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Improvement of Lactic Acid Production from Cassava by *Streptococcus bovis* Using Two-Stages Membrane Bioreactor

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The investigation of improvement for lactic acid production from fresh cassava roots by *Streptococcus bovis* using two-stages membrane bioreactor with purging was performed to increase the concentration and productivity of the lactic acid. The result showed that by the use of two-stages membrane bioreactor, the productivity and concentration of lactic acid were 5.03 and 50 g/L, respectively which were obtained at dilution rate of 0.25 h^{-1} and 30 g/L feeding sugar concentration. Based on the data obtained, it is shown that the two-stages membrane bioreactor system was very effective and efficient and it could be potentially used for the production of lactic acid from fresh cassava roots by *S. bovis*.

Keywords: Cassava roots, Lactic acid, Two-stages reactor, *S. bovis*.

INTRODUCTION

Lactic acid is natural organic acid with long history in food, chemical and organic acid, pharmaceutical personal care industries. Polymerization of lactic acid produces poly(lactic acid) (PLA), a biodegradable plastic primarily used as surgical implants, drug delivery system and artificial scaffold materials biomedical application. However, the cost of poly(lactic acid) production from biomass is several times higher than production of conventional plastics from fossil resources[1]. In an attempt to reduce the production cost, a new medium of fresh cassava roots (FCR) in tofu liquid waste was used for lactic acid production. However the productivity of lactic acid is very low[2]. In order to increase the productivity, the concentrate *meguro* waste (CMW) was added to new media. The productivity in new media was comparable to those in the standard media by batch fermentation[3].

Lactic acid is commonly produced by batch and continuous fermentation process from refined sucrose, starch corn, whey permeate and other carbon resources. Lactic acid is a primary metabolite and it is well known that the production of lactic acid is strictly dependent on cell growth and the final biomass. But it suffers relatively low productivity due to end-product inhibition[4,5]. Several attempts have been made to replace batch fermentation with continuous processes to reduce end-product inhibition and improve lactic acid productivity. Improvements in productivity have been achieved using higher cell densities, employing cell immobilization[6,7] and by using cell-recycling modules[8], different fermentation feedstocks and different lactate-producing strains.

Membrane bioreactor (MBR) could solve these problems satisfactorily. The efficiency of the membrane bioreactor was demonstrated in a number of previous studies on the enhancement of lactic acid productivity[4,9,10]. The membrane bioreactor can achieve and maintain a high cell density in the fermentor, which allows for high throughputs[11]. Furthermore, the microorganism and residual substrate are recycled for further use and the lactate (an end-product inhibitor) is continuously removed[5,12].

Following our success on batch and continuous acid fermentation, study on lactic acid fermentation in single stage membrane bioreactors has also been conducted[10]. The objective of this study was to evaluate the lactic acid fermentation performance on these two stage of membrane bioreactor. This research aimed to investigate the improvement of lactic acid production using the effect operating variables: two stages fermentation, membrane performance characteristics which will be used for membrane bioreactor of lactic acid fermentation.

EXPERIMENTAL

Fermentation: *S. bovis* JCM 5802 (RIKEN, Saitama, Japan) was used for L-lactic acid fermentation[5,10]. The bacteria was culture in 5 mL of MRS medium (Difco, Sparks, MD, USA), Fresh cassava roots (FCR) used as substrate and

Tryptosoya broth (Nissui Pharmeutical, Tokyo) was used as basal medium. The substrate and medium were mixed and autoclaved at 121 °C for 20 min.

Two-stages membrane bioreactor: The experimental setup of two-stages membrane bioreactor with purging is shown in Fig. 1. The fermentation was carried out in a 1 L fermentor (ABLE, Type BMJ-01, Japan) with a 750 mL working volume (fermentor 1) and 1 L flask. The temperature was maintained at 39 °C for both fermentors and only fermentor 1 used an agitation of 175 rpm. The culture was maintained at pH 5.5 by the automatic addition of a 5.5 N NH_4OH solution. Batch fermentation was conducted for 24 h. Subsequently, continuous fermentation or fed-bath fermentation was implemented by feeding an FCR solution with 30 g/L sugar. The membrane used was the same with the single-stage operations. Micro tube pumps (Eyela MP, Tokyo Rikakikai Co. Ltd., Japan) were used to convey neutralizing agent, fermentation solution for recycling and purging and permeate as product.

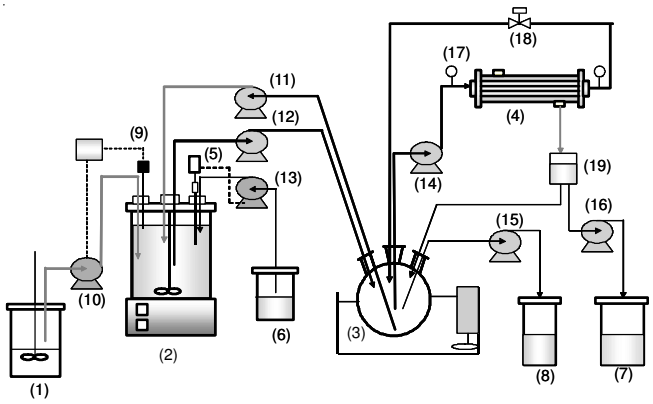

Fig. 1. Experimental setup of two-stages membrane bioreactor. (1) feeding tank; (2) fermentor 1; (3) fermentor 2; (4) membrane module; (5) pH electrode; (6) neutralizing agent; (7) product tank; (8) purging; (9) level controller; (10) feeding pump; (11) recycle pump 1; (12) recycle pump 2; (13) neutralizing pump; (14) recycle pump 3; (15) purging pump; (16) product pump; (17) pressure gauge; (18) pressure valve; (19) permeate separator

Analysis: The concentrations of lactic acid and residual starch were analyzed using a biosensor (Oji Scientific Instruments, Osaka). Aliquots of the fermented broth samples were aseptically collected. Viable cells of *S. bovis* (CFU/mL) in the diluted samples (0.1 mL) were counted by the plate count method using bromocresol purple agar medium (Nissui Pharmaceutical, Tokyo) after anaerobic incubation for 72 h at 37 °C.

RESULTS AND DISCUSSION

Comparison of continuous and membrane bioreactor operation: Fig. 2 shows the comparison of lactic acid concentration (a) residual sugar concentration (b) and viable cells (c) for continuous and membrane bioreactor operations at initial sugar concentration of 40 g/L and dilution rate of 0.12 h^{-1}. After batch operation conducted and lactic acid concentration of 30 g/L and no residual sugar were achieved, the continuous operation and membrane bioreactor were carried out. After 48 h operation, lactic acid concentration in membrane bioreactor was higher than that in continuous operation, while the residual starch concentration in membrane bioreactor was lower than that in continuous operation. It can be understood that membrane bioreactor operation allows cell recycle so that the viable cells in membrane bioreactor was higher than that in continuous operation. Therefore, the lactic acid concentration in the membrane bioreactor was higher than that in continuous operations. This values was similar to that of continuous lactic acid production from soluble starch using immobilized amylase and immobilized cells[13,14] have reported the lactic acid concentration was higer in membrane bioreactor.

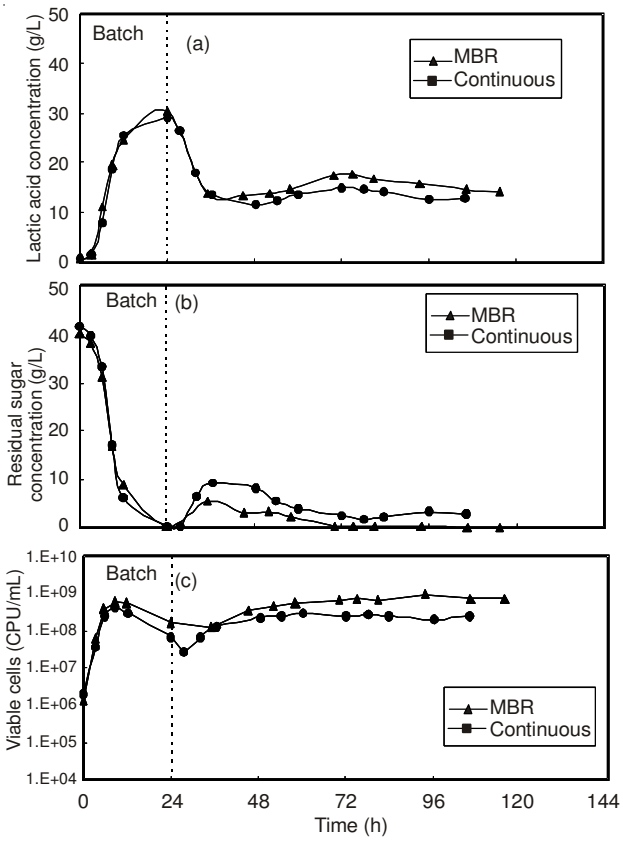

Fig. 2. Comparison of continuous and membrane bioreactor (MBR) for lactic acid concentration (a), residual sugar concentration (b) and viable cells (c) at C_{Sf} = 30 g/L and D = 0.12 h^{-1}

Fermentation performance of two-stages membrane bioreactor: Fig. 3 shows the performance in the two-stages fermentation in terms of lactic acid concentration, residual sugar concentration and viable cells at initial sugar concentration (C_{So}) of 40 g/L and feeding sugar concentration (C_{Sf}) of 30 g/L. Batch fermentation was first conducted for 12 h until 30 g/L lactic acid concentration and no residual substrate were achieved. Subsequently, continuous fermentation was started at fermentor 1 and the outflow from fermentor 1 was pumped to fermentor 2 at dilution rate of 0.17 h^{-1}. After 12 h, the fermentor 2 was filled with 1000 mL of fermentation broth. Then, the recirculation pump was started, the fermentation broth flows through the membrane, the permeate was then collected. The hold-up volume in the membrane and the connection pipes was about 400 mL. The dilution rate in fermentor 2 becomes 0.13 h^{-1} because the working volume which is about 1000 mL (including the hold-up volume) is higher than that in fermentor 1 which is about 750 mL.

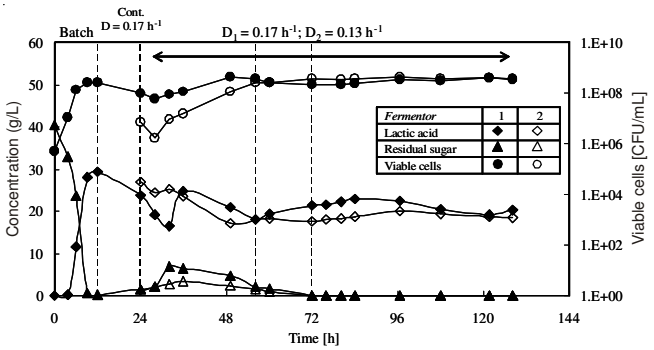

Fig. 3. Two-stages membrane bioreactor at C_{So} = 40 g/L and C_{Sf} = 30 g/L

The fermentation broth in fermentor 2 which contained high cell density were then pumped to the fermentor 1 after 56 h operation at the flow rate about 40 mL/h. The purging was then started after 72 h operation with the flow ratio to permeate flow was about 0.17. The dilution rate in fermentor 2 was calculated by adding permeate flow and purge flow and divided with the working volume of fermentor 2 and hold-up volume. The results show that lactic acid concentration in fermentor 1 was similar with that in fermentor 2 after 108 h operation and there was no residual sugar in both fermentor from 74 h operations.

The fermentation properties were then summarized in Table-1. It can be seen that the lactic acid at steady state ($C_{La,ss}$) in fermentor 1 and fermentor 2 was about 20.2 and 18.4 g/L, respectively. The productivity of lactic acid ($P_{La,ss}$) in fermentor 1 and fermentor 2 were about 3.43 and 2.39 g/Lh, respectively. Meanwhile, the viable cells in both fermentors were almost the same. Shibata *et al.*[15] conducted membrane bioreactor of 20 g/L sago starch concentration by *Enterococcus faecium*. They found that the highest lactic acid productivity was 2.86 and 1.91 g/L residual sugar concentration and 17.9 g/L lactic acid concentration were obtained at dilution rate of 0.16 h^{-1}.

Fig. 4 shows the performance in the two-stages fermentation at initial sugar concentration (C_{So}) of 50 g/L and feeding sugar concentration (C_{Sf}) of 30 g/L. The batch fermentation was first conducted for 24 h until 43 g/L lactic acid and no residual sugar were achieved. Subsequently, fed-batch operation was started. 500 mL fermentation broth from the working volume of 750 g/L was taken from fermentor 1 and pumped to fermentor 2 for 20 min. The feeding media (fresh cassava roots and TSB) with 50 g/L sugar concentration was added with the same volume (500 mL). The batch fermentation was conducted for 12 h until no residual sugar was achieved. The second fed-batch operation was carried out with the same procedure as before, while the 500 mL fermentation broth was pumped to fermentor 2. The total fermentation broth in fermentor 2 was about 1000 mL. At 53 h operation, the continuous membrane bioreactor was started. The dilution rate was first set-up at 0.15 and 0.12 h^{-1} for fermentor 1 and fermentor 2, respectively. At 82 h operation, the dilution rate was increased at 0.25 and 0.18 h^{-1} for fermentor 1 and 2, respectively. Purging ratio (purging flow divided by total outflow from fermentor 2) was set-up at 0.3.

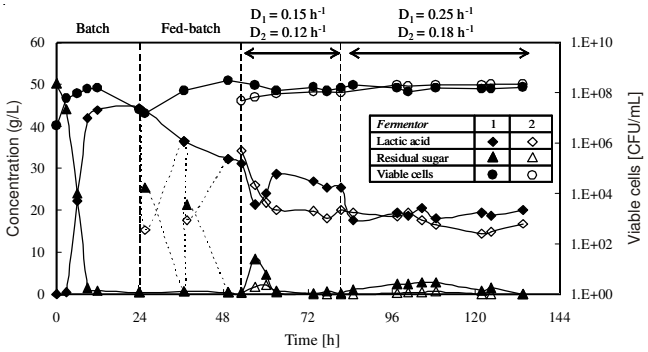

Fig. 4. Two-stages membrane bioreactor at C_{So} = 50 g/L and C_{Sf} = 30 g/L

The fermentation properties for this process are then summarized in Table-2. It can be seen that lactic acid concentration at fermentor 2 was higher at dilution rate of 0.12 h^{-1} than that in 0.18 h^{-1}. Meanwhile the lactic acid productivity was higher at dilution rate of 0.18 h^{-1} than that in 0.12 h^{-1}. The yield in fermentor 1 at dilution rate of 0.15 h^{-1} was higher than that in dilution rate of 0.25 h^{-1}. Viable cells in fermentor 1 were not changed as dilution rate increased. However, the viable cells in fermentor 2 increased two times as the dilution increased from 0.12 to 0.18 h^{-1}. Dilution rate of 0.12 h^{-1} was proposed for the optimum operating conditions in which moderate lactic acid concentration of 20.1 g/L with 2.41 g/Lh of productivity was obtained. Increasing the dilution rates resulted in a decrease in both lactic acid and biomass and an increase in residual sugar concentrations. The presence of excess sugars in higher dilution rate cultures and their complete utilization at lower dilution rates have also been reported by Major and Bull[16] from *L actobacillus delbrueckii* on glucose

TABLE-1
FERMENTATION PROPERTIES OF TWO-STAGES MEMBRANE BIOREACTOR C_{So} = 40 g/L, C_{Sf} = 30 g/L

	Working volume (mL)	Dilution rate (h^{-1})	$C_{La,ss}$ (g/L)	$C_{RS,ss}$ (g/L)	$Y_{P/S}$ (g/L)	$P_{La,ss}$ (g/Lh)	Viable $cell_{ss}$ (CFU/mL)
Fermentor 1	750	0.17	20.2	0	0.67	3.43	3.88×10^8
Fermentor 2	1000	0.13	18.4	0	–	2.39	3.39×10^8

TABLE-2
FERMENTATION PROPERTIES OF TWO-STAGE MEMBRANE BIOREACTOR AT C_{So} = 50 g/L and C_{Sf} = 30 g/L

	Working volume (mL)	Dilution rate (h^{-1})	$C_{La,ss}$ (g/L)	$C_{RS,ss}$ (g/L)	$Y_{P/S}$ (g/L)	$P_{La,ss}$ (g/Lh)	Viable $cell_{ss}$ (CFU/mL)
Fermentor 1	750	0.15	25.3	0	0.84	3.80	1.61×10^8
Fermentor 2	1000	0.12	20.1	0	–	2.42	1.07×10^8
Fermentor 1	750	0.25	20.1	0	0.67	5.03	1.66×10^8
Fermentor 2	1000	0.18	16.7	0	–	3.01	2.18×10^8

and Naby *et al.*[17] for *L. lactis* on starch. High glucose concentration in fermentation broth results in inhibitory effect of substrate on fermentation and in low yield of lactic acid whereas glucose limitation leads to the end of lactic acid production and anabrupt cessation of cell growth[18].

Membrane performance in two-stage membrane bioreactor: Fig. 5 shows the performance of membrane bioreactor at initial sugar concentration (C_{So}) of 50 g/L and feeding sugar concentration (C_{Sf}) of 30 g/L. The recirculation rate was first set up at 4.8 L/min which is equal to 0.31 m/s of cross-flow velocity and *trans*-membrane pressure was initially operated at 5 kPa. It can be seen that cross-flow velocity slightly decreased to 0.25 m/s and then remained constant until more than 120 h operations. *trans*-Membrane pressure slightly increased to 10 kPa and remained constant. Hence, the fermentation was expected can be continued for long period as the cross-flow velocity and transmembrane pressure kept constant.

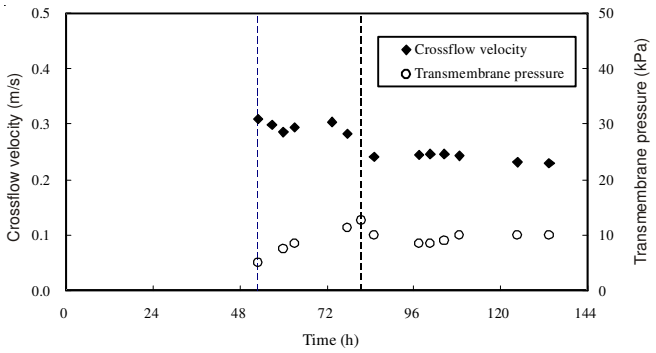

Fig. 5. Membrane performance of membrane bioreactor operation at C_{So} = 50 g/L and C_{Sf} = 30 g/L

Conclusion

High lactic acid productivity (5.03 g/Lh) and diution rate 0.25 h^{-1} were achieved during continuous fermentation by *S. bovis* in a two-stage process. The process could be operated in a stable manner. However, after continuous culture operation with very low or no residual sugar for several days, loss of productivity was observed in the second reactor due to loss of biomass activity. Long time operation of two-stages membrane bioreactor is expected because of the stability of the membrane used.

Nomenclature

C_{La}	Lactic acid concentration (g/L)
C_{RS}	Residual sugar concentration (g/L)
C_{Sf}	Feeding sugar concentration (g/L)
C_{So}	Initial sugar concentration (g/L)
D	Dilution rate (h^{-1})
P_{La}	Lactic acid productivity (g/Lh)
$Y_{P/S}$	Yield product to substrate (-)
ss	Steady state

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