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

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## ABSTRACT

**Abdullah,** in paper recycling of pineapple waste using *lactobacillus delbroeckii* to lactic acid, explain that The pineapple wastes juice contains 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 $^{o}$ 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

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 conditions (Buckle, 1989). . The solid waste from pineapple canning process was estimated about 40 - 50 % from fresh fruit as pineapple peals and cores. If these wastes discharge to the environment intreated they could cause a 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 (Kroyer, 1991). This waste contains valuable components which are mainly sucrose, glucose, fructose and other nutrients (Busairi and Mat, 1998; Sasaki et al.1991). 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; Bardiya et al.,1996; Vimal and Adsole, 1976). 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. Recently, lactic acid has been considered to be an important raw material for production of biodegradable lactide polymer (Goksungur and Guvenc,1997).

The objective of this study was to examine the potential of pineapple waste juice as a carbon source for lactic acid production using *Lactobacillus delbrueckii*

## MATERIALS AND METHODS

### Substrate

The substrate used to carry out the fermentation process was pineapple waste juice obtained from Malaysian Cannery of Malaysia Sdn. Bhd. The pretreatment of substrate was described by Lazaro (Lazaro, 1989). .

### Strain

The micro-organism 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.

### Inoculum Media

The culture was transferred to liquid MRS medium and than incubated in incubator shaker at 37$^{o}$C, 150 rpm for 24 hours (Goksungur and Guvenc,1997; Sakamoto and Komagata, 1996).

### Fermentation Experiment

The shake flask fermentation were conducted in a temperature controller shaker. The shake flasks were performed by transferring 5 ml of Inocolum 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 (Mercier and Yerushalmi, 1992; Vahvaselha, and Linko, 1987)

### 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) were used. The eluent used was 0.2 M phosphoric acid at flow rate 0.8 ml per minute and temperature 25 $^{o}$C. The sugar content was 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 acetonitrile : water (80:20) at flow rate of 2 ml per minute and temperature 25 $^{o}$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. However the last pH value of initial pH 6.0 and 6.5 is 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 44.97 g/l). With increasing initial pH 7.0 to 7.5 during fermentation the last pH increase from 6.15 to 6.4 and lactic acid production decreased from 44.20 to 36.25 g/l. This indicated that the adding $CaCO_3$ (3% w/v) was effective for control pH value about 6.0.

Table 1. Effect of Initial pH at The end Result of Fermentation

| 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 |

### 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 70g/l of sugar were used. Fermentation was performed in shake flask at 40$^{o}$C, pH 6.0, 5% yeast extract, 150 rpm and Inocolum 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 6.00 in fermentation process. The result can be seen in Table 2.

Table 2. Effect of Substrate Concentration at The end Result of Fermentation

| 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 were 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 utilised 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 thermophilic or meshopilic. *L. delbrueckii* is meshopilic bacteria which grow at 17-50 $^{o}$C and have optima growth between 20 to 40 $^{o}$C (Buchta, 1983). The temperature was studied at 30, 35 , 40, 45, and 50 $^{o}$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 $^{o}$C). The lactic acid production decrease above temperature 45 $^{o}$C, it might be due at this temperature the growth not optima therefore the yield become smaller, and the highest yield at 79.8 %, was achieve at 40 $^{o}$C.

Table 3. Effect of Temperature at The end Result of Fermentation

| Temperature ($^{o}$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 |

Among the different nitrogen sources supplemented to the substrate which having the same elemental nitrogen level, yeast extract was the

best (Hujanen and Linko, 1996; Arasaratnam,et al., 1996).

Yeast extract with different amount was added to pineapple waste juice to obtain the final concentration: 5, 10, 15, 20, and 25 g/l. All experiment the 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 Addition of Yeast Extract at The end Result of Fermentation

| 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 increasing concentration yeast extract 10 g/l, the lactic acid production was increased 81.29 g/l or yield 79 % to 81 %. 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 (Table 4). 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 (Hujanen and Linko, 1996); Arasaratnam,et al.,1996; Lund et al., 1992).

However the high cost of yeast extract has a negative impact on the economics of its use in industrial scale.

## 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. The highest yield was 85.65 % achieved at 40 $^{o}$ C, pH of 6.00, 52.5 g/l sugar concentration with 5 g/l yeast extract.

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