

CONVERSION OF PINEAPPLE JUICE WASTE INTO LACTIC ACID IN BATCH AND FED – BATCH FERMENTATION SYSTEMS

Abdullah Mochamad Busairi

Department of Chemical Engineering,
Faculty of Engineering, University of Diponegoro
Jl. Prof. Soedarto, SH, Tembalang, Semarang, Indonesia, Telp/Fax: +62-24-7460058 ; +62-24-76480675
E-mail: abd_busairi@yahoo.com

Abstract

Pineapple juice waste contains valuable components, which are mainly sucrose, glucose, and fructose. Recently, lactic acid has been considered to be an important raw material for the production of biodegradable lactide polymer. The fermentation experiments were carried out in a 3 litres fermentor (Biostat B Model) under anaerobic condition with stirring speed of 50 rpm, temperature at 40°C, and pH of 6.00. Effect of feed concentration on lactic acid production, bacterial growth, substrate utilisation and productivity was studied. The results obtained from fed- batch culture fermentation showed that the maximum lactic acid productivity was 0.44 g/L.h for feed concentration of 90 g/L at 48 hours. Whereas the lactic acid productivity obtained from fed-batch culture was twice and half fold higher than that of batch culture productivity.

Keywords: *batch and fed-batch culture, lactic acid, pineapple juice waste*

Abstrak

Buangan jus nanas mengandung komponen yang berharga terutama sukrosa, glukosa, dan fruktosa. Asam laktat adalah bahan baku yang terbaru dan penting untuk dibuat sebagai polimer laktat yang dapat terdegradasi oleh lingkungan. Percobaan dilakukan pada fermentor 3 liter (Model Biostat B) di bawah kondisi anaerob dengan kecepatan pengadukan 50 rpm, temperatur 40°C, dan pH 6,00. Pengaruh konsentrasi umpan terhadap produksi asam laktat, pertumbuhan mikroba, penggunaan substrat dan produktivitas telah dipelajari. Hasil yang didapatkan pada fermentasi dengan menggunakan sistem fed-batch menunjukkan bahwa produktivitas asam laktat maksimum adalah 0,44 g/L,jam dengan konsentrasi umpan, 90 g/L pada waktu 48 jam. Bahkan produktivitas asam laktat yang didapat pada kultur fed-batch lebih tinggi 2,5 kali dari pada proses menggunakan sistem batch.

Kata kunci: *kultur batch and fed-batch, asam laktat, buangan jus nanas*

INTRODUCTION

Batch culture fermentation is still the most important process operation used in bio-processing industries due to its simplicity which can be carried out without advanced measurement and control. Batch culture becomes less suitable when substrate inhibition on cell growth by product formation occurs (Hammes and Whiley, 1993). Some problems of batch culture fermentation can be overcome by fed batch operation. Fed batch culture is a batch culture, which substrate nutrients are fed continuously or sequentially without the removal of fermentation broth. It is widely used for the production of microbial biomass, ethanol, organic acids, antibiotic, vitamins, enzymes, and other

compound (Roukas and Kotzekidou, 1998). The important rationale of fed batch operations are the excess of carbon caused inhibition of cell growth and the excess of carbon or nitrogen caused the catabolite repression (Wang *et al.*, 1986).

Pineapple juice waste contains valuable components, which are mainly sucrose, glucose and fructose (Abdullah and Mat, 1998). Lactic acid was chosen as the desirable product, as it is one of the most important organic acids widely used in the food of industries. Recently, lactic acid has been considered to be an important raw material for the production of biodegradable lactide polymer (Goksungur and Guvenc, 1997). Abdullah and Mat (2005) studied the

effect of some parameters, such as initial pH, substrate concentration, temperature, and yeast extract concentration on the yield of lactic acid production from pineapple juice waste by employing batch culture. The highest yield and productivity obtained from batch fermentation at 40°C, pH 6.00, sugar concentration of 52.5 g/L with 5 g/L of yeast extract were 79% and 0.32 g/L.h, respectively. Pineapple solid waste utilisation for lactic acid production had also been studied, with only 56% yield and productivity of 0.1 g/L.h were achieved (Abdullah, 2007).

The aim of this investigation was to examine the potential of fed batch culture as fermentation system for lactic acid production from pineapple juice waste.

EXPERIMENTAL METHODS

Substrate

The fermentation media contained pineapple juice waste was obtained from Malaysian Cannery Sdn. Bhd. Before being used as a substrate, the pineapple juice waste was boiled for 5 minute to deactivate the existing enzymes and followed by filtration to remove the solid particles. It was then stored at -18°C for other batches of fermentation as suggested by Lazaro (1989).

Strain

The microorganism used in this study was *Lactobacillus delbrueckii subsp. delbrueckii* ATCC 9649 obtained from DSMZ, Germany. The strain was maintained on MRS agar at 4°C and transferred to fresh medium every month.

Inoculum Media

Each fermentation process was initiated by transferring a small amount of biomass to 250 mL Erlenmeyer flasks containing 50 mL of liquid MRS medium. Anaerobic condition was prepared by flushing with nitrogen and sealing the flasks with thigh-fitting rubber stopper. The inoculum was constantly shaken in an incubator shaker at 37°C and 150 rpm for 24 hours as suggested by Sakamoto and Komagata (1996).

Batch Fermentation

The batch fermentation was carried out in a 3 litres fermentor (Biostat B Model). The fermentor was equipped with pH, temperature and dissolved oxygen controllers. The fermentor containing 950 mL of substrate was firstly sterilised at 121°C for 15 minutes. Fifty millilitres of inoculum was sterilised separately and added aseptically into the fermentor. Anaerobic system was generated by sparging the fermentor with nitrogen at 6.5 mL/minute and homogenisation was done by stirring at 50 rpm (Lund *et al.*, 1992). Samples of 10 mL were withdrawn from the fermentation broth at regular time interval. The

microbial cells were separated by centrifugation for dry biomass determination. The supernatant was immediately frozen for further determination of the lactic acid, glucose, fructose, and sucrose concentrations (Mercier and Yerushalmi, 1992).

Fed-batch Fermentation

The fermentation was performed in a 3 litres stirred fermentor with effective working volume of 2.5 litres. The fermentation was performed in two steps. In the first step, 700 mL of pineapple juice waste containing 65 g/L of sugar was inoculated with 5% inoculum, while the lactic acid bacteria were grown in a batch culture for 24 hours. In the second step, the production medium containing 65, 90, and 115 g/L of sugar was continuously added into the fermentor from it's above at constant rate of 8.5 mL/h.

Analysis

The organic acid content in the sample was measured by HPLC (Waters TM 600). A 250 mm × 4.6 mm ID Spherisob Octyl column (Waters) and UV detector (210nm) were used. The eluent used was 0.2 M phosphoric acid at flow rate of 0.8mL per minute and temperature of 25°C.

The sugar content was also measured by the same apparatus, using a 300 mm × 4 mm ID μ Bondapak/Carbohydrate column (Waters) with RI detector. The eluent used was a mixture of acetonitrile : water (80:20) at flow rate of 2 mL per minute and temperature of 25°C.

Cell concentration was quantified by constructing a calibration curve of optical density as a function of dry cell weight. The optical density was measured on spectrophotometer Model (UV-1601) at 620 nm. Dry weight determined by centrifugation of fermentation broth at 4000 rpm for 15 minute, washing it twice with distilled water and dried at 103°C for 24 hours and followed by weighing (Abdullah, 2007).

RESULTS AND DISCUSSION

Effect of Feed Concentration on Lactic Acid Production and Bacterial Growth

The feedings were begun at exponential growth rate (24 hour). The effect of the feeding concentration on lactic acid production and bacterial growth were shown in Figure 1.

Figure 1a shows that the lactic acid production increased with the increase of sugar content in the feed. The highest concentration of lactic acid of 59.69 g/L was obtained at sugar concentration in the feed of 115 g/L, and almost similar result was obtained at 57.76 g/L lactic acid for substrate feeding containing 90 g/L of sugar. The lactic acid yields were 78.83%, 64.17% and 51.90% at feeding concentration of 65 g/L, 90 g/L and 115 g/L, respectively.

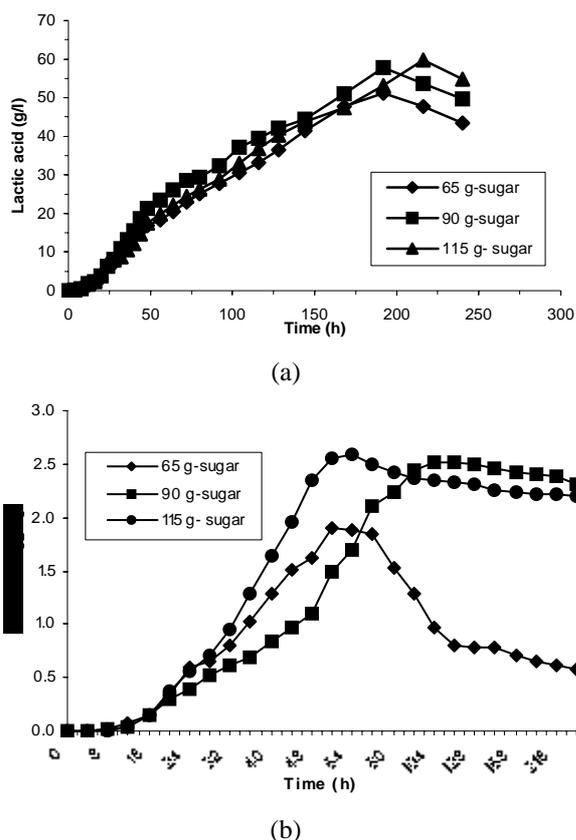


Figure 1. a) Lactic acid production and b) bacterial growth on pineapple waste juice fermentation using fed batch culture with different of feed concentration. Experimental conditions: $T=40^{\circ}\text{C}$, $\text{pH}=6.0$, 5% inoculum and stirring speed=50 rpm.

Figure 1b shows that at feed concentration of 90 g/L the bacteria growth faster than that of 65 and 115 g/L, due to inhibition by high sugar concentration in medium. The maximum concentration of biomass obtained at substrate feedings of 90 and 115 g/L was obtained similar of 2.5 g/L. At substrate feeding containing 65 g/L of sugar, the maximum biomass concentration was only 2 g/L. Surprisingly, the bacteria growth at this feeding condition was faster than that at 115 g/L, even though it then declined gradually after 80 hour. This indicated that at feed concentration of 65 g/L, the concentration of nutrient in medium was not sufficient to maintain the growth of the cell.

Effect of Feed Concentration on Substrate Utilization

Figure 2 shows that the concentration of residual sugar decreased in batch fermentation, while it increased during fed-batch culture, except at feeding substrate concentration of 65 g/L. After 24 hours feeding, the concentration of sugar at feed concentration of 65 g/L decreased, but at feed concentration of 95 and 115 sugar g/L the sugar concentration increased. This indicated that at feed concentration of 65 g/L, the rate of lactic acid

production much faster than any other substrate feeding rates studied. The sugars seemed to be not completely utilised. This may reflect the complex of nature in pineapple waste juice where the sugar may not be easily metabolised by bacteria.

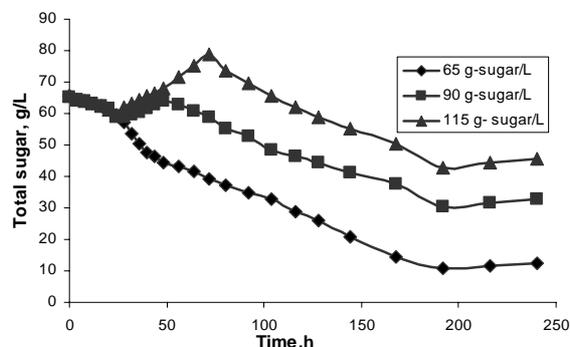


Figure 2. Sugar consumption on lactic acid fermentation of pineapple waste juice using fed batch culture with different of feed concentration. Experimental conditions: $T=40^{\circ}\text{C}$, $\text{pH}=6.0$, 5% inoculum and stirring speed=50 rpm.

Effect of Feed Concentration on Lactic Acid Productivity

Effect of feed concentration on lactic acid productivity can be shown in Figure 3.

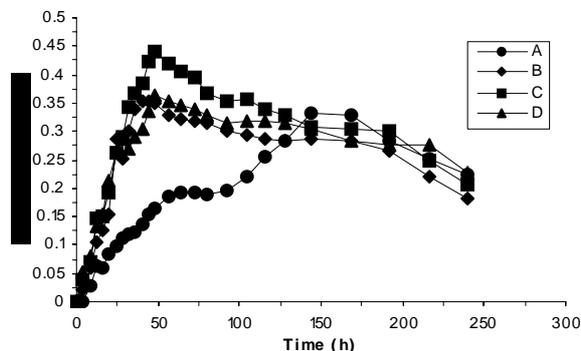


Figure 3. Volumetric productivity on lactic acid fermentation of pineapple waste using batch and fed batch culture. a) batch, b) fed batch (65 g/L), c) fed batch (90 g/L) and d) fed batch (115 g/L). Experimental conditions: $T=40^{\circ}\text{C}$, $\text{pH}=6.0$, 5% inoculum and stirring speed=50 rpm.

By increasing the feed concentration (65-90) g/L, the productivity of lactic acid also increased. However, if sugar concentration in the feed was further increased to 115 g/L, the productivity decreased due inhibition by excess sugar in the culture media. If the results obtained from 24 hours of fed-batch fermentation were compared to that of batch culture, the lactic acid productivity was twice and half fold higher. The fed-batch culture also generated higher lactic acid concentration, lactic acid yield, lactic acid productivity and biomass concentration.

Figure 3 also presents the results for the volumetric productivity of lactic acid at different substrate concentration of feedings. The maximum productivity was 0.44 g/L obtained from feeding of 90 g/L.h at 48 hour. Roukas and Kotzekidou (1998) obtained lactic acid maximum productivity of 4 g/L h with feeding rate of 97.2 g/L at 19 hour. The difference of this finding is due to substrate used in this fermentation. In this work, the substrate contained materials inhibiting the lactic acid production causing lower volumetric productivity.

CONCLUSION

The maximum productivity of lactic acid production from fed-batch of pineapple juice waste fermentation by *Lactobacillus delbrueckii* obtained was 0.44 g/L with feed concentration of 90 g/L.h at 48 hour. This result was twice and half fold higher than batch fermentation productivity.

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