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Original research

Comparative study on chemical pretreatment (acid and ozone) methods for improving enzymatic digestibility of sugar cane bagasse

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A B S T R A C T

Sugarcane bagasse contains cellulose, lignin and hemicellulose, 39-42%, 20-25% and 25-27% respectively. So it is can be used as a sugar source in many processes. Lignin and hemicellulose must be removed before hydrolysis of cellulose. Several different pretreatment approaches have been studied. The purpose of this research is comparison of acid, ozone and combination of ozone-acid as pretreatment methods for improving enzymatic digestibility of sugar cane bagasse in pilot plant scale. Sugar cane Bagasse was washed and pretreated by sulfuric acid (BA) 0.1 %, sodium hydroxide (BSH) 0.1% and (BS) steam was done at pressure 2 bar. Sugar cane bagasse was also treated by sulfuric acid (1, 2, 2.5, and 5%) at 121 ºC and 151 ºC. Ozone and ozone-acid methods also was used for delignification of bagasse. Particle diameter of bagasse in all pretreatment methods was 3-4 mm. All pretreated bagasse was hydrolyzed by cellulose enzyme complex and beta-gluocosidase at pH 5 and temperature 45 ºC. Glucose and xylose content of hydrolyzed sample was analyzed by high performance liquid chromatography (HPLC). Acid is the best media for pretreatment of bagasse in comparison with steam and base. The data showed that high concentration of acid had indirect effect on yield of sugar production in hydrolysis step. Furthermore ozonolysis pretreatment of bagasse led to higher amount of glucose in comparison with acid and acid-ozone methods. Moisture content and duration of ozonation had significant effect on sugar content of hydrolyzed solution and sugar content of hydrolyzed samples was 6%.

Keywords: Bagasse, Pretreatment, Ozone, Acid

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1. Introduction

Sugarcane bagasse is one of the largest agro-industrial by product in several countries. Sugarcane bagasse contains cellulose, lignin and hemicellulose, 39-42%, 20-25% and 25-27% respectively (Hamelinck, et al., 2005). So it is a source of monosaccharide such as glucose and xylose (Gamez et al., 2006) and can be used as a sugar source in many processes. Nevertheless, it has been used as animal feed and only few companies have been succeed to hydrolyze it (Badger et al., 1994). Lignin and hemicellulose must be removed before hydrolysis of cellulose. Several different pretreatment approaches have been studied. They grouped into physical, physicochemical, chemical and biological methods. In physical methods, efficiency is low in comparison with cost. Biological pretreatments also require low investment and energy, but process rate is very low. It seems chemical and/or physicochemical methods are suitable more than two previous styles. In these methods, acid, alkali, hydrogen peroxide, ammonia, carbon dioxide and steam have been used as pretreatment media. Lignin and hemicellulose elimination are high and cellulose crystalinity structure convert to amorphous structure (suitable for hydrolysis enzymes action). Cost of operating and investment are not high, although enzyme inhibitor material has been formed during pretreatment process (Weil et al., 1994).

In recent years, due to increasing price of energy, new approaches has been initiated that are consist of ultrasonic, ozone and microwave. Many scientists studied them in lab scale and stated that efficiency of these approaches is fairly good (Imai et al., 2004; Keshwani et al., 2010; Quesada et al., 1999; Teresa et al., 2009). But it has been proven that these methods are expensive and cannot be used alone. For this reason, two or more methods were merged. Hu and Wen (2008) founded microwave-assisted alkali pretreatment had more efficient than microwave or alkali pretreatment. Up to now, ozone and acid were not used for delignification of sugar cane bagasse. Thus the purpose of this research is comparison of acid, ozone and combination of ozone-

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acid as pretreatment methods for improving enzymatic digestibility of sugar cane bagasse.

2. Material and Methods

2.1. Material

Bagasse was obtained from implant & industry Company of Haft tape, Ahvaz, Iran. Cellulase complex (NS22086) and beta-glucosidase enzyme (NS22118) was provided by Novozyme Company. All of the chemical reagent were analytical grade and was purchased from Merck Co.

2.2. Bagasse preparation

Raw materials were washed and stored at room temperature (23-28 ºC) for 3 days. Their moisture content reached to 6-7%. After that, they was milled and sieved by 6 and 20 mesh size. Dried samples were stored for next analysis.

2.3. Chemical pretreatment

2.3.1. Acidic, steam and alkali treatment

H_2SO_4 (0.1%), NaOH (0.1%) and steam were used at 121 ºC. Bagasse was immersed in these media at pressure 2.5 bar in steel storage tank designed by Fars Engineering Research Center, Shiraz, Iran. After 60 min, the mixtures were filtered and the obtained permeate analyzed for sugar content. Supernatant was washed with distilled water to reach the neutralize pH. Then the delignified bagasse was dried at 100 ºC to reach MC 7% and stored at - 40 ºC for hydrolysis steps.

2.3.2. Acid treatment

Sugar cane bagasse was treated by sulfuric acid (1, 2, 2.5, and 5%) at 121 ºC and 151 ºC in steel storage tank designed by Fars Engineering Research Center, Shiraz, Iran. After 1 h, the obtained mixture was washed with distillated water similar to 2.3.1 and dried for further analysis.

2.3.3. Ozone treatment

10 gram of bagasse (MC 40 % & 50%) were placed in ozone chamber (ozone concentration= 10000 ppm) (COW-020 Ozone generation manufactured by Azarbaijan Engineering Research Center, Tabriz, Iran) for 90, 150 min. Due to consuming of ozone by bagasse during process, ozone concentration was controlled carefully by ozone analyzer (ozone analyzer BMT 964; Germany) and fixed at 10000 ppm. Obtained material after ozone processing was also washed for removal of released lignin and stored for next steps.

2.3.4. Acid-ozone treatment

10 Bagasse was immersed in sulfuric acid solution 1% at 121 ºC for 180 min. Then the samples were washed and pH neutralized. Moisture content of pretreated bagasse was adjusted to 40 % and 50 % and then was processed by ozone for 60, 90 and 120 min at 10000 ppm. After washing, they were dried and stored at freezer – 40 ºC. Particle diameter of bagasse in all pretreatment methods was 3-4 mm.

2.3.5. Hydrolysis

Dried bagasse and distilled water were mixed in the ratio of 1 to 10. Cellulase complex (NS22086) and beta-glucosidase enzyme (NS22118) were added to this mixture (5 and 1% of bagasse weight respectively). PH was adjusted to 5 and mixture was incubated at 45 ºC for 24 h. After hydrolyzing, obtained mixture was filtered by watman paper and buchner funnel. Obtained turbid solution centrifuged at 600 rpm for 15 min. Control sample (raw bagasse) was also hydrolyzed under same condition. The amount of enzyme and temperature of incubation step were selected in accordance with novozyme catalogue.

2.4. Sugar analyzing

The clear solution obtained from hydrolysis step was filtered by 0.22µ filter and injected to HPLC (Knauer, Wissenschatiche Gerätebau) equipped with vertex column 300 × 8 mm and detector RI. Acid sulfuric 0.01 N at flow rate 0.4 ml/min was selected as mobile phase. The column was adjusted at 75 ºC. Standard curve of glucose and xylose obtained by injection of different concentration of them (glucose: 10-30 mg/ml and xylose 5-25 mg/ml).

2.5. Statistical analysis

All experiments were performed in triplicates. The effect of different treatment methods on sugar content of hydrolyzed samples was determined by analysis of variance, using statistical analyses with SPSS (SPSS Inc., Chicago, IL).

3. Results and Discussion

3.1. Acid, alkali and steam pretreatment

Pretreatment of bagasse by sulfuric acid (BA) 0.1 %, sodium hydroxide (BSH) 0.1% and (BS) steam was done at pressure 2 bar. As depicted in Fig. 1, glucose yield in acid pretreatment method was higher than steam and sodium hydroxide methods, but xylose content of hydrolyzed sample in acidic method was lower than two others methods. Hemicellulose is one of the components of bagasse and had fragile structure that easily break down in low concentration of acid or base and mostly release xylose as its monosaccharide. For these reason, xylose content of leakage obtained from sample pretreated by acid is high, while steam and base could not act as well as acid. Therefor hemicellulose content of sample BSH and BS was high. It was broken by enzyme in hydrolysis step and leaded to increase in xylose content of hydrolyzed sample. In fermentation step, saccharomyses applied as convertor of sugar to ethanol. It cannot use xylose as substrate (Jeffries, 2006; Hahn-Hagerdal et al., 1999). So, xylose is an inhibitor for fermentation process and reduced process yield. Therefore, acid is the best media for pretreatment of bagasse in comparison with steam and base.

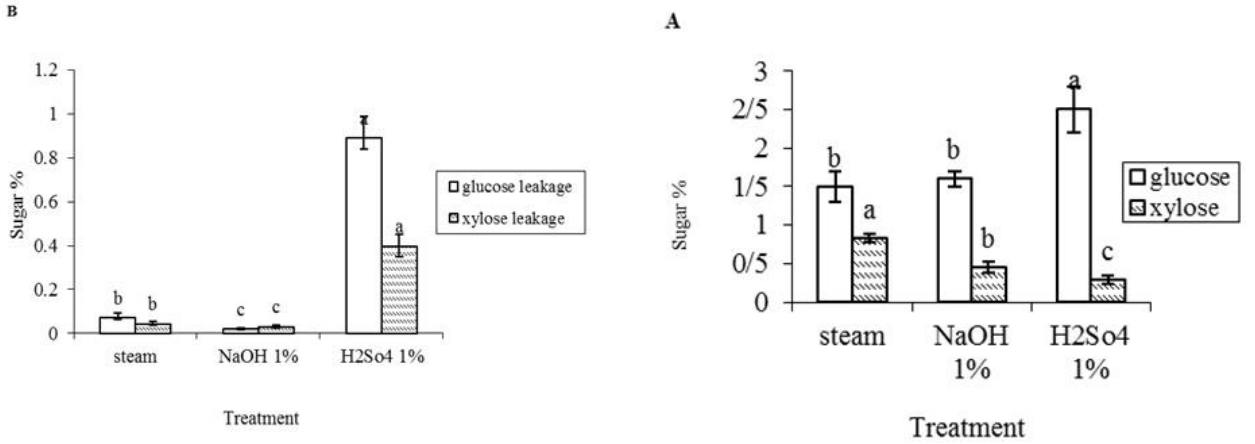

Fig. 1. Sugar content of pretreated bagasse by acid, base and steam at 150 ºC (A) after enzyme hydrolysis and obtained leakage from pretreatment step.

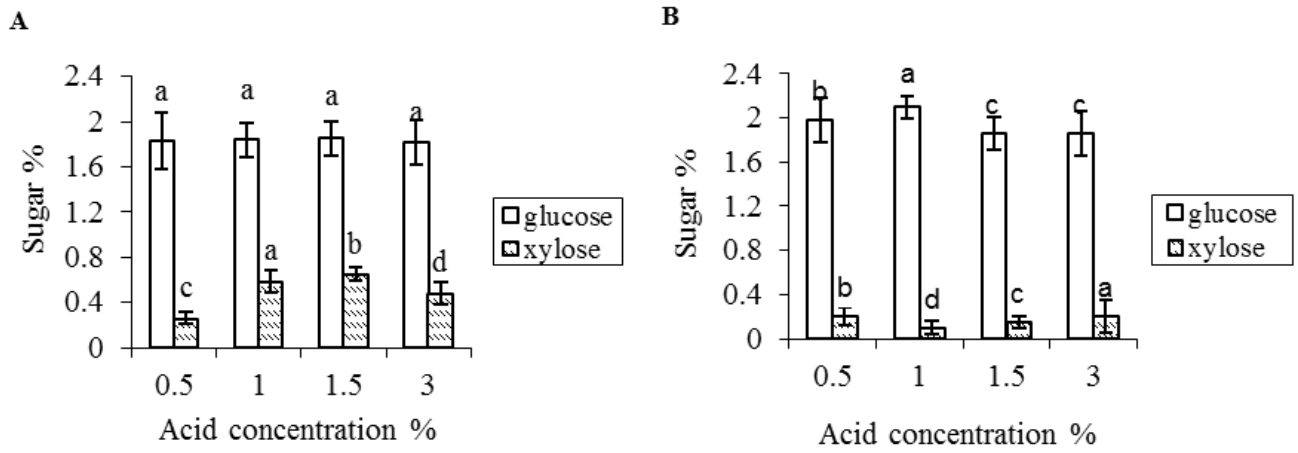

Fig. 2. Sugar content of pretreated bagasse at121 ºC (A) and 150 ºC (B) by acid solution after enzyme hydrolysis.

3.2. Acid pretreatment

Sugar (glucose and xylose) obtained from hydrolysis of sample pretreated by acid in 121ºC has been shown in Fig 2. Different concentration of acid had no significant effect on sugar content of hydrolyzed bagasse or pretreatment of bagasse. In other hand, acid pretreatment could remove lignin and increase hydrolysis ability of enzymes in comparison with control sample. In next step, pressure 5 bars or temperature 150 ºC was selected for acid pretreatment. As shown in Fig. 2 acid solution 0.5 and 1% could prepare better material for hydrolysis at 150 ºC comparison with 121 ºC but acid solution 2 and 3.5 % could not act as well as acid solution 0.5 and 1%. Reason of this change at temperature 150 ºC may be production of inhibitor ingredient in harsh condition of pretreatment method. Xylose content of hydrolyzed solution in this condition is less than 121 ºC. Application of higher concentration of acid and temperature resulted in hydrolysis of more hemicellulose as single source of xylose through pretreatment process. Gamez et al. (2004) used acid phosphoric 2-6% for removal of lignin and preparation of sugar cane bagasse for hydrolysis step. They could produce 1.7 % glucose by using acid 4% at 300 min. They found that increasing of acid concentration and duration time of processing resulted in reduction of xylose content and increasing of glucose content of hydrolyzed solution. In high concentration of acid, hemicellulose was broken down and lost during washing step. These results are in agreement with the result obtained by Sun and Cheng (2005) that pretreated sugar bagasse by acid sulfuric 0.6-1.5 % at 121 ºC and hydrolyzed the obtained samples. They could convert 20 % of bagasse by 1.5 % acid for 60 min. we got same yield in this research.

3.3. Ozone pretreatment

As shown in Fig. 3, moisture content and duration of ozonation had significant effect on sugar content of hydrolyzed solution. Sugar content of hydrolyzed samples was 6%. While, obtained sugar from sample with lower moisture content or lower processing time is between 5-5.75%. Color of samples pretreated by ozone was become very light in comparison with raw bagasse and after washing, finny and dark materials sediment. They may be lignin that separated from cellulose.

Several scientists found similar results. They observed ozone can remove lignin from lignocellulosic materials and increased convention of cellulose to sugar to 80 % during hydrolysis process

(Vidal & Molonier, 2005; Teresa et al., 2009). Their results show thatmoisture content and biomass type are main factors in yield of pretreatment method. Amount of ozone that was consumed in this method is very high and was not as suitable media economically. Sun and Cheng (2002) also found same result and reported that ozone cannot be used alone for delignification of bagasse.

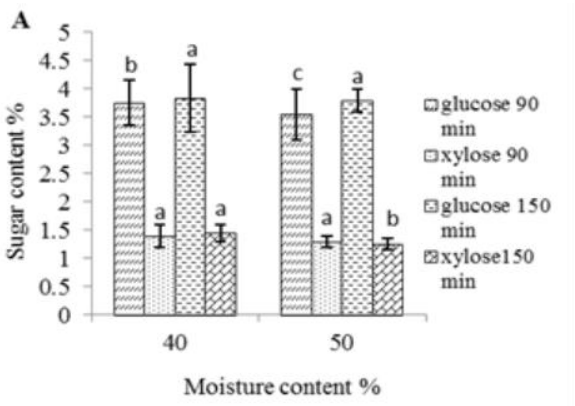

Fig. 3. Sugar content of pretreated bagasse by ozone at different moisture content and time after enzyme hydrolysis.

3.4. Acid-ozone pretreatment

Sugar content of samples pretreated by combination of acid and ozone pretreatment methods is shown in Fig. 4. Duration of ozone processing had significant effect on yield of hydrolyzing results. Duration time 90 and 120 min is more appreciable in comparison with 60 min. In these set of treatment, moisture content did not have significant effect on sugar content of hydrolyzed bagasse containing 40 and 50% MC was not different.

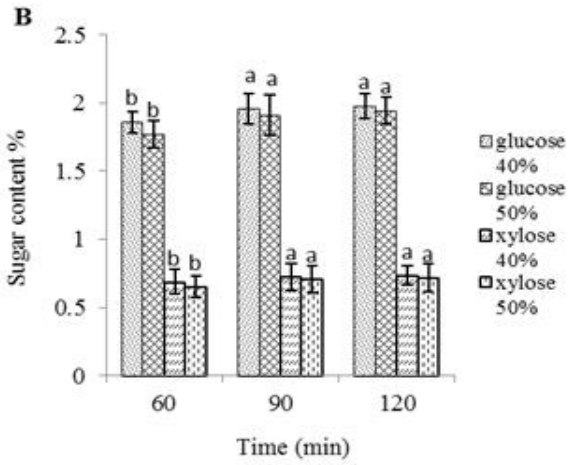

Fig. 4. Sugar content of pretreated bagasse by acid-ozone at different moisture content and time after enzyme hydrolysis.

4. Conclusion

Comparing the methods used in this research shown ozone processing is a suitable procedure for delignification of bagasse. But economic evaluations illustrate that this procedure is not suitable for industry scale now. For this reason we decided to combine ozone and acid. No research has ever been done about this combination. And in this research, the results obtained by acid and ozone were not noticeable and sugar content after hydrolyzing is lower than single ozone procedure. It seems that some inhibitor ingredient produce in the mentioned condition. Hence, the yield of hydrolysis step may be decreased.

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