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UTILIZATION OF PINEAPPLE WASTE AS CARBON SOURCE FOR LACTIC ACID FERMENTATION

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Abstract

The liquid pineapple waste contains mainly sucrose, glucose, fructose and other nutrients. It therefore can potentially be used as carbon source for organic acid fermentation. The objective of this work is to evaluate the use of pineapple waste as substrate for lactic acid fermentation under variables of aerobic, anaerobic condition and pH controlling. Initial results showed that the liquid pineapple waste can be used as carbon source for lactic acid fermentation using Lactobacillus delbrueckii. In the anaerobic condition growth of bacteria and lactic acid production better than aerobic condition. In the anaerobic condition and the controlled pH the production of lactic acid are found to be 54.79 g/l (78.27% yield) at 40^{o}C, pH 6, 50 rpm and 70 g/l sugar concentration. In contrast, only 13.87g/l lactic acid produced if the fermentation pH was not controlled even though the fermentation parameters were kept at the same conditions

Keywords: *carbon source, lactic acid, Lactobacillus delbrueckii, liquid pineapple waste*

Abstrak

Buangan nanas cair dari industri pengalengan masih mengandung sukrosa, glukosa , fruktosa dan nutrien lain, oleh karena itu sangat potensial untuk digunakan sebagai sumber karbon pada fermentasi asam organik. Tujuan dari penelitian ini adalah untuk mengevaluasi pemanfaatan buangan nanas sebagai substrat untuk fermentasi asam laktat pada kondisi aerob, anaerob dan pengendalian pH. Hasil awal menunjukkan bahwa buangan nanas cair dapat digunakan sebagai sumber karbon untuk fermentasi asam laktat menggunakan Lactobacillus delbrueckii. Pada kondisi anaerob pertumbuhan bakteri dan produksi asam laktat lebih baik dari kondisi aerob. Pada kondisi anaerob dan pengendalian pH didapatkan produksi asam laktat sebesar 54,79 g/l (78,27% yield) pada suhu 40^{o}C, pH 6, kecepatan pengadukan 50 rpm dan konsentrasi gula 70 g/l. Sebaliknya apabila pH fermentasi tidak dikendalikan, asam laktat yang dihasilkan hanya 13,87g/l walaupun parameter fermentasi dijaga pada kondisi yang sama.

Kata kunci: *sumber karbon, asam laktat, Lactobacillus delbrueckii, buangan nanas cair*

INTRODUCTION

Food processing operation uses enormous quantities of water which are consequently discharged as a polluted effluent. The waste contains high concentration of biodegradable organic material and suspended solid. As a result it has a high BOD and extreme of pH conditions (Buckle, 1989). The solid waste from pineapple canning process was estimated about 40-50% from fresh fruit as pineapple peals and cores while the waste water are generated is respectively 1.89 million litre per ton fresh fruit, 4.8 g/l BOD and 2.4 g/l suspended solid (Moon and Woodroof, 1986). If these wastes discharge to the environment in treated, they may cause a serious environment problem. Besides their pollution and hazard aspects in many cases, pineapple waste might have a potential as a raw material for higher value added products, or even as raw material for food or feed after biological treatment (Kroyer, 1991). This waste contains valuable components which are mainly sucrose, glucose, fructose and other nutrients (Abdullah, 2008). An attempt has been made by many researchers to utilise the waste for producing high value added chemicals such as Single Cell Protein (SCP), ethanol, acetic acid, oxalic acid and biomethanation process (Sasaki, *et al.*, 1991; Vimal and Adsole, 1976; Bardiya, *et al.*, 1996). Based on physico-chemical properties of the pineapple waste, it can potentially be used as carbon sources for

organic acid fermentation such as lactic acid fermentation.

Lactic acid, a normal organic acid, has long been used in the pharmaceutical, chemical, cosmetic and food industry. Recently, lactic acid has been considered as an important raw material for production of biodegradable lactide polymer (Wang, *et al.*, 1995). Lactic acid is generally produced from glucose, maltose, sucrose or lactose. Starches, especially those from corn and potatoes are hydrolysed by enzymes or acid to maltose and glucose before using for lactic acid fermentation (Atkinson and Mavituna, 1991). The most important producers of lactic acid are belonging to the family of *Lactobacillae.* The selection of organism producing lactic acid depends primarily on the type of carbohydrates as a substrate for fermentation (Buchta, 1983).

This paper will study effect of aerobic and anaerobic condition under controlled and uncontrolled pH on the lactic acid fermentation of pineapple waste using *Lactobacillus delbrueckii* ATCC 9649.

MATERIALS AND METHODS

Substrate

The substrate used to carry out the fermentation process was liquid pineapple waste juice obtained from Malaysian Cannery of Malaysia Sdn. Bhd. The liquid pineapple waste was boiled for 5 minute to remove existing enzymes and followed by filtering to remove the solid particles. It was also stored at -18^{o}C for other batches of fermentation (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^{o}C and transferred to fresh medium every month.

Inoculums Media

Each fermentation process was initiated by transferring a small amount of biomass to a 250 ml Erlenmeyer flask containing 100 ml of liquid MRS medium. The bacteria were aerobically cultivated in a cotton plugged Erlenmeyer flasks by shaking. The aerobic system was incubated at 37^{o}C and shaked at 175 rpm for 24 hour (Mercier and Yerushalmi, 1992). Anaerobic condition were maintained by flushing with nitrogen and sealing them with a tight-fitting rubber stopper (Sakamoto and Komagata, 1996).

Experimental Procedures

The fermentation was carried out in 3-litre fermentor (Biostat B Model). The fermentor was equipped with pH, temperature and dissolved oxygen controllers. The fermentor containing 900 ml substrate was first sterilised at 121^{o}C for 15 minutes. About 100 ml of inoculums was sterilised separately and added aseptically to the fermentor. The experiment was performed in batch mode under atmospheric pressure, temperature 40^{o}C, speed 250 rpm and pH 6. The fermentation was carried out at controlled and uncontrolled pH. The anaerobic system was produced by nitrogen sparges to the fermentor at 6.5 ml/minute and stirring speed at 50 rpm (Lund, *et al.*, 1992). Samples of 10-20ml were with drawn from the fermentation broth at specified 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).

Fermentation Product Analysis

Dry cell weight

Cell concentration was determined 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 was determined by centrifugation at 4000 rpm for 15 minute, washing twice with distilled water and dried at 103^{o}C for 24 hours. The dry cell was weighed using analytical balance (Aeschlimann. and.Stockar, 1987).

Organic acids

The lactic acid and other organic acid content were measured by HPLC (Waters TM 600). A 250 mm x 4.6 mm ID Spherisob Octyl column (Waters) with UV detector (210nm) was used. The eluent used was 0.2 M phosphoric acid at flow rate of 0.8 ml per minute and temperature of 25^{o}C.

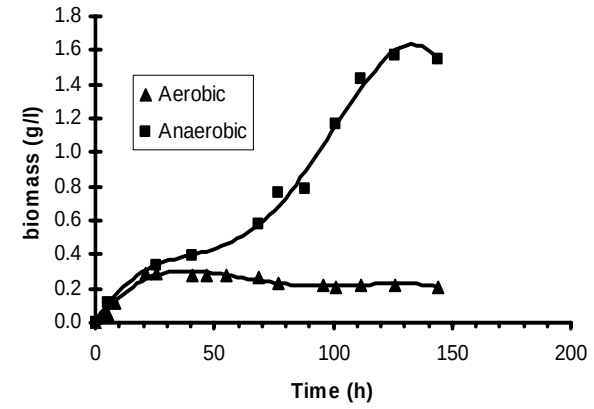
Sugars

The glucose, fructose and sucrose content were also measured by the same HPLC, using a 300 mm x 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^{o}C.

RESULTS AND DISCUSSION

Aerobic and Anaerobic Fermentation

The growth of *L. delbrueckii* in aerobic and anaerobic condition can be shown in Fig. 1. *L. delbrueckii* grows well under anaerobic than aerobic condition, After 20 hours aerobic fermentation the growth decreases because the strains are accumulated a high concentration of hydrogen peroxide in the substrate , which caused the growth to case due to its toxicity. The mechanism of oxygen toxicity is through the formation of single oxygen, super oxide radicals O_2^-, peroxide O_2^{2-} or hydroxy free radical OH^- which are destructive to many cell components (Teuber, 1993).



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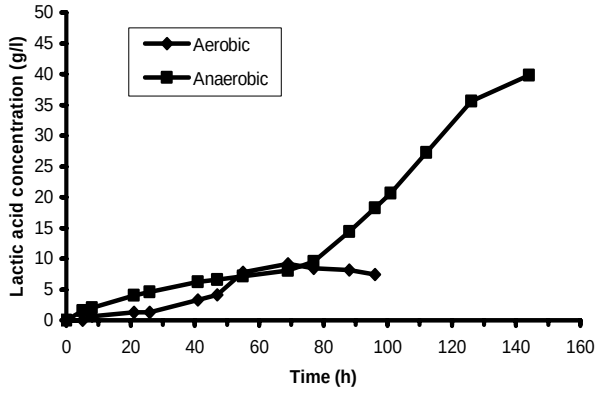

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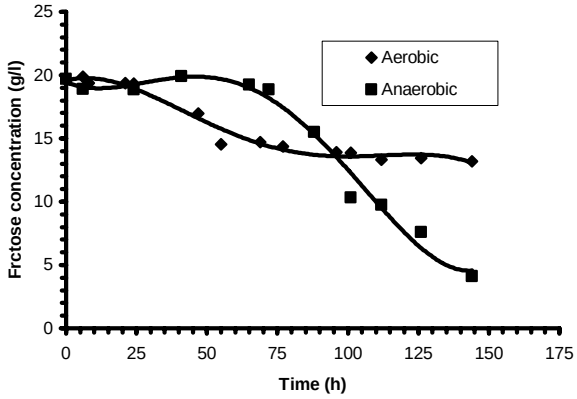
Figure 1: a) Biomass concentration and b) Lactic acid production on lactic acid fermentation (T=40^{o}C; pH=6.0; and 10% inoculums)



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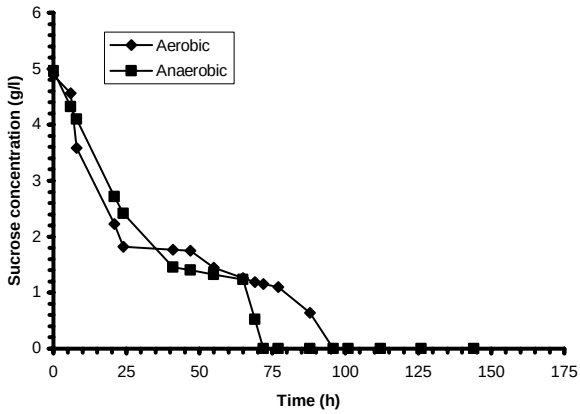
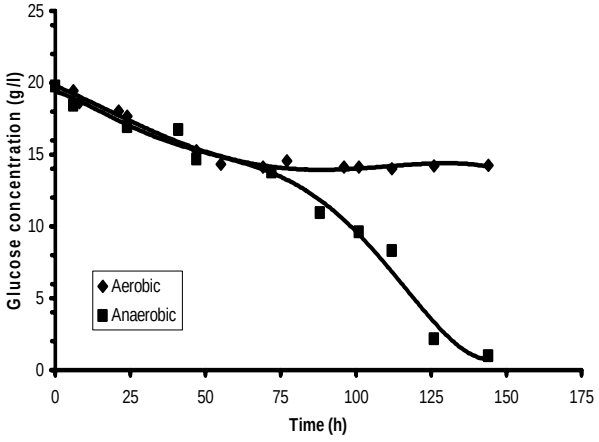


(c)

Figure 2: a) Glucose, b) fructose and c) sucrose consumption on lactic acid fermentation (T=40^{o}C; pH=6.0 and 10% inoculums)

The concentration of lactic acid increases with time, while the concentration of sugar decreases (Fig.1 and 2). Glucose consumption during lactic acid fermentation is higher than fructose. In the anaerobic system sucrose consumption is higher than the aerobic system. The concentration of glucose and fructose remains constant after 75 hours fermentation in the aerobic system and almost no lactic acid is produced.



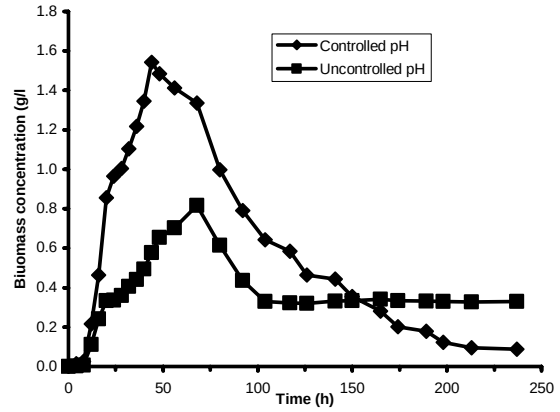
(a)

The lactic acid yield for the anaerobic system is higher than the aerobic system, for this cases the yield was about 79% and 11%. In the anaerobic process the end product of sugar metabolism is only lactate or homofermentative, but the aerobic process produce lactate and acetate or heterofermentative (Sakamoto and Komagata, 1996).

Controlled and Uncontrolled pH

The optimal growth of *lactobacillus* at pH range between pH 5.5-6.0. Fermentation is strongly inhibited at pH 5.0 and ceases at pH 4.5 (Buchta,K. 1983). In lactic acid fermentation, there is an inhibition that causes decreasing of lactic acid productivity. The effect of lactic acid inhibitor on cell growth of *L. delbrueckii* is very significant, therefore the production of lactic acid must be neutralised with alkaline (Tyagi *et al.*, 1991). The lactic acid inhibition is higher than ammonium lactate, sodium lactate or calcium lactate. Calcium carbonate can not be used because the solubility in water is limited and interfered with bacterial density measurement. Therefore sodium hydroxide is the suitable alkaline for pH control in fermentation process.

The concentration of lactic acid product depends on whether pH of the fermentation was controlled or not. If the pH was controlled, lactic acid production after 165 hours are found to be 54.97 g/l or 78.2% yield, while for uncontrolled pH the production of lactic acid is only about 13.52 g/l or 19.31% yield. If the pH was not controlled (Fig. 3), the pH dropped from 6.0 to 5.1 within 165 hours. After that there was no more lactic acid produced. This invention is a better finding as compared to literature, by using 106 g/l glucose with *Lactococcus lactis* as bacteria, the pH dropped from 5.85 to 3.2 in 24 hour and only 3.3 g/l lactate was produced or 2.1% yield (Hofvendahl and Hagerdal, 1997).



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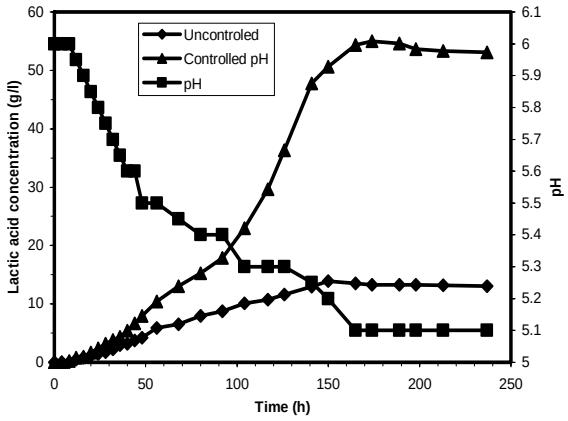

(b)

Figure 3 a) Biomass concentration and b) lactic acid Production in lactic acid fermentation with controlled and uncontrolled pH (50 rpm; 40^{o}C and 5% inoculum).

CONCLUSIONS

The chemical composition of the pineapple waste appears to be a good nutrient for cultivation of lactic acid bacteria. It can potentially be used as carbon source for lactic acid fermentation. In the anaerobic condition growth of bacteria and the lactic acid production is better than aerobic condition. The lactic acid obtained under controlled pH was 54.79 g/l, while only 13.87 g/l of lactic acid was produced when pH was not controlled.

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