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RESEARCH ARTICLE

A Catalytic Hydrothermal Pretreatment process for sugar recovery from pearl millet biomass for Biofuels Production

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ABSTRACT

Pearl millet biomass represents an unused source for biofuel production. Bioconversion of pearl millet biomass to ethanol is significantly hindered by the structural complexity of the biomass. Pretreatment is required to prepare the pearl millet biomass for enzymatic hydrolysis. The efficiency of combination of chemical (acid, alkaline hydro- gen peroxide and lime) with hydrothermal pretreatment on pearl millet was investigated in the present study. Combination of acid with hydrothermal pretreated hydrolysate (160˚C – 10 min, 12.5 % - TS) showed maximum recovery of fermentable sugars 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 remov- al of the hemicellulose and lignin for maximum recovery of fermentable sugars. At elevated temperature, lignin particles migrate from inner side of the biomass to outer surface of the biomass. The effect of pretreatment on the pearl millet biomass was characterized by SEM.

Key words: structural complexity, Lime + hydrothermal pretreatment, Enzymatic hydrolysis

Introduction

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 pro- cess is required for depolymerization of lignin before recovery of fermentable sugars (Balan *et al*., 2009; Adsul *et al*.,2011). Many pretreatment methods have been studied and are still in development. The pre- treated samples enzymatically hydrolysed in order to assess the efficiency of the different pretreatment methods on sugar yield.

Materials and Methods

Pearl millet biomass (ICMV 05222) obtained from ICRISAT, Hyderabad was used for the study. As preparatory processing biomass drying and size re- duction was done. The biomass was air dried to re- duce the moisture content prior to milling. Wherever ash content was more than 10 % size reduction and sieving was performed. As particle size influences ash had been done as optimized by National Renewable Energy Laboratory (NREL) LAPs for the compositional analysis of the lignocellulosic biomass. The prepared sample were analyzed immediately or otherwise it could be stored in airtight container or polyethylene bag to prevent the entry of moisture and other con- taminants and kept at -20°C until needed.

The catalytic hydrothermal pretreatment exper- iment was carried out in 1 L stainless steel reactor. Combination of previously optimized catalytic con- centration at three different temperature regimes (140, 150 and 160˚C) and three different solid load- ing (7.5, 10 and 12.5%) was studied for reaction time of 10, 20 and 30 min. The catalysts optimized under previous studies were o-phosphoric acid, lime and al- kaline hydrogen peroxide. Steam generator was used to supply heat for the pretreatment process. After pretreatment, the reactors were immediately cooled by quenching in a water containing vessel for 30 min. Liquid and solid fractions were separated by centrifu- gation (12000 g, 5 min). The residual substrate (water insoluble fiber) was washed with distilled water until neutral pH. The neutralized biomass was pressed manually to remove the water. The biomass was dried in an oven until the constant weight reached and the dried biomass was measured to determine the weight loss during pretreatment which corresponds to the lignin reduction percentage and digestibility of other components. The amount of solid and liquid fractions of the pretreated pearl millet biomass was quantified after each pretreatment process.

The sugar release was estimated in the liquid sample using dinitrosalicyclic acid assay (DNSA meth- od) according to the method of Miller, (1959). The proximate analysis of raw and pretreated sample *viz*., moisture content, ash, lignin content was carried out according to the laboratory analysis protocol (LAP) of National Renewable Energy Laboratory (NREL), Colorado, USA (NREL, 2004). Cellulose and hemicellulose content was carried out EN ISO 16472 -2006 (ISO, 2006). Enzymatic saccharification of pearl millet bio- mass was measured by standard procedure described in NREL/TP-510-42629 (Selig *et al*.,2008).The pearl millet biomass was characterized by SEM.

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%), glucan (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 lig- nin (5.49±0.08%). Based on the pretreatment experi- mental results a mathematical model was developed for each of catalytic hydrothermal pretreatment and prediction of the amount of total reducing sugar yield. Acid pretreated biomass subjected to hydrothermal pretreatment (160˚C – 10 min, 12.5 % TS) showed maximum sugar recovery of 41.78 g / 100 g of bio- mass because of exposure of surface area increased and breakdown of complex structure of biomass which might be due to pretreatment. The hydrother- mal pretreatment are mainly dependent on the size of the biomass, moisture content, reaction time and temperature (Duff and Murray,1996). Acid pretreat- ed biomass subjected to hydrothermal pretreatment showed maximum cellulose content of 75.54 % fol- lowed by lime pretreatment 68.92 % and shown in the Table 1. Further, the pretreated hydrothermal pretreatment removed 69 % of hemicellulose and lignin loss ranged from 51.08 to 66.42 %. Enzymat- ic hydrolysis rate mainly depends on the substrate characteristics (cellulosic accessible surface area, porosity, degree of polymerization and crystallinity), environmental condition (pH, temperature)' and en- zyme loading. Enzymatic hydrolysis of cellulose was enhanced due to better adsorption of enzymes onto the surface of the biomass.

In SEM analysis, drastic changes were observed in the surface of the pretreated biomass. These re- sults suggested that pretreated biomass exhibited peeled off with high porosity (Fig.1). The removal of hemicellulose from the biomass, makes the tightly packed cellulosic structure tends to become loose. The globular or uneven shape of the lignin deposits were found to be in the pretreated biomass which is accordance with Poggi *et al*., (2005);Simola *et al*.,(2000). Removal of hemicellulose from pearl mil- let biomass, increase the porosity and improves the rate of enzymatic digestibility. Furfural (0.11 to 1.56 %) and acetic acid (0.97 to 3.03 %) was observed in acid pretreated hydrolysate as acetic acid is primarily produced from hydrolysis of hemicellulose. Furfural, a heterocyclic aldehyde compound which is produced from xylose monomeric sugar may be converted to

jet and diesel fuel blendstock which will create the de- mand for these fuels.

Conclusions

A reduction of the cost of the ethanol produc- tion can be achieved by reducing the cost of the raw materials, pretreatment cost and the enzyme cost. A designer biomass with higher carbohydrate content combined with improved bioconversion technology could reduce the cost of ethanol production. Though hydrothermal treatment incurs high energy use, there is a significant quantity of removal of lignin and a rise in the cellulose content. Minimizing energy, input cost, preserving cellulose and hemicellulose fractions, avoiding size reduction and limiting formation of in- hibitors are key issues to develop cost-effective pre- treatment methods

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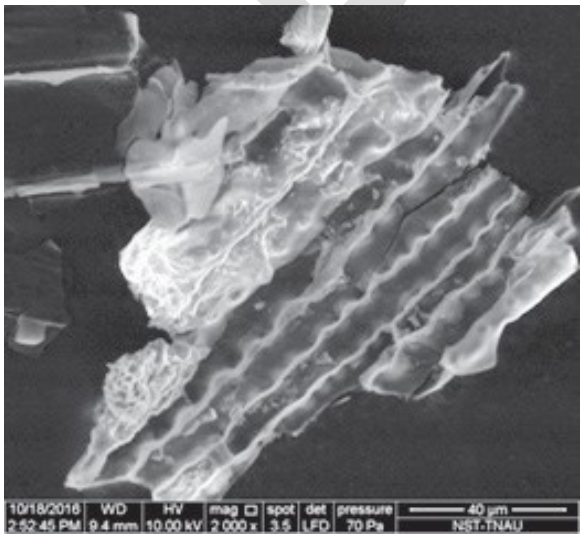
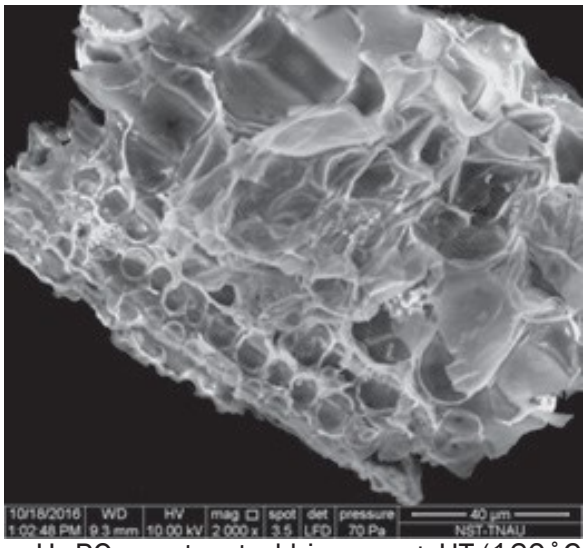
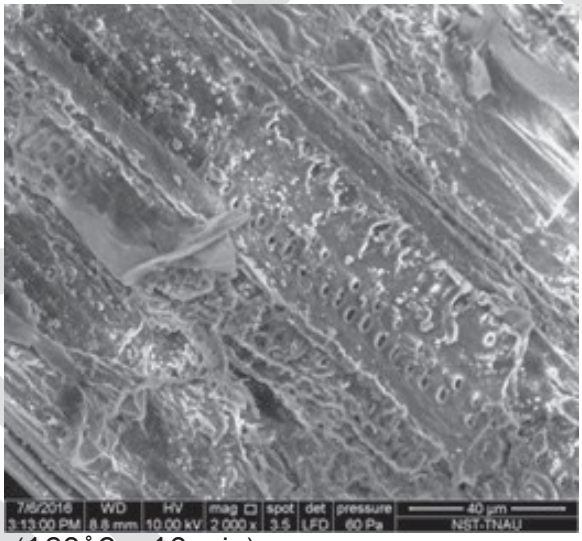




H_3PO_4 pretreated biomass + HT (160˚C–10 min) H_2O_2 pretreated biomass + HT (160˚C – 10 min)



Lime pretreated biomass + (160˚C – 10 min)