EFFECT OF NITROGEN SOURCE AND INITIAL SUGAR CONCENTRATION ON LACTIC ACID FERMENTATION OF PINEAPPLE WASTE USING *L.DELBRUECKII*

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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 lactic acid fermentation. The lactic acid is utilised in food technology as pH regulator, microbial preservative, buffering agent and in the chemical industry. Recently, lactic acid has been considered to be an important raw material for production of biodegradable lactate polymer. The experiments were carried out in batch fermentation at anaerobic condition with stirring speed: 50 rpm, temperature: 40 ^oC, pH: 6.0, and inoculum size: 5%. Effect of nitrogen source and initial sugar concentration were studied. The effect of nitrogen source on lactic acid production shows that the yeast extract is highest yield , followed by urea , corn steep liquor, malt sprout and ammonium sulphates with the yield of 78.52; 26.68; 19.14; 14.10 and 5.6 %, respectively. The highest yield of initial sugar concentration on lactic acid production obtained was 78.52 % (54.97 g/l) at 70 g/l, if the concentration of sugar was increased to 110 g/l , the lactic acid production or yield decrease to51.53 g/l or 54.24%.

Key words: lactic acid, l. delbrueckii, nitrogen source , pineapple waste, sugar concentration.

Introduction

Pineapple canning industries that are located in tropical region such as Malaysia, Thailand and Indonesia producing large quantity of solid and liquid waste. In the canneries nearly 75% of the fruit in the form of peeled skin, core, crown end, etc. is no utilised and is discharged as wastage involving problems of disposal and pollution. The dry matter content of pineapple waste is around 10% and constitutes about 96% organic matter and 4 % inorganic matter (Chandapillai and Sel-varajah, 1978). 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). Beside their pollution and hazard aspects in many cases, food processing waste such as pineapple waste might have a potential for recy-cling 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 (15.5 g/l), glucose (28 g/l), fructose (27.5 g/l), and soluble protein (1.1 g/l). The chemical composition of the pineapple waste appears to be a good nutrient for cultivation of lactic acid bacteria (Abdullah and Hanafi Mat, 2008). Lactic acid, a normal organic acid, has long been of use in the pharmaceutical, chemical, cosmetic and food industry. Recently, lactic acid has been considered to be 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 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).

This paper will study the effect of nitrogen source and initial sugar concentration on the lactic acid fermentation of pineapple waste using *Lactobacillus delbrueckii* ATCC 9649.

Materials and Methods

Materials

Substrate

The fermentation media contained liquid pineapple waste 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 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

Each fermentation process was initiated by transferring a small amount of biomass to a 250ml Erlenmeyer flask containing 50ml of liquid MRS medium. Anaerobic condition were produced by flushing with nitrogen and sealing them with thigh-fitting rubber stopper . The inoculum was shaken in incubator shaker at 37 ^{o}C, 150 rpm for 24 hours (Sakamoto and Komagata, 1996).

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Methods

Batch Fermentation

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 950 ml substrate was first sterilised at 121^{o}C for 15 minutes. 50 ml of Inocolum was sterilised separately and added aseptically to the fermentor. Anaerobic system were produced by sparged the fermentor by nitrogen 6.5 ml/minute and stirring speed at 50 rpm (Lund *et al*., 1992). Samples of 10-20ml 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).

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 250mm x 4.6mm ID Spherisob Octyl column (Waters) with UV detector (210nm) were 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.

Results And Discussion

Effect of Nitrogen Source

There are 5 nitrogen source were chosen for test fermentation's on the basis of their nitrogen quantity and cost. To improve the economic parameters of lactic acid fermentation comparable nitrogen source were substituted with 5g/l yeast extract (11% nitrogen) or 0.55 gr nitrogen g/l in the medium. If the nitrogen content in of corn steep liquor (3.5 %) malt sprout (4.0%), ammonium sulphate (21.0 %) and urea 46.7, to give the equivalent nitrogen of 0.55 g/l in substrate the amount of each nitrogen source which added per litre were: corn steep liquor (15.71 g) , malt sprout (13.75 g), ammonium sulphate (2.61 g) and urea 1.17 g.

Effect of nitrogen source to lactic acid production can be seen in Fig. 1a , yeast extract is the highest lactic acid production followed by urea , corn steep liquor, malt sprout and ammonium sulphates with the yield of 78.52, 26.68, 19.14, 14.10 and 5.6 % respectively. The maximum lactic acid production using yeast extract at 168 hour or volumetric productivity of 0.327 g/l h. The bacterial growth using the yeast extract is faster than other, the lag phase at 4 hour, corn steep liquor, malt sprout, ammonium sulphate and urea have lag phase at 12 hour (Fig. 1b). The yeast extract is the best of nitrogen source for growth of *L. delbrueckii*, this according to all published reports previously. The yeast extract supplementation not only increased the bacterial yield , but reduce the time required for the completion of fermentation. This could be due to the substances as amino acid, peptides, vitamins, and several organic acids including pyruvic and glyseric acid in yeast extract.

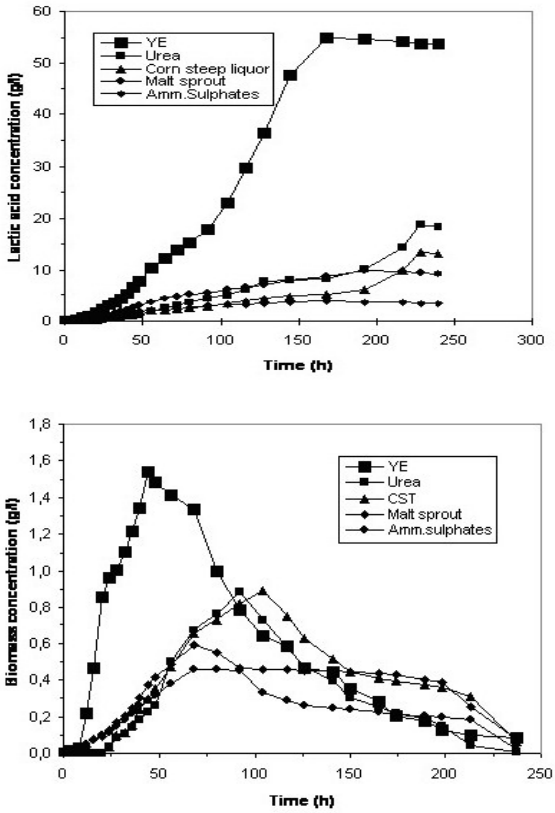

Figure 1 :

a) Lactic acid production and
b) bacterial growth on lactic acid fermentation of pineapple waste with different nitrogen source. Experimental conditions: T : 40 ^{o}C, pH: 6.0, inoculum: 5% and agitation rate: 50 rpm.

Effect of Initial Sugar Concentration.

The effect of initial sugar concentration on cell growth and product formation can be seen in Figure 2. The initial sugar concentration varying between 70 g/l and 150 g/l addition of pure sugar to liquid pineapple waste with proportional quantity according to composition of sugar at original pineapple waste. The highest lactic acid production and yield was obtained 54.97 g/l lactic acid and yield value was 78.52 % at 70 g/l sugar concentration, if the concentration of sugar was increased to 110 g/l , the lactic acid production or yield decrease to51.53 g/l or 54.24%. When initial sugar concentration was in-

creased to 150 g/l , lactic acid production or yield decreased rapidly and were obtained 19.92 g/l or 14.75 % (Fig. 1a). The decreasing of yield value might be due to inhibition produced by high sugar concentration, this characteristic of a batch culture. The effect of initial sugar to bacterial growth are illustrated at Fig. 1b. If the concentration of sugar were increased give increasing of lag phase and a decreasing specific growth rate. Although the cell concentration increases with increasing of initial sugar concentration but the yield of lactic acids decrease, this indicates that any inhibition effect of initial sugar to lactic acid production effect.

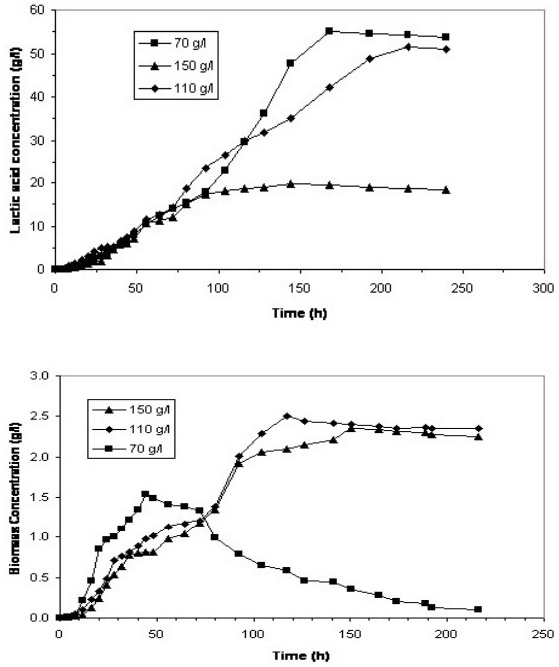

Figure 2:
a) Lactic acid production and
b) bacterial growth on lactic acid fermentation of pineapple waste with different initial sugar concentration. Experimental conditions: T: 40 ^{o}C, pH: 6.0, inoculum : 5% and stirring speed: 50 rpm.

Conclusions

The effect of nitrogen source on lactic acid production shows that the yeast extract is highest yield , followed by urea , corn steep liquor, malt sprout and ammonium sulphates with the yield of 78.52; 26.68; 19.14; 14.10 and 5.6 %, respectively. The yeast extract is the best of nitrogen source for growth of *L. delbrueckii*. The yeast extract supplementation not only increased the bacterial yield , but reduce the time required for the completion of fermentation. The highest yield of initial sugar concentration on lactic acid production obtained was 78.52 % (54.97 g/l) at 70 g/l initial sugar concentration, if the concentration of sugar was increased to 110 g/l , the lactic acid production or yield decrease to51.53 g/l or 54.24%.

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