Combo Catalytic Hydrothermal Pretreatment for Lignocellulosic Biomass Biofuels Production

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Due to the depletion of the world petroleum energy reserves and the increased public concern about global warming are strongly attracting worldwide interest in alternative fuels. Bioconversion of pearl millet biomass to ethanol is significantly hindered by the structural complexity of the biomass. Pretreatment is required to prepare pearl millet biomass for enzymatic hydrolysis. The efficiency of combination of chemicals (acid, alkaline H_2O_2 and lime) with hydrothermal pretreatment on pearl millet was investigated in the present study. Combination of acid with hydrothermal pretreated hydrolysate (160ºC -10 min) showed maximum recovery of fermentable sugar (41.78±0.64 g /100 g of biomass) even though, lime + hydrothermal pretreated biomass were found to be effective in the enzymatic hydrolysis rate. The main aim of the pretreatment is to remove hemicellulose and lignin for maximum recovery of fermentable sugars. At elevated temperature, lignin particles migrated from inner to outer surface of the biomass. The effect of pretreatment on the pearl millet biomass was characterized by SEM.

Key words: Thermal treatment, Lignocellulosics, Biofuel

Pearl millet biomass (*Pennisetum glacum L.*) is generally known as bajra. It is one of the most important millet crop in India and ranks third position in terms of area (7.32 mha) after rice and wheat. Biofuel is primarily derived from sugar, starch and lignocellulosic biomass. Now-a-days biofuel production is mainly obtained from lignocellulosic biomass. A carbohydrate rich biomass resource is required for biofuel production and these polymers are plentiful in bajra biomass. In the raw biomass; cellulose, hemicellulose and lignin are closely associated with each other. Lignin is formidable barrier for enzyme adsorption on to the substrate. Lignin encloses the cellulose in the cell wall hindering cellulase from reaching cellulosic fibrils. The enzymatic hydrolysis of the biomass is limited by the complex structure. So, an ideal pretreatment process is required for depolymerization for lignin before recovery of fermentable sugars (Balan *et al*., 2009 and Adsul *et al.,* 2011). Many pretreatment methods have been studied and are still in development. It is more difficult to evaluate and compare the pretreatment methods because they involve upstream, and downstream processing cost, capital investment, chemical recycling and waste treatment systems (Jeoh *et al*., 2007). The pretreated samples enzymatically hydrolysed in order to assess the efficiency of the different pretreatment methods on sugar yield.

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Material and Methods

Pearl millet biomass (ICMV 05222) obtained from ICRISAT, Hyderabad was used for the study. As a preparatory processing, biomass drying and size reduction was done. The biomass was air dried to reduce the moisture content prior to milling. Wherever ash content was more than 10 %, size reduction and sieving were performed. The particle size influences on compositional analysis of the lignocellulosic biomass had been optimized by National Renewable Energy Laboratory (NREL). The prepared samples were analyzed immediately or stored in airtight containers or polythene bags to prevent the entry of moisture and other contaminants and kept at -20ºC until needed.

The catalytic hydrothermal pretreatment experiment was carried out in 1 L stainless steel reactor. Combination of previously optimized catalytic concentration (for each pretreatment chemical concentration and solid loading) at three different temperature regimes of 140, 150 and 160˚C with reaction time was 10, 20 and 30 min (Medina *et al*., 2016). The bajra biomass sample was loaded in a one-liter reactor. The desired temperature with moist steam was passed inside the reactor for 10, 20 and 30 min. time interval. After pretreatment time has elapsed, the steam valve was immediately closed and reactor was kept in a water containing vessel to terminate the reactions.

After each pretreatment, the resulting slurry was collected from the reactor; the solid and liquid

fractions were separated by filtration technique and neutralized with acid or alkali. The residual or water insoluble fibrous biomass was washed with distilled water until neutral pH. The neutralized biomass was pressed manually to remove the water. The residual biomass was dried in an oven at 40°C until constant weight. The dried residual biomass weight was measured for determining the weight loss during pretreatment. The neutralized solid and liquid fractions were stored at -20˚C for further analysis.

Pretreatment efficiency was calculated as the amount of total sugar release measured after each pretreatment. The pretreated bajra biomass hydrolysate sample sugar release was performed according to the method of Miller (1959). The proximate analysis of raw and pretreated sample *viz*., moisture content, ash, and lignin (acid soluble and in soluble lignin) content was carried out according to the laboratory analysis protocol (LAP) of National Renewable Energy Laboratory (NREL), Colorado, USA (NREL, 2014) Cellulose and hemicellulose content were analyzed by following Van Soest Method and Wine (1967). The byproducts and degradation products formed during the pretreatment were analyzed by using GC-MS. The pretreated pearl millet biomass was characterized by SEM. The crystallinity index of the raw and pretreated biomass was calculated according to the method of Zhao *et al*. (2008).

Results and Discussion

The proximate analysis of pearl millet biomass revealed that moisture (8±0.32 %), ash 6.27±0.08 %), total solids (92±0.14%), water extractives (6.43±0.12 %), ethanol extractives (5.72±0.34 %)cellulose (41.6±0.16%), hemicellulose (22.32 ± 0.65), glucan

Table 1.Comparison of catalytic hydrothermal pretreatment for sugar release from pearl millet biomass sample

Temperature (ºC)	Duration (min.)	Total sugar (g/L)		
		PA+HT (16 %)	AHP+HT (3 %)	Lime+ HT (1.1 %)
140	10	26.87±0.12	19.99±0.25	22.43±0.33
	20	27.78±0.44	22.87±0.62	23.56±0.21
	30	28.22±0.12	23.45±0.48	24.89±0.66
150	10	28.62±0.15	20.22±0.39	23.44±0.45
	20	29.99±0.17	21.82±0.67	24.98±0.02
	30	30.06±0.18	23.55±0.24	26.12±0.06
160	10	41.78±0.02	27.12±0.15	28.46±0.04
	20	42.29±0.05	28.09±0.23	29.93±0.15
	30	43..07±0.23	29.23±0.66	30.55±0.34
Inhibitor	Acetic acid	0.97	2.25	2.63
	Furfural	0.11	-	0.38
	Formic acid	-	1.24	-
Optimised condition biomass 160 ºC - 10 min for saccharification				
Cellulase saccharification		72.65±0.01	73.57±0.01	75.76±0.01
Crude enzyme saccharification		69.24±0.01	70.16±0.01	72.27±0.01
Crystallinity Index (CrI)	60.43	67.61	69.18	70.18

PA + HT - H_3PO_4+Hydrothermal, AHP - Alkaline H_2O_2+Hydrothermal, CA- Cas(OH)$_2$ +Hydrothermal Standard errors are shown in parenthesis

(28.47±2.88%), galactan (17.24±0.46), arabinan (3.78±0.04), xylan (5.12±0.46), acid insoluble lignin (16.32±0.49) and acid soluble lignin (5.49±0.08%). Acid pretreated biomass subjected to hydrothermal pretreatment showed maximum 41.78g /100 g of biomass (Table 1). The break down and exposure surface area of the biomass is high which might be due to pretreatment. The hydrothermal pretreatment is mainly dependent on the size of the biomass, moisture content, reaction time and temperature (Duff and Murray, 1996).
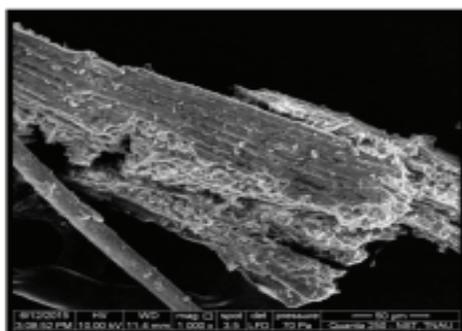
Inhibitors in the pretreated liquid sample

The pretreated liquid fraction contains complex innumerous compounds such as soluble lignin, total reducing sugars and inhibitor, which were increasing with solid loading, reaction time and temperature (Table 1). Lignin fraction is oxidized and digested into formation of carboxylic acid and phenolic compounds (Martin *et al*., 2007). The pretreatment efficiency is based on the pH, temperature, solid loading and chemical concentration and size of the biomass (Karagoz *et al*., 2013). Ortho-phosphoric acid + hydrothermal pretreated liquid showed acetic acid (0.97 %) and furfural (0.11 %). Alkaline hydrogen peroxide + hydrothermal pretreated hydrolysate revealed that acetic acid (2.45%), formic acid (1.56 %) and HMF (2.97 %). Lime + hydrothermal pretreated liquid contained acetic acid (3.03 %) and furfural (0.38 %). Acetic acid is primarily derived from hydrolysis
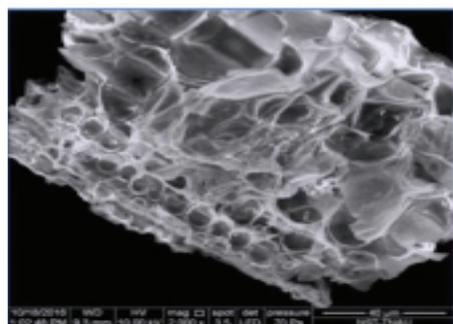
of hemicellulose, formic acid is released from degradation of sugar product of furfural compound. Furfural is a heterocyclic aldehyde compound which is produced from degradation of monomeric sugars.
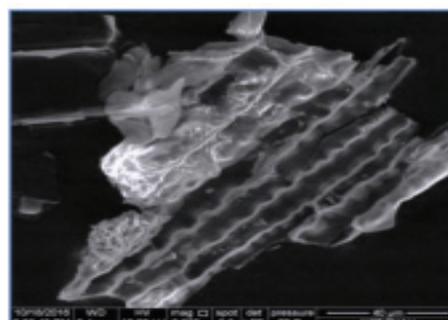
SEM image

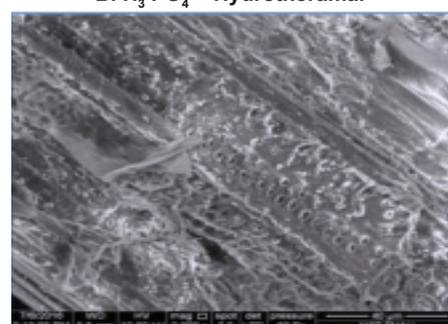In SEM analysis, cell wall changes were observed in the surface of the pretreated biomass. The bajra biomass with catalytic hydrothermal pretreatment showed numerous holes on the surface of the biomass which might be due to removal of fibrous components Fig. 1. (A, B, C and D). The removal of hemicellulose and lignin from the biomass, makes the tightly packed cellulosic structures tends to become loose. The globular or uneven shape of the lignin deposits were found to be in the pretreated

A. Raw biomass

B. H_3PO_4 + Hydrotheramal

C. H_2PO_2 + Hydrotheramal

D. Ca (OH)$_2$ + Hydrotheramal

Fig.1. SEM micrographs of combination of catalytic hydrothermal pretreatment

biomass which is accordance with Poggi *et al*. (2005). Removal of hemicellulose from pearl millet biomass, increase the porosity and improves the rate of enzymatic digestibility. *Ortho*-phosphoric acid + 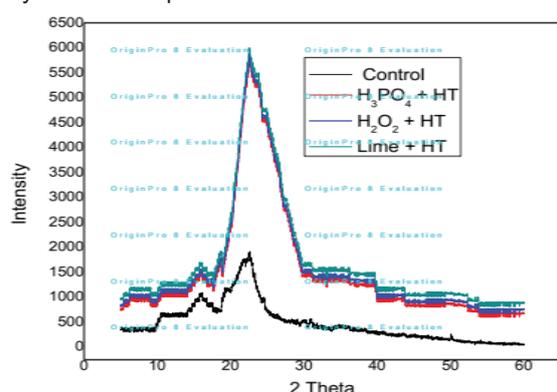hydrothermal pretreated biomass exhibited dark brown color (Kristensen *et al*., 2008).Raw bajra biomass samples were very hard and pretreated samples were softened in nature, which indicates the removal of lignin and acetyl groups. Compositional analysis was carried out before and after pretreatment showed that a significant fraction of lignin and hemicellulose removed during pretreatment (Asgher *et al*, 2013).

Crystallinity index (CrI)

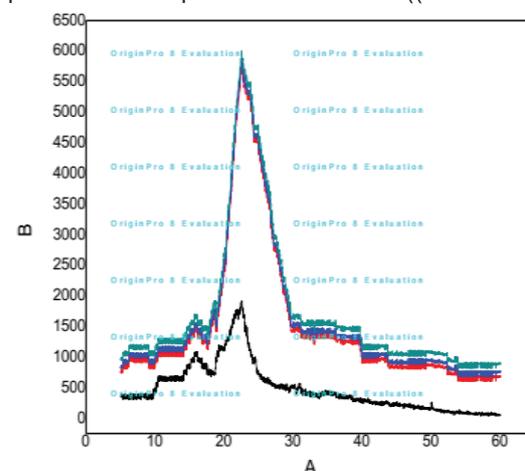The crystallinity index (CrI) of the raw and pretreated sample was calculated ((Table1. and

Fig.2a. Crystallinity index of the raw and catalytic hydrothermal pretreated biomass

Fig.2b. Crystallinity index of the raw and pretreated biomass
1. Control; 2. H_3PO_4 + HT pretreated biomass
3. H_2O_2 + HT pretreated biomass; 4. Lime + HT Pretreated biomass

Fig.2). Cellulose exist in amorphous (lignin and hemicellulose) and crystalline (cellulose) form. The crystallinity index is believed to be the obstacles for enzymatic hydrolysis. So, the effective pretreatment is required for enhancing the enzymatic hydrolysis rate. The crystallinity index was high in all the pretreated biomass (67.61 -70.18 %). These results confirmed that the higher amount digestion of amorphous cellulose. Hence, the pretreated biomass was swollen in nature. The concentration of the cellulose was increased which might be due to the removal of lignin and hemicellulose.The acid + hydrothermal pretreatment resulted in maximum recovery of fermentable sugars (Nazarphour *et al*., 2013)

Enzymatic saccharification

Enzymatic saccharification was more from 69.24–75.76 % in pretreated biomass than in raw biomass (32.42 %) (Table 1). Accessible surface area plays an important role in enzymatic saccharification. Amorphous form of cellulose is more accessible than in crystalline form of cellulose (Kumar *et al*., 2012). In the present study, enzymatic hydrolysis rate was high in the lime and hydrothermal pretreated sample when compared to other pretreatments. The studies confirmed that lime + hydrothermal pretreatment was promising as it had high crystallinity index (70.18 %), fermentable sugar release (28.46g.100 g^{-1} of biomass), minimal production of inhibitors, required less energy input and environmentally safe. Lime has also be recovered through the kiln process.

Conclusion

A reduction of the cost of the ethanol production can be achieved by reducing the cost of the raw materials, pretreatment cost and enzyme cost. A designer biomass with higher carbohydrate content combined with improvement technology could reduce the cost of ethanol production. Though hydrothermal treatment incurs high energy use, there is a significant quantity of lignin removal and a rise in the cellulose content. Minimizing energy, input cost, preserving cellulose and hemicellulose fractions, avoiding size reduction and limiting formation of inhibitors are key issues to develop cost-effective pretreatment methods. The overall optimized parameters were found to be lime and hydrothermal, 60 FPU/g of substrate of enzyme, and 72 h. The actual and predicted value of total sugar yield (17.10 g/l) of biomass and saccharification (75.42 %) and (75.13%) was obtained for the optimized conditions. Enzymatic hydrolysis was considered as a key parameter of pretreatment efficiency (Kim *et al*., 2014).

Acknowledgment

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