Enzymatic Saccharification of Bagasse: Effects of Different Pre-treatment Methods

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Abstract- About 800,000 tons of bagasse is obtained per year as a by-product from sugar industries in Bangladesh. Bagasse is one of the most readily available lignocellulosic biomass that is a potential source for ethanol production through enzymatic saccharification and hydrolysis. Pretreatment enhances saccharification by removing lignin and hemicellulose. In this study pretreatment of bagasse was done using hot compressed water (HCW), (HCW+H_3PO_4) and (HCW+NH_3). Effects of different pretreatment conditions such as temperature, residence time, concentration of NH_3 and H_3PO_4 were studied in detail. (HCW+H_3PO_4) system was found to be more effective compared to others investigated in this study. A maximum yield of glucose (60%) and xylose (71%) was obtained in case of (HCW+H_3PO_4) system at temperature180°C and 160°C and residence time of 30 min and 15 min, respectively.

Keywords- Saccharification, Bagasse, Biofuel, Pretreatment, Hot compressed water.

1. Introduction

The production of ethanol from lignocellulosic materials has been of interest because of several sustainable reasons such as scarcity of fossil fuel, mitigation of green house gas emissions, several economic and environmental concerns [1]. Lignocellulosic materials are renewable and can be produced domestically from cheap feedstocks which are available in large quantities. Lignocellulosic materials comprise a wide range of materials such as agricultural residues, forest products and dedicated crops. Cellulose, hemicellulose, lignin and pectin attribute to around 90% of the dry weight of most plant materials [2]. Among them sugarcane bagasse possesses high potential for the biotechnical conversion process. A huge amount of bagasse is obtainable from the sugar mills in our country. Sugarcane bagasse contains 34.5% cellulose, 24% hemicellulose, and 22-25% lignin [3-4].

Production of ethanol from lignocellulosic biomass consists of the following major operations: pretreatment, enzymatic hydrolysis, fermentation and ethanol separation or purification. The presence of lignin in the biomass lowers the biodegradability both of the cellulose and hemicelluloses [5]. An ideal pretreatment would remove only the lignin portion without loss of hemicellulose or cellulose. An effective pretreatment improves the availability of sugars, prevent degradation of carbohydrate, reduce unfavorable by-products, and be low cost [4]. Numerous pretreatment methods including physical, physico-chemical, chemical, and biological methods have been developed for separation of lignocellulosic materials to cellulose, hemicellulose, and lignin [6-10]. This preconditioning makes cellulose more available to hydrolytic enzymes which thereupon facilitate the conversion of carbohydrate polymers to fermentable sugar more efficiently accompanied by greater yield.

In this study sugarcane bagasse was used as a raw lignocellulosic material to be pretreated with hot compressed water HCW, (HCW+H_3PO_4) and (HCW+NH_3) at various conditions. These treatments disrupt cellulose-hemicellulose-lignin network and is known to promote the enzymatic

hydrolysis of bagasse. The effects of various pretreatment conditions such as temperature, residence time and concentration of H_3PO_4 and NH_3 were studied in detail to find out optimum conditions for pretreatment of bagasse.

2. Materials and Methods

2.1. Sugarcane Bagasse

Sugarcane bagasse used in this study was collected from Rajshahi Sugar Mills Limited (An enterprise of Bangladesh Sugar and Food Industries Corporation, BSFIC). The bagasse was washed to remove the unwanted particles and was dried. The flow diagram for saccharification of bagasse was shown in Figure 1.

2.2. Cutter Mill

Dried bagasse was milled by cutter mill (Fretsch, Germany) in to desired size. The bagasse of 2.0 mm size was used in pretreatment while 0.25 mm size was used for acid hydrolysis for determining composition of bagasse. The composition of bagasse used in this study was determined by its acid hydrolysis and the data are tabulated in Table 1.

Table 1. Composition of bagasse

Item	Composition (%)
Glucose	44.22
Xylose	29.52
Galactose	1.37
Arabinose	2.81
Mannose	1.80
Ash	4.13

2.3. Hot Compressed Water (HCW)

The HCW reactor (PARR 4565, PARR Instrument Company-Moline, Illinois, USA) made of stainless steel autoclave (with a volume of 100 ml) equipped with a mechanical stirrer, a PARR 4842 temperature controller and a tachometer was used in this study.

2.4. Pretreatment of Bagasse

3.0 g of bagasse chips (2 mm size) and 30 ml of deionized water/(deionized water + dil. H_3PO_4)/(deionized water + dil. NH_3) were charged in the reactor. The initial pressure of the reactor was maintained at 20 atm with nitrogen gas, and the stirrer was rotated at a speed of 120 rpm. The reactor was heated by an electric mantle heater at a rate of approximately 4.5°C/min.

The pretreatment was controlled at different temperature and different pretreatment time. Upon completion of operation for desired time the reactor was cooled by immersing into room temperature water immediately, and cooled down to 30°C. Ice was used for faster cooling of the reactor. Then approximately 10 ml of deionized water was used for washing reactor chamber and the pretreated sample (approximately 40 ml) was stored in a vial and was kept in refrigerator for saccharification.

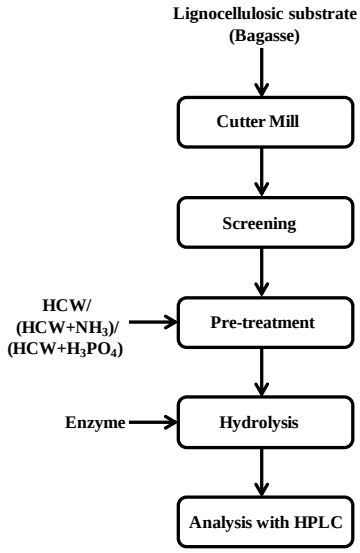

Fig. 1. Schematic diagram for pre-treatment of bagasse.

2.5. Enzymatic Saccharification

Enzymatic saccharification was performed in a 40 ml vial equipped with a mechanical stirrer with a reaction volume of 20 ml. The saccharification was carried out at different temperatures, residence times and concentrations of H_3PO_4 and NH_3 to determine the optimum conditions. In all experiments 10% substrate concentration and 4 FPU (Filter Paper Unit)/g enzyme loadings (Acremonium cellulase, Optimash BG) were used. Hydrolysis was carried out in 50 mM acetate buffer (pH 5) at 50°C for 72 hours. After hydrolysis the hydrolysates were centrifuged to remove solid particles and the supernatant was analyzed using HPLC to determine the composition of released sugars. Sugars obtained from pretreatment of HCW, (HCW+H_3PO_4) and (HCW+NH_3) were termed as glucose/xylose-1, glucose/xylose-2 and glucose/xylose-3, respectively.

2.6. Analytical Method

Sugar compositions of the raw materials were determined according to the procedure of National Renewable Energy Laboratory (NREL) [11]. Ash content was determined by placing the known amount of sample in a muffle furnace at 600°C for 24 h and subsequent cooling in a desiccator. Sugars and degradation products in the liquid faction samples were analyzed by the NREL procedure [11]. Monomeric sugars in liquid fraction were analyzed by HPLC (JASCO, Japan) using Aminex HPX-87P column (7.8 mm I.D. x 30 cm, Biorad) equipped with a refractive index detector [12]. Degassed and deionized water was used as a mobile phase at a flow rate of 1.0 ml/min. The column temperature was maintained at 80°C. Sulfuric acid hydrolysis was used to determine monomeric and oligomeric sugars followed by HPLC determination [12]. Hydrolysate sugars concentration in hydrolysis liquid fraction was determined by

comparison its peak area detected by HPLC with peak area of 1% standard sugar which consists of 5 sugars namely glucose, xylose, galactose, arabinose and mannose. Analyses were carried out in duplicate, and the results were expressed as mean values.

2.7. Calculation of Sugar Yield

All sugar yields mentioned in this manuscript are in comparison with acid hydrolysis which are termed as theoretical yield. Therefore,

3. Results and Discussion

Lignocellulosic biomass is composed mainly of cellulose, hemicellulose, and lignin [13]. Pretreatment increases surface area, decrease crystallinity, break the lignin barrier and remove hemicelluloses [14]. HCW pretreatment involves using hot water at elevated temperature ranging from 140°C to 200°C and high pressure. HCW acts as an acid and solubilizes hemicelluloses and lignin [15]. (HCW+NH_3) and (HCW+H_3PO_4) are also physico-chemical process in which biomass material is subjected NH_3 and H_3PO_4 under high pressures and moderate temperatures. In case of (HCW+NH_3) system, ammonia breaks down lignin by ammonolysis and solubilizes hemicellulose.

3.1. Effect of Temperature on Digestibility of Pretreated Bagasse

Temperature plays an important role in pretreatment of bagasse. In order to observe the effect of temperature of pretreatment system of HCW, (HCW+H_3PO_4) and (HCW+NH_3) on enzymatic saccharification of bagasse pretreatment experiments were carried out at different temperatures ranging from 140°C to 200°C for 15 minutes residence time. Samples were hydrolyzed for 72 h with enzyme dose of 4 FPU/gm of substrate. Figure 2 shows the effects of temperature of pretreatment solvents on glucose and xylose yields. It was found that both glucose and xylose yields increased with an increase in temperature. In case of HCW system glucose yield increased with an increase in temperature but xylose yield decreased after 180°C. As a result total sugar yield (glucose + xylose) is decreased after 180°C. In case of (HCW+H_3PO_4) and (HCW+NH_3) systems glucose and xylose yields decreased after 180°C. The highest yields of glucose (44%) and xylose (55%) were obtained at 180°C when HCW pretreatment method is performed. At the same temperature highest yields of glucose (44%) and xylose (42%) were obtained when (HCW+NH_3) pretreatment method was used. The highest yields of glucose (60%) and xylose ((71%) were obtained at 160°C using (HCW+H_3PO_4) pretreatment system.

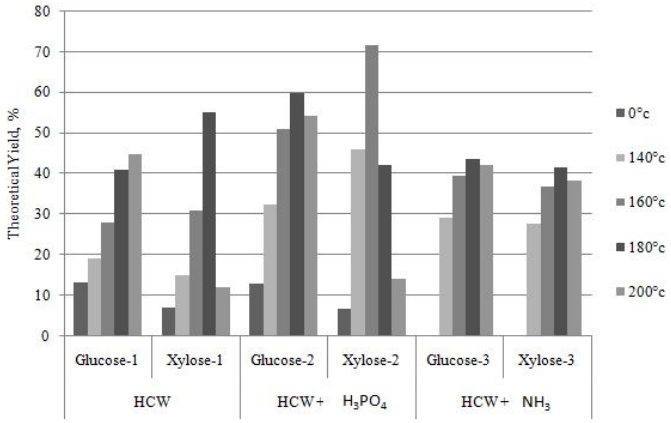

Fig. 2. Effect of temperature of pretreatment system on yields of glucose and xylose.

3.2. Effect of Residence Time on the Digestibility of Pretreated Bagasse

Figure 3 illustrates the effects of residence time on enzymatic saccharification of bagasse. In order to determine the effect of residence time of HCW, (HCW+H_3PO_4) and (HCW+NH_3) systems on enzymatic saccharification of bagasse, pretreatment experiments were carried out for 5 min, 15min, 30 min and 60 min at 180°C. The samples were hydrolyzed for 72 h with enzyme dose 4 FPU/gm of substrate. It was found that glucose yield is increased up to 30 min residence time whereas xylose yield decreased after 15 min when HCW pretreatment is performed. So highest yield of glucose (58%) is obtained for 30 min residence time and highest xylose yield (55%) is obtained for 15 min pretreatment. But in case of (HCW+NH_3) pretreatment method there is no significant change in this limit. But after 30 min residence time xylose yield is sharply increased from 51% to 72% where as glucose yield is little bit decreased. So the highest yield, 57% of total sugar is obtained for 60 min residence time. The highest yield of total sugar is obtained when HCW pretreatment is performed in presence of H_3PO_4. In this case, the highest yield of total sugar 60% is obtained for 15 minute residence time.

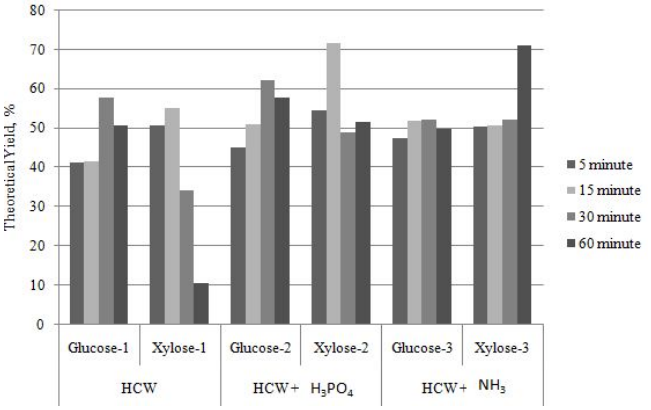

Fig. 3. Effect of residence time of pretreatment of bagasse on yields of glucose and xylose.

3.3. Effect of Concentration of H3PO4 on Enzymatic Saccharification

Dilute acid generally increases surface area, removes hemicelluloses and alters lignin structure. The effect of concentration of H_3PO_4 on the digestibility of pretreated bagasse is shown in Figure 4. The enzymatic hydrolysis was carried out with 0.25%. 0.50%, 0.75% and 1.0% H_3PO_4 solution at a temperature of 160°C for 15 min and same enzyme concentration (4 FPU/g) was used for enzymatic hydrolysis. The digestibility of glucose and xylose were estimated. Glucose is little bit decreased with an increase in acid concentration but xylose is gradually increased from 38% to 62% with an increase in H_3PO_4 concentration from 0.25% to 1.0%. The yield of total sugar is increased only 4% with an increase in acid concentration from 0.25% to 0.5%. No significant effect was observed at a concentration of more than 0.5%.

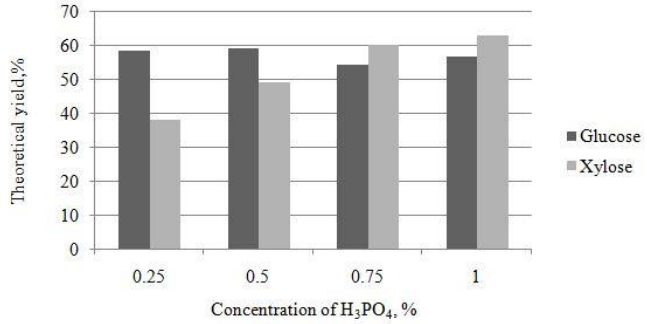

Fig. 4. Effect of concentration of H_3PO_4 on enzymatic saccharification of bagasse.

3.4. Effect of the Concentration of NH3 on Enzymatic Saccharification

The effect of concentration of ammonia in (HCW+NH_3) system on digestibility of pretreated bagasse is shown in Figure 5. Yields of glucose and xylose as well as total sugar were almost same for 0.25%, 0.50% and 0.75% NH_3. Glucose yield is only increased about 6.0% by increasing ammonia concentration from 0.5% to 1.0% but xylose yield remain almost constant. The results revealed that the presence of 1.0 % ammonia in HCW is enough to obtain better yield from enzymatic hydrolysis.

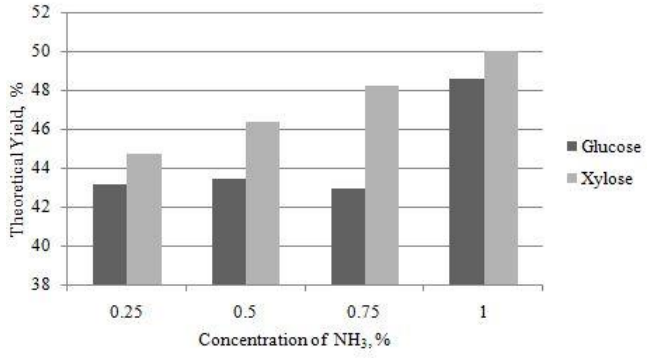

Fig. 5. Effect of concentration of NH_3 on enzymatic saccharification of bagasse.

4. Discussion

The above data reveal that pretreatment with HCW, (HCW+NH_3) and (HCW+H_3PO_4) are very much significant for enzymatic saccharification of bagasse. Recently Jamal et al reported that 0.18L of ethanol can be obtained from one Kg of substrate (bagasse) at 4 FPU/g enzyme loading for 24 h [17]. As mentioned earlier most of the bagasse produced in our sugar mills in Bangladesh is used as fuel or for paper manufacturing. Moreover, it is reported that energy production based on cellulosic biomass have almost zero greenhouse gas emissions [18-19]. Therefore, production of biofuel from bagasse will be cost effective and environmentally friendly.

5. Conclusion

Bagasse is a potential lignocellulosic biomass that can be used to produce considerable quantities of bioethanol, a substitute for gasoline. Hot compressed water (HCW) pretreatment has a significant effect on enzymatic saccharification of bagasse but it depends on various parameters of HCW system. Pretreatment temperature of 180°C was found to be optimum for HCW, (HCW+NH_3) system while it was found to be 160°C for (HCW+H_3PO_4) system. (HCW+H_3PO_4) system was found to be more effective compared to HCW and (HCW+NH_3) system. The effects of concentration of H_3PO_4 and NH_3 were not significant. Only 0.5% H_3PO_4 or 1% NH_3 with HCW was found to be optimum to have better yields. However, the conversion of lignocellulosic material into fermentable sugar is complicated and not yet a commercial business.

Dedication

The authors would like to dedicate this research article to the memory of late Professor Manoranjan Saha of Applied Chemistry and Chemical Engineering, University of Dhaka, Bangladesh.

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