RESEARCH ARTICLE

Advanced Science Letters
Vol. 23, 2533–2535, 2017

Sugar-Rich Hydrolysates of Palm Oil Empty Fruit Bunch Production Through Two Step Solid State Fermentations and Its Conversion to Ethanol

KaharMuzakhar[1,*], Masruroh[1], Siswoyo[2], RudjuWinarsa[1], and Sutoyo[1]

[1]*Biology Department, Faculty of Mathematic and Natural Sciences, University of Jember, 68121, Indonesia*
[2]*Chemistry Department, Faculty of Mathematic and Natural Sciences, University of Jember, 68121, Indonesia*

An improvement hydrolysis to produce a sugar-rich palm oil empty fruit bunches (POEFB) through a two-step solid state fermentation using *Aspergillus niger* and *Tricodhermaviride* has been investigated. Initial solid state fermentation was done in a hundred grams of water saturated POEFB medium in the 5L flaskwithout the addition of any nutrient. The medium was sterilized, inoculated with *A. niger*, and incubated at 30 °C for 5 days. The sugar-rich hydrolysates in POEFB medium were harvested through filtration by adding 100 ml distilled water. Then POEFB sugar-rich free hydrolysates were subsequent re-sterilized, inoculated with *T. viride* and at the same temperature. After for 4 days incubation,sugar-rich hydrolysates also extracted and pooled. The analysis proves that the sugar-rich hydrolysates containing 6.52% (33.4 g sugar in a total volume of 512 ml or 65.2 mg/ml) as a reducing sugar. Further analysis by Gas Chromatograph (GC)astransmethylated sugar as alditol acetates revealed that 63% of POEFB hydrolysates are monosaccharides (41.1 mg/ml). Anaerobic fermentation of sugar-rich hydrolysates using *Saccharomyces cerreviseae* at 30 °C for 36 hours gave yield maximum 19.1 mg/ml ethanol concentration with efficiency 46.5%, respectively.

Keywords: Ethanol, Fermentation, Hydrolyzates, POEFB.

1. INTRODUCTION

Indonesia have some palm oil plantation with huge amount of biomass wastes in the form of palm oil empty fruit bunches (POEFB) were released.[1] This lignocellulosic POEFB is a complex structure consisting mainly of cellulose (52%), hemicellulose (28%) and lignin (17%), and currently one of the most abundant renewable resource on earth.[2,3] One possible approach headed for microbial utilization of lignocellulosic POEFB by converting into fermentable saccharides to produce ethanol and other value-added products.[4,5] Regarding to environment issues, the conversion of this biomass as a raw material for bioenergy and biomaterial is encouraged by the need for a secure energy supply and a reduction of greenhouse gas emissions.[6] However, conversion of this low economic value POEFB to fuels has received less attention due to a sustainable system for commercializing the various developed technologies especially in bioconversion of POEFB to ethanol is very limited and yet to be studied.[3,6] In this research, microbial utilization of POEFB through two steps solid state fermentation to improve hydrolysis and an ethanol production was reported.

*Author to whom correspondence should be addressed.

2. EXPERIMENTAL DETAILS

2.1. Solid State Fermentation

One hundred gram of water saturated POEFB in 5L flask was sterilized, inoculated with *A. niger*, and then incubated at 30 °C for 5 days. The sugar rich hydrolysates was harvested by extracting 100 ml water contain 0.01% natriumazide (v/v), shaking at room temperature for 6 hours, filtered using filter paper, then followed by centrifugation 8000 rpm to remove remaining cells and debris. The POEFB then subsequent inoculated with *T. viride* for 4 days and the sugar rich hydrolysates was harvested using the same method. All hydrolysates were pooled and stored at 4 °C till used for next step analysis.

2.2. Degree of Hydrolysis and Total Sugar Content Analysis of POEFB Hydrolysates

Total reducing sugars production in hydrolysates during solid state fermentation was quantified using the method of Nelson[7] and Somogy.[8] Then the degree of hydrolysis during solid state fermentation was calculated as formula shown below.

$$\text{Degree of hydrolysis (\%)} = \frac{\text{Total reducing sugarofhydrolyzates } \mu\text{g/ml}}{\text{Total substrate } \mu\text{g/ml}} \times 100\%$$

The total sugar content of POEFB hydrolysates was also measured by the phenol-sulphuric-acid method.[9]

2.3. Sugar Component Analysis of POEFB Hydrolysates

Analysis for sugar composition of POEFB hydrolysates was done by using Hitachi Gas Chromatography (GC) as alditol acetates.[10] A sample of 10 mg of freeze dried POEFB hydrolysates was fully hydrolyzed with 2 ml 2 N HCl for 9 hours at 100 °C. The sample was filtered, evaporated to dryness, added with 1 mg of 2-deoxy-D-glucose as an internal standard, and reduced with 2 ml of 0.2 M $NaBH_4$ at room temperature overnight. The mixture added with dowex resin H type 100–200 mesh (Bio-Rad Laboratories, CA) 5–6 drops, incubated for 1 hour at room temperature, followed by filtration. The filtrate was evaporated to dryness and residual boric acid removed by repeated evaporation with methanol. The sugar alcohol was acetylated in 2 ml of acetic anhydride:pyridine (1:1) at 100 °C for 10 min. The mixture was then diluted with chloroform:water (1:4), shaken well and the upper layer removed after centrifugation at 2000 rpm for 5 minutes. The resulting alditol acetate of sugar was dried and dissolved in chloroform to an appropriate volume. The GC equipped with stainless steel column, 2 mm I.D. × 1.83 m, packed with 3% (w/w) ECNSS-M on Gas Chrom Q 100–120 Mesh (GL Sciences, Tokyo Japan). Nitrogen as carrier was adjusted at flowing rate 30 ml/min, while initial column temperature GC 190 °C for 5 minutes and increased gradually to 210 °C at a rate of 1 °C/min. Then an amount 0.5 μl of transmethylated sample was injected to GC for analysis.

2.4. Ethanol Production

The production of ethanol was conducted in sterilized condition using mini fermenter using POEFB hydrolysates medium 50 ml inoculated with *S. cerreviseae* without any nutrients added. This fermentation was done in double. For each fermenter containing 50 ml POEFB hydrolysates was inoculated with 1 ml suspension of *S. cerreviseae* (cells population ≈ 0.1 mg/ml) and aerobically fermentation was maintained at 30 °C for 48 hours. Ethanol production was analysed every 12 hours using QuantiChrom Kit DIET-500 colorimetric method at OD 580 nm. Ethanol concentration was also analysed using GC above equipped with Trace GOLD TG-1301MS GC column with sample volume 0.5 μl. The GC machine was setup at 250 °C with carrier gas helium at flow rate 35 cm/sec.

3. RESULTS AND DISCUSSION

Hydrolysis of POEFB experiment using crude enzyme from *A. niger* and *T. viride* has been reported that gave low degree of hydrolysis.[11] To increase and make improvement decomposition to produce sugar from POEFB has been reported in some investigation, further expected can be converted to another advantageous product efficiently and may lead to increase economic value of POEFB. Production of sugars from POEFB can be improved through steam pretreatment,[12] enzymatically saccharification.[13–15] In this investigation, improvement of sugar production gave significant value under two steps (subsequent) solid state fermentation using *A. niger* and *T. viride.* At Figure 1 showed that when initial solid state fermentation was done by *A. niger*, the sugar production was increased gradually and reach

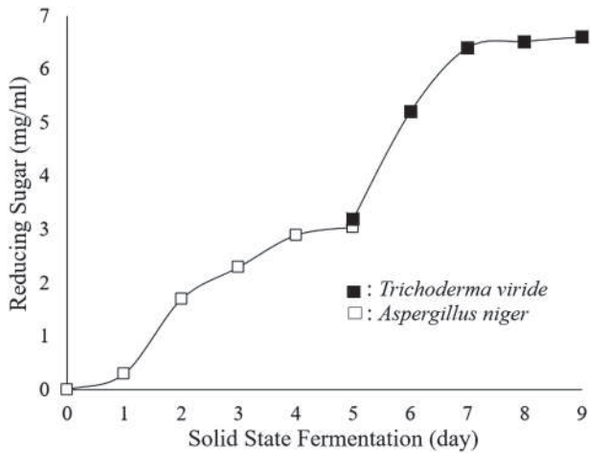

Fig. 1. Reducing sugar production during two step solid state fermentation of POEFB by *A. niger* and *T. viride*.

optimum about 3.02 mg/ml to 3.2 mg/ml after 4 to 5 days fermentation. No significant increasing in sugar production after 5 days incubation. The second step of solid state fermentation of POEFB is subsequent inoculated with *T. viride*. In this case reducing sugar was produced and reached maximum at 65.2 mg/ml after 4 days fermentation. Total reducing sugar was 33.4 g sugars in 512 ml total volume.

Referring to Figure 1, increment of reducing sugar production significantly after inoculation by *T. viride* on the fifth day showed that the enzyme released by among the two microorganisms *A. niger* and *T. viride* had dissimilarities, even though they reported as lignocellulolityc species.[16, 17] It was observed that in day fifth and ninth fermentation, small amount liquid phase was released, indicating hydrolyzation process happened. Accordingly, some extracellular enzymes were released by both *A. niger* either *T. viride* during solid state fermentation.

Table I showed that during solid state fermentation two monosaccharides glucose and xylose were produced by *A. niger* and *T. viride* with significant quantity. However, from the total reducing sugars, only 63% are detected as monosaccharides (41.10 mg/ml). Also, indicates that these two species secreted exctracellular enzymes as carbohydrase were cellulases and xylanases. These two enzymes seem acted exowise which capable releasing monomer glucose from reducing-end of polysaccharides. Others monosaccharides detected with small amount were arabinose and fucose. Indicates that during fermentation, *A. niger* and *T. viride* not only secreted cellulase and xylanase but also arabinosidase and fucosidase. It was reported that these two species able to utilize and grow some organics matter including POEFB.[18, 19] To ensure ethanol concentration, analysis using GC was done. This analysis also gave similar results 18.87 at

Table I. Sugar component of sugar-rich POEFB hydrolysates.

Sugar component	mg/ml	
	A. niger	*T. viride*
—Glucose	16.62	21.12
—Xylose	2.1	0.38
—Other	0.8	0.08
Sub total	19.52	21.58
Total	41.1	

Table II. Concentration profile of sugar-rich POEFB hydrolyzatesduring ethanol production.

Anaerobic fermentation (hour)	Initial concentration of sugar-rich POEFB hydrolyzates (mg/ml)	GC analysis total monosaccharide and component (mg/ml)				Ethanol production (mg/ml)
		Total	Glucose	Xylose	Other	
0	65.2	41.1	37.7	2.48	0.88	0
12	60.2	34.65	32.02	1.98	0.65	3.4
24	29.9	15.1	13.02	1.43	0.65	17.2
36	28.5	6.79	5.27	0.89	0.63	19.1
48	24.8	2.62	1.37	0.62	0.63	19.13

36 hours and 19.64 mg/ml at 48 hours incubation. It was clearly during anaerobic fermentation *S. cerrevisae* fermented reducing sugar as monosaccharide and produced ethanol in between 12 to 36 hours incubation progressively where at that time monosaccharides concentration decrease sharply up to 82% from initial concentration, shown at Table II.

Further, monosaccharides remain (2.62 mg/ml) here after 48 hours fermentation. The production of ethanol 3.4 mg/ml was detected after 12 hours fermentation. Ethanol concentration increased sharply in period of 12 to 24 hours where ethanol production reached at 17.2 mg/ml. And optimum ethanol production at 36 hours fermentation where the concentration was 19.1 mg/ml as shown at Figure 2. Glucose was easily fermented and after 48 hours fermentation 1.37 mg/ml remain. Seventy five percent xylose was fermented and only 0.62 mg/ml remain, but other monosachharides was not suitable sources for this fermentation even though they available as monosaccharide in small amount in POEFB hydrolyzates. Amount of 0.63 mg/ml other monosaccharide remain or only 28% was fermented. These evidence showed that fermentation of POEFB hydrolyzates gave good results with calculation of ethanol production efficiency was 46.5%, even though some monosaccharides still remain in POEFB hydrolysates medium were not consumed during fermentation by *S. cerrevisae*.

Based on these results, improvement microbial utilization of POEFB to increase the yield of sugar as monosaccharides POEFB itself still needed, and either improvement of this POEFB hydrolysates conversion to ethanol.[18] It were reported that fermentation temperature has a direct effect on the biochemical

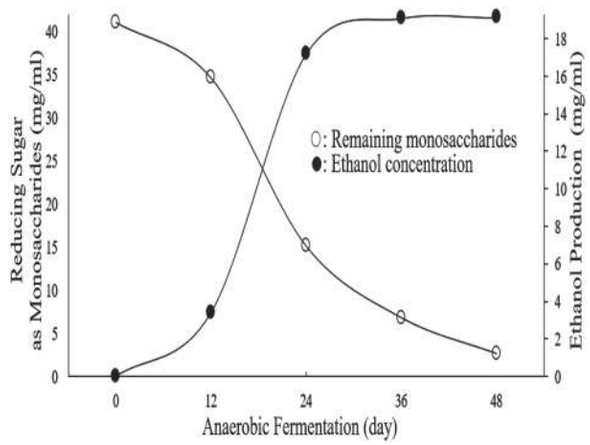

Fig. 2. Reducing sugar as monosaccharides and ethanol production during anaerobic fermentation using *Saccharomyces cerreviseae*.

reactions of yeastand affecting ethanol production. The alcohol fermentation increased as the temperature increased. It was also recorded that the fermentation at 35 °C had no lag phase but alcohol production had a quick exponential phase and reached the maximal level of fermentation earlier.[17] Some parameters such as substrates properties, optimum pH and stability, optimum temperature and stability, and other optimizing environment factors in fermentation such as sugar concentration, pH of medium and temperature as well must be investigated.

4. CONCLUSION

Two step solid state fermentation which subsequently inoculated with *A. niger* and *T. viride* could improve production of sugar-rich POEFB hydrolyzates where 63% of it are monosaccharides. Anaerobic fermentation of this hydrolysates using *Saccharomyces cerreviseae* optimum at 36 °C and gave yield of ethanol 19.1 mg/ml ethanol with efficiency 46.5%, respectively.

Acknowledgments: This research partly funded by grant of STRANAS 2016, Ministry of Research, Technology, and Higher Education Republic of Indonesia granted to first author.

References and Notes

1. Y. L. Chiew and S. Shimada, *Biomass and Bioenergy* 51, 109 **(2013)**.
2. D. Zhang, Y. Ling, Z. Li, and J. Chuan, *Chem. Eng. J.* 181, 636 **(2012)**.
3. S. Y. Ting, A. Hermanto, and K. L. Peh, *Biocatal. Agric. Biotechnol.* 3, 310 **(2014)**.
4. Y. Sudiyani, D. Styarini, and E. Triwahyuni, *Phys. Procedia* 32, 31 **(2013)**.
5. L. Ye, M. Sufian, B. Hudari, Z. Li, and J. Chuan, *Biochem. Eng. J.* 83, 16 **(2014)**.
6. J. J. Thambirajah, M. D. Zulkali, and M. A. Hashim, *Bioresour. Technol.* 52, 133 **(1995)**.
7. N. Nelson, *J. Biol. Chem.* 153, 375 **(1944)**.
8. M. Somogyi, *J. Biol. Chem.* 70, 599 **(1926)**.
9. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.* 28, 350 **(1956)**.
10. S. Arai and M. Murao, *Agric. Biol. Chem.* 42, 1651 **(1978)**.
11. K. Muzakhar, *Int. J. Biosci. Biotechnol.* 2, 32 **(2014)**.
12. S. Shamsudin, U. Kalsom, H. Zainudin, and S. Abd-aziz, *Biomass and Bioenergy* 36, 280 **(2011)**.
13. Z. Wang, H. Bay, K. Chew, and A. Geng, *Process Biochem.* 49, 673 **(2014)**.
14. S. Y. Ting, H. Tay, K. Lin, W. Shang, and C. Siang, *Biocatal. Agric. Biotechnol.* 2, 162 **(2013)**.
15. Y. Zhang, W. Sun, H. Wang, and A. Geng, *Bioresour. Technol.* 147, 307 **(2013)**.
16. M. Z. Alam, M. N. Bari, S. A. Muyibi, and P. Jamal, *Procedia Environ. Sci.* 8, 396 **(2011)**.
17. J. Cheng, S. Leu, J. Y. Zhu, and T. W. Jeffries, *Bioresour. Technol.* 164, 331 **(2014)**.
18. F. Hamzah, A. Idris, and T. K. Shuan, *Biomass and Bioenergy* 35, 1055 **(2011)**.
19. R. Martín-sampedro, A. Rodríguez, A. Ferrer, L. L. García-fuentevilla, and M. E. Eugenio, *Bioresour. Technol.* 110, 371 **(2012)**.

Received: 12 October 2016. Accepted: 2 November 2016.