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Glucose Enzymatic Hydrolysis from Pretreated Low Lignin and Low hemicellulose Sugarcane Leaves

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Abstract : One of the second-generation alternative energy and renewable energy which is relatively cheap in its production aspects is bioethanol from cellulosic biomass. Waste sugarcane leaves contain high enough cellulose that can be converted into sugars that are then fermented into ethanol in several steps, i.e. pretreatment, delignification, hydrolysis, fermentation and product purification. The existence of hemicellulose and lignin will decrease the efficiency of hydrolysis, therefore pretreatment procedure should be done prior to enzymatic hydrolysis. Dilute sulfuric acid and sodium bisulfite pretreatment generate high cellulose content material. Enzymatic hydrolysis has several advantages over acid hydrolysis. It will not degrade sugar hydrolysis, can be operated under milder conditions and more environmentally friendly. This study aims to determine the effect of lignin content and hemicellulose content in enzymatic hydrolysis. The best result of pretreatment procedure (low lignin and low hemicellulose) was hydrolyzed using cellulase enzymes derived from *Trichodermareseei*. Three grams powdered sugarcane leaves was dissolved in 100 mL of buffer solution and was addedwith distilled water until 200 mL, then set its corresponding variable pH level (4; 4.5; 5; 5.5 and 6) by using a solution of citric acid 1 M. After pH level conditions were achieved, cellulase enzymes was added in accordance with a variable ratio of substrate-enzyme (1:0.01, 1:0.03, 1:0.06 and 1:0.09 in gram substrate/ gram enzyme). A mixture of sugarcane leaves and enzyme was hydrolyzed with stirring for 45 hours. The highest glucose content (4.777 mg/ mL) was obtained from the enzyme-substrate ratio of 1:0.09, pH level of 5.5 and40.832% glucose yield to the sugarcane leaves powder. Enzymatic hydrolysis at various stages of the sample contained sugarcane leaf powder (without pretreatment), pretreated sulfuric acid material, Na-bisulfite delignification material and pure cellulose which produced glucose respectively 4.36%, 5.13%, 7.70% and 8.45%. The higher cellulose content was hydrolyzed, the greater glucose was produced. This suggests that the presence of lignin and hemicellulose was inhibiting hydrolysis process.
Keywords : sugarcane leaves, lignin, hemicellulose, enzymatic hydrolysis.

1. Introduction

Bioethanol from cellulosic biomass is one of the second-generation alternative energy and renewable energy source which isrelatively cheap in its production. Waste sugarcane leaves contain high enough cellulose that can be converted into sugars that are then fermented into ethanol. There are several steps for efficient

ethanol formation, i.e. pretreatment, delignification, hydrolysis, fermentation and productpurification. The existence of hemicellulose and lignin will decrease the hydrolysis efficiency,thereforepretreatment procedure should be done prior to enzymatic hydrolysis[1]. Dilute sulfuric acid and sodium bisulfite pretreatment generate high cellulose content material (80% of cellulose, 3% of hemicellulose and 10% of lignin)[2]. Enzymatic hydrolysis has several advantages over acid hydrolysis. It will not degrade sugar hydrolysis, can be operated under milder conditions (low temperature, low pressure and neutral pH level), and more environmentally friendly.

Hydrolysis process convert sugar monomer from cellulose and hemicellulose that can take place through by acid or or enzymatic hydrolysis. In addition, the lignin can be further degraded to inhibitors compounds such as furfural and hydroxymethyl furfural[3]. Acid saccharification can also lead to degradation of glucose so that the glucose and ethanol yield decreased. Enzymatic hydrolysis has several advantages over acid hydrolysis, such asit will not degrade sugaras hydrolysis product, milder process conditions (low temperature, low pressure andneutral pH level), more environmentally friendly[4]. Enzymatic hydrolysis has the ability to produce relatively high glucose content (75-95%).

Putri et al. conducted a study to determine the effect of pH on the *Chorella vulgaris* microalgae hydrolysis process into glucose using cellulase enzymes. Hydrolysis carried out for 48 hours, the stirring speed of 100 rpm with the ratio of raw material to the cellulase enzyme is 1:0.06 in the corresponding variable pH level (5, 6 and 7). The optimum conditions obtained the highest glucose content of 5.35 g/L at pH 6[5]. Sedlak et al conducted anethanol production study from hydrolyzedcellulosic biomass using Saccharomyces yeast to convert glucose and xylose. Optimal results of glucose of 32.0 g/L and xylose content of 18.1 g/L and produce ethanol 22.0 g/L and the yield of ethanol/total sugar fermented of 86.1%[6]. Thongkhew hydrolyzedsugarcane leaves enzymatically, with 3% sulfuric acid pretreatment at 35^0C for 48 hours and the concentration of enzyme 10 FPU/g substrate, produced 13.52 g/L of glucose[7].

Boopathy produced cellulosic bioethanol from sugarcane leaves and sugarcane bagasse without going through enzyme hydrolysisprocess. Both of materials were pretreated in a hydrogen peroxide-alkali and sulfuric acid. The optimum condition was acid hydrolysis pretreatment with 0.8 M acid concentration, a 24-hour hydrolysis, and 12 days fermentation time for sugarcane leaves and 18 days for bagasse. Ethanol produced from sugarcane leaves is 335.67 mg/L and from bagasse 395.5 mg/L of ethanol[8,9]. Enzymatic hydrolysis of sugarcane bagasse was also done prior to delignification hydrogen peroxide pretreatment. Optimum conditions were obtained at 50^0C, the raw cellulase enzymes from Aspergillus niger, pH level of 4.8 for 120 hours. Reduced sugar produced was 54.47 mg/100 mL[4]. Betancur studied the production of bioethanol from bagasse with two main treatment to find the optimum conditions of acid hydrolysis and fermentation. The optimum conditions are acid concentration of 1.09% (v/v) or 0.2 M, the ratio of solid:liquid 1:2.8 (g:ml), and 27 minutes hydrolysis time at 121^0C. This condition produces 50 g/L xylose. Optimum fermentation time to produce 20 g/L of ethanol was 40 hours[10,11]. This method took shorter time than on Boopathy'sresearch[8,9]. Based on the procedures and operating conditions of the Boopathy'sstudy, Lalitha conducted a study on ethanol from waste fruit. With the 15-day fermentation produced 330 mg/L ethanol[12]. Meanwhile,a study of the optimum conditions for rice straw hydrolysis reaction using *Trichoderma reseei* for bioethanol production was conducted. The variation was enzyme-substrate ratio (from 1: 1 to 1:1.75), pH level (4-5), hydrolysis time (3-7 days) and the ratio of rice straw-water (2-5%). The results showed that the greater the ratio of the enzyme-substrate, increasing glucose content. The optimum condition was achieved at pH 4.2 and enzyme-substrate ratio of 1:1.4; with the 18.01% glucose produced[13]. Biomass MSW (Municipal Solid Waste) gave the highest 4.093% glucose through a process of hydrolysis using cellulase enzymes from *Trichoderma viride*. Trichoderma extraction performed in a stationary phase at 139 hours, with a temperature of 50^0C hydrolysis and MSW weight of 5 grams[14,15].

Wood fir with dilute sulfuric acid pretreatment (diluted acid/ DA) and sulfite pretreatment was used to overcome the barrier on lignocellulose (SPORL). Pretreatment DA release almost all of the hemicellulose, while SPORL at pH 4.5 releases large amounts of lignin (20-25%). But both of them providedlow enzymatic saccharification digestibility (Substrate Enzymatic Digestibility / SED). DA provides SED values of 25-40% while the SED SPORL value of about 27%. The combination of the two treatment reduced about 90% hemicellulose and 10-20% lignin, with 50-60%SED value[16].

2. Experimental

Raw materials such as sugarcane leaves taken from sugarcane plantation in the district Turen, Malang. Dried sugarcane leaves was crushed using a grinder (disc mill) and sieved with a mesh size of 60 passes and 80 mesh held.

Dilute sulfuric acid pretreatment used pure sulfuric acid analysis (p.a.) from Merck. This treatment released cellulose and hemicellulose from the lignin walland then dissolved hemicellulose. This process is carried out at 121^0C in an autoclave with the solid/acid 1:30, the operation time of 30 minutes and sulfuric acid concentration of 2.5%. Bisulfite pretreatment used a solution of Sodium Hydrogen Sulfite ($NaHSO_3$) p.a. from Merck. This treatment dissolved the lignin that was still mixed in cellulose in treatment before. The process was done in a 12% Na-bisulfite solution at 180^0C for 30 minutes.

Cellulose with the lowest lignin and hemicellulose was hydrolyzed to glucose. Three grams of pretreated sugarcane leaves was hydrolized in a 100 mL citrate buffer at 45^0C for 45 hours using cellulase enzymes *Trichoderma reseei* (Sigma-Aldrich). The process variations were substrate-enzyme ratio (1:0.01, 1:0.03, 1:0.06 and 1:0.09 in gram substrate/ gram enzyme) and pH level (4; 4.5; 5; 5.5; 6). In addition, this study compared the hydrolysis performance for the pure cellulose, acid pretreated results (high lignin) and pretreated bisulfite results (low content of lignin and hemicellulose) and powder sugar cane leaves.

The initial analysis of sugarcane leaf powder, acid and bisulfite pretreated sugarcane leaf powder analysis with Chesson-Datta method performed at the Laboratory of Chemical Engineering, ITN Malang. Analysis of glucose (phenol-sulfate method) and cellulase enzyme activity test conducted at the Chemical Laboratory of Universitas Islam Negeri Maulana Malik Ibrahim, Malang. Ethanol content, the results of fermentation was analyzed in Bioprocess Laboratory at the Universitas Surabaya.

3. Results and Discussion

Analysis of sugarcane leaves powder composition included Hot Water Soluble (HWS), hemicellulose, cellulose, lignin and ash were performed using Chesson-Dattamethods. This analysis was also performed foracid and bisulfite pretreated sugarcane leaves powder. The analysis results are shown in Table 1.

Table 1.The composition of initial and pretreated sugarcane leaves powder

Component	Composition (%)		
	Sugarcane leaves powder	**Diluted sulfuric acid pretreatment**	**Na-bisulfitPretreatment**
HWS	2.0	5.6	3
Hemicellulose	34.5	23	8
Cellulose	43.5	47.4	78
Lignin	18.0	21.3	9
Ash	2.0	2.7	2
Total	100	100	100

Cellulase enzyme activity test showed an average enzyme activity of 3.2924×10^{-2} units/gram. The number of enzyme activity is much smaller than the listed one on the packaging, but still can be used for further processing.

Enzymatic hydrolysis of the sugarcane leaves powder were already separated from hemicellulose and lignin, produced solution with glucose content that are presented in figure 1. The highest glucose levels (4.777 mg/mL) were obtained from the hydrolysis of the enzyme-substrate ratio 1:0.09 (3 gram sample: 0.27 grams enzyme) and pH level of 5.5.

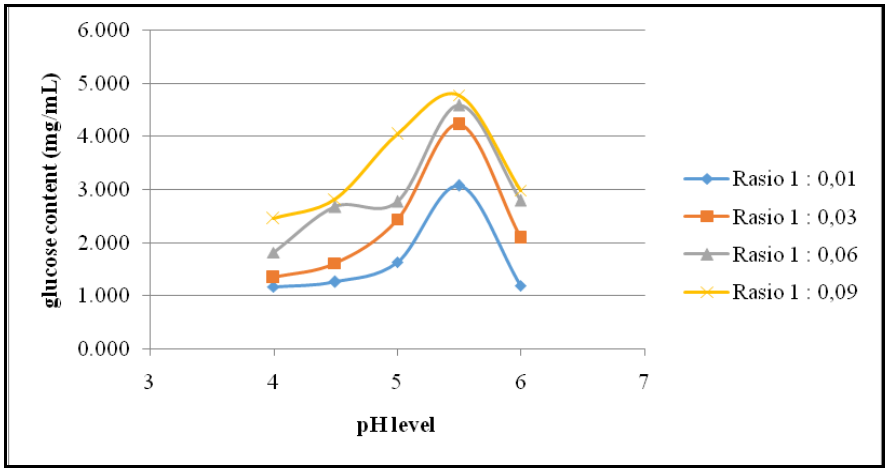

Figure 1.The effect of pH level for glucose content in various substrate-enzyme ratio

Figure 1 shows that in various substrate-enzyme ratio, the highest sugar content was obtained when pH level of 5.5. The optimum pH level conditions would help the enzyme in catalyzing a reaction well. Enzymes could not work on too low pH level (acidic) or too high pH level (alkaline) because the enzymes would be denatured. The higher pH level the the amount of the enzymes carboxyl group will increase and form the glycosidic oxygen protonation to facilitate glycosyl enzyme complex formation. This will increase the enzyme activity. On higher from optimal pH level, the enzymes excessed OH^- ions in solution so that the fraction of active thiol groups (-SH) lossed positive charge and formed an-S-group. This wouldinhibit the protonated thiol functional group so that the substrate and enzyme interaction could not take place properly and complex formation E-S was becoming weaker. These conditions decreased glucose formation.

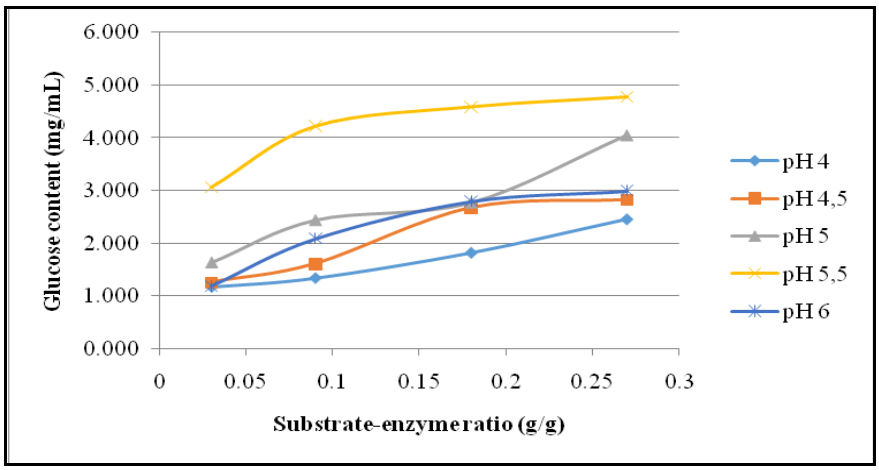

Figure2.The effect of substrate-enzyme ratio to glucose content in various pH level

Figure 2 shows the effect of enzyme addition (by increasing the ratio of substrate-enzyme) of the glucose produced at pH level of 4-6. The addition of enzymes would increase glucose content during the hydrolysis process at various pH level. The maximum value was reached on substrate-enzyme ratio of 1:0.09 and still showed an upward trend although the reaction rate tend to decrease. The greater the ratio of enzyme-substrate increased collisions between reactant molecules to the enzyme, so the absorption chances of the enzyme molecules into substratesincreased.

Glucose yield to the sugarcane leaves powder was obtained by comparing the weight of glucose produced to the weight of the cellulose contained in the material powder sugarcane leaves. Glucose was obtained from enzymatic hydrolysis process with a 3 grams sample weight and 78%cellulose content.Hence, the cellulose weight contained in the sugarcane leaves powder was 2.34 grams, with a volume of 200 mL hydrolyzate. Glucose yield calculation are shown in Table 2. The highest glucose yield (40.832%) was obtained

on samples with a glucose concentration of 4.777 mg/mL with a substrate-enzyme ratio of 1:0.09 and pHlevel of 5.5.

This study also compared the enzymatic hydrolysis and fermentation at various stages of the sample. Hydrolysis carried out on sugarcane leaves powder (without pretreatment), sulfuric acid pretreatment sample, bisulfite delignification sample and pure cellulose. Table 2 shows that the higher cellulose content material was hydrolyzed, the greater glucose was produced. This had proven that the presence of lignin and hemicellulose contained in the initial sample would inhibit hydrolysis process.

Table 2. Comparison of hydrolyzed glucose content from various cellulose content

No.	Sample	Cellulose content (%)	Glucose content (mg/mL)
1	Without pretreament	43,5	4,3618
2	Acid Pretreatment	47,4	5,1263
3	Na-Bisulfite Delignification	78	7,6959
4	Selulosa murni	100	8,4576

4. Conclusions

The enzymatic hydrolysis efficiency of cellulose is affected by the presence of lignin and hemicellulose in the substrate. Lignin and hemicellulose would inhibit hydrolysis reaction. The sugarcane leaves powder enzymatic hydrolysis with 78% cellulose content produced the highest glucose content (4.777 mg/mL) on the addition of enzyme-substrate ratio of 1:0.09 and a pH level of 5.5. At this condition, yield glucose comparing to initial sugarcane leaves was 40.832%.

References

1. Jimmy, Poespowati, T., Noertjahjono, S. (2015a). “Delignifikasi Lignoselulosa Daun Tebu Menggunakan Asam Sulfat Encer”. Prosiding Seminar Nasional Kimia 2015, Jurusan Kimia FMIPA Universitas Negeri Surabaya
2. Jimmy, Poespowati,T., Noertjahjono, S. (2015b). “Lignin Removal From Sugarcane Leaves Lignocellulose Using Sodium Hydrogen Sulfite As Glucose Enzymatic Hydrolysis Feedstock”. International Journal of ChemTech Research, 8(11):188-193.
3. Taherzadeh, M. J., and Karimi K. (2008). “Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review”. Int. J. Mol. Sci. 9, 1621-1651.
4. Gunam, I. B. W., Wartini N. M., Anggreni A. A. M. D., danSuparyana P. M. (2011). “Delignifikasi Ampas Tebu Dengan Larutan Natrium Hidroksida Sebelum Proses Sakaraifikasi Secara Enzimatis Menggunakan Enzim Selulase Kasar Dari Aspergillus Niger Fnu 6018”. LIPI Teknologi Indonesia Vol.34, 24-32.
5. Putri, W.O., Amri, A., danUtami, S.P. 2015. “Pengaruh pH Pada Proses Hidrolisis Mikro Alga Chlorella Vulgaris Menjadi Glukosa Menggunakan Enzim Selulase”. JOM FTEKNIK Volume 2 No. 1.
6. Sedlak, M., Nancy, W.Y.H. (2004). “Production of Ethanol from Cellulosic Biomass Hydrolysates Using Genetically Engineered Saccharomyces Yeast Capable of co-Fermenting Glucose and Xylose”. Humana Press Inc. 0273-2289/04/114/0403–0416.
7. Thongkheaw, S., Pitiyont, B. (2011). “Enzymatic Hydrolysis of Acid-Pretreated Sugarcane Shoot”. World Academy of Science, Engineering and Technology 60, 454-458.
8. Boopathy, R. and Dawson, L. (2007). “Use of Post-Harvest Sugarcane Residue for Ethanol Production”, Bioresource Technology 98, 1695-1699.
9. Boopathy, R. and Dawson, L. (2008). “Cellulosic Ethanol Production From Sugarcane Bagasse Without Enzymatic Saccharification”. Bioresource 3(2), 245-460.
10. Betancur, Gabriel J. Vargas and Pereira Jr., Nei. (2010). “Sugar cane bagasse as feedstock for second generation ethanol production. Part I: diluted acid pretreatment optimization”. Electronic Journal of Biotechnology, Vol.13(3).

11. Betancur, Gabriel J. Vargas and Pereira Jr., Nei. (2010). "Sugar cane bagasse as feedstock for second generation ethanol production". Part II: Hemicellulose Hydrolysate Fermentability. Electronic Journal of Biotechnology, Vol. 13(4).
12. Lalitha, G. and Sivaraj, R. (2011). "Use of Fruit Biomass Peel Residue For Ethanol Production". International Journal of Pharma and Bio Sciences, Vol 2(2), 15-23.
13. Yulianto, M. E., Diyono, Hartati I., Santiko R., Putri F. (2009). "Pengembangan Hidrolisis Enzimatis Biomassa Jerami Padi Untuk Produksi Bioetanol". Simposium Nasional RAPI VIII, 66-73.
14. Poespowati, Tyasing-Putri (2009), "Pretreatment Process of MSW (Municipal Solid Waste) Conversion into Glucose", International Symposium on Sustainable Energy and Environmental Protection (ISSEEP) 2009, UGM Yogyakarta, Indonesia. 23-26 November 2009.
15. Putri, W.O., Amri, A., danUtami, S.P. 2015. "Pengaruh pH Pada Proses Hidrolisis Mikro Alga Chlorella vulgaris Menjadi Glukosa Menggunakan Enzim Selulase". JOM FTEKNIK Volume 2 No. 1.
16. Zhang, Chao., Lei, Xiaochum., Scott, Tim.C., Zhu, J.Y., Li, Kecheng. (2013). "Comparison Of Dilute Acid and Sulfite Pretreatment For Enzymatic Saccharification of Earlywood and Latewood of Douglass Fir", Spinger Science and Business media New York

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