Enzymatic Hydrolysis of Sorghum Bagasse to Readily Fermentable Sugar for Bioethanol

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Abstract

Production of sugar from sorghum bagasse using enzyme of cellulase and cellobiase in a batch culture was conducted. The purpose of this experiment was to study of the effect of sorghum baggase loadings and lime pretreatment time on production and yield of sugar. Lime pretreatment was carried out in a 1000 ml three-neck flask with a lime loading of 0.1 g $Ca(OH)_2$ /g sorghum bagasse and added with 500 ml distilled water. Effects of pretreatment time course (1, 2, 3, and 4 h) at temperature of 100°C and biomass loading (5, 10, 15 % w/v) were observed to produce sugar. The results showed that maximum concentration of sugar obtained was 28.04 g/l with a *pretreatment* time of 4 h; and the maximum yield of sugar obtained was 0.4 g glucose/ g biomass with a biomass loading of 5% (w/v).

Keywords: Batch culture, cellulose, cellobiase, Enzymatic hydrolysis, ime pretreatment, sorghum bagasse .

Abstrak

Produksi gula dari bagase sorghum menggunakan enzim selulase dan selobiase dilakukan dalam kultur batch. Tujuan percobaan adalah mempelajari pengaruh beban bagase sorghum dan waktu *pretreatment* kapur terhadap produksi gula dan yield gula. Pretreatment kapur dilakukan dalam 1000 ml labu leher tiga dengan beban kapur 0,1 g $Ca(OH)_2$ /g sorghum bagasse dan ditambah dengan 500 ml air distilasi. Pengaruh waktu pretreatment (1, 2, 3, dan 4 jam) pada suhu 100°C dan pengaruh beban biomassa (5, 10, 15 % w/v) pada hidrolisis enzim untuk menghasilkan gula. Hasil penelitian menunjukkan bahwa konsentrasi gula maksimum dicapai sebesar 28,04 g/l dalam waktu *pretreatment* 4 jam; dan yield maksimum gula diperoleh 0,4 g glukose/ g biomassa dengan beban biomassa 5% (w/v).

Kata kunci: Bagase sorghum, selulase, selobiase, hidrolisis enzim, kultur batch, *pretreatment* kapur

Introduction

An increasing demand for energy and continuous depleting fossil fuel resources, globally there is an increased interest in alternative fuels (Wyman, 2007; Lynd et al., 2008). Also, increasing environmental concerns associated with the use of fossil-based fuel, such as carbon dioxide (CO_2) gas emission, have triggered the interest in alternative sources of fuels and chemicals. Current production of bioethanol depends on ethanol from starch and sugars, this is called first generation. However, there has been considerable debate about its sustainability.

One of the most promising options to meet this challenge is the production of bioethanol from lignocellulosic biomass, for example agricultural residues, using second-generation technologies (Galbe and Zacchi, 2007).

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Bioethanol produced from lignocellulosic biomass is an interesting alternative since lignocellulosic biomass do not compete with food crops and they are also less expensive than conventional agricultural feedstocks. Lignocellulose is the most abundant renewable biomass; Lignocellulosic biomass from energy crops, wood and agricultural residues, represent the most abundant global source of renewable biomass (Lin and Tanaka, 2006).

One of major hurdles in production of bioethanol from lignocellulosic biomass is low solubilization of cellulose and hemicelluloses during hydrolysis process to produce sugar. *Lignocellulosic* biomass consisting of celluloses, hemicelluloses and lignin are linked and formed a strong structure which is difficult to be broken down. The conversion of lignocellulosic biomass to fermentable sugar using enzymes has been technically achievable for decades. However, the use of lignocellulosic biomass involves several technical and economical challenges (Mosier et al., 2005a and 2005b) and pretreatment of these

biomass remains one of the key hurdles. The pretreatment is a prerequisite to make cellulosic biomass available to cellulosic enzymes. Biomass pretreatment can be classified into different categories, e.g. physical, chemical, biological, and combination of these methods (Silverstein et al., 2007). Physical pretreatment alters the structure of biomass by applying mechanical stress without addition of chemical or biological reagent. The pretreatment process will increase the active surface area and pore sizes of the biomass.

Alkaline pretreatment is one of the current leading chemical pretreatment methods, particularly for removing lignin, acetyl and other uronic substitutions present on the hemicellulosic portion of the biomass (Mosier et al., 2005a and 2005b). In addition, acetyl groups and various uronic acid substitutes, which lower susceptibility of hemicellulose and cellulose to hydrolytic enzymes, are also removed by alkaline pretreatment (Mosier et al., 2005a dan 2005b). Agricultural residues have been shown to be more suitable to alkaline pretreatment than woody biomass (Galbe and Zacchi, 2007). Alkaline soaking at room temperature instead of elevated temperatures was also found to be effective in improving cellulose digestibility of corn stover (Li et al., 2008). As alkaline pretreatment does not need a complicated reactor, there is an increased interest in applying alkaline pretreatment on-farm (Digman et al., 2007). The major problem of chemical pretreatments is washing and/or neutralization of the pretreated slurry. In order to reduce the environmental impacts of chemical waste disposal, hydrolysates comprise aliphatic acids, furaldehydes, aromatics, must be treated before being discharged into the environment. During alkaline pretreatment some of the alkaline is converted into irrecoverable salts that become associated with biomass, which can cause the cellulose to become denser and more stable than native cellulose and is the limitation of alkaline pretreated biomass (Hendriks and Zeeman, 2009). The best conditions of alkaline pretreatment for switchgrass were determined by residence time of 6 h, NaOH loading 0.1 g/g biomass and $Ca(OH)_2$ loading of 0,02 g/ g biomass at which glucose and xylose yields were 59.4% and 57.3%, respectively (Xu and Cheng, 2011).

The purpose of the experiments was to study of the effect of sorghum loadings and lime pretreatment time course on glucose production.

Materials and Methods

Sorghum Bagasse

Sorghum stalks were obtained/purchased from a farm located in village, Lamongan, East Java, Indonesia. They were cut off in small sizes and dried under sunshine, then was blended using a blender to obtain bagasse with 60-80 mesh particle size. These experiments were carried out at the Unit Operations Laboratory, the Department of Diploma Chemical Engineering, Sepuluh Nopember Institute of Technology, Surabaya, Indonesia.

Enzymes

Enzymes of cellulase (celluclast 1.5 L) having activity of 700 units/g and cellobiase or β-glucosidase (Novozyme 188) having activity of 250 units/g from Novo Inc. were purchased from Sigma-Aldrich.

Lime Pretreatment

Concentrations of sorghum bagasse 10%(w/v) and lime, $Ca(OH)_2$ 1% (w/v) were prepared in a 1000 ml Three-neck flask using distilled water and then was heated to temperature of approximately 100^{o}C in a palm oil batch for 1 - 4 h. After pretreatment, solids and liquid were separated using a Buchner funnel. The pretreated biomass was washed with distilled water until neutral at pH 7.0, dried at temperature 50°C in an oven for 48 h, and stored in a plastic bag, if not used immediately. While, the liquid was analysed for reducing sugar concentration as a glucose using Lane and Eynon methods.

Enzymatic Hydrolysis of Sorghum Bagasse

The pretreated sorghum bagasse with a loading of 10% (w/v) was prepared in a 250 ml Erlenmeyer flask using a solution of 50 mM sodium citrate and pH adjusted to 4.8-5 with 2 N HCl. To this slurry, enzymes of cellulase and cellobiase were added at loading of 10% (v/w) to the biomass, then was incubated at temperature of 50^{o}C in a oven for 24 – 27 h. Liquid sample was withdrawn every 24 h for glucose analysis.

Determination of Glucose Concentration

Lane and Eynon method (ISI, 1999) was used to determine glucose/sugar concentration. Ten millilitres of mixed Fehling solutions A and B was pipetted into Erlenmeyer flask 250 ml and 4 drops of methylene blue at concentration of 10 g/l was added. Then, the solution was heated to

boil. While boiling, a standard glucose solution at concentration of 5 g/l was added from burette until the blue colour disappeared to red one. The titration was repeated using a sample solution instead of the standard glucose solution. The concentration of glucose in the samples was calculated as seen in Eq. (1).

$$C_{spl} = \frac{C_{std} \times V_{std}}{V_{spl}} \left(\frac{g}{l}\right) \quad (1)$$

Results and Discussion

Pretreatment of $Ca(OH)_2$

Pretreatment aims to facilitate the enzymes to access and contact with the cellulose in order to obtain a sugar production. The results are shown in Figure (1), indicating that concentrations of sugar lost during the pretreatment of 1 h to 4 h increased from 2.24 g/l - 2.39 g/l. This was because the longer the process the more compounds in lignocellulosic biomass decomposed by the pretreatment of $Ca(OH)_2$.

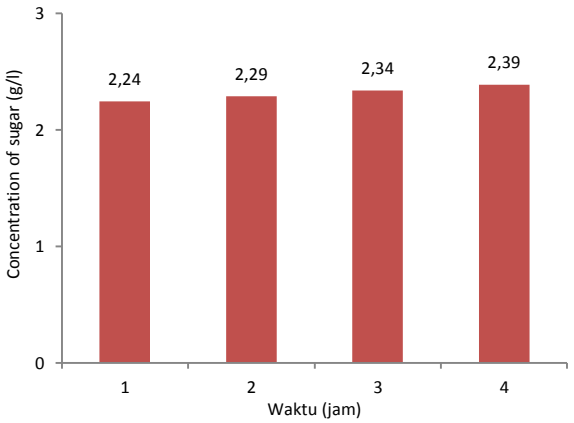

Figure 1. Profile of Sugar concentrations were lost during the pretreatment of $Ca(OH)_2$.

Enzymatic Hydrolysis

Effect of Sorghum Bagasse Loading on sugar yield

Biomass used in the experiments was pretreated for 4 h. Figure (2) shows relashionship between yield of sugar and biomass loadings. Each enzyme of celluase and cellobiase introduced to the 100 ml of slurry was 0.5 ml. The results show that during enzymatic hydrolysis for 72 h at a variety of biomass loadings, a maximum yield of sugar achieved was 0.4 g sugar/g biomass with sorghum loading of 5% (w/v); and was followed by loading of sorghum of 10% and 15% with sugar yield of 0.18 and 0.13 g sugar/g biomass, respectively. These results suggested that production of sugar from those loadings was relative the same as during hydrolysis process, as a result of the same amount of enzymes used. Thereafter, increasing in addition of biomass loadings would not affect on the production of sugar, however would reduce the yield of sugar. These results were lower than those found by McIntosh and Vancov (2010). They found that a maximum yield of sugar was 0.95 g/g biomass using a high enzyme dosage combinations of 5 FPU/7.5 CBU/1.5 FXU.

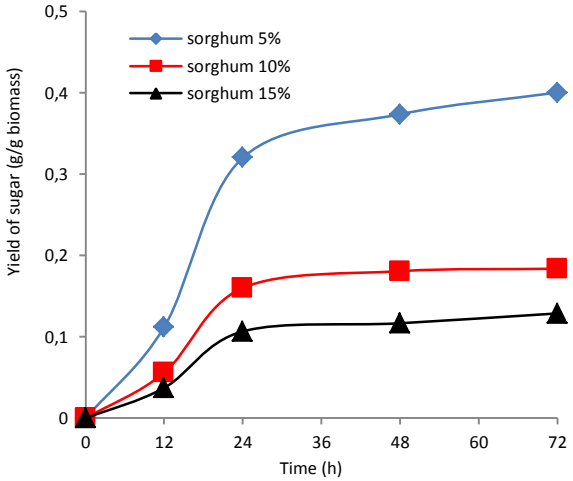

Figure 2. Effect of biomass loading of 5-10% on the yield of sugar with the addition of cellulase enzyme 700 U/ml and cellobiase 250 U/ml.

Effect of Pretreatment Time on Glucose Production

Figure (3) shows effects of time course of lime pretreatment on sugar production in enzymatic hydrolysis. Loading of sorghum bagasse and enzymes used in the experiments was 5% (w/v) and 0.5 ml/5 g biomass, respectively. The results show that the production of sugar had the same trends. Concentrations of sugar increased with time, at time course from 0-24 h, the sugar increased sharply, after that from time 24 to 72 h it increased slightly. Lime pretreatment of biomass before enzymatic hydrolysis affected on production of sugar. A maximum concentration of sugar achieved was 28.04 g/l with pretreatment time of 4 h; and the results were followed by pretreatment time of 3, 2, and 1 h with sugar production of 27.35 g/l, 26.70 g/l and 25.49 g/l, respectively. When biomass was not pretreated, the production of sugar was 11.68 g / l .

In enzymatic hydrolysis, cellulases hydrolyze cellulosic chains and produce the formation of cellobiose, which can be further broken down into glucose by cellobiase (β -glucosidase). The

existence of cellobiose may inhibit the cellulase due to the weaker enzyme to end product inhibition by cellubiose than those by cellulose (Duff and Murray, 1996). In order to reduce the influence of inhibitors and the increase of glucose yield, cellobiase (Novozyme - 188), which contained a high activity of β - glucosidase, was supplemented in the hydrolysis system.

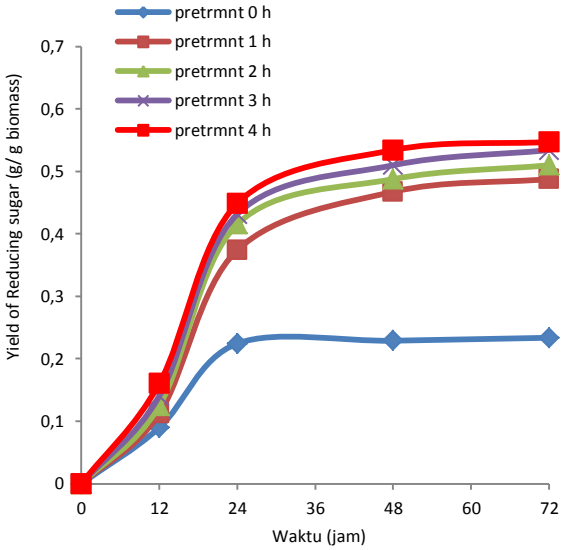

Figure 3. Effect of time course of pretreatment time of Ca(OH)2 on the production of sugar with the addition of cellulose 700U/g and cellobiase 250U/g.

Conclusions

Lignocellulotic biomass have a good potential for production of glucose as a raw material for bioethanol, as it contained a high concentration of cellulose and hemicellulose. This cellulose content was comparable to the other materials, such as corn, wheat, and rice.

Lime pretreatment was effective for improving enzymatic hydrolysis of sorghum bagasse. The lime pretreatment of sorghum bagasse (0.1 g $Ca(OH)_2$/ g biomass at temperature of 100^o C, and for 4 h increased the access of polymer of carbohydrates to enzymatic hydrolysis producing sugar. However, during the pretreatment, the lost of sugar increased from 2.24 g/l - 2.39 g/l with time from 1-4 h. A maximum yield of sugar obtained was 0.4 g sugar/ g biomass with sorghum loading of 5% (w/v). The time course of lime pretreatment was achieved 4 h to obtain sugar concentration of 28.04 g/l. when the biomass was not pretreated, the production of sugar was 11.68 g/l.

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Notation

CBU	=	Cellobiase Unit
C_{std}	=	Concentration of standard glucose solution, g/l
C_{smpl}	=	Concentration of glucose in samples, g/l
FPU	=	Filter Paper Unit
FXU	=	Farvet Xylan Unit
h	=	Time, hour
v	=	Volume, ml
m	=	Mass of biomass, g
V_{std}	=	Volume of standard glucose solution for titration, ml
V_{spl}	=	Volume of sample glucose solution for titration, ml

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