 5% (w/v) as soaking agent for residence time 20 minute at 121 ^{0}C temperature and 15 psi pressure. The contents after cooling were filtered with double layer of cheese cloth and the residues were washed several times with water to neutralize the pH followed by a final rinse in distilled water. Then the residue was air dried by spreading on tray at 80 ^{0}C for 8 hour for further analysis.

C. Hydrogen peroxide pretreatment:

The solid subtract 5% (w/v) was pretreated in an autoclave with H_2O_2 at concentration of 2% (w/v) as soaking agent for residence time 20 minute at 121 ^{0}C temperature and 15 psi pressure. Then the cooling contents were filtered with double layer of cheese cloth. The residues were washed several times with water to neutralize the pH followed by a final rinse in distilled water. Finally the residue was air dried by spreading on paper at 80 ^{0}C for 8 hour for subsequent use [5].

Analytical methods:

The cellulose, hemicelluloses and lignin content in the given water hyacinth sample were estimated by the method of Goering Van Soest [6]. Estimation of total reducing sugars after hydrolysis in different methods was done by DNS methods [7].

Statistical method:

Each pre-treatment experiment i.e. acid, alkali and hydrogen peroxide was repeated at least two times and each analytical experiment results were means based on data. Standard deviations were showing by errors bars at figures.

III. RESULTS AND DISCUSSION

Estimation of water hyacinth composition:

In the present study pretreatment of water hyacinth plants were investigating with NaOH, H_2SO_4 and H_2O_2 under optimum conditions (20 minute, 121^{0}C and 15 psi pressure). The contents of lignin, cellulose and hemicellulose were investigated and compare with the original untreated sample. The results indicated in **Table-1** represent the composition of the pretreated water hyacinth under the optimum pretreating conditions. Pretreatment typically breaks down macroscopic rigidity of biomass and reduces the physical barriers to mass transport [8].

In the present study, three pretreatment i.e. acid, alkali and hydrogen peroxide were compared for their effect on water hyacinth composition. **Table-1** shows the composition (20.5 % cellulose, 40.1% hemicelluloses and 13.9% lignin) of raw water hyacinth and effect of pretreatment on water hyacinth composition. Dilute acid pretreatment did not remove lignin significantly from the substrate but only modified the lignin carbohydrate linkage and high concentration of acid resulted in the lost of polysaccharides [9]. In the present study, during dilute acid treatment removal of lignin 54% and hemicelluloses removal 22% where observe as shown **Table-1.** Alkali pre-treatment removed lignin without affecting other components [10]. NaOH treatment causes lignocellulosic biomass to swell, leading to an increase in the internal surface area, decreases the degree of crystallinity and disruption of lignin structure. The increase in cellulose during alkali pretreatment where predominantly attributed to decreases in lignin**.** Lignin decreases because of its solubilization in alkali aqueous solution. Alkali pretreatment reduce lignin in biomass, increase the surface area, allowing the penetration of water molecules to inner layers and break the bonds between lignin and hemicellulose carbohydrate [11]. Alkali pereatment is regarded as an efficient pretreatment method for removing lignin from lignocellulosic biomass [12]. The efficiency of delignification was 63.24% with 4% NaOH shown in **Table-1**.

Hydrogen peroxide pretreatment removes the hemicellulose and lignin to increase the accessibility of cellulose. H_2O_2 pretreatment utilizes oxidative delignification to detach and solubilize the lignin and loosen the lignocellulosic matrix. **Table-1** shows the effect of removal efficiency of H_2O_2 on water hyacinth.

All pretreatments can remove the lignin in biomass to retrieve the comparatively purer cellulose with different percentage.

Table: 1 Effect of pre-treatment on Water hyacinth composition (cellulose, hemicellulose and lignin content)

Component	**Without pre-treatment**	**Acid pre-treatment (5% H_2SO_4)**	**Alkali pre-treatment (4% NaOH)**	**Oxidative pre-treatment (2% H_2O_2)**
Cellulose	20.5	35.1	33.3	20.2
Hemicelluloses	40.1	31.5	39.6	26.2
Lignin	13.9	6.3	5.1	7.1

Trearment of water hyacinth by acid, alkali and hydrogen peroxide for saccharificatons:

After pretreatnent the residue in each case was farther treated with acid, alkai and hydrogen peroxide for saccharification.

The substrate at a solid loading of 5% (w/v) was treated in an autoclave with NaOH, H_2SO_4 and H_2O_2 at concentration of 2% and 5% (w/v) respectively as soaking agent. The residence time 15, 20 and, 30 minute at 10, 15, 20 psi pressure of each sample separately. After cooling, the content were filter with Whatman filter paper and the filtrate, after neutralizing of pH was used for estimation of reducing sugar content .

Saccharificatons of Water Hyacinth by treatment with acid, alkali and hydrogen peroxide:

Effect of acid treatment:

The effect of acid concentration (2% and 5%) on sugar content of sample in different temperature and pressure is shown in **Fig-1.** With an increases acid concentration up to 5% the sugar content in sample increases up to 30 minute treatment with 20 psi pressure. Higher acid concentrations have negative effect of sample with increases of time at 20 psi pressure. This may be due to hydrolysis of sugar molecule in furfural and hydroxyl methyl furfural at high acid concentration at elevated temperature with increases time [13]. The present investigation shows that the autoclavation of water hyacinth at 20 psi pressure for 30 minute using 5% acid is suitable method for conversation of lignocelluloses to sugar. The maximum reducing sugar obtain 80.60% (w/v) with 5% acid at 30 minute treatment time and 20 psi pressure.

Effect of alkali treatment:

The highest sugar content of 97.5% (w/v) could be achieved after treatment with 5% alkali at 30 minute and 20 psi pressure. That is higher than the sugar obtained by similar acid treatment. Farther increases of alkali concentration with time at 20 psi pressure have a reverse effect on sugar content of alkali treated samples [13]. It is highly effective for saccharificatons of the cellulosic material to reducing sugar as shown in **Fig-2**.

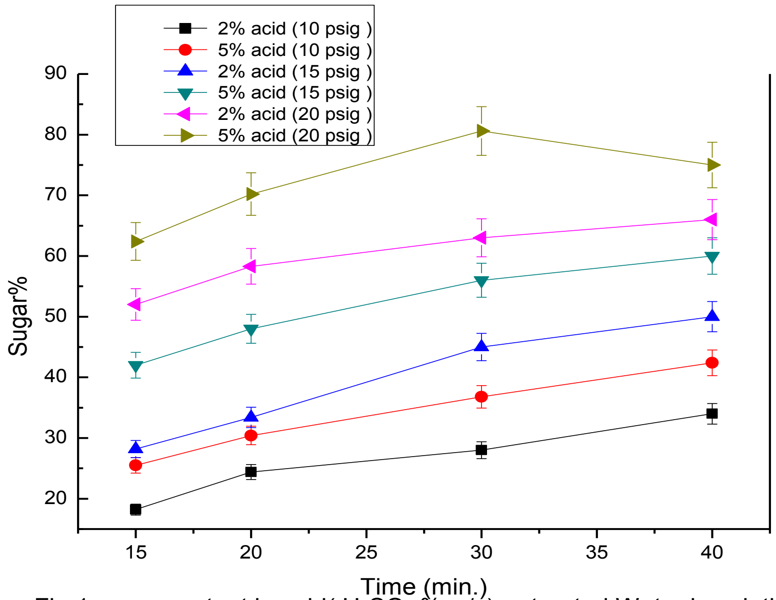

Fig 1:sugar content in acid(H_2SO_4 % w/v)pretreated Water hyacinth

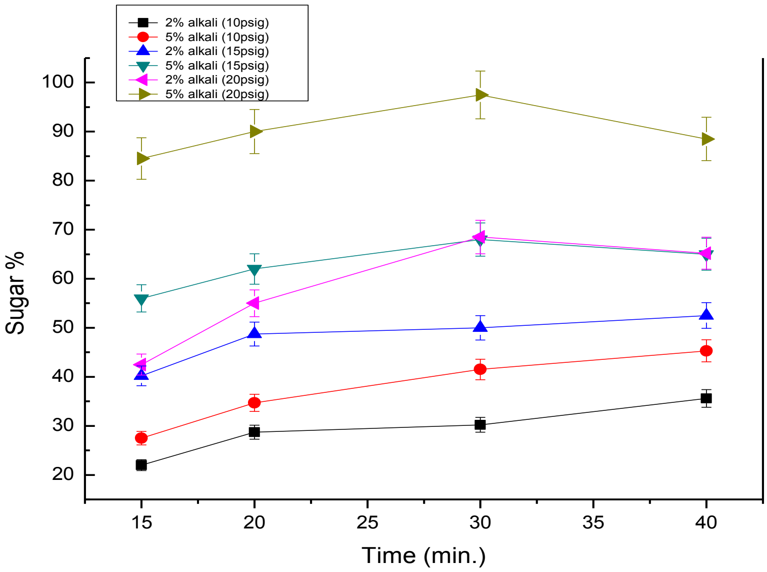

Fig 2: Sugar content after alkali(NaOH % w/v)pretreated Water hyacinth

Effect of hydrogen peroxide treatment:

The H_2O_2 treatment at a concentration of 2% and 5% (w/v) has a lower effect of sugar realises from water hyacinth in contrast to acid and alkali with 30 minute treatment time and a pressure of 20 psi. With increase of time decreases the sugar concentration. During the H_2O_2 treatment several reactions can take place like electrophilic substitution, displacement of side chains and the oxidative cleavage of aromatic nuclei. H_2O_2 treatment utilise oxidative delignification to detach and solubilise the lignin and loosen the lignocellulosic materials, thus improving saccharificatons of biomass. With further increases of time at 20 psi pressure decreases saccharificatons of biomass as shown in **Fig-3**.

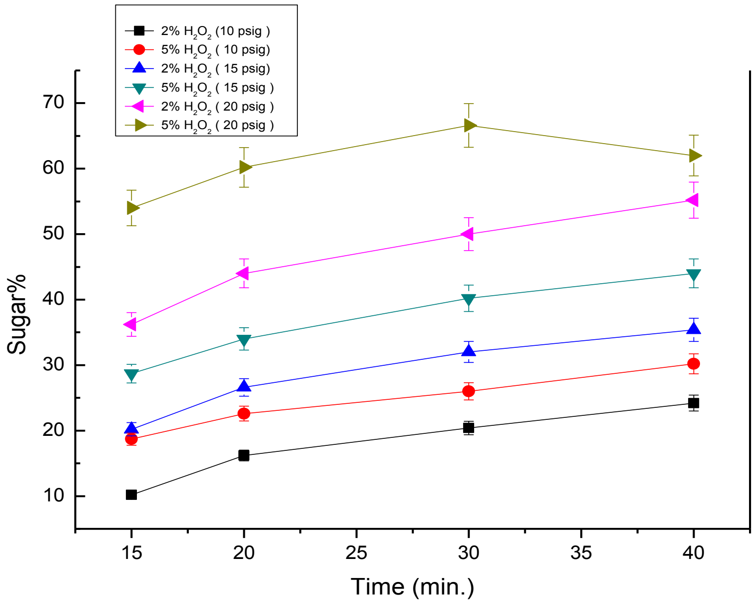

Fig 3: sugar content in H_2O_2(% w/v) pretreated Water hyacinth

Comparative Study:

Fig-4 shows comparative study by the different methods, such as acid, alkali and hydrogen peroxide at different concentration and different time of period with a pressure of 20 psi. It has been found from **Fig-4** that the alkali treatment is superior for saccharificatons of cellulose, hemicelluloses from water hyacinth at a concentration of 5% with 30 minute treatment time and a pressure of 20 psi.

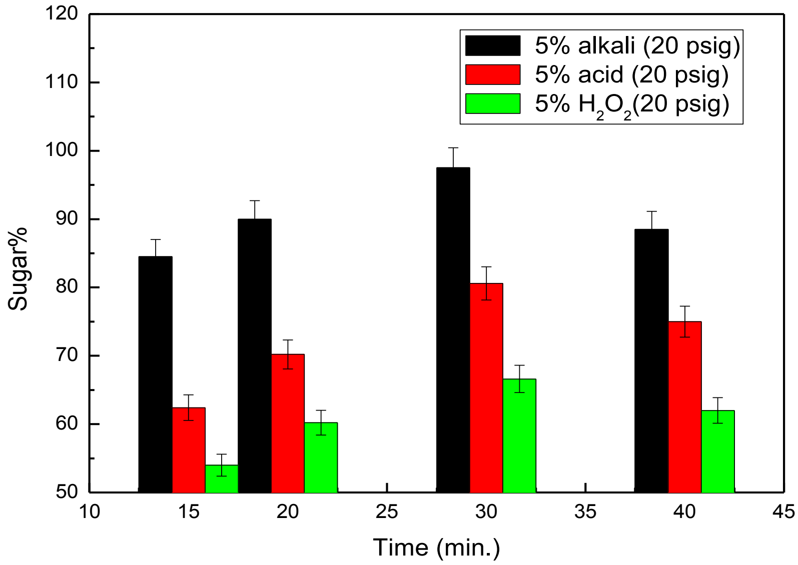

Fig 4: Effect of different pretreatment methods on conversion of lignocellulose to sugar

Effect of alkali treatment on morphology of water hyacinth:

Water hyacinth samples before and after pretreatment processes were view under light microscope to detect changes on their morphologies. Good morphological comparisons of water hyacinth samples ware obtained 10.00 kx magnifications. In the specimen of untreated water hyacinth, it may be observe the organelles randomly positions within the cells' cytoplasm. The cell walls are intact and not damaged as shown in **Fig-5a**. After being steamed at 20 psi pressure for 20 minutes, the cells were seen to be swollen and overlapped with the adjacent cell. Some cells were burst resulting to swelling, induced by the constant heat supply from the steaming process. As the cells collapsed, the biomass structure had weakened and the cells content i.e. moisture and organelles being removed as shown in **Fig-5b**. Moisture remove benefits the processing of biomass by increasing the porosity level of the material and collapsing the structure [14].

In acid hydrolysis, the moisture removal will avoided, the acid being diluted as it pre-treatment through the substrate. This is proven when dried sample has produced higher sugar production in alkali hydrolysis against wet sample **Fig-5b**. This morphology inspection have discover alteration of water hyacinth structure resulting from pretreatment processes.

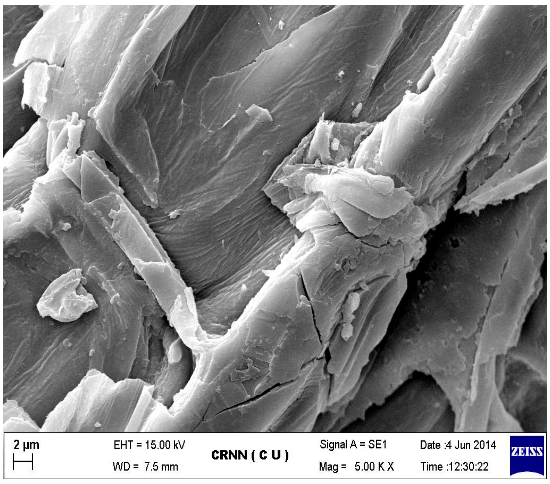

Fig 5 a: Scanning Electron Microscopic Structure Of normal Water hyacinth

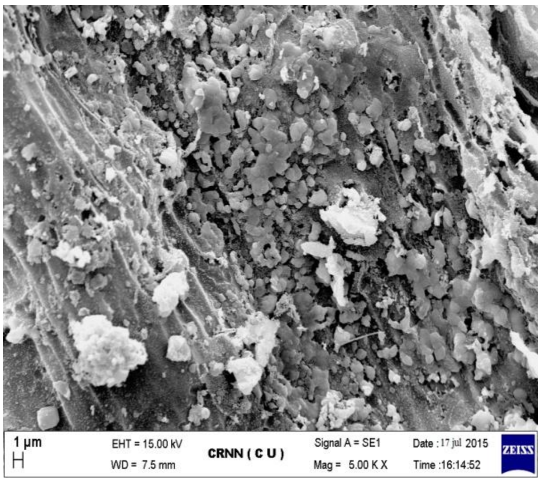

Fig 5b: Scanning Electron Microscopic Structure of alkali pretreated Water hyacinth

IV. CONCLUSION

The present results had demonstrated the ability of physical treatment on water hyacinth for improving the yield of sugar and processing time in dilute alkali hydrolysis. The best treatment method concluded in this study was combination of drying, grinding and steaming with high pressure, with yield of 97.50% sugar in alkali treatment process which is higher than the acid treatment process 80.60%. Morphology inspections have revealed disruptions of water hyacinth structure which explain the enhancement of alkali hydrolysis process under high pressure.

V. ACKNOWLEDGEMENT

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