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RECYCLING OF PINEAPPLE WASTE USING *LACTOBACILLUS DELBROECKII* TO LACTIC ACID

Abdullah [*)] **and H.B. Mat** [**)]

Abstract

The liquid pineapple wastes contain mainly sucrose, glucose, fructose and other nutrients. It therefore can potentially be used as carbon source for organic acid fermentation. Recently, lactic acid has been considered to be an important raw material for production of biodegradable lactate polymer. The experiments were carried out in shake flask fermentation using Lactobacillus delbrueckii. Effect of some parameters such as temperature, initial pH, initial substrate concentration, yeast extract concentration and fermentation time to the yield have been studied. The highest yield was 85.65 % achieved at 40° C, pH 6.00, 52.5 g/l sugar concentration with 5 g/l yeast extract. There was no significant increasing in lactic acid production was observed if supplementation of yeast extract above 10%

Keywords: *Lactic acid fermentation, liquid pineapple waste, Lactobacillus delbrueckii*

Introduction

World canned pineapple production in 1997 was estimated at about 954666 tones as against 800000 tonnes in 1996, a increase of almost 16%. Major world producers of canned pineapple are Thailand (39%), Philippines (23%), Indonesia (13%) and Kenya (8%) which are together contribute to more than 80% of total world canned pineapple production in 1997. Malaysia's production amounting 31265 tonnes would be equivalent to 3.3% total world production (Regensteine, *et.al.*, 1984). Food processing operation also uses enormous quantities of water, which are consequently discharge as a polluted effluent. The waste are contain high concentration of biodegradable organic material and suspended solid. As a result it has a high BOD and extremes of pH condition (Buckle, *et. al.*, 1989). The solid waste from pineapple canning process was estimated about 40 – 50% from fresh fruit as pineapple peals (Sakamoto, *et.al.*, 1996). The pineapple canning process and wastes production were describe previously (Abdullah and Mat, 1998).

If these wastes discharge to environment untreated, they could cause serious environment problems. Beside their pollution and hazard aspects in many cases, food processing waste such as pineapple waste might have a potential for recycling to get raw material or for conversion into useful product of higher value added products, or even as raw material for other industries, or for use as food or feed after biological treatment (Lazaro, 1989). This waste contains valuable components, which are mainly sucrose, glucose, fructose and other nutrients (Vimal, *et. al.*, 1976, Abdullah, and Mat, 1998). An attempt has been made by many researchers to utilize the waste for producing high value added chemicals such as Single Cell Protein (SCP), ethanol, acetic acid, oxalic acid and biomethanation process (Vimal, *et. al.*, 1976; Winarno, 1984; 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 was chosen as the present product, it is one of the most important organic acids is widely used in the food of industries, it exhibits preservative properties such as inhibiting microbial spoilage in meats sea foods, mayonnaise, salad dressing and soft drink. Emulsifiers derivatives from lactic acid improve the quality of breads, cake mixes, filling and topping, powdered coffer, shortening and whiteners. This organic acid functions as flavor enhancer in beer, wine, cider, soft drink, candies, frozen dessert, jellies, margarine and pickles (Oda, *et. al.* 1997). Recently, lactic acid has been considered to be important raw material for production of biodegradable lactic polymer (Clesceri, *et. al.* 1989). Lactic acid is generally, produced from glucose, maltose, sucrose or lactose. Straches, 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).

*) Dept. of Chemical Engineering, Diponegoro University
Jl. Prof. Sudharto, SH., Tembalang, Semarang, 50239 Telp. (024) 7460058
**) Faculty of Chemical and Natural Resources, University Teknologi Malaysia,
Skudai, Johor Bahru, Malaysia, 81310

The most important producers of lactic acid are belong to the family of *Lactobacillae*. The selection of organism producing lactic acid depends primarily on the type of carbohydrates to be fermented (Buchta, 1983). The present paper report the feasibility of lactic acid production from liquid pineapple waste as a substrate using *Lactobacillus delbrueckii*.

Materials

The substrate used to carry out the fermentation process was liquid and solid pineapple waste obtained from Malaysian Cannery of Malaysia Ltd. Co. The pretreatment of substrate was described by Lazaro (Mercier and Yerushalmi, 1992). The micro-organism used in this study was *Lactobacillus delbrueckii supsp. Delbrueckii ATCC* 9649 obtained from DSMZ, Germany. The strain was maintained on MRS agar at 4°C and transferred to fresh medium every month.

The preparation of inoculum media starts with transferring of the lyophilized culture (Freeze dried) to aliquid MRS medium. The composition of 1 liter MRS medium are as follows: yeast extract 5g; meat extract 5g; peptone 10g; K_2HPO_4 2g; diammonium citrate 5g; glucose 20g; sodium acetate 2g; $MgSO_4.7H_2O$ 0.58g; $MnSO_4.4H_2O$ 0.25g and 1 ml of Tween-80. The culture was transferred to solid MRS medium after a visible growth of microorganism was observed which normally takes one day. The plates were incubated at 37°C for 24 hours in order to allow sufficient growth of colonies. The grown colonies were either used to initiate the fermentation process or stored at 4°C for later used. Each fermentation process was initiated by transferring a small amount of biomass to a 250ml Erlenmeyer flask containing 50ml of liquid MRS medium (Moon and Woodroof, 1986). Anaerobic condition were produced by flusing with nitrogen and sealing with thigh-fitting rubber stopper (Vahvaselha and Linko, 1987).

Methods Of Fermentation Experiment

Shake flask fermentation

The shake flask fermentation was conducted in a temperature controller shaker. The shake flasks were performed by transferring 5 ml of inoculums to a 250 ml Erlenmeyer flask containing 95 ml of substrate by adding $CaCO_3$ (3% w/v) for pH control in the shake flask fermentation (Wang, *et. al.* 1995; Freeman, 1985).

Batch fermentation

The fermentation was carried out in 3-liter fermentor (Biostat B model). The fermentor was equipped with pH, temperature and dissolved oxygen controllers. The fermentor containing 950 ml substrate was first sterilized at 121°C for 15 minutes. 50 ml of inoculum was sterilized separately and added aseptically to the fermentor. Anaerobic system were produced by sparged the fermentor by nitrogen 6.5 ml/minute and speed at 50 rpm. Samples of 10-20 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 concentration (Moon and Woodroof, 1986).

Chemical analysis

The organic acid content was measured by HPLC (waters TM 600). A 250-mm x 4,6-mm ID Spherisob Octyl column (Waters) with UV detector (210 nm) was used. The eluent used was 0.2 M Phosporic acid at flow rate 0.8 ml per minute and temperature 25°C. The sugar content was measured by the same HPLC, using a 300 mm x 4 mm ID Bondapak/Carbohydrate column (Waters) with RI detector. The eluent used was a mixture acetonitrile : water (80 : 20) at flow rate 2 ml per minute and temperature 25°C.

Results and Discussion

Effect of initial pH to the end product

The product of lactic acid fermentation was studied at four different initial pH values of 6.0, 6.5, 7.0 and 7.5. The result of these fermentation also used to express effect of adding $CaCO_3$ (3% w/v) for pH control in the shake flask. The effect of initial pH on the yield of lactic acid can be shown in Table 1.

Table 1. Effect of Initial pH at the end result of fermentation, Experiment condition: Substrate 70 g/l; T: 40°C; Yeast Extract: 0.5% and 5% Inoculum

Initial pH	Lactic Acid (g/l)	Yield (%)	Glucose Left (g/l)	Fructose Left (g/l)	Last pH
6.0	55.36	79.80	3.26	7.70	6.02
6.5	46.23	66.04	4.68	15.52	6.05
7.0	44.20	63.10	5.43	17.40	6.15
7.5	36.25	51.78	8.81	18.84	6.40

However, the last pH value of initial pH 6.0 and 6.5 are similar but the yield is different. It might be due the microbial growth at initial pH 6 better than 6.5 or concentration of bacteria more higher so the lactic acid production at initial pH 6 higher than pH 6.5 (55.36 and 46.23 g/l). With increasing initial pH 7.0 to 7.5 during fermentation the last pH increase from 6.15 and 6.4 these indicates that was effect of addition of Sodium Hydroxide to adjust the initial pH. The process of neutralization can be expressed by reaction of alkali in the substrate with lactic acid

production by decreasing pH and further more the Calcium Carbonate maintain the pH was constant. Thus, Calcium Carbonate was effective in controlling pH value at 6.0.

Effect of substrate concentration

In order to determine the effect of sugar concentration on the final concentration of lactic acid produced, dilute pineapple waste containing 23.3, 35, 52.5 and 70 g/l of sugar were used. Fermentation was performed in shake flask at 40°C, pH 6.0, 5% yeast extract, 150 rpm and inoculum 5%. For control the pH, the substrate must be added Calcium Carbonate 3% (w/v). After 144 hours, the end of pH with different concentration of sugar were constant and this indicates that by adding Calcium Carbonate 3% to the substrate could control the pH at 6.0 in fermentation process. The result can be seen in Table 2.

Table 2. Effect of Initial Substrate Concentration at the end result of fermentation. Experiment condition: T: 40°C; Yeast Extract: 0.5%; pH: 6.0 and 5% Inoculum

Initial Sugar (g/l)	Lactic Acid (g/l)	Yield (%)	Glucose Left (g/l)	Fructose Left (g/l)
70.00	55.36	79.80	3.26	7.70
52.50	44.97	85.65	2.30	3.88
35.00	28.94	82.60	0.68	5.83
23.33	19.19	82.25	0.00	2.02

The yield increased with increasing of initial sugar concentration, and the yield value was 82.25, 82.6, 85.65 and 79.8%. When the initial sugar concentration exceeded 52.5 g/l, yield values decreased due to inhibition produced by high sugar concentration, a characteristic of a batch culture. The glucose utilisation better than fructose, but both the sugar were not completely utilized exception for level initial sugar concentration 23.3. g/l. The biomass concentrations were not measured because Calcium Carbonate in shake flask fermentation was not completely soluble in substrate, so interfered with bacterial density measurement.

Effect of temperature

Lactic acid bacteria such are classified as thermofilic or meshopilic. *L. delbrueckii* is meshopilic bacteria which grow at 17-50°C and have optima between 20 to 40°C (4). The temperature was studied at 30, 35, 40, 45 and 50°C using 70 g/l of sugar concentration, 5% yeast extract at pH 6.0. The effect of temperature to the yield can be seen in Table 3. The yield increased with each increase at temperature level of fermentation (30 to 40°C). The lactic acid production decrease above temperature 45°C, it might be due at this temperature the growth was not optima, therefore the yield become smaller and the highest yield at 79.8% was achieved at 40°C.

Table 3. Effect of Temperature at the end result of fermentation. Experiment condition: substrate: 70 g/l; Yeast Extract: 0.5%; pH: 6.0 and 5% Inoculum

Temperature °C	Lactic Acid (g/l)	Yield (%)	Glucose Left (g/l)	Fructose Left (g/l)
30	14.03	20.04	20.18	31.47
35	42.90	50.47	9.35	16.43
40	55.36	79.08	3.26	7.70
45	53.06	75.80	5.11	9.61
50	32.71	46.71	13.64	21.89

Effect of supplementation substrate with different concentration of yeast extract

Among the different Nitrogen source supplemented to the substrate which having the same elemental Nitrogen level, yeast extract was the best (8, 25, 29). Yeast extract with different amount was added to pineapple waste to obtain the final concentration 5,10, 15, 20 and 25 g/l. All experiment initial sugar level was kept at 70 g/l. The effect of different concentration yeast extract to production of lactic acid can be seen in Table 4.

Table 4. Effect of Yeast Extract Concentration at the end result of fermentation. Experiment condition: T: 40°C; substrate: 70 g/l; pH: 6.0 and 5% Inoculum

Yeast Extract (g/l)	Lactic Acid (g/l)	Yield (%)	Glucose Left (g/l)	Fructose Left (g/l)
5	55.36	79.08	3.26	7.70
10	56.90	81.29	2.18	7.34
15	57.49	82.14	2.04	7.66
20	57.53	82.19	2.46	7.28
25	57.75	82.35	1.89	6.79

When pineapple waste was supplemented with 5 g/l of yeast extract the production of lactic acid is 55.36 g/l, with incresing concentration yeast extract 10 g/l, the lactic acid production was increased 56.0 g/l or yield increased from 79.08% to 81.29%. No effect after addition concentration of yeast extract above 15% and the highest yield was achieved if the substrate supplemented 15 g/l of yeast extract as Nitrogen source. However the high cost of yeast extract has a negative impact on the economics of its use in industrial scale. Similar with all Researcher have reported that the highest production of lactic acid were found with addition of 5-15 g/l of yeast extract (12,30). However the high cost of yeast extract has a negative impact on the economics of its use in industrial scale.

Effect of fermentation time

The fermentation was carried out in 3-liter fermentor (Biostat B model). The fermentation time was studied at 1 to 10 days using 70 g/l of sugar concentration, 5% yeast extract at pH 6.0. Effect of the time of fermentation to lactic acid production can be seen in Figure 5. During fermentation lactic acid production acid increases with time, while the concentration of sugar decrease. After 72 hours, the yield increased rapidly and then constant at 168 hours (7 days). The Glucose consumption during lactic acid fermentation is higher than fructose, but the sucrose consumption is higher than their both. After 168 hours the concentration of glucose and fructose is constant and no production of lactic acid. The lactic acid yield this cases was found to about 79%.

Table 5. Effect of Fermentation Time at the end result of fermentation. Experiment condition: Substrate 70 g/l; T: 40°C; Yeast Extract: 0.5%; pH: 6.0 and 5% Inoculum

Time (hours)	Lactic Acid (g/l)	Yield (%)	Glucose Left (g/l)	Fructose Left (g/l)	Sucrose Left (g/l)
0	0.00	0.00	19.56	20.97	15.53
24	0.95	1.35	25.61	24.03	5.10
48	2.95	4.21	29.17	26.87	2.24
72	6.15	8.79	30.13	27.83	0.00
96	17.86	25.51	29.44	26.54	0.00
120	29.63	42.33	17.55	16.29	0.00

Conclusion

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. The highest yield was 85.65% achieved at 40°C, pH 6.00, 52.5 g/l sugar concentration with 5 g/l yeast extract.

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