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Optimization of pretreatment conditions of sorghum bicolor straw, a substrate for bioethanol production: a pilot study

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Abstract: A demand paradigm shift from fossil fuel to Biofuel has become inevitable not only due to the scarcity of the resources but also due to renewability and environment friendly nature of Bioethanol. Lignocellulosic biomass is a potential feedstock for production of bioethanol. Pretreatment of biomass, which breaks the complex structures of biomass hence making it available for hydrolysis, is a very critical step in bioethanol production from the aspect of determining its economic viability. Sorghum *bicolor* straw being one of the potential lignocellulosic feedstock was used in this study to evaluate the effect of Acid concentration, Time and Temperature on pretreatment. Samples were analyzed by HPLC using Refractive Index detector and UV detector. Best results with maximum combined sugar value (Glucose+Xylose) of 0.43 g/g of dry mass were observed with 2% H_2SO_4 at 121 ^{0}C for 10 minutes. These results help us to suggest these optimized pretreatment conditions could be an effective prerequisite for the production of monomer sugars which could be subsequently fermented to Bioethanol-a renewable and environment friendly fuel.

Keywords: Sorghum bicolor, bioethanol, dilute acid pretreatment, lignocellulose.
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INTRODUCTION

The uses of Biofuel dates as old as the Second World War when petrol was short in supply, many German vehicles were run on petrol made from potatoes. Brazilians having deficits in oil reserves had devoted a part of their land to grow sugarcane to be fermented and used as a fuel for their cars. The present scenario indicates an inverse relationship between demand/consumption and the fossil reserves which leaves the world to explore into alternative resources. Moreover usage of fossil fuels also leaves a negative impact on environment by emitting green house gases (GHGs). Due to these problems the focus has now shifted to the development of renewable energy resources[1].

Bioethanol is a good substitute of fossil fuel as it is cheap, renewable and environment friendly emitting about 90% less green house gases (GHGs) than gasoline. Bioethanol can be produced by the process of fermentation using different sugars such as starch[2], glucose[3,4], xylose[5] etc.

One of the major research objectives in the production of commercially viable bioethanol is the selection of biomass because it affects the economy of the end product directly, by cost of raw material and indirectly by processing methods[6-9].

The feedstock for bioethanol production is mainly divided into three categories, i.e. starch based, sugar based and cellulosic material.[10] Presently commercial bioethanol is produced by using starch from crops like wheat, corn etc. but there is a drawback that these sources are also used as food and their increased use in bioethanol production is creating a threat to human food supply. Keeping this in view now the research has been focused on producing bioethanol by lignocellulosic material of agriculture, forestry and crop biomass, municipal and industrial wastes etc[3,4,11].

Sorghum *bicolor* is the one of the best crop sources that can be used for bioethanol production. It is cultivated in summer for mostly animal fodder but its grains are also used for human food. It is a very potential crop for bioethanol production due to its capability to grow in dry conditions, tolerance to heat and nutrient stress and short life span[1].

Plant biomass is composed of cellulose, hemicellulose and lignin. For fermentation monomeric sugars are needed. To release these monomeric sugars from cellulose and hemicellulose pretreatment of biomass is needed. Pretreatment is the most critical and cost determining step for bioethanol production. Pretreatment breaks lignin and hemicellulose, breaks crystalline cellusose increasing the porosity and surface area of biomass resulting in enhanced chances of efficient enzymatic hydrolysis[11-14].

Different physical and chemical methods of pretreatments are used. Physical methods include, steam explosion[15], ammonia fiber explosion[16], mechanical disruption etc. while chemical methods include hot water treatment[17], alkaline[18] and acid hydrolysis[19].

Different acids have been used for acid hydrolysis including sulphuric acid[1], hydrochloric acid[20] and Phosphoric acid[7]. Acid concentration, temperature and treatment time are important factors affecting the efficiency of acid pretreatment. Agricultural biomass and grasses have been reported to give better results with dilute H_2SO_4 pretreatment as compared to alkali pretreatment[21-23]. Dilute acid decreases the time and temperature of the reaction by increasing the hemicellulose hydrolysis and solubilization of lignin. One study has reported degradation of hemicelluloses up to 80% and that of cellulose below 10% varying with pretreatment conditions[7].

Some of the monosaccharides such as Hexoses and pentoses produced during pretreatment may be converted, depending upon pretreatment conditions, to 5-hydroxymethylfurfural (HMF) and furfural respectively which act as inhibitors later on modulating the activity of fermentation enzymes of yeast[24]. Therefore the ideal pretreatment conditions should be those producing maximum sugars with less inhibitory compounds.

During pretreatment, acid concentration and temperature have stronger effect on yield of sugars than reaction time. With increase in acid concentration and temperature the yield of sugars increase up to a certain level and then declines due to the degradation of these sugars in to inhibitors[8].

Previous studies were mostly focused on the pretreatment of forage sorghum[1,8,9,14,23, 25, 26,] but there are very few studies on residues of grain varieties of Sorghum[28]. Due the scarcity of the findings as per to my knowledge, this pilot study was designed to evaluate and provide a data of dilute acid pretreatment of sorghum straw investigating the effect of Temperature, Time and Acid concentration on the sugar recovery and production of phenolic and acetate compounds.

MATERIALS AND METHODS

Sample preparation

Post harvested Sorghum Straw was obtained from the Millet Research Station Rawalpindi. The biomass was air dried, grinded with Waring laboratory Blender (LB20EG) and sieved through 80 mesh pores to get particle size of 178µm or less, according to the laboratory analytical procedure[27]. After preparation, dried samples were stored in sealed bags at -20°C for further analysis.

Dilute acid pretreatment

20% solid loading of biomass (w/v) was used for reaction with sulfuric acid. Sulfuric acid was used in concentrations of 0.5%, 1% and 2% (w/w). The biomass was treated with acid at two different temperatures of 121°C and 140°C in an autoclave for 10, 30 and 60 minutes. Total 18 (3×3×2) combinations of these parameters were performed. After pretreatment, samples were stored at -20°C for further analysis.

Analytical procedure

Glucose, Xylose, determined by HPLC using Refractive Index detector while furfural, Hydroxymethyl furfural (HMF) Acetate, and Lactate by HPLC using UV detector.

RESULTS AND DISCUSSION

This pilot study was performed using different pretreatment parameters of acid concentration, temperature and reaction time in order to optimize the pretreatment conditions. Our results demonstrate that maximum glucose released was 0.25g/g of dry mass with 2% acid at 140°C for 30 minutes (Figure 1) while maximum Xylose released was 0.205g/g of dry mass with 2% acid at 121C for 10 minutes (Figure 2). Maximum collective value of sugar (glucose+xylose) observed was 0.43 g/g with 2% acid at 121°C for 10 minutes (Figure 7). Figures 3-6 demonstrate the production of inhibitors i.e. Furfural, HMF, Lactate and Acetate in the above mentioned pretreatment conditions.

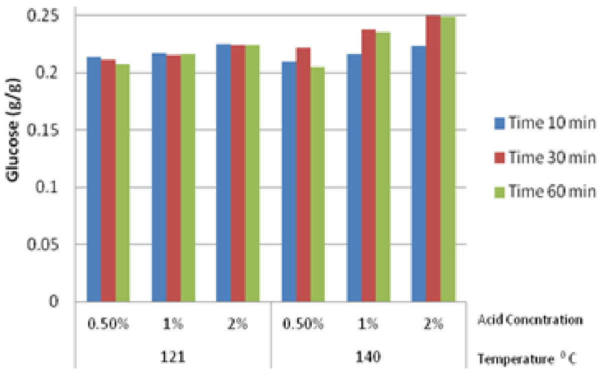

Figure 1: Concentration of Glucose at different pretreatment parameters of acid, temperature and reaction time.

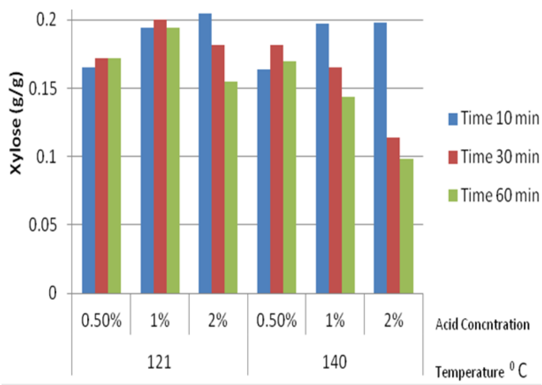

Figure 2: Concentration of Xylose at different pretreatment parameters of acid, temperature and reaction time.

Pertaining to the best suited pretreatment conditions our results depict a greater productivity than Vancova and Mclntosha[1] who performed their experiment with 2% acid at 121°C for 60 minutes for xylose and 30 minutes for Glucose on Mr. Buster variety of Sorghum. Moreover maximum collective value of sugar (glucose+xylose) as observed in our experiment was 0.43g/g with 2% acid at 121°C for 10 minutes (Figure 7), slightly higher as compared to 0.32g/g with 2% H_2SO_4 at 121°C for 30 minutes reported by Vancova and Mclntosha[1]. The reason for the slightly increased productivity in our case could be attributed to the difference of Sorghum variety and its composition. Composition along with the other factors is one of the important determinants for selecting feedstock for Bioethanol production. Biomass containing higher content of cellulose and Hemicellulose are a potential source of bioethanol[9]. *Mehmood et al*[28] have reported 35.01±0.71% of cellulose and 24.40±1.06% of Hemicellulose and 59.41% of total carbohydrate contents from sorghum straw.

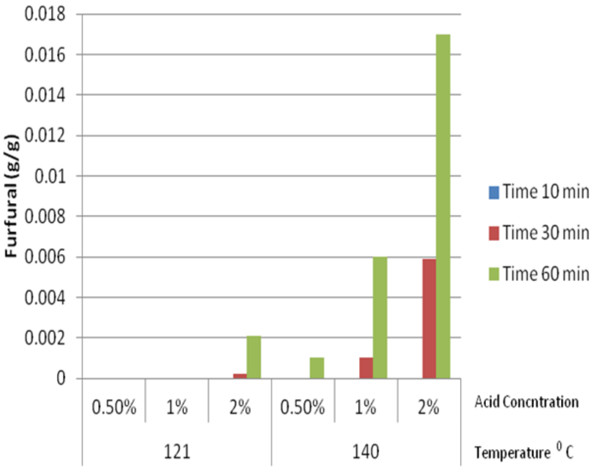

Figure 3: Concentration of Furfural at different pretreatment parameters of acid, temperature and reaction time.

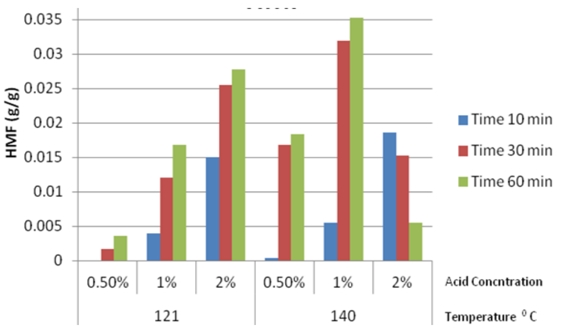

Figure 4: Concentration of HMF at different pretreatment parameters of acid, temperature and reaction time.

Thus we could suggest that this variety could have rendered more biomass. Biomass is a compact structure of cellulose, hemicelluloses and lignin. In order to release monosaccharides from cellulose and hemicelluloses these polymers need to be broken down[10]. Bioethanol production process involves the steps of pretreatment, enzymatic hydrolysis and fermentation. In order to make cellulose available for enzymatic hydrolysis, first lignin and hemicellulose barrier need to be broken down and this is done by the pretreatment.

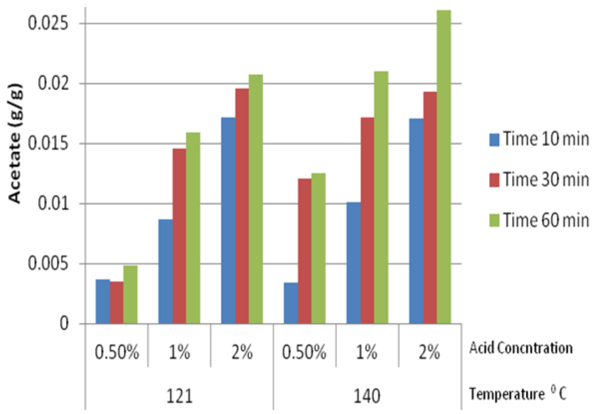

Figure 5: Concentration of Acetate at different pretreatment parameters of acid, temperature and reaction time.

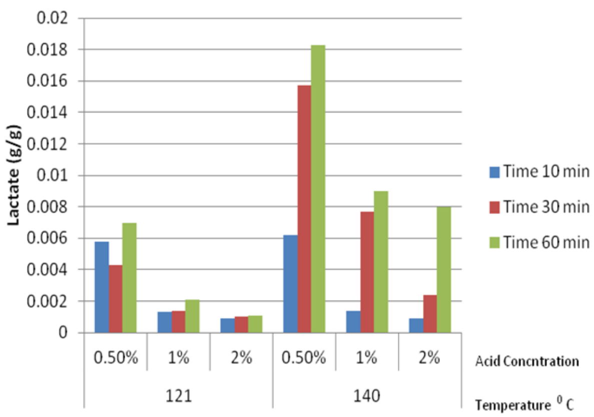

Figure 6: Concentration of Lactate at different pretreatment parameters of acid, temperature and reaction time.

Efficiency of pretreatment in the form of higher yield of monosaccharides is the most critical and cost evaluating step. Different methods of pretreatments are used [10,15-19] but dilute acid pretreatment is considered to be most effective because it can be used for different feedstock. It makes lignin and hemicellulose soluble exposing cellulose for enzymatic hydrolysis[21,22].

Along with composition, reaction time also affects the yield. Moreover a less reaction time employed (limiting the degradation of sugars[8,29]) in our pretreatment process could have accounted to the maximum availability of sugars as compared to the yield reported previously[1] , hence our results demonstrate that sugar concentration was increased with increase in temperature and acid concentration suggesting their dominant role along with the factors mentioned previously.

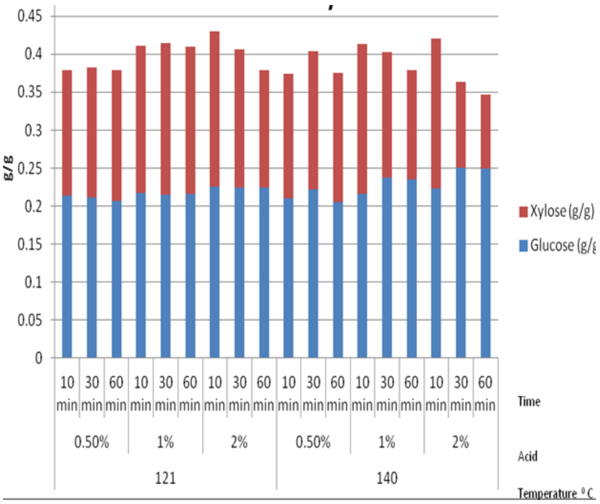

Figure 7: Combine concentration of sugar (Glucose+Xylose) at different pretreatment parameters of acid, temperature and reaction time.

Our pretreatment experiment yielded monosaccharides and some degradation compounds such as Furfural, HMF, Acetate and Lactate which are responsible for limiting the rate of reaction[24]. Although minimal production of inhibitors i.e. Furfural, HMF, Lactate and Acetate were observed at less severe pretreatment conditions i.e. minimum acid concentration, temperature and reaction time (Figures 3-6) but were not chosen as the optimized conditions because the yield of sugars was also at its lowest level contrary to demand of maximum sugar yield for this experiment. We need pretreatment conditions for fermentation thus at these optimized conditions of 2%, 121°C, 10 minutes, observed values of Furfural, HMF, Acetate and Lactate were 0, 0.015, 0.0172 and 0.0009 g/g respectively (Figures 3-6).

This study was conducted as pilot study for future large scale research work on Sorghum bicolor and Pearl Millet for bioethanol production.

CONCLUSION

Sorghum bicolor is a very attractive and prospective candidate for bioethanol production. Pretreatment is a crucial step for the yield of fermentable sugars to produce commercially viable bioethanol. The best pretreatment parameters observed in this study giving maximum yield of fermentable sugars were 2% H_2SO_4 at 121 ^{0}C for 10 minutes. It would be of interest to investigate and probe into other pretreatment optimization conditions for bioethanol production.

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