AENSI Journals

Australian Journal of Basic and Applied Sciences

ISSN:1991-8178

Journal home page: www.ajbasweb.com

The Potential of Supernatant and Sludge of POME in ABE Fermentation by *Clostridium acetobutylicum*

Azima, S.J., Mohd Sobri, T., Jamaliah, M.J. and Abd. Amir, H.K.

Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600, UKM Bangi, Selangor, Malaysia

ARTICLE INFO

Article history:
Received 15 April 2014
Received in revised form 22 May 2014
Accepted 25 October 2014
Available online 10 November 2014

Keywords:
Acetone-butanol-ethanol (ABE) fermentation; Palm oil mill effluent; Product recovery

ABSTRACT

Production of acetone, butanol and ethanol (ABE) as well as the cell growth in non-sterilized supernatant and sludge of palm oil mill effluent (POME) by *C. acetobutylicum* NCIMB 619 were investigated. The fermentation was carried out anaerobically in 250 mL conical flask with working volume of 170 mL in batch mode for 72 hours at a temperature of 37°C. Total solvents concentration obtained were 0.4187 g/L and 0.3027 g/L in POME supernatant and POME sludge, respectively. The product recovery was conducted by liquid-liquid extraction using oleyl alcohol as extractant and calculated based on the partition coefficient, K_d. POME supernatant showed the highest recovery with 70.8% while POME sludge was able to recover 67% of solvents.

To Cite This Article: Azima, S.J., Mohd Sobri, T., Jamaliah, M.J. and Abd. Amir, H.K.., The Potential of Supernatant and Sludge of POME in ABE Fermentation by Clostridium acetobutylicum. ***Aust. J. Basic & Appl. Sci.,*** *8(19): 44-46, 2014*

INTRODUCTION

One of the limitations in conventional acetone, butanol and ethanol (ABE) fermentation process is the high cost of conventional renewable carbohydrates substrates for instance, molasses and maize mash. One possible solution is to use the industrial waste such as palm oil mill effluent (POME) that can be obtained easily and able to reduce the cost of raw materials. POME is well known for its high values in biological oxygen demand (BOD) and chemical oxygen demand (COD) that can be reduced by the fermentation process (Idris, Z.M., 2012). Previous studies showed that POME has the potential to become the main carbon source for ABE fermentation (Kalil, M.S., 2003; Somrutai, W., 1996). The study that was conducted in sterilized POME supernatant and sterilized POME sludge were able to produce the total ABE of 0.3985 g/L and 1.1253 g/L, respectively.

This study aims to investigate the cell growth of non-sterilized POME supernatant and non-sterilized POME sludge as well as their potential in ABE production.

MATERIALS AND METHODS

The inoculum of C. acetobutylicum NCIMB 619 was added into POME supernatant and POME sludge that was collected from Sime Darby's East Palm Oil Mill in Carey Island. The fermentation process was carried out anaerobically in 250 mL conical flask with working volume of 170 mL at temperature of 37°C for 72 hours. The pH of both medium was adjusted to 5.8 using 5M NaOH (Kalil, M.S., 2003) and was spurted by purified nitrogen gas. The ratio of inoculum to medium was 1:10. The cell growth was determined based on the pH values, cell concentration and reducing sugar during the fermentation process. The pH values were determined using pH meter, while cell concentration and reducing sugar in the samples were determined by volatile suspended solid (VSS) and dinitrosalicylic acid (DNS) methods, respectively.

Liquid-liquid extraction was conducted to recover ABE from the fermentation media before the samples were injected to gas chromatography. Oleyl alcohol is one of the chemical that was reported to have the potential to extract solvent for ABE (Boudreau, T.M., A.H. Gordon, 2006). After an amount of oleyl alcohol was added into the samples with the ratio of 1:1 (oleyl alcohol:sample), the solution was mixed using vortex mixer at 1600 rpm for a minute to increase the interaction between two fluids with different density. Lastly, the samples were

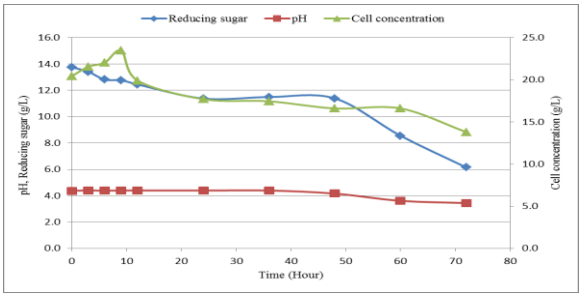
Corresponding Author: Mohd Sobri Takriff, Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia
E-mail: sobri@eng.ukm.my

centrifuged at 2000 rpm for 5 minutes before 1.5 mL sample was taken from each phase and transferred into sealed gas chromatography vial tubes for ABE analysis in the gas chromatography.

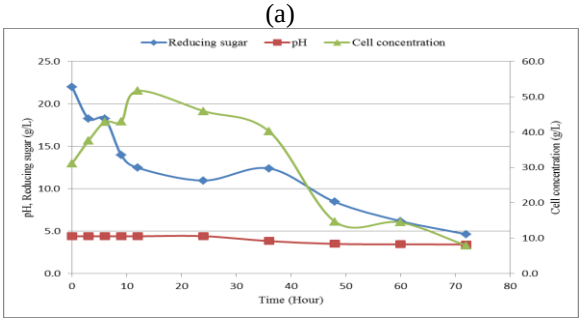
RESULTS AND DISCUSSION

Cell growth profiles of C. acetobutylicum NCIMB 619:

Figure 1 (a) and (b) show the growth profile of C. acetobutylicum NCIMB 619 in POME supernatant and POME sludge. A short vaguely lag phase was observed in POME supernatant that occurred after 3 hours of the fermentation process while for the POME sludge, the lag phase could be seen clearly occurred after 6 hours of fermentation. This indicated that the bacteria needed time to adapt with the new environment before the cell started to grow. The reducing sugar started to reduce after 12 hours and 3 hours of fermentation for POME supernatant and POME sludge, respectively. It showed that the bacteria were starting to utilize the sugar and assist them to produce ABE in the fermentation. The bacteria in POME supernatant utilized 65.5% of reducing sugar while in POME sludge, the bacteria consumed 72.1% of reducing sugar. The pH values in both media were starting to decrease within 3 hours after fermentation. It showed that the culture entered the acidogenic phase where the production of organic acids; acetate and butyrate were starting to occur. The metabolism of the cells started to shift to solventogenic phase to produce solvents. However, there were only few organic acids that successfully converted into ABE since the pH values were fairly constant and could be due to the low concentration of sugars in both media.



(a)



(b)

Fig. 1: Cell growth profiles of *C. acetobutylicum* NCIMB 619 in (a) POME supernatant; and (b) POME sludge.

ABE Production and recovery:

Figure 2 shows the amount if solvent produced during ABE fermentation and its distribution in organic and aqueous phase for POME supernatant and POME sludge. It showed that most of the solvent produced was distributed in the organic phase. Ethanol is the major product with the production of 57% and 90% from total ABE produced in POME supernatant and POME sludge, respectively. The maximum solvent production in POME supernatant and POME sludge occurred after 3 hours and 6 hours respectively after the ABE fermentation. Total solvents produced in POME supernatant and POME sludge were 0.4187 g/L and 0.3028 g/L, respectively. According to Jones and Woods (Jones, D.T., 1986), the solvent ratios varies based on the fermentation condition and the type of strain used in the fermentation, but a ratio of 3:6:1 (acetone:butanol:ethanol) is common for the Weizmann fermentation. This study produced a ratio of 3:40:57 for POME supernatant and 3:7:90 for POME sludge. This ratio was calculated based on the total solvent produced. Table 1 shows the comparison of fermentation performance for both media.

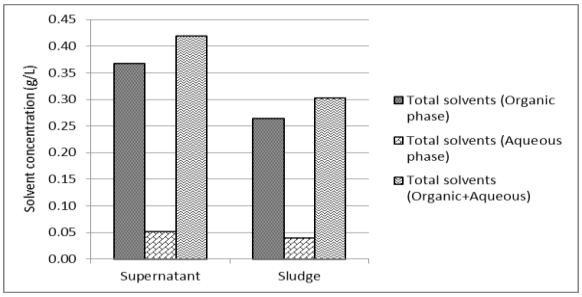


Fig. 2: Total solvent produced and its distribution in organic and aqueous phase.

Table 1: Comparison of fermentation performance.

Parameter	POME Supernatant	POME Sludge
Maximum cell concentration (g/L)	23.45	51.70
Maximum acetone concentration (g/L)	0.0048	0.0021
Maximum butanol concentration (g/L)	0.1099	0.0151
Maximum ethanol concentration (g/L)	0.0933	0.2099
Total solvent concentration (g/L)	0.4187	0.3028
A:B:E ratio	3:40:57	3:7:90
$Y_{P/S}$ (g ABE/g substrate)	0.05	0.02
$Y_{P/X}$ (g ABE/ g cell)	0.06	0.01
Productivity (g/L/h)	0.07	0.10

Liquid-liquid extraction using oleyl alcohol as an extractant was used to recover ABE. The percentage of recovery was determined based on the partition coefficient. K_d. When two fluids with different density and solubility were shaken together, the solute tends to distribute itself in a ratio roughly proportional to its solubility (Pahlavan, 2010). The percentage of recovery for POME supernatant and POME sludge were 70.8% and 67%, respectively.

Conclusion:

Palm oil mill industrial waste was proven to be one of the most potential carbon sources that can be converted into useful product despite its negative effect to the environment. This study was able to produce 0.4187 g/L and 0.3028 g/L of ABE in POME supernatant and POME sludge, respectively with ethanol as the major product. Liquid-liquid extraction using oleyl alcohol as extractant was able to recover 70.8% of ABE in POME supernatant and 67% of ABE in POME sludge. For the non-sterilized condition, POME supernatant proved to be the best substrate to produce ABE with solvent ratio of 3:40:57.

ACKNOWLEDGEMENT

The authors wish to thank Ministry of Science, Technology and Innovation Malaysia for funding this project under grant number 03-01-02-SF0710.

REFERENCES

Boudreau, T.M., A.H. Gordon, 2006. Improved ethanol-water separation using fatty acids. Process Biochemistry. 41**:** 980-983.

Idris, Z.M., P. Jamal, M.Z. Alam, 2012. Evaluation of palm oil mill effluent treatment with concomitant phenolics production by Aspergillus niger IBS-103ZA. Australian Journal of Basic and Applied Sciences, 6(1): 55-61.

Jones, D.T., D.R. Woods, 1986. Acetone-butanol fermentation revisited. Microbiological Reviews, 50(4): 484-524.

Kalil, M.S., P.W. Kit, W.M.W. Yusoff, Y. Sadazo And R.A. Rahman, 2003. Direct fermentation of palm oil mill effluent to acetone-butanol-ethanol solvent producing clostridia. Pakistan J. Biol Sci, 6(14): 1273-1275.

Pahlavan, 2010. Extraction Process: Determination of distribution coefficient. Houston Community College.

Qureshi, N., I.S. Maddox, 1995. Continuous production of acetone-butanol-ethanol using immobilized cells of Clostridium acetobutylicum and integration with product removal by liquid-liquid extraction. Journal of Fermentation and Bioengineering, 80(2): 185-189.

Somrutai, W., M. Takagi, T. Yoshida. 1996. Acetone-butanol fermentation by Clostridium acetobutyricum ATCC 17777 from a model medium for palm oil mill effluent. J. of Fermentation and Bioeng, 81(6): 543-547.