



Semicontinuous Fermentation of Cassava by *Streptococcus bovis* Using Membrane Bioreactor to Produce Lactic Acid

SURIPTO D. YUWONO^{1,*}, WASINTON SIMANJUNTAK¹, FITRIANI², TAKAO KOKUGAN², SUTOPO HADI¹

¹Department of Chemistry, University of Lampung, Jl. Prof. Dr. Sumantri Brojonegoro No. 1, Bandar Lampung, Indonesia

²Department of Chemical Engineering, Tokyo University of Agriculture and Technology, Tokyo 183-0057, Japan

Abstract: *The main objective of this study is to compare the production of lactic acid from cassava by Streptococcus bovis in semicontinuous system with and without membrane separation. Fermentation was run for 72 h, and membrane filtration was performed using micro filtration membrane (MF). The results obtained demonstrate that higher productivity of lactic acid was achieved using fermentation coupled with membrane separation. It was also found that dilution rate (D) of 0.02 h⁻¹ led to slight increase in the lactic acid productivity. Despite this small increase, this investigation shows that membrane separation has the potential to improve the performance of semicontinuous fermentation system.*

Keywords: *cassava, fermentation, lactic acid, micro filtration (MF), semi continuous, S. bovis*

1. Introduction

Lactic acid is recognized as a very valuable compound for its wide range of applications, from traditional uses such as chemical, soft drinks, medicine, and foodstuff, to more advanced materials such as biodegradable polymer and medical ingredients [1]. Both chemical route and fermentation process can be used to produce lactic acid; however the latter is more widely used for several advantageous reasons. Fermentation process requires cheaper raw materials and lower production cost since the process can be run in low temperature, thus reducing the cost for energy. In addition, fermentation is a highly specific process, enabling the production of pure L and D-lactic acid, which are the desired stereoisomers [2-6].

Lactic acid by fermentation can be produced from different types of raw material, among them are glucose, xylose, sucrose, lactose, and cassava [7-11], therefore offering the flexibility in selecting raw material considering the availability and necessary pretreatment, as well as the cost of fermentation [12]. In Indonesia, cassava is a very promising raw material for production of lactic acid, since this agriculture commodity is abundantly available in different regions, in which the Province of Lampung is one of the main producing regions [13,14]. The use of cassava is also supported by the existence of several species of bacteria capable to carry out the fermentation of this raw material, such as *S. bovis* [15,16] and *L. manihotivorans* [17].

Productions of lactic acid through fermentation of starch by *S. bovis* have been reported in previous studies, using batch system [11] and fed batch and continuous modes [18,19]. The results of these previous studies indicate that each system has its own advantages as well as disadvantages. For batch system, the main drawback observed was decreased concentration and productivity of lactic acid, most likely due to inhibition by starch in high concentration, which is acknowledged as a main obstacle of batch culture [11]. In this respect, the variation in fermentation processes should be acknowledged by taking into account the type and characteristics of raw materials, microbial growth, and the media of fermentation.

The present investigation was conducted as an attempt to develop an efficient fermentation process for production of lactic acid from cassava in single stage [18] and two stages [19]. The main objective of the study was to compare the performance of semicontinuous system with and without membrane separation (MF).

*email: suripto.dwi@fmipa.unila.ac.id

2. Materials and methods

2.1. Materials

Cassava was used as a substrate (5g/L), while Tryptosoya broth (TSB) (Nissui Pharmaceutical, Tokyo) was used as the basic medium. TSB consisted of the following (per 2 4, 2 4, liter of distilled water): 17 g peptone, 3 g soybean peptone, 2.5 g glucose, 2.5 g K HPO 2.5 g KH PO 5 g NaCl. The basic medium and substrate solution were then sterilized by autoclaving at 121°C for 15 min. The seed culture of *S. bovis* JCM 5802 used was similar to the one that has been used in our previous work [19, 20].

2.2. Semicontinuous Fermentation

Anaerobic fermentation experiment was conducted in 3.5 L chamber, at fixed temperature of 39 °C. During the experiment, the pH of the broth was controlled using pH meter (pH meter type HM-50G-DKK-TOA Corp., Japan), and maintained at specified value, by adding 6 N NH₄OH solution to neutralize the acid. The sample was agitated with the speed of 390 rpm. Membrane separation was carried out using 0.1 μm thickness MF membrane. Substrate and medium were supplied into the chamber using plunger pump and fermentation broth was regulated by reciprocating pump.

A series of experiments were conducted at pH of 5.5 to study semicontinuous technique with membrane separation. Before commencing semicontinuous experiments, batch fermentation was carried out for 24 h, during which time the concentration of remaining glucose was estimated to decrease to 5 - 10 g L⁻¹, based on previous batch fermentations. After the completion of batch experiment, semicontinuous fermentation experiments with membrane separation was undertaken by removing an aliquot of 1440 mL of fermentation broth from the system and fresh media contained 30 and 50 g L⁻¹ cassava were added every 24 h of interval time. Samples were taken before and after filling fresh media every 24 h. During the continuous fermentation with membrane separation, permeate was continuously removed through membrane separation with constant flow rate of at 0.066 L h⁻¹ by regulation valve, and fresh medium was added to the system with flow rate about 0.068 L h⁻¹.

2.3. Analysis

For analysis, an aliquot of broth was drawn aseptically from the system and the concentrations of lactic acid and residual starch were determined with a biosensor (Oji Scientific Instruments, Osaka). Since the permeate side of the membrane was not sterile, the number of *Streptococcus bovis* cells (CFU (mL)⁻¹) in 0.1 mL diluted sample was estimated by plate count technique. This counting was conducted using bromocresol purple agar medium (Nissui Pharmaceutical, Tokyo) after incubating the sample for 72 h at 37 °C in anaerobic condition.

3. Results and discussions

The results after 24 h batch experiment followed by semicontinuous treatment with membrane separation at different times and 0.02 h⁻¹ dilution rate are shown in Figure 1. As can be seen, increased viable cell in the reactor was observed up to 72 h of experiment. It was also found in the experiment fed with substrate contained 50 g.L⁻¹ cassava, gradual increase of lactic acid concentration took place from transition period of 24 h and reached 19 g.L⁻¹ after 48 h, and about 7.8 g.L⁻¹ residual starch in the filtrate. Semicontinuous culture commenced when the cassava reached lactic acid produced has almost constant concentration at 19 g L⁻¹ until 7 days of operation. Lactic acid produced from this operating mode (semicontinuous with membrane separation) has similar concentration every day. This constant concentration differs from batch mode which has more variation in lactic acid produced. There was also an increasing of viable cell because of batch feeding of new substrate and cell was recycled back to the vessel by membrane separation. The experiment fed with substrate contained 30 g L⁻¹ cassava was found to produce very low yield (Table 1, row 2)

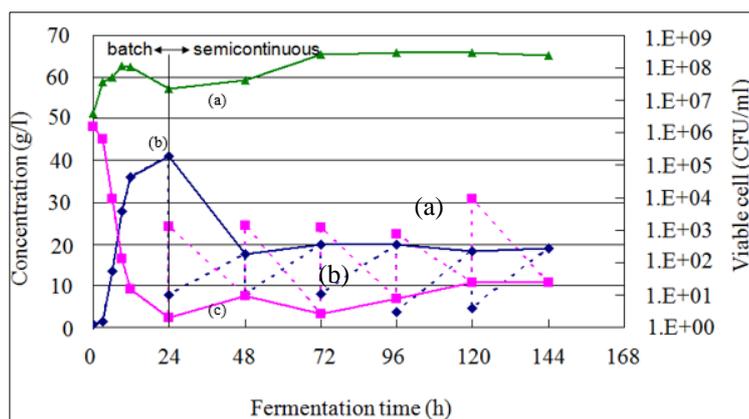


Figure 1.
Semicontinuous
fermentation with
membrane separation
 $D = 0.02 \text{ h}^{-1}$: (a) viable
cell; (b) lactic acid
concentration;
(c) starch concentration

Typical example of the results obtained from semicontinuous experiment without membrane for experimental time of 144 h is presented in Figure 2. As can be seen, the concentration of lactic acid has reached the value of about $13 \text{ g} \cdot \text{L}^{-1}$ after 72 h, and the concentration of lactic acid increased up to $18 \text{ g} \cdot \text{L}^{-1}$. As a result, the productivity achieved was $0.33 \text{ g} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ for the third and fourth stage of fermentation, or equivalent to specific productivity of 1.2 h^{-1} . Higher productivity than that achieved in this study was reported in previous investigation with the use of continuous processes coupled with membrane separation [19]. It is then clear that the use of MF to prevent the buildup of lactic acid in the bioreactor will increase the reaction rate of lactic acid formation, leading to higher productivity.

During the experiments, the conditions were controlled to ensure the concentration of starch was within the range of $19 - 20 \text{ g} \cdot \text{L}^{-1}$ in bioreactor and less than $5 \text{ g} \cdot \text{L}^{-1}$ in the permeate effluent. This step was taken because if the concentration of starch reaches $25 \text{ g} \cdot \text{L}^{-1}$ in the reactor, the fermentation was hindered by inhibiting effect of the concentration, leading to suppressed formation of lactic acid. Application of recycled cells after membrane separation, to obtain high cell density, was found to result in higher concentration and yield of lactic acid produced. As can be seen in Table 1, dilution rate of 0.02 h^{-1} resulted in lactic acid with concentration of $19 \text{ g} \cdot \text{L}^{-1}$ and yield of $0.75 \text{ g} \cdot \text{L}^{-1}$. The concentration of lactic acid achieved in this study is relatively higher than that reported in previous study [21], in which *S. cremoris* was used for fermentation of whey permeate with the concentration of $5.38 \text{ g} \cdot \text{L}^{-1}$. In another study [22], production of lactic acid from cane molasses was investigated. In the study, *L. plantarum* was used to determine the performance of conventional continuous culture. The results obtained demonstrated that maximum volumetric productivity achieved was closely related to low concentration of lactic acid high concentration of unreacted molasses total sugars [22].

Figure 3 displays the results of experiment carried out with starch concentration of $50 \text{ g} \cdot \text{L}^{-1}$ and dilution rate of 0.02 h^{-1} , resulting in $0.39 \text{ g} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ as the maximum productivity for lactic acid. For batch system, concentration, productivity, and yield of lactic acid are $42 \text{ g} \cdot \text{L}^{-1}$, $0.84 \text{ g} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$, and $3.44 \text{ g} \cdot \text{L}^{-1}$, respectively (Table 1). In Table 1, the initial cassava concentration $[C_{S0}]$, dilution rate (D), lactic acid concentration $[C_{LA}]$ and residual starch concentration $[R_S]$ were directly measured as described in analysis section, while the calculation of the productivity (P_{LA}), specific growth rate (μ) and yield of lactic acid ($Y_{P/S}$) the following formula as in Equations 1 to 3 were used.

$$(P_{LA}) = [C_{LA}] \times D \quad (1)$$

$$(\mu = \ln (X_2/X_1)/(t_2-t_1)) \quad (2)$$

where X_2 = cell concentration at time t_2

X_1 = cell concentration at time t_1

$$Y_{P/S} = [C_{LA}]/[C_{S0}] \times 100\% \quad (3)$$

As seen in Table 1, both concentration and yield of lactic acid obtained from continuous system are analogous to those obtained using semicontinuous fermentation, but continuous fermentation resulted in much higher productivity compared to that of semicontinuous fermentation.

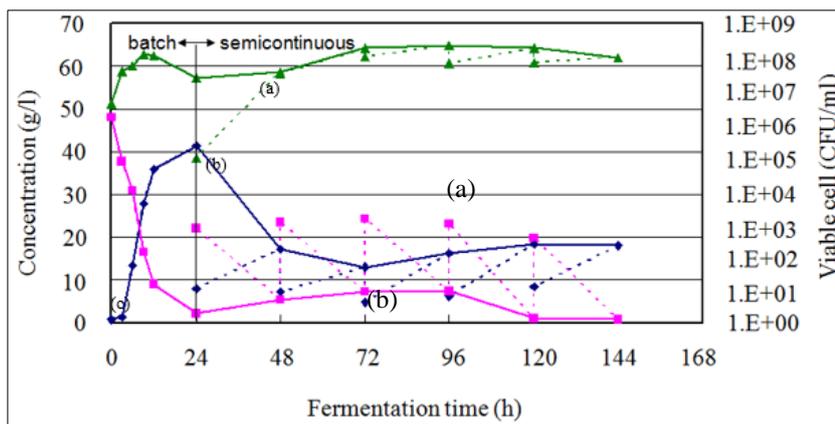


Figure 2. Semicontinuous fermentation without membrane separation, $D = 0.02 \text{ h}^{-1}$: (a) viable cell; (b) lactic acid concentration; (c) starch concentration

Table 1. Kinetic parameters of each fermentation mode

No	Operating mode	Time [days]	C_{S_0} [g L ⁻¹]	D [h ⁻¹]	C_{LA} [g L ⁻¹]	R_S [g L ⁻¹]	P_{LA} [g L ⁻¹ h ⁻¹]	μ [h ⁻¹]	$Y_{P/S}$ [-]
1	Batch	3	50	-	42	5	3.44	0.85	0.84
2	Semicontinuous with membrane	7	30	0.01	16.5	4.4	0.16	-	0.22
3	Semicontinuous with membrane	7	50	0.02	19	7.8	0.38	-	0.75
4	Semicontinuous without membrane	7	50	0.02	13	4.4	0.33	-	0.52
5	Continuous with membrane	3	50	0.02	19.7	9.4	0.39	-	-

Note: $[C_{S_0}]$ = initial cassava concentration, D = dilution rate, $[C_{LA}]$ = lactic acid concentration, $[R_S]$ = residual starch concentration, P_{LA} = productivity, μ = specific growth rate, $Y_{P/S}$ = Yield of Lactic acid

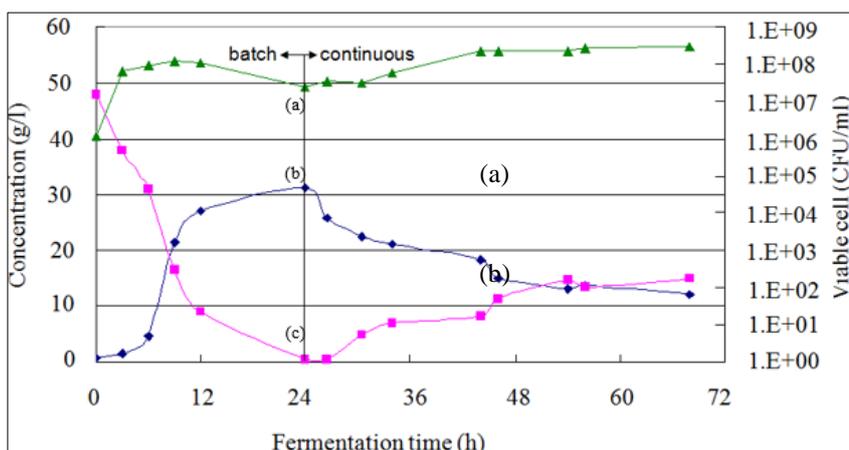


Figure 3. Continuous fermentation with membrane separation: (a) viable cell; (b) lactic acid concentration; (c) starch concentration

The results obtained from semicontinuous system with and without membrane, at different rates of dilution are compiled in Table 1. As can be seen, the same D of 0.02 h^{-1} was observed for both systems, but higher concentration of lactic acid in the filtrate (19 g.L^{-1}) was produced by the system with membrane. These results demonstrated that the high performance of membrane last for long period of time, thus supporting high activity of cells in the fermenter to conduct fermentation. Kinetic parameters for semicontinuous fermentation with and without membrane, and dilution rates are included in Table 1. To achieve better fermentation results, the inhibitory effect of lactic concentration should be



suppressed, which can be done by running the system at low dilution rate and using comparatively lower viable cell [11].

As observed in previous study [15], lactic acid concentration of 50 g L⁻¹ caused severe damage of *S. bovis*. This finding is in agreement with the result of the present study, which revealed that suppression of lactic acid to less than 50 g L⁻¹ during the fermentation process is required order to maintain high viability of the cell. Concentration of lactic acid produced using *S. bovis* is appreciably lower than that achieved using *L. amylovorus*. In investigation by others [23], it was reported that lactic acid production directly from cassava with initial liquefied starch concentration of 120 g L⁻¹ using *L. amylovorus* in a batch fermenter resulted in lactic acid concentration of 96.2 g after 20 h. The study also revealed that lactate with concentration of 92.5 g L⁻¹ was produced from raw starch of the same concentration in 39 h. Conversion of starch might proceed either through primary hydrolysis of starch molecule or direct conversion of starchy substrate to lactic acid by amylolytic organisms [24]. In direct conversion of starch, it was acknowledged that different results were obtained from different substrates. As examples, utilization of corn starch produced lactic acid with concentration 0.68 g L⁻¹ [25], and production of lactic acid from liquefied starch using *L. plantarum* NCIM 2084 was reported to produce lactic acid with concentration of 0.729 g L⁻¹ [26].

4. Conclusions

In conclusion, this study showed a maximum lactic acid productivity of 0.38 g L⁻¹ h⁻¹, where the optimal lactic acid production conditions were starch feed concentration of 50 g L⁻¹ and 0.02 h⁻¹ dilution rate. Furthermore, since the semicontinuous process was shown to produce a similar amount of lactic acid to the continuous culture experiment.

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