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FERMENTATION OF SUGARCANE (*SACCHARUM OFFICINARUM L.*) BAGASSE HYDROLYZATE BY *PICHIA STIPITIS*, *SACCHAROMYCES CEREVISIAE*, *ZYMOMONAS MOBILIS* TO ETHANOL

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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 bio-ethanol from hydrolizate of sugarcane (Saccharum officinarum L.) bagasse which hydrolyzed by combination of acid and enzymeand further fermented by three types of microorganisms, respectively P. stipitis CBS 5773, or S. cerevisiae D1/P3GI, or Z. mobilis 0056 FNCC. The experiment employed descriptive analyses in triplicates. The result were as follows: effectiveness of Z. mobilis 0056 FNCC was highest for producing bio-ethanol, respectively 18.99 g/L within 3 hours. S. cerevisiae D1/P3GI produced 17.05 g/L bio-ethanol within 12 hours, and P. stipitis CBS 5773 13.03 g/L bio-ethanol within 24 hours.

Key words: *bagasse, P. stipitis, S. cerevisiae, Z. mobilis, ethanol, fermentation.*

INTRODUCTION

Sugarcane bagasse (*Saccharum officinarum*) is a byproduct of the extraction process or milking cane liquid. From one mill generated bagasse approximately 35-40% of the weight of the milled sugar cane. Bagasse contains most of ligno-cellulose are composed of lignin, cellulose and hemicellulose. Lignocellulose is a substrat that can be renewed, most are not used and is available in abundance (Taherzadeh and Karimi, 2007). Chemical hydrolysis with sulfuric acid can be formed on lignocelluloses materials with specific time and temperature will produce four mayor components namely carbohydrate polymers (cellulose, hemicellulose), lignin, extractive materials, and ash. Further elaborate polysaccharide polymers into a single sugar monomers (Morohoshi, 1991), so the enzyme is more easily hydrolyze these compounds into monomers more simple sugars (Taherzadeh and Karimi, 2007). Enzyme hydrolysis of sugarcane bagasse can be done with the addition of cellulase and hemiselulase to hydrolyze cellulose and hemicellulose into sugar monomers. But using a combination of acid hydrolysis and enzyme more effectively and efficiently produce DE value of about 65% (Tjokroadikoesoemo, 1986). The last stage is fermentation of hydrolizate with culture *P. stipitis, S. cerevisiae,* and *Z. mobilis*. Sugar cane bagasse hydrolysates containing C-5 and C-6. P. stipitis and Candida sheatae they use xylosa and able to ferment hexoses and give a high yield, low tolerant, to ethanol and can produce ethanol concentrations above 30-35 g/ l. According Rouhollah (2007), *S. cerevisiae* has the ability to ferment glucose, maltose, and trehalosa to ethanol. However, *S. cerevisiae* is not tolerant to high concentrations of ethanol produced during fermentation. In addition, *S. cerevisiae* is not able to ferment xylosa because they do not have xylosa xyilitol reductase and dehydrogenase (Gaur, 2006). *Z. mobilis* is more tolerant to ethanol with the concentration levels of 2.5-15% since its membrane plasma structure containing a hopanoid and sterols (Gunasekaran et al., 1986). *Z. mobilis* is more

tolerant to high of concentration of sugar and give higher ethanol production, fermentation at low pH (Kompala et al., 2001).The aim of this research was to study ability of microbes to produce bio-ethanol from hydrolyzate of sugarcane (*Saccharum officinarum L.*) bagasse. In this study, sugar cane bagasse was hydrolyzed by a combination of acid with enzyme then fermented by *P. stipitis, S. cerevisiae*, and *Z. mobilis* to ethanol.

MATERIALS AND METHODS

Preparation of hydrolyzates

Sugarcane bagasse (*Saccharum officinarum*) were powdered by size of 30 mesh and dried in an oven at a temperature of 80^{o}C for 10 minute then add water at a ratio of 1: 20; (w/ v). Further baggase is heating in steam at a temperature of 120^{o}C for 30 and 45 minutes at a pressure of 1 atm using the autoclave. In erlenmeyer, 250 ml, suspension of baggase is added with H2SO4 as much as 2% (w/w) of the weight of sugar cane bagasse. Then it heated by autoclave at a temperature of 120 º C, for 60 minutes. After its cooling at 25 º C temperature and pH is ajusted to 6.0. Hemicellulase with dose 0,001 g/g, is added then it incubated at 55 º C temperature for 4.5 hours with agitation speed of 150 rpm. Further, hydrolysates is cooling to 25 º C and add cellulose enzymes with dose 0,83 µl/g pH to 4.8 then hydrolysates incubated at 60 º C for 48 hours with agitation speed of 130 rpm. DE (Dextrose Equivalent) was measured and type of sugar is formed is analyzed by HPLC.

Preparation of culture starter

The microorganism used was S. D1/P3GI *S.cerevisiae, P. stipitis* CBS 5773, and *Z. mobilis* FNCC 0056. S. cerevisiae, and P. stipitis was cultured in YEPD agar, containing per liter: 3 g yeast extract, 10 g Peptone, 20 g dextrose, 15 g agar-agar, and 1 L aquades. For the culture of *Zymomonas mobilis* used medium containing per liter: 10 g yeast extract, 10 g Peptone, 20 g glucose, 15 g agar-agar, and 1 L aquades (Atlas, 1993). Starter was cultured in agar slant and incubated at 30^{o}C for 24 hours. For starter, cultures was growing in YEPD broth at pH 7, incubated at 30^{o}C using a shaker with agitation speed 100 rpm, culture is incubated 39 hours for *P. stipitis* for 39 hours, 18 hours for *S. cerevisiae*, and 9 hours for *Z. mobilis*.

Fermentation

Sugarcane bagasse hydrolyzate with high DE is filtered using filter paper no.4 Whatman. Hydrolyzate added with medium containing (/ L): 4 g yeast extract, 2 g KH_2PO_4, 3 g $(NH_4)_2SO_4$, 1 g MgSO4.7H_2O, and 3.6 g peptone, pH was adjusted at 7,0 (Sanchez et al., 2002), then hydrolyzate is sterilized by autoclave for 15 minutes. After sterilized, hydrolyzate is cooling and add with starter with concentration of starter 10%. Fermentation is incubated at 30^{o}C, at pH 7, and agitation speed of 150 rpm for 84 hours for *P. stipitis*, and *S. cerevisiae* and 21 hours to *Z. mobilis*.

Analitical Methode

pH is measured with a pH meter. Total reducing sugars is measured by DNS (Apriyantono et al., 1989), and type of sugar is measured and analyzed by HPLC column HPX-87H AMINEX. The ethanol concentration is measured by bichromate oxidation method (Caputi et al., 1968). Type of organic acids is formed during fermentation is measured by HPLC column HPX 87H Aminex.

RESULTS AND DISCUSSIONS

Fermentation was conducted to determine the ability of each straint to ferment the sugar cane bagasse hydrolyzate to ethanol.

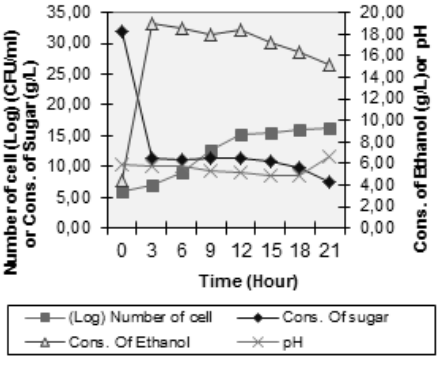

Figure 1.a

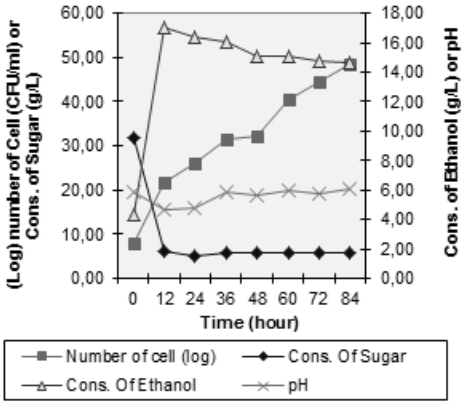

Figure 1b.

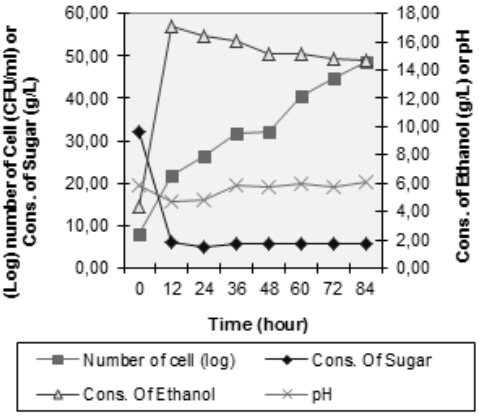

Figure 1 c

Figure 1 a, b, c. Production of ethanol from sugarcane bagasse hydrolysates by (a) *Z. mobilis* (b) *S. cerevisiae* D1/P3GI, (c) *P. stipitis* CBS 5773.

Based on the results shown in Figure 1, show that *S. cerevisiae* D1/P3GI, *P. stipitis* CBS 5773, and *Z. mobilis* FNCC 0056 need adaptation in three hours than begin to log phase. It proves that the three microorganisms capable of using reducing sugar from sugarcane bagasse hydrolyzate as a nutritional source of growth. Based on the results of HPLC analysis of hydrolysed cane is used for the fermentation process containing 23.78% glucose, 56.91% xylosa, and 19.31% arabinose. Based on the results of HPLC, the final stage, fermentation produced lactic acid and acetic acid using a culture of *S. cerevisiae* D1/P3GI and acetic acid from culture *Z. mobilis* 0056 FNCC. Concentration of ethanol produced in line with the growth of each culture and the amount of reducing sugar consumed. In this study, *Z.* 0056 FNCC *mobilis* able to ferment sugar into ethanol hydrolyzate with highest yield of ethanol compared to *P. stipitis* CBS 5773 and *S. cerevisiae* D1/P3GI. Fermentation was more fast, which is 3 hours produced ethanol at 18.93 g / L. The results of this study in line with more reference stated that *Z. mobilis* can convert mixture of sugar into ethanol 92%-94% is greater compared to the yeast which only reached 88-90% (Silalahi, 1987). This possibility is caused that ethanol fermentation of *Z. mobilis*, conversion of glucose into two molecules of ethanol produced one molecule of ATP. The low energy generated ATP resulted in cell mass produced low and high ethanol produced. From Fig. 1c also show that in the first 12 hours, fermentation is constantly increasing. This means that *S. cerevisiae* capable of using sugar cane bagasse hydrolysates as a source of nutrition. This can be seen from the decrease in the amount of sugar in the hydrolyzate accompanied with the production of ethanol amounted to 17.05 g / L. While the research Rouhollah et al. (2007), fermentation using microorganisms Fermentative *S. cerevisiae* in sugar mixture to produce ethanol 0.32 g / L per gram of glucose or by 62% with ethanol concentrations of 14.25 g / L and ethanol productivity was 0.88 g / L / hour in 48 hours fermentation time. This proves that *S. cerevisiae* able to ferment sugar cane bagasse hydrolysates with a shorter time which is 12 hours with levels of ethanol produced 17.00 g/ L. Okur and Nurdan (2006) conducted research on the production of ethanol from waste and the fermentation process needs 55 hours and produced of ethanol concentration 9.66 g / L. While the fermentation of sugar mixed media using *P. stipitis* produces ethanol 0.40 g / L per gram xylose and ethanol concentration 30.23 g / L with productivity of ethanol is 0.95 g / L / hour in 72 hours fermentation time (Rouhollah et al., 2007). While in this study, *P. stipitis* CBS 5773 is able to ferment sugar cane bagasse hydrolyzate to ethanol as much as 13.03 g / L in 24 hours fermentation time. Overall, *P. stipitis* CBS 5773, *S. cerevisiae*, and *Z.* 0056 FNCC *mobilis* use sugarcane bagasse hydrolyzate as a source of nutrition in the period 0-24 hours in line with the ethanol production.

CONCLUSIONS

Z. mobilis 0056 FNCC able to ferment sugar cane bagasse hydrolyzate to ethanol 18.99 g L in 3 hours, *S. cerevisiae*, is able to ferment the sugar cane bagasse hydrolyzat to ethanol amounted to 17.05 g / L in 12 hours, whereas *P. stipitis* CBS 5773 is able to ferment sugar cane bagasse hydrolyzate to ethanol 13.03 g / L in 24 hours.

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