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UTILIZATION OF ONGGOK TAPIOCA FOR LACTIC ACID PRODUCTION BY *STREPTOCOCCUS BOVIS*

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ABSTRACT

Onggok tapioca is a low value byproduct from tapioca industry. It provides a cheap source of starch and has potential for being applied as substrate in fermentations to produce lactic acid. Lactic acid production from onggok tapioca by *Streptococcus bovis* was investigated in order to reduce the manufacturing cost of lactic acid. Lactic acid yields were inversely proportional to initial onggok tapioca concentration within the experimental area (30-100 g/L). The highest lactic acid productivity in batch fermentation, 1.70 g/L.h was obtained with 50 g/L onggok tapioca. Lactic acid fermentation using onggok tapioca was significantly affected by yeast extract concentration. The maximum lactic acid productivity was obtained 2.13 g/L.h at 20 g/L of yeast extract.

Keywords: onggok tapioca, lactic acid, *Streptococcus bovis* and fermentation.

INTRODUCTION

Lactic acid is a valuable raw material for numerous industrial processes (Bozoglu and Ray, 1996), for example, it is used as feedstock for chemical and biotechnological production of other organic acids; flavoring and acidulant in the food industry; an intermediate product in the manufacture of cosmetics; and used in the plastics industry as biodegradable polymer (Kharas *et al*., 1994; Lipinsky and Sinclare, 1986). Lactic acid has been produced commercially either by chemical synthesis or fermentation. However, the production of lactic acid through biotechnological fermentation has recently gained a great interest due to the environmental pollution caused by petrochemical industries and the depletion of petrochemical resources. Furthermore, fermentation has the advantage of able to produce optical pure lactic acid. In the technological synthesis of poly lactic acid, the lactic acid is mostly used, because it gives crystalline poly lactic acid.

A number of different substrates, so far, have been used for the production of lactic acid, such as wheat (Shamala and Sreekantiah, 1988), corn (Hoshino *et al*., 1991), potato (Ray *et al*., 1991), sorghum (Richter and Trager, 1994), barley (Childs and Welsby, 1977), and glucose (Dermici and Pometto, 1995), however, in recent years, there has been an increasing trend towards more efficient utilization of agro-industrial residues such as molasses (Wee *et al*., 2004; Dumbrepatil *et al*., 2008), corncobs (Hang, and Woodams, 1998), and sugar beet pulp (Pandy *et al*., 1998). Applications of agro-industrial residue in bioprocesses on the one hand provide alternative substrate, and on the other hand help in solving pollution problems, which their disposal may otherwise cause.

Lampung province, Indonesia, is a large producer of cassava root. The production in 2003 was estimated to be 2.1 million tons. Onggok tapioca is a solid residue from the starch extraction process, which is generated during the separation stage. Thus, there is an urgent need to find suitable applications and disposal of this waste. One alternative to its economic utilization is to use it as substrate in fermentation processes for the production of lactic acid. The objective of the present work is to study the effect of various fermentation parameters, i.e. onggok tapioca concentration, and nitrogen source concentration on lactic acid fermentation from onggok tapioca.

METERIALS AND METHODS

Seed Culture

Streptococcus bovis JCM 5802 was obtained from Institute of Physical and Chemical Research (RIKEN Japan). *Streptococcus bovis* JCM 5802 is a facultative anaerobic and homofermentative bacteria producing mainly L-lactic acid. The strain was stored in deMan, Rogosa and Sharpe (MRS) broth with skim milk at –80 ^{o}C. The medium composition was as follows (g l^{-1}): peptone, 10; meat extract, 10; yeast extract, 5; glucose, 20; K_2HPO_4, 2; sodium acetate, 5; diamonium citrate, 2; $MgSO_4$.7H2, 0.1; $MnSO_4.H_20$, 0.05; Tween 80 (poly sorbit-80). In preparation for each experiment, a stock culture was inoculated into 5 ml MRS broth incubated for 18 h on a shaking water bath maintained at 37^{o}C.

Medium Preparation

Onggok tapioca was used as substrate, while trypto soya broth was used as the basic medium. The basic medium (trypto soya borth) consisted of the following (per liter of

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distilled water): 17 g peptone, 3 g soybean peptone, 2.5 g glucose, 2.5 g K_2HPO_4, 2.5 g KH_2PO_4, 5 g NaCl. The basic medium and substrate solution were then sterilized by autoclaving at 121°C for 15 min.

Fermentation

Batch culture was carried out at 37°C in a membrane bioreactor with volume total of 500 mL, the working volume used for experiment in this vessel was 300 mL. At the bottom of bioreactor vessel, a 2 μm micro filtration membrane sheet whit 76 mm diameter (Advantec, Toyo Roshi Kaisha) was incorporated. Before fermentations, the bioreactor was sterilized. Temperature of fermentation was kept constant at 37°C while pH of fermentation was maintained at 5.00 by controlling with the addition of 6 M NH_4OH.

Analytical Methods

Lactic acid and glucose were determined by Biosensor (Bio Flow BF4, Oji Scientific Instruments Ltd). The biosensor is an analytical device of flow injection method (Bio Flow) using enzyme column and hydrogen peroxide. Two columns with different enzymes were used for the measurements. One column was packed with lactic acid dehydrogenase to measure the lactic acid concentration, while the other one was packed with glucose oxide to measure the glucose concentration. High-performance liquid chromatography (HPLC) was employed to analyze organic compounds, including succinic acid, present in the fermentation broth. The HPLC system (Tosoh UV-8010) was equipped with UV detector 210 nm. The eluent was $NH_4H_2PO_4$ + H_3PO_4 (pH 2.5) at flow rate of 1 ml min^{-1}. Cell growth during fermentation was determined aseptically by sampling an aliquot of the cultures. Viable count samples were taken regularly, plated on a BCP agar medium and incubated at 39°C for 48 h. After 48 h incubation the colonies were counted by colony counting method in Colony Forming Unit (CFU/mL).

RESULTS AND DISCUSSION

Influence of Onggok Tapioca Concentration

In an attempt to evaluate the influence of onggok tapioca concentration on lactic acid fermentation, the medium containing 30, 50, 70 and 100 g/L onggok tapioca was used (Fig. 1), the final lactic acid concentration increases with the increase of initial onggok tapioca concentration. The maximum amount of lactic acid and the productivity were obtained at 42.5 g/L and 1.70 g/L.h, repectively after 96 h of fermentation at 50 g/L of onggok tapioca. However, lactic acid production rates decreased when the initial onggok tapioca concentrations exceeded 70 g/L. This could be probably due to substrate inhibition a traditional property of batch fermentations.

Table 1 shows the fermentation parameters of experiments at various concentrations of onggok tapioca, i.e. specific growth rate and productivity within fermentation time of 98 h. The specific growth rate reached the maximum level at 50 g/L onggok tapioca. When the initial onggok concentration exceeded 70 g/L, the specific growth rate also decreased significantly due to inhibition by high substrate concentration. The maximum specific growth rate was obtained at 0.65 h^{-1} at onggok tapioca concentration 50 g/L.

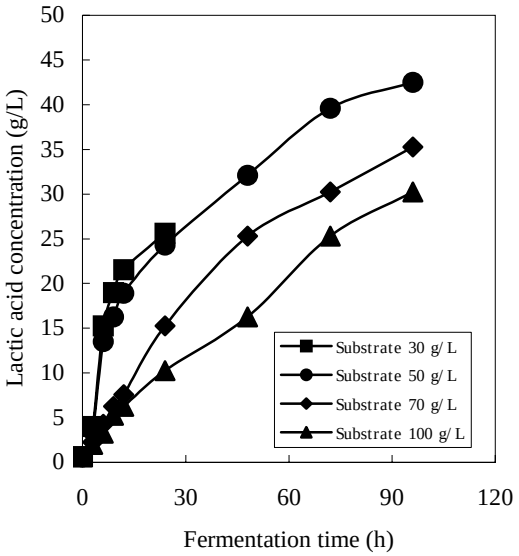

Fig. 1. Lactic acid production in lactic acid fermentation with different concentrations of initial onggok tapioca by *Streptococcus bovis.*

Table 1. Effect of initial onggok tapioca concentration on specific growth rate and productivity in lactic acid fermentation by *Streptococcus bovis.*

Onggok tapioca (g/L)	Specific growth rate (h^{-1})	Productivity (g/L.h)
30	0.62	1.65
50	0.65	1.70
70	0.60	0.51
100	0.59	0.31

Influence of Yeast Extract Concentration

Lactic acid bacteria are generally fastidious organisms, which require complex nutrients such as amino acids and vitamins for their cell growth (Oh *et al*., 2003). Yeast extract, the most commonly used nitrogen source, provides complex nutrients for lactic acid bacteria (Aksu and Kutsal, 1986). Yeast extract was added in different concentrations to study the effect on growth and lactic acid formation. Figure 2a and figure 2b show the effect of yeast extract on cell growth and lactic acid production. As can bee seen in figure 2, the final lactic acid concentration and the growth of cells increase with the increase of yeast extract. Exponential growth can be observed when yeast extract is added remain almost on the level of inoculums.

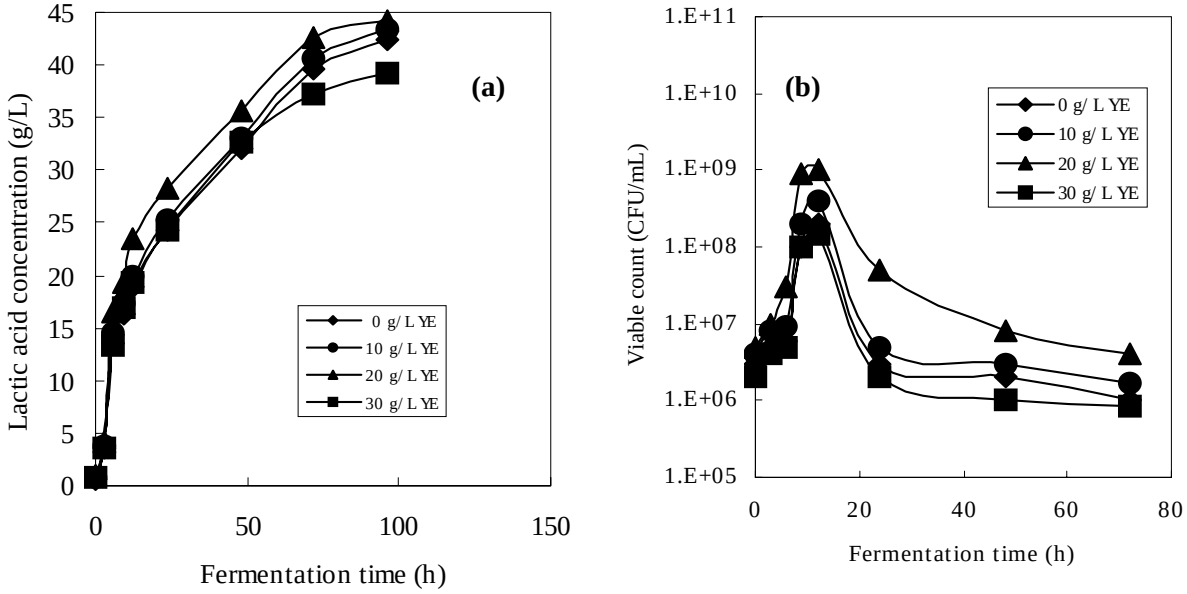

Fig. 2. Effect of yeast extract (YE) addition on (a) lactic acid production; (b) cell concentration.

Table 2. Effect of yeast extract concentrations on lactic acid produced, lactic acid yield, specific growth rate and productivity in lactic acid fermentation from onggok tapioca by *Streptococcus bovis.*

Yeast extract (g/L)	Lactic acid (g/L)	Yield (%)	Specific growth rate (h^{-1})	Productivity (g/L.h)
0	42.50	91.00	0.59	1.70
10	43.50	93.14	0.64	1.79
20	44.25	94.75	0.70	2.13
30	39.25	84.04	0.51	1.55

Table 2 summarizes the fermentation parameter of experiments at various of yeast extract, such as lactic acid produced, lactic acid yields, and productivities. The highest yield (94.75%) was found at 20g/L of yeast extract. Yuwono and Kokugan, (2008) who investigated the effect of yeast extract on lactic acid fermentation from cassava root, also reported that lactic acid fermentation was considerably simulated by increase of yeast extract.

CONCLUSIONS

Onggok tapioca can be used as an effective substrate for *Streptococcus bovis* to produce lactic acid. Through the optimization of the fermentation conditions, a rate of lactic acid production was obtained which accomplished complete utilization of the onggok tapioca within a fermentation time of 98 h. Lactic acid productivity and cell growth in onggok tapioca fermentation were severely effected by yeast extract concentrations added to the medium. Supplementation with yeast extract at a level of 20 g/L was found to be optimum to exploit the contribution of cell growth to lactic acid production.

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