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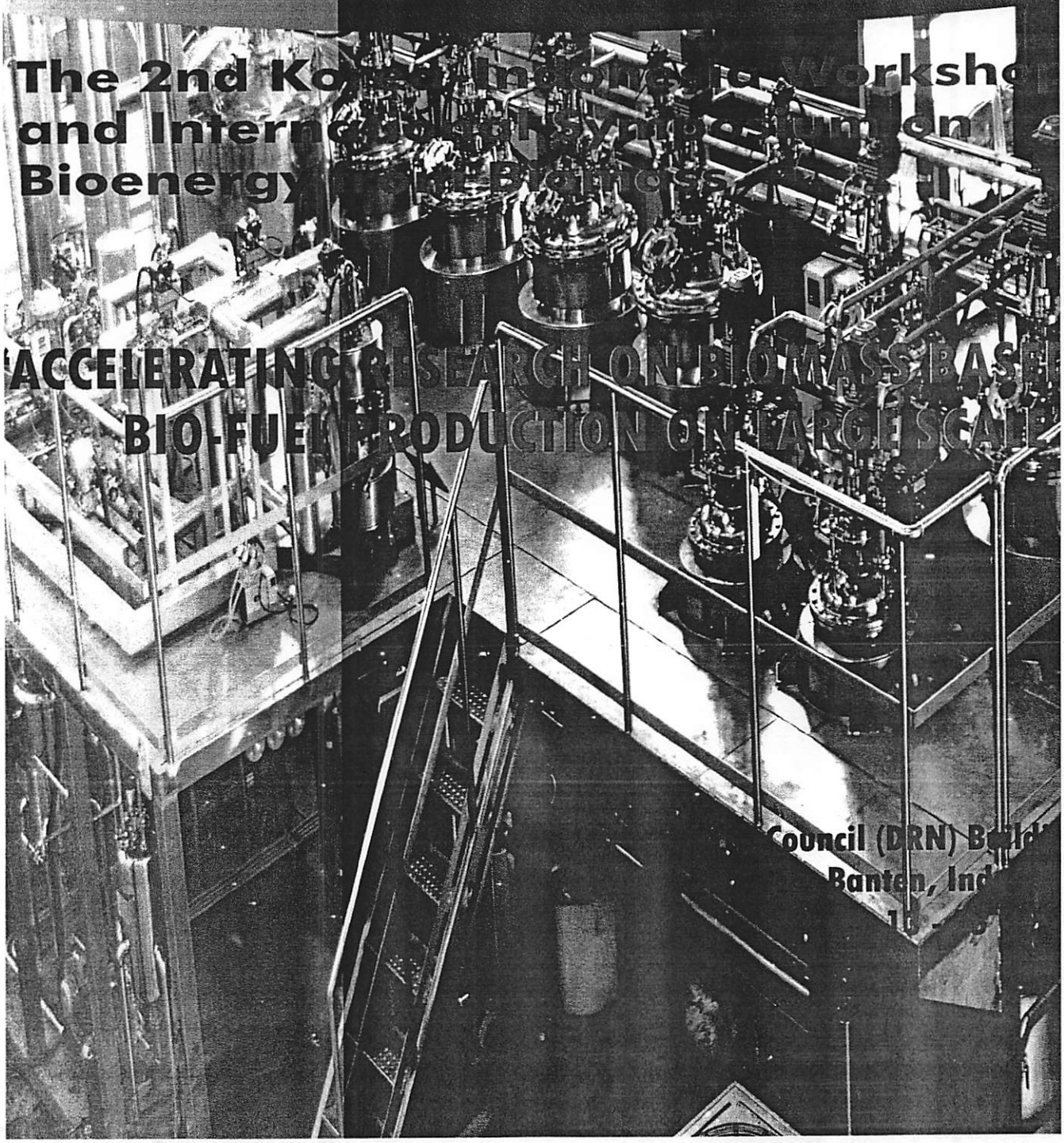

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Effect of Pre-Treatment and Hydrolysis of Sugarcane Bagasse (Saccharum officinarum L.) with Combination of Acid-Enzyme of A Reducing Sugar

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Abstract: Sugarcane bagasse (Saccharum officinarum L.) is a readily available waste product of cane-sugar processing. The major components of bagasse are cellulose and hemicellulose. The objective of the research was to produce fermentable sugar using bagasse, involving optimization of pretreatments, sulphuric acid hydrolyses, enzym hydrolyses using respectively cellulose and hemicellulase. The experiment employed descriptive analyses in triplicates. The result were as follows: Pretreatments of 10% (w/v) sugarcane bagasse in particle size 30 mesh required temperature 120°C during 30 minute. The acid hydrolysis was best by using a sulphuric acid 2% (w/w) and heating steam temperature at 120°C; for 60 minute. The enzymatic hydrolyses was best when using hemicellulase in dosage of 0,001 g/g, followed by cellulase hydrolyses at dose of 0,083 µL/g. The enzymatic was able to hydrolyze the lignocelullose sugarcane bagasse by 65,78% with reducing sugar content 26,38 g/L.

Keywords: Bagasse, hemicellulase. Lignocellulose, hydrolysis, pretreatments cellulose

1. Introduction

Sugar cane bagasse (*Saccharum officinarum* L*.*) is a byproduct of the sugar factory. From the factory the bagasse produced approximately 35-40% by weight of sugar cane milled (Indriani & Sumiarsih, 1992). Bagasse mostly contains ligno-cellulose which are composed of lignin, cellulose, and hemicellulose. Lignocellulose is a renewable substrate, mostly are not used, available and abundant (Taherzadeh & Karimi, 2007). However, lignocellulosic biomass such as sugarcane bagasse and rice straw can not be hydrolyzed into sugars without pre-treatment. A good pre-treatment can reduce the amount of enzyme used in the hydrolysis process.

Pre-treatment of physical, chemical, and biological can produce fuel ethanol from lignocellulosic (Taherzadeh & Karimi, 2008). Physical and chemical methods can be used to separate the cellulose from the lignin by minimizing the size of cellulose particles and swelling of the particles through the pre-treatment (Roehr, 2001). Pre-treatment by grinding the sugar cane bagasse can damage tissues-lignin cellulose-hemicellulose of plant cell walls. Grinding cellulose effectively reduces the formation of crystalline cellulose to 80-90%, then cellulose in sugar cane bagasse more easy to hydrolyze (Inoue *et al.*, 2006). Chemical hydrolysis with sulfuric acid to the lignocellulosic material with an optimal time and temperature can be produced four major components namely carbohydrate polymers (cellulose, hemicellulose), lignin, extractive material, and ash. Further enzymes hydrolyze easier polysaccharide polymer compounds into monomers sugars (Morohoshi, 1991, Taherzadeh and Karimi, 2007). Lignocellulose hydrolysis using sulfuric acid at a concentration of 0.7 M and a temperature of 90°C for 3.5 hours is able to produce sugars from hemicellulose about 90% (Okur & Nurdan, 2006). Enzymatic hydrolysis of sugarcane bagasse to be done with the addition of cellulase and aimed to hydrolyze hemicellulose and cellulose into sugar monomers. Combination of acid and enzyme hydrolyze lignocellulose more effective and efficient produce DE value about 65% (Langlois & Dale (1940) in Tjokroadikoesoemo, 1986). In this study, sugar cane bagasse through the stages of size reduction, then do heat (steam) at a temperature of 120oC for 30 and 45 minutes at a pressure of 1 atm by using the autoclave. Pre-treatment, a combination of acid hydrolysis - enzyme is expected to produce maximum sugar hydrolysates.

2. Research Method

Materials and Methods.

Preparation of Sugarcane Bagasse Sugar cane is obtained from PT. RNI (Rajawali Nusantara Indonesia) located in Cirebon, then reduced in size by grinding until a size of 30 mesh, dried in an oven at a temperature of -80 ° C for 10 minutes.

Sugarcane bagasse powdered has been dried added with water like slurry at a ratio of 1: 20, 1: 13.3; and 1: 10 (w / v) in 100 ml distilled water respectively. Subsequently heatied by steam at a temperature of 120°C for 30 and 45 minutes and reducing sugar was measured by DNS method.

Suspension with highest reducing sugar oconcentration (suspension I) put in a 250 ml erlenmeyer, added with H_2SO_4 as much as 1%, 1.5%, 2% (w / w) of sugar cane bagasse weight. Furthermore each steamed at a temperature of 100°C; 110 ° C; 120°C, for 60; 90 minutes, in order to obtain a suspension II. Suspension of sugarcane bagasse with optimum concentration of the acid hydrolysis (suspension II) is then performed cooling to a temperature of 25oC and pH adjust to 6.0 by using a solution of HCl and NaOH 1 N. Hemiselulase enzyme is then added as much as 0.00033 g / g (dosages1 / 3), 0.00067 g / g (dosage2 / 3), and 0.001 g / g (dosage 3 / 3) of the weight

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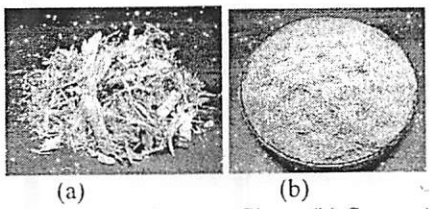
of the enzyme / g substrate, and then incubated at temperature of 55°C for 4.5 hours with agitation speed of 150 rpm At this stage of sugarcane bagasse hydrolyzate III is obtained. The suspension (Hydrolyzate III) then is cooled to 25°C temperature and pH is adjusted to 4.8 by using a solution of HCl and NaOH 1 N. Cellulase enzyme is then added as much as 0.277 ml / g (dosis1 / 3), 0.553 ml / g (dosis2 / 3), and 0.83 ml / g (dosis3 / 3) of the volume enzyme / g substrate, and enzyme as much as 0.123 ml amiloglukosidase / g (dosis1 / 3), 0.247 ml / g (dosis2 / 3), and 0.37 ml / g (dosis3 / 3) of the volume enzyme/ g substrate. Hydrolysates were incubated at 60 ° C for 48 h with agitation speed of 130 rpm.

Analytical methods

pH is measured bya pH meter. Total reducing sugars is measured by DNS method (Apriyantono, et al., 1989).

3. Results and Discussions

Sugarcane bagasse fiber has a length between 1.7 to 2 mm with a diameter of about 20µ. Bagasse fibers are not soluble in water and is mostly composed of cellulose, pentosan, and lignin (Husin, 2007). Size reducing will destroy most of the fiber bond by extending fiber surface and open the cell wall structure, damaging the crystal structure of cellulose and break the chemical bonds of long-chain molecules. Further size reduction leads to the breakdown of carbohydrates than lignin (Murni, dkk., 2008; Inoue *at al.*, 2006). Sugarcane bagasse used in this study can be seen in Figure 1.



(a) (b)

Fig. 1. (a) Sugarcane bagasse fiber ; (b) Sugarcane bagasse fiber in 30 mesh (0,595 mm)

Sugarcane bagasse consists largely of lignocellulose-containing polymers cellulose and hemicellulose, lignin, extractive material, and ash. Chemical composition of sugarcane bagasse is analyzed are presented in Table 1.

Tabel 1. Compositin of Sugarcane bagasse

Content	(%)
Hemicelulose	27,90
Cellulose	23,77
Lignin	35,62
Abu	1,98
Ash	0,69
Water	4,06
Total Reducing Sugar	40,11

Pre-treatment in various of concentration of substrate and time incubation of heating steam of Sugarcane bagasse. Concentration of a reducing sugar was calculated is presented in Figure 2.

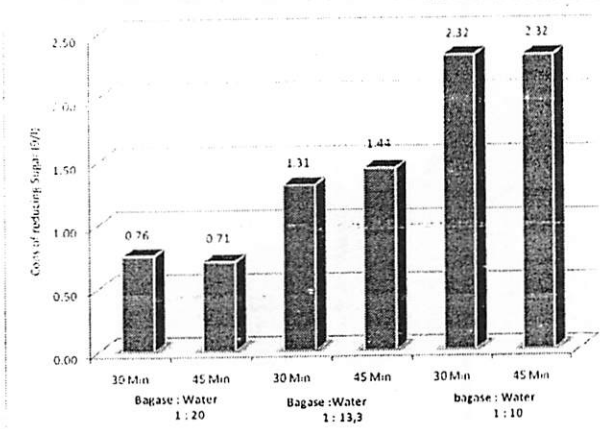


Figure 2. Effect of substrate concentration and time c heating steam to the concentration .. c reducing sugar (g/L)

Based on the research shows that concentrations reducing sugar cane bagasse is highgest is obtained in rat of bagasse and water 1: 10 (w / v) with a time of heatir 30 and 45 at minutes 120°C obtained reducing sugar concentration 2.32 g / L (4.61 DE %).

Pre-treatment with high-pressure can lead to swelling fiber making it easier for acid and enzymatic hydrolys (Fengel, 1995). Steam heat can destroy the bonds betwe cellulose, hemicellulose, and lignin whereas the chemic composition has not changed. High pressure steaming ab to increase the availability of energy due to the increas solubility of cellulose and hemicellulose, and degradab substances release from lignin (Murni, dkk., 200 Concentration, temperature and hydrolysis time Sugarcane Bagas hydrolyzate by H_2SO_4 The process hydrolysis in this study used of H_2SO_4 concentrated. Da of reducing sugars obtained by acid hydrolysis sta calculated is presented in Figure 3.

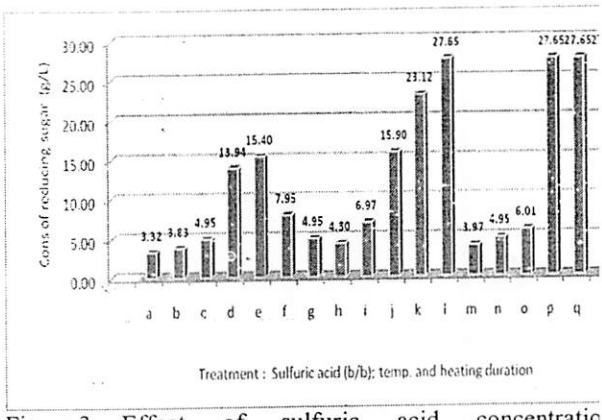


Figure 3. Effect of sulfuric acid concentratio temperature, and steam heating time reducing sugar concentration (g / L)

Caption :

a. 1%, 100°C, 60 min
b. 1%, 100°C, 90 min
c. 1%, 110°C, 60 min
d. 1%, 110°C, 90 min
e. 1%, 120°C, 60 min
f. 1%, 120°C, 90 min
g. 1,5%, 100°C, 60 min
h. 1,5%, 100°C, 90 min
i. 1,5%, 110°C, 60 min
j. 1,5%, 110°C, 90 min
k. 1,5%, 120°C, 60 min
l. 1,5%, 120°C, 90 min
m. 2%, 100°C, 60 min
n. 2%, 100°C, 90 min
o. 2%, 110°C, 60 min
p. 2%, 110°C, 90 min
q. 2%, 120°C, 60 min
r. 2%, 120°C, 90 min

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Treatment with H_2SO_4 2% (w / w), temperature 120oC for 60 minutes produced the highest concentration of reducing sugars 27.65 g / L with a DE value of 54.88%.
This means that 45.12% reducing sugar was not hydrolyzed. These results prove that the use of higher concentrations of H_2SO_4 can reduce the length of time of hydrolysis, while the use of lower temperature hydrolysis hydrolysis takes longer.

In the study Guraf & Geeta (2007), the pre-treatment with Phanerochaete chrysosporium and Pleurotus sp concentration of sugar produced from sugarcane bagasse by 2.54 mg / g. Whereas in this study, pre-treatment with sulfuric acid hydrolysis acid 2% (w / w) with a heating temperature of 120°C for 60 minutes produced a reducing sugar concentration of 27.65 g / L. That is, the hydrolysis of lignocellulosic material with sulfuric acid is more effective than the use of fungi that take a long time. According to Inoue (2006), acid hydrolysis is very important to the efficiency of the enzyme hydrolysis step. Cellulose is naturally bound by hemicellulose and lignin are protected by this structure which causes the biomass is difficult to dihirolysis. The purpose of the acid hydrolysis of lignocellulosic structure in order to unlock the cellulose becomes more accessible to the enzymes that break down the polysaccharide polymers into monomeric sugars. Because one of the hemicellulose components (glucose) has the nature of cellulose is first degraded hemicellulose than cellulose.

The combination of the Acid Hydrolysis - Enzymes in various Dosages of Enzymes. Hydrolysed by the addition of H2SO4 acid hydrolysis of 2% (w / w), temperature of 120°C for 60 minutes for heating was continued for an enzyme hydrolysis. This study used an enzyme hemiselulase and followed by cellulase enzymes. At this stage of optimization has been done hemiselulase and cellulase enzyme dosage.

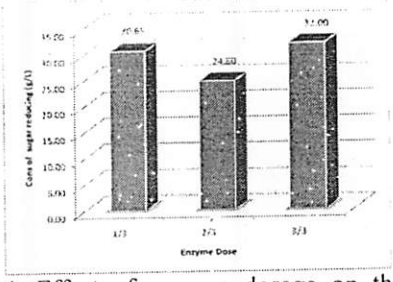

Figure 4. Effect of enzyme dosage on the reducing sugar concentration (g / L)

From the calculation result data obtained by a reducing sugar concentration of reducing sugar concentration (g / L) of enzyme dosage on enzyme hydrolysis.

Based on the results shown in Figure 4, the highest sugar concentration obtained in the treatment and the addition of cellulase enzyme dosage hemiselulase 3/3, amounting to 32.00 g / L with a DE value of 63.52%.

Sugar polymer that has not been hydrolyzed presumably due to the persistence of sugarcane bagasse lignin in the acid hydrolysis, so the cellulase enzymes and hemiselulase difficult to degrade into a reducing sugar monomers. According to Taherzadeh (2008), lignin is a complex compound that is bound to each other with cellulose and hemicellulose. This bond causes the enzyme is difficult to degrade cellulose and hemicellulose into a reducing sugar monomers. Degradation of lignin in berlignoselulosa materials will affect the amount o biodegradable cellulose into reducing sugars. The highe the lignin is degraded, it is increasingly easy to break down the cellulose to form reducing sugars during the enzym hydrolysis (Harlina, 2002).

4. Conclusion

1. In the pre-treatment concentrations of reducing suga produced in the best sugar cane bagasse and water rati of 1: 10 (w / v), heating steam at a temperature o 120oC for 30 minutes the concentration of suga peredukasi 2.32 g / L with 4.61% DE.
2. Hydrolisis by sulfuric acid at concentration of 2% (w w), temperature of the steam heating 120oC for 6 minutes produces a reducing sugar concentration o 27.65 g / L with a DE value of 54.88%.
3. Hydrolisis by cellulase and hemiselulase able t increase the hydrolysis of lignocellulose to sugar can bagasse to reach 63.52% DE with a reducing sug content of 32.00 g / L

Suggestion
Further studies should be conducted to determine th treatment that is able to degrade lignin berlignoselulos 100% of the materials, both chemical and microbiological

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References

Apriyantono, A., Dedi Fardiaz, Ni Luh Puspitasa Sedarnawati, & Slamet Budiyanto. 1989. Analis Pangan. Pusat Antar Universitas Pangan dan Gi Institut Pertanian Bogor.

Fengel, D. 1995. Kayu. Kimia, Ultrastruktur, Reaks Reaksi. Penerbit Gadjah Mada University Pres Yogyakarta.

Gurav, M.S., & G.S. Geeta. 2007. Effectivenes of Fung Pretreatment of Agro Residues on Etanol Productio by Yeast and *Zymomonas mobilis*. Karnataka Agric.Sci, 20(2) : 301-304.

Indriani dan Sumiarsih. 1992. Analisis Serat Bag http://bioindustri.blogspot.com/ 2008/04/ampa tebu.html. Diakses tanggal 22 Juli 2009.

Inoue, H., Sutipa, T., Akira, K., & Shinichi Y. 200 Biomass Technology Research Center. Natu Institute of Advanced Industrial Science a Technology. AIST, http://unit.aist.go.jp/btrc/ci/

Judoamidjojo, M., A. A. Darwis, & E. G. Sa'id. 19 *Teknologi Fermentasi*. Penerbit Rajawali. Jakarta.

Morohoshi, N. 1991. Journal of Wood Chemistry a Technology (4): 111–128.

ISSN: 2302-012

Murni, R., Suparjo, Akmal, & BL. Ginting. 2008. Buku Ajar Teknologi Pemanfaatan untuk Pakan. Laboratorium Makanan Ternak Fakultas Peternakan. Universitas Jambi.

Okur,Telli & Nurdan Eken S. 2006. Etanol Production from Sunflower Seed Hull Hydrolysate by *Pichia stipitis* under Uncontrolled pH Conditions in a Bioreactor. Turkish J. Eng. Env. Sci. (30): 317 – 322.

Roehr, M. 2001. The Biotechnology of Etanol. The Biotechnology of Etanol: Classical and Future Applications. ISBN: 3-527-30199-2. WILEY-VC Verlag GmbH, Weinheim.

Taherzadeh, M.J. & K. Karimi. 2007 Acid Bas Hydrolysis Processes For Etanol From Lignocellulo Materials : A Review. Bio Resources, 2(3) : 472-499

Taherzadeh, M.J. & K. Karimi. 2008. Pretreatment Lignocellulosic Wastes to Improve Etanol and Biog Production: A Review. International Journal Molecular Sciences, 9: 1621-1651.

Tjokroadikoesoemo, P. S. 1986. *HFS dan* Industri U Kayu Lainnya. Penerbit Gramedia. Jakarta.