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Batch and Continuous Lactic Acid Production from Cassava by *Streptococcus bovis*

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Abstract. Process variables were optimized for the production of lactic acid from cassava by Streptococcus bovis for batch and continuous fermentations. In the batch fermentation, maximum yield 82.5% and maximum lactic acid productivity 2.43 was achieved at 39 ^{o}C, pH 5.5 with 50 g/l cassava concentration. In the continuous fermentation maximum productivity lactic acid 1.25 g/l.h was obtained at dilution rate 0.05 h $^{-1}$. Copyright © 2006 Teknik Kimia UNSYIAH

Keywords: fermentation lactic acid, cassava, streptococcus bovis

INTRODUCTION

Lactic acid is a natural organic with long history in food, pharmaceutical, chemical and personal care industries [Beninnga, 1990]. Polymerization of lactic acid will produce poly lactic acid (PLA), a biodegradable plastic primarily used as surgical implants, drug delivery system and artificial scaffold materials in biomedical application [O'Connor, A. Riga and J.F. Turner, 1994]. Lactic acid can be commercially manufactured either by chemical synthesis or by biotechnological fermentation. However, the production of lactic acid through biotechnological fermentation has recently gained a great interest due to environmental pollution caused by petrochemical industries and depletion of petrochemical resource. The fermentative production of lactic acid can be regarded as a green technology not only because renewable substrates are used for its production, but also because CO_2, a greenhouse gas, is in corporate into lactic acid during the fermentation.

Although a number of different substrate have been used for the biotechnological production lactic acid, most studies on raw materials for lactic acid production have been focused on the pure substrate, such as wheat [Ray, L., G. Mukherjee, S.K. Majumdar, 1991], sorghum [Richter, K. and A. Trager, 1994] and Barley [Childs, C.G and B. Welsby, 1977]. Herein, starch is fisting enzymatically liquefied and sacharified to glucose, which is fermented by microorganisms to lactic acid. The direct conversion of starch to lactic acid by bacteria with amylolytic and lactic acid producing characteristics will eliminate the liquefaction and scharification processes.

S. bovis is a homofermentative lactic aid microorganism; therefore it produces lactic acid. Very recently, it has been proved that *S. bovis* is very effective for production of lactic acid from raw starch [Narita, J, S. Nakahara, H. Fukuda and K. Akhihiro, 2004]. We have also conducted batch lactic acid fermentation from glucose by *S. bovis* using various basal media and gave same interesting result [Ghofar, A, S. Ogawa and

T. Kokugan, 2005]. In this paper, we report on the production of lactic acid by batch and continuous fermentation of *S. bovis* from cassava.

METHODOLOGY

1) *Seed Culture*: *S. bovis* JCM 5802 was obtained from Institute of Physical and Chemical Research (RIKEN Japan). *S. 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.

2) *Medium Preparation*: Cassava was as substrate, while trypto soya broth was used as the basic medium. The basic medium (trypto soya broth) consisted of the following (per liter of 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 ^{o}C for 15 min.

3) *Fermentation*: Batch culture was carried out at 37 ^{o}C in a bioreactor (ABLE BMJ-01, Japan) with volume of 1L. It consists of a glass vessel with stainless steel endplates and three equally spaced vertical baffles. Agitation was provided by six plate-blade impeller located 4 cm above the bottom of the vessel. The temperature was controlled at 37 ^{o}C and agitation speed at 100 rpm. NH_4OH 6 N was used to maintain the pH at 5.5.

Continuous cultures were carried out with working volume 750 mL in a 1 L fermentor. Other fermentation conditions were the same as those described for the batch culture. After 24 h of batch operation, feeding solution was added at several different dilution rates, while an equal volume of spent medium was removed from the fermentor.

4) *Analytic 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 dehydroganase to measure lactic acid concentration, while the other one was packed with glucose oxide to measure 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 sampling an aliquot of the cultures. Viable count samples were taken regularly, plated on a BCP agar medium and incubated at 39 ^{o}C for 48 h. After 48 h incubation the colonies were counted by colony counting method in Colony Forming Unit (CFU/mL).

RESULTS AND DISCUSSIONS

Batch cultures

Analysis of fermentation broth using HPLC showed the lactic acid fermentation by *S. bovis* produced L-lactic acid (with 100% optical purity) in major amount and organic acids in minor amount (data not shown). Due to this reason, pH of fermentation sharply decreases as the fermentation progress. Maintaining pH fermentation at a value 5.5 or above is

necessary, because pH lower than 5.0 will decrease microbial activity. pH control was carried out by addition neutralization. It can be Figure 1 that the control of pH worked well. It is also important to note that the sudden rise of pH in continuous fermentation was due to sudden addition of medium whose pH is higher than 5.5.

Anaerobic batch cultures of *S. bovis* have been studied for the production lactic acid from cassava. When 50 g/L of cassava were used, starch was consumed completely, and specific growth rate grew to 0.57 h^{-1} in 12 h of culture (Figure 1). The concentration lactic acid, yield, and productivity of lactic acid obtained at the end of fermentation were 38.5, 82.5 %, and 2.43 g/l.h, respectively. Moreover, the batch experiment revealed that sufficient concentration of lactic acid can be achieved in 24 hours. The viable cell concentration followed a pattern similar to lactic acid with viable cells number observed at the same time as the maximum concentration of lactic acid was observed. The maximum concentration of viable cell in fermentation by *S. bovis* was 4.27 x 10^8 after 10 h of fermentation.

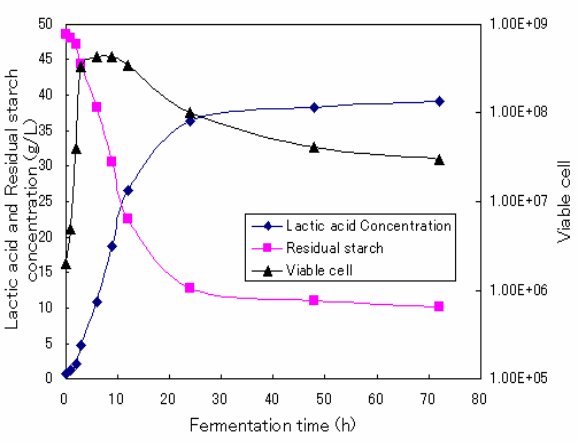

Fig. 1. Time course of lactic acid, glucose and cell concentration of broth during batch fermentation

Production of lactic acid using continuous culture fermentation

Although traditional batch fermentation appears to dominate the industrial today, there is much interest in developing more rapid methods of producing lactic acid, like continuous culture. In order to provide information on substrate utilization and volumetric productivities over a wide range rates, continuous culture of *S. bovis* was investigated in bioreactor. The substrate concentration was 50 g/l. Dilution rates analyzed were between 0.03 and 0.06 h^{-1}. Figure 2 shows the effect of dilution rate on the rate both lactic acid and specific growth rate. Both lactic acid and specific growth rate increased gradually with increase of dilution rate to a maximum at D = 0.05 h^{-1}. Maximum productivity (1.25 g/l.h) and specific growth rate (0.59 h^{-1}) were obtained at dilution rate of 0.05 h^{-1}.

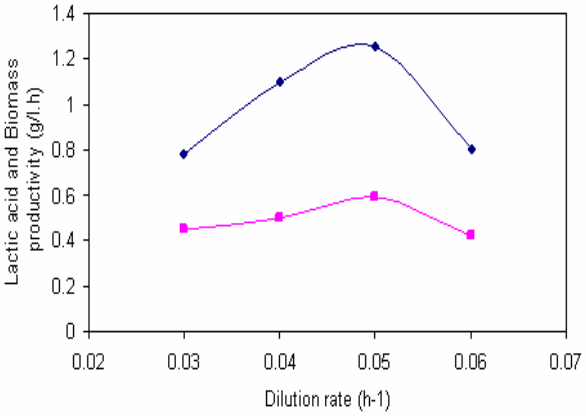

Fig. 2. Time course of lactic acid, glucose and cell concentration of broth during batch fermentation

CONCLUSION

In this study batch and continuous fermentations of lactic acid from cassava by *S. bovis* were conducted. In batch mode, lactic acid production and yield (38.5 g/l and 82.5%) and maximum productivities (2.43 g/l.h) were observed at pH 5.5 and 39 ^oC with 50 g/l initial cassava concentration. In continuous mode, maximum lactic acid concentration was obtained at low dilution rate, while the maximum productivity was recorded at D= 0.5 h^{-1}.

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