http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 6 (14): 1273-1275, 2003
ISSN 1028-8880

Direct Fermentation of Palm Oil Mill Effluent to Acetone-butanol-ethanol by Solvent Producing Clostridia

Mohd Sahaid Kalil, [1]Pang Wey Kit, [1]Wan Mohtar Wan Yusoff, [2]Yoshino Sadazo and Rakmi Abdul Rahman
Department of Chemical and Process Engineering, [1]Center for Bioscience and Biotechnology
Faculty of Science and Technology, University Kebangsaan Malaysia, 43600 UKM Bangi Selangor, Malaysia
[2]Laboratory of Microbial Technology, Department of Bioscience and Biotechnology
Kyushu University, 6-10-1 Hakozaki, Higashi-ku Fukuoka 812-8581, Japan

Abstract: Studies on direct use of palm oil mill effluent (POME) as fermentation medium for acetone-butanol-ethanol (ABE) production by *Clostridium acetobutylicum* NCIMB 13357 and *C. saccharoperbutylacetonicum* N1-4 have been carried out in batch culture system. Investigations were carried out on the effect of concentration of sedimented POME, the effect of initial culture pH and the use of immobilized cells for ABE production. It was found that *C. acetobutylicum* NCIMB13357 grown in 90% sedimented POME with initial pH 5.8 produced highest total ABE (4 g L^{-1}). However, butanol production was maximum (1.82 gL^{-1}) in the culture with the initial pH of 6.0. Results obtained from these experiment with immobilized cells of *C. saccharoperbutylacetonicum* N1-4 indicated that ABE production from POME could be improved when high concentrations of cells at solventogenic growth phase were used.

Key words: Palm oil mill effluent (POME), acetone-butanol-ethanol (ABE), clostridia, immobilized cells

Introduction

Palm oil is one of the world's chief edible oils produced by South East Asian and African countries, used for producing various food products, cosmetic and pharmaceutical products and oleo-chemicals. Its production generates various wastes chief among which is palm oil mill effluent (POME). POME is produced from production of crude palm oil which involves extraction process where the fresh palm oil fruit bunches undergo sterilization, digestion and extraction of the oil, which is then clarified. POME is produced in vast amounts throughout the year, in particular in palm oil-producing countries such as Malaysia, could be a kind of sustainable resources. Previous report showed that *C. acetobutylicum* P262 produced total ABE up to 0.94 g L^{-1} when grown in POME as the fermentation medium (Khaw *et al*., 1999). POME is a highly concentrated industrial wastewater with BOD of up to 40,000 mg L^{-1} thus may pose serious pollution potential if not properly managed. The production of ABE by solvent-producing strains of Clostridium was one of the first large-scale industrial fermentation processes developed. During the first and second world war, acetone was required for ammunition manufacture. However, in the 1960s everything have change when ABE could also produced cheaply from petroleum; driven also the increase in the price of sugarcane which have increased the production cost of ABE by fermentation (Jones and Woods, 1986; Volesky *et al*., 1981; Durre, 1998). Therefore, one of the main factors causing why ABE fermentation could not survive was due to the cost of the raw materials for medium preparation. In any fermentation process the cost of the substrate (fermentation medium) will be about 60% of the overall cost (Ross, 1961).

Palm oil Mill effluent (POME) has great potential as a substrate for ABE fermentation because it contains a mixture of carbohydrates including starch, hemicellulose, sucrose and other carbohydrates that can be utilized by saccharolytic clostridia (Kwon *et al*., 1989; Mun *et al*., 1995). Such utilization would further increase profitability of palm oil industry besides solving an environmental problem. This paper discussed on the use of POME as a cheap raw material for ABE fermentation.

Materials and Methods

C. acetobutylicum NCIMB 13357 was purchased from a British culture collection, NCIMB Ltd. Scotland, UK. The bacterium was cultivated in anaerobic condition in Reinforced Clostridial Medium (RCM) for 48 h at 30°C. Liquid medium of RCM was used for inoculum preparation. The growth of culture in RCM was monitored by measuring an optical density at 680 nm using a spectrophotometer. Only inoculum with optical density (OD) values greater than 0.7 after 18 h cultivation was used as inoculum. An inoculum of 10% v/v was used throughout this work.

Samples of POME were obtained from Sri Ulu Langat Palm Oil Mill, Dengkil, Selangor, Malaysia. Fresh POME was sedimented passively in a cool room at 4°C for 24 h before use. The supernatant layer (upper part) was decanted and sedimented POME sludge (lower part) was sterilized at 121°C for 20 minutes and used directly as fermentation medium without additional nutrient. Sedimented POME was diluted with distilled water to obtain required concentration before deoxidizing by gassing with nitrogen gas for a few minutes.

C. saccharoperbutylacetonicum N1-4 was obtained from a laboratory stock culture maintained at Kyushu University, Japan. The bacterium was grown and maintained in potato glucose (PG) medium. *C. saccharoperbutylacetonicum* N1-4 was grown anaerobically in TYA medium at 30°C until growth of cells has reached late log growth phase. The culture was harvested by centrifugation and cells were suspended in 50% less phosphate buffer solution at pH 6.0. The cells suspension was then mixed with equal volume of 2% sodium alginate solution and dropped into 400 mL of 2.5% $CaCl_2$ solution to form alginate beads.

ABE production by *C. acetobutylicum* NCIMB 13357 was studied in 250 mL conical flask culture without mixing. The effect of concentration of sedimented POME was studied at pH 5.8 using POME concentration at 70, 80 and 90% v/v. While the effect of initial pH 4.5, 5.0, 5.5, 5.8, 6.0 and 6.5 was studied using POME 90%. Investigations on the use of immobilized cells of *C. saccharoperbutylacetonicum* N1-4 for ABE production using POME were carried out in 250 mL glass bottle without mixing.

The concentration of ABE was measured by gas chromatography (Shimadzu 17-A) fitted with a flame ionization detector (FID) using capillary column BP1 with nitrogen as the carrier gas. The temperature was programmed by initial temperature of 40°C for 5 min and increased to 170°C at a rate of 20°C min^{-1}.

Results and Discussion

Effect of concentration of POME on ABE fermentation: From initial studies it was found that *C. acetobutylicum* was able to grow in POME without addition of nutrients and produced nearly 1 g L^{-1} ABE after 48 h incubation. It was also found that sedimented POME at 50% concentration gave better results. Sedimentation of POME helped to remove traces of oil and soluble toxic substances leaving less inhibitory POME which is more suitable for growth of Clostridia. With reduction of water content, sedimented POME contains higher concentrations of lignocellulose and other insoluble materials which supported growth of *C. acetobutylicum*. The following experiment was done to find out suitable concentration of sedimented POME for ABE production. POME 90% produced highest ABE compared to POME 70% and POME 80% (Fig. 1).

However, *C. acetobutylicum* NCIMB 13357 produced ABE with a different ratio (5:4:1) compared to reported ratio of 3:6:1 (Jones and Woods, 1986). The ABE obtained was 1.5-2.0 g L^{-1}; this is higher than that reported by Khaw *et al.* (1999) which was only 0.94 g L^{-1} ABE produced when *C. acetobutylicum* P262 was grown in POME. That lower production of ABE might be due to the use of glucose derived from POME instead of the whole POME as the medium.

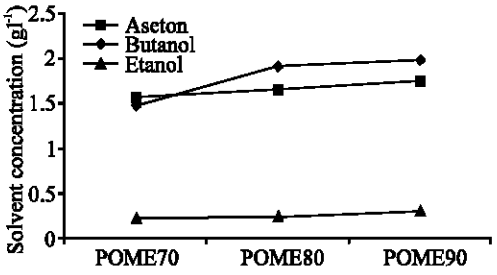

Fig. 1: The concentration of ABE produced after 48 h cultivation using different concentration of POME (POME 70, 80 and 90%)

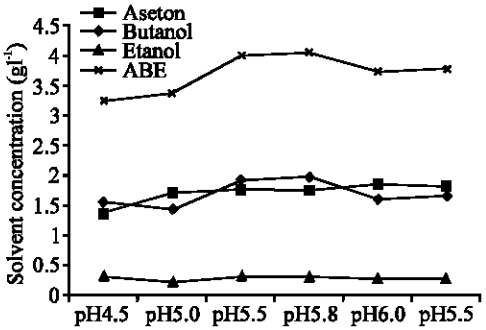

Fig. 2: ABE production by immobilized cells of N1-4 using POME

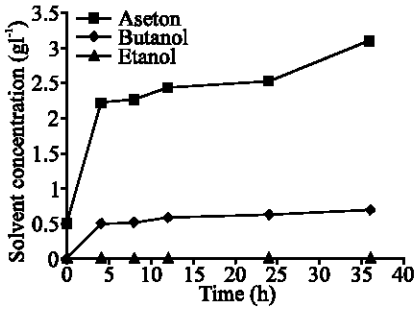

Fig. 3: ABE production by immobilized cells of N1-4 using POME

Effect of initial pH on ABE fermentation: The concentration of ABE increases with the increase of initial pH up to pH 5.8; thereafter the concentration of ABE decreased at higher initial pH values (Fig. 2). Highest ABE concentrations were obtained at initial pH of 5.5 to 5.8 where ABE concentration reached 4 g L^{-1}. It was found that butanol concentration was high (1.86 g L^{-1}) in the culture with initial pH 6.0 compared to other cultures, although the total ABE in the culture was only 3.7 g L^{-1}. Previous reports using other clostridial strains showed that production of ABE was optimum at the initial pH values of 5.0-6.5 (Jones and Woods, 1986).

ABE fermentation using immobilized cells of *C. saccharoperbutylacetonicum* N1-4: Initial run for ABE production by immobilized cells of N1-4 using TYA medium produced maximum ABE concentration of 12 gL^{-1} after 12 h incubation, which is four times faster than using growing culture. The color of gel beads turned to deep cream yellow from light cream yellow just after preparation. In the second experiment the immobilized cells were incubated with sterile POME. Fig. 3 shows that rapid production of solvent was observed within 4 h of incubation in POME medium. The concentration of total ABE reached 3.8 g L^{-1} after 36 h incubation and the amount of butanol produced was 2.5 g L^{-1}. Previous report showed that cultures of *C. acetobutylicum* P262 could only produced total ABE up to 0.94 g L^{-1} when grown in POME as the fermentation medium (Khaw *et al*., 1999). In order to increase product yield, several options may be tried. Among these are enzymatic hydrolysis of insoluble materials in POME or addition of cheap starchy substrate, which may also be from a waste source. The production of solvent was much more rapid with immobilized cells compared to growing cells; this might be due to cells being at late log phase when harvested, where most of them were in the solventogenic phase (ready for solvent production). When cultivating Clostridial cells in a culture medium, two phases of growth will occur. Acidogenic phase will occur first where cells produce organic acids (acetic and butyric acid), followed by solventogenic phase where cells assimilate the organic acids formed to produce solvents. The same beads can be used repeatedly. Experience from this study shows that they can be reused for at least 5 times for ABE production from POME without losing their performance. These studies have shown that POME can be effectively fermented by using immobilized cells in a continuous fermentation systems to produce high yields of ABE. These results show great potential for POME to be used as raw material for ABE production, thereby yielding a valuable product as well as solving our environmental problem.

Sedimented POME at 90% concentration is suitable for ABE production by fermenting Clostridia. The initial pH of the POME must be increased to pH 5.8 before used for ABE fermentation medium in order to get high yield of solvent. Either free growing cells or immobilized cells of Clostridia can ferment POME to produce ABE. High yield of ABE can be achieved by using immobilized cells when high concentration of solventogenic cells were used.

Acknowledgements

The authors would like to thank Yayasan Felda for their financial support through a grant D/9/99. Apart of this work was done under research collaboration between Kyushu Univesity and UKM.

References

Dürre, P., 1998. New insights and novel developments in clostridial acetone/butanol/isopropanol fermentation. Appl. Microbiol. Biotechnol., 49: 639-648.

Jones, D.T. and D.R. Woods, 1986. Acetone-butanol-ethanol revisited. Microbiol. Rev., 50: 484-524.

Khaw,T.S., A. Arbakariya, H. Mohd. Ali and A.K. Mohd. Ismail, 1999. Utilization of fermentable sugars from saccharification of palm oil mill effluent (POME) and oil palm empty fruit bunch (OPEFB) fibers for acetone-butanol-ethanol (ABE) fermentation. Seminar on efficient use of palm oil waste as renewable resource for energy and chemical, Bali, Indonesia.

Kwon, G.S., B.H. Kim and A.S.H. Ong, 1989. Studies on the utilization of palm oil residues as the substrate for butanol fermentation. ELAEIS, 1: 91-102.

Mun, L.T., A. Ishizaki, S. Yoshino and K. Furukawa, 1995. Production of acetone, butanol and ethanol from palm oil waste by Clostridium saccharoperbutylacetonicum N1-4. Biotechnol. Lett., 17: 649-654.

Ross, D., 1961. The acetone-butanol fermentation. Prog. Ind. Microbiol., 3: 73-85.

Volesky, B., A. Mulchandani and J. Williams, 1981. Biochemical production of industrial solvents (acetone-butanol-ethanol) from renewable resources. Ann. N.Y. Acad. Sci., 369: 205-218.