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Lactic Acid Production from Fresh Cassava Roots Using Single-Stage Membrane Bioreactor

Suripto Dwi Yuwono & Sutopo Hadi

Department of Chemistry, University of Lampung, Bandar Lampung 35145, Indonesia

E-mail: yuwen_5774@yahoo.com; sutopohadi@unila.ac.id

Fitriani & Takao Kokugan

Department of Chemical Engineering, Tokyo University of Agriculture and Technology 22416, Nakamchi Koganei, Tokyo 1848588

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Abstract

The production of lactic acid from fresh cassava roots by *Streptococcus bovis* using membrane bioreactor was investigated. Single-stage membrane bioreactor (MBR) without purging was conducted to find out the concentration and productivity of the lactic acid obtained. The result showed that in a single stage MBR, the lactic acid productivity and concentration were 1.88 g/Lh and 15.7 g/L, respectively which were obtained at dilution rate of 0.12 h⁻¹ and 30 g/L feeding sugar concentration. The result also indicated that the MBR bioreactor operation showed excellently and potentially for the production of lactic acid directly from fresh cassava roots by *S. bovis*.

Keywords: Lactic acid, Single-stage reactor, *S. bovis*, Cassava roots

1. Introduction

Over the last decade, lactic acid production has attracted considerable attention, because lactic acid is a raw material for synthesis of polylactic acid (PLA) which is an essential bioplastics material (Datta *et al.*, 1995). The production of PLA from biomass is expected to be environmentally friendly, effective for reducing CO₂ gas emission and for saving fossil resources. However, the use of PLA is still limited because its production cost is 3 to 10 times higher than that of conventional plastics from fossil fuel resources. In order to reduce the production cost of PLA, we proposed an onsite cell plant method for the production of PLA by fermentation, separation and polymerization in sequential production step. For fermentation, a cheap fresh cassava roots was used as raw material and also the direct fermentation without saccharification was conducted by previous study (Ghofar, *et al.*, 2005; Yuwono and Kokugan, 2007). For polymerization process, direct polycondensation method without catalyst and solvent was used to synthesize PLA as replacing of the high cost ring opening polymerization method (Achmad, *et al.*, 2009).

Conventional methods of fermentation that use *fretee* cells in batch process have several limitations, such as low productivity, product inhibition and batch to batch variation in the product (Mehaia and Cheryan, 1987). The effect of product inhibitions on lactic acid fermentation has been reported by Yuwono *et al.* (2008). Continuous fermentation, on the other hand, although has been known to overcome some of the problems associated with batch processes, is limited by cell wash-out (Tejayadi and Cheryan, 1995). In order to increase the productivity significantly, it is necessary to use high cell concentrations and to remove the inhibitory product (lactic acid).

Membrane bioreactor, an integrated process of fermentation and separation, as a compact process has the valuable advantages in order to increase lactic acid productivity. This process is expected to reduce product inhibition, reuse unconverted substrate, obtain high cell density culture, and decrease separation load of the next separation process (Tejayadi and Cheryan, 1995). By the optimization of these advantages, it is expected to reduce cost production of PLA in on-site cell plant.

In this research, membrane bioreactor with a ceramic monolith-type microfiltration membrane was used for production of lactic acid from fresh cassava roots as raw material. The efficiency of membrane bioreactor in

terms of maximum lactic acid concentration and lactic acid productivity was investigated. One case of membrane bioreactor, single stage without purging was conducted.

2. Experiment

2.1 Microorganism and Culture Conditions

Streptococcus bovis JCM 5802 (RIKEN, Saitama, Japan) was used for L-lactic acid fermentation in this study. The bacteria was cultured in 5 mL of MRS medium (Difco, Sparks, MD, USA) consisting of 10 g of peptone (casein peptone tryptic digest), 10 g of meat extract, 5 g of yeast extract, 20 g of glucose, 2 g of K_2HPO_4 , 5 g of sodium acetate, 2 g of diammonium citrate, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.05 g of $MnSO_4 \cdot H_2O$, 1 g of Tween 80 (poly sorbit-80) per liter of distilled water. After 18 h of incubation at 37 °C, the cultures were divided into 0.3 ml of aliquots (around 106 CFU/ml) per microtube and the cells were resuspended in skim milk, and then stored at -80°C. Precultures were prepared by transferring a stock culture to 5 ml of MRS medium and incubated at 37 °C for 18 h and used as an inoculum for the fermentation process.

2.2 Media Fermentation

Fresh cassava roots (FCR) used as substrate, directly supplied from Indonesia, were crushed and then stored in a freezer. A basic medium (glucose) and tapioca (cassava starch) were also used for comparison. Tryptosoya broth (Nissui Pharmaceutical, Tokyo) which consisted of 20 g of peptone, 2.5 g of glucose, 2.5 g of K_2HPO_4 , 2.5 g of KH_2PO_4 and 5 g of NaCl per liter of distilled water, was used as basal medium. The substrate and medium were mixed and autoclaved at 121°C for 20 min.

2.3 Single-Stage Membrane Bioreactor

A fermentor having a working volume of 3 L was used in the following operating modes: batch, conventional continuous fermentation, complete cell recycling. The fermentation was carried out under anaerobic conditions at 39°C and pH was maintained at 5.5 by automatic addition of 5.5 N NH_4OH solution. Constant stirring at 360 +/- 3 rpm was kept during the fermentation.

The batch fermentation set-up consisted of a simple pH-controlled system. The set-up for cell recycling with membrane separation (membrane bioreactor) is shown in Figure 1. The system was initially operated in batch mode and switched to continuous mode or cell recycling mode after 24 h when the residual starch concentration was about 0.2-0.5 g/L. The fresh medium was feeding continuously with feeding pump which connected to the level controller to maintain a constant working volume. For continuous fermentation mode, the fermentation broth was then pumped to the stored tank at certain flow rate. For continuous cell recycling mode, the amount of fermentation broth was pumped by magnetic gear pump (Iwaki, Type MDG-H15KA200, Japan) at certain flow rate to a membrane system. A ceramic monolith-type membrane (Cefilt, NGK Filtech, Japan) with 50 cm long, 37 holes with 3 mm diameter and a surface area 0.174 m² was used. A hold-up volume in the connection pipe and the membrane system was about 400 ml. The retentate was returned back to the fermentor and the permeate was then collected. The pressure was adjusted manually with pressure regulating valve and monitored with pressure gauge.

2.4 Analysis

The concentrations of L-lactic acid and residual starch (as glucose concentration) were analyzed using a biosensor (Oji Scientific Instruments, Osaka). Residual starch was measured by conversion to glucose by addition of glucoamylase enzyme and put in water bath at 50 °C for 4 h. 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.

3. Results and Discussion

3.1 Single Stage Membrane Bioreactor: Fermentation of Various Carbon Sources

Figure 2 shows the results of fermentation of various carbon sources (FCR, tapioca, glucose) in terms of lactic acid concentration (a), residual sugar concentration (b) and viable cells (c). For all variations, batch fermentation was first conducted for 24 hr at initial sugar concentration (C_{S0}) of 40 g/L. Subsequently, continuous membrane bioreactor (MBR) was carried out with feeding sugar concentration (C_{Sf}) of 30 g/L and dilution rate (D) of 0.12 h⁻¹. It can be seen that for batch operation, after 24 h, lactic acid concentration for FCR was the highest among the other carbon sources. Moreover, residual sugar concentration of FCR was nearly zero after 72 h operation and the lowest among other carbon sources. On the other hand, lactic acid concentration for glucose was the lowest among the other carbon sources and the residual sugar concentration of glucose was the higher among the

other carbon source. For subsequent MBR, the results show the same tendency with that in batch operation.

This result is in agreement with the study by Ghofar et al. (2005) where they conducted batch fermentation of FCR, tapioca, and glucose in different basal media by *S. bovis*. FCR was found to be more suitable for *S. bovis* compare to other carbon sources. This is due to *S. bovis* has the ability to directly produce lactic acid from starch because *S. bovis* secretes α -amylase (Narita, et al., 2004; Satoh, et al., 1993). The starch is saccharified by extracellular α -amylase to maltooligosaccharide. Subsequently, intracellular α -amylase liquefies the oligosaccharides to glucose inside the cell (Cotta, 1997, Satoh, et al., 1997). Then, *S. bovis* simultaneously converts glucose into lactic acid. It was known that *S. bovis* grown in a medium containing starch, as the carbon source, the organism has more α -amylase activity than when cultured in a medium containing glucose (Cotta, 1988). It can also be seen that the viability cells of FRC is the highest among other carbon sources for the consecutive operation, batch and MBR (Figure 5).

3.2 Effect of Feeding Sugar Concentration

Figure 3 shows the effect of feeding concentration of sugar concentration (FCR) on lactic acid concentration (a), residual sugar concentration (b) and viable cells (c). The initial sugar concentration (C_{S0}) was 40 g/L, and the dilution rate (D) was kept at 0.07 h^{-1} . The results show that after 24 h of batch operation, 32 g/L lactic acid concentration and no residual sugar concentration were obtained. When MBR operation started, lactic acid concentration suddenly decreased and the steady-state value at about 14 g/L was obtained after about 48 h operation for both varied sugar concentrations. Residual sugar concentration increased when MBR operation started and decreased after about 30 h for feeding sugar concentration (C_{Sf}) of 30 g/L. However, residual sugar concentration was almost constant at about 14 g/L for feeding sugar concentration (C_{Sf}) of 50 g/L. It can be understood the sugar can not be completely converted to lactic acid at higher feeding sugar concentration. This could be due to the reaction time to complete sugar consumption has not yet been achieved at the given dilution rate. The substrate inhibition occurred at 50 g/L sugar concentration was also presumed. Zhang and Cheryan (1994) have reported the study of starch fermentation by membrane bioreactor with *L. amylovorus* as strain. They found that lactic acid concentration was higher at 50 g/L sugar concentration than that at 90 g/L sugar concentration.

Viable cells count increased until 12 h operation and then decreased due to lack of sugar concentration for batch operation (Figure 3c). When MBR started from 24 h operation, the viable cells stayed decreasing until 30 h operation and it started growing until the steady-state value at about 8.69×10^8 CFU/mL (feeding sugar concentration of 30 g/L) and 6.72×10^8 CFU/mL (feeding sugar concentration of 50 g/L) were achieved. It was generally known that the cell concentration, in terms of dry cell weight, can be increased as the operating time increased at MBR operation because cell was rejected by membrane and recycled back to the fermentor as reported by several studies (Hayakawa, et al., 1990, Xavier, et al., 1995, Hu, et al., 2006). However, the viable cells have not been mentioned clearly. Our study shows that the viable cells remained constant even cell concentration (in term of cell dry weight) increased. Because during the process, the living cells undergo stress, which result in change in physiology (decrease of global activity and of viability) and possibly in morphology (appearance of cell damage and lysis) (Toma, et al., 2000).

3.3 Effect of Dilution Rate

Figure 4 shows the effect of dilution rate on lactic acid concentration (a), residual substrate (b) and viable cells (c) at initial sugar concentration (C_{S0}) of 40 g/L and feeding concentration (C_{Sf}) of 30 g/L. The results show that about 30 g/L lactic acid concentration and no residual sugar concentration were obtained after 24 h of batch operation. At the operation MBR, it shows that lactic acid concentration at dilution rate (D) of 0.12 h^{-1} (15.7 g/L) was higher than that of 0.07 h^{-1} (14.0 g/L) and the residual concentration at dilution rate of 0.12 h^{-1} was lower than that of 0.07 h^{-1} . The viable cells was remain constant with similar degree for both dilution rates, about 8.69×10^8 CFU/ml for dilution rate of 0.07 h^{-1} and about 7.44×10^8 CFU/ml for dilution rate of 0.12 h^{-1} . Hipolito et al. (2002) have studied the fermentation of lactic acid from glucose by *L. lactis* with sugar concentration of 53 g/L. They found that lactic acid concentration increased from 36.8 g/L to 38.6 g/L, residual sugar decreased from 0.6 g/L to 0.24 g/L as dilution rate increased from 0.44 h^{-1} to 0.5 h^{-1} . They used high cell density at higher dilution rate so that higher lactic acid was obtained.

3.4 Membrane Performance in Single-Stage Membrane Bioreactor

Figure 5 shows the performance of the membrane in MBR operation at feeding sugar concentration (C_{Sf}) of 30 g/L and dilution rate (D) of 0.12 h^{-1} . The recirculation flow was set-up at 4.8 L/min which is equal to 0.31 m/s cross-flow velocity. The membrane was first operated at low transmembrane pressure at 5 kPa. The result shows that cross-flow velocity decreased after 58 h operation and continued to decrease until very low cross-flow

velocity after 106 h operation. This was due to the increase of fermentation broth viscosity since the cells were recycled back to the fermentor. The fermentation was stopped after 120 h. Meanwhile the transmembrane pressure slightly increased during the operation and kept constant at about 10 kPa. Because there was no purging conducted during operation, the fermentation process can not be operated for long period. Therefore, we purposed two-stage membrane bioreactor with purging which will be mentioned in the following section.

In addition, during the membrane bioreactor operation, it was found that lactic acid and residual sugar passed through the membrane, the cell was completely rejected because no viable cells found in the permeate solution.

4. Conclusions

The production of lactic acid from fresh cassava roots by *Streptococcus bovis* using membrane bioreactor was investigated. Single-stage membrane bioreactor (MBR) without purging and two-stages MBR with purging was conducted and the lactic acid concentration and productivity was then compared to batch, fed-batch and continuous fermentation. For single stage MBR, 1.88 g/L.h lactic acid productivity and 15.7 g/L lactic acid concentration were obtained at 0.12 h⁻¹ dilution rate and 30 g/L feeding sugar concentration. For two-stages MBR, at the same dilution rate and feeding concentration, 2.41 g/L lactic acid productivity and 20.1 g/L lactic acid concentration were obtained as the best results at purging ratio of 0.3. Long time operation of two-stages MBR is expected because of the stability of the membrane used. In comparison with other operation modes (batch, fed-batch, continuous), high productivity of lactic acid was obtained by MBR. Therefore, MBR bioreactor operation shows the excellent potential for the production of lactic acid directly from fresh cassava roots by *S. bovis*.

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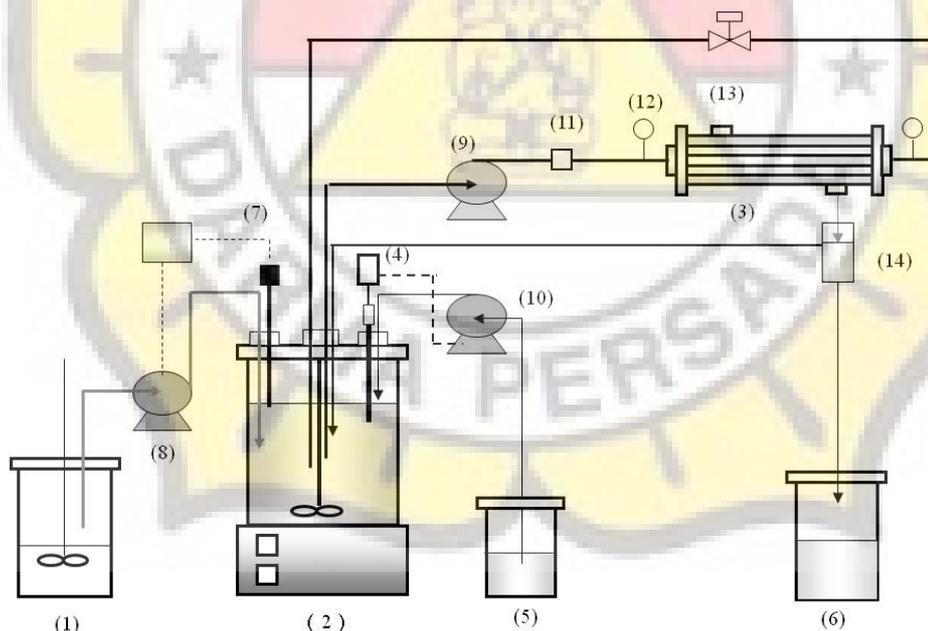


Figure 1. The experimental setup of single-stage membrane bioreactor

(1) feeding tank; (2) fermentor; (3) membrane module; (4) pH electrode; (5) neutralizing agent; (6) product tank; (7) level controller; (8) feeding pump; (9) recycle pump; (10) neutralizing pump; (11) flow meter; (12) pressure gauge; (13) pressure valve; (14) permeate separator

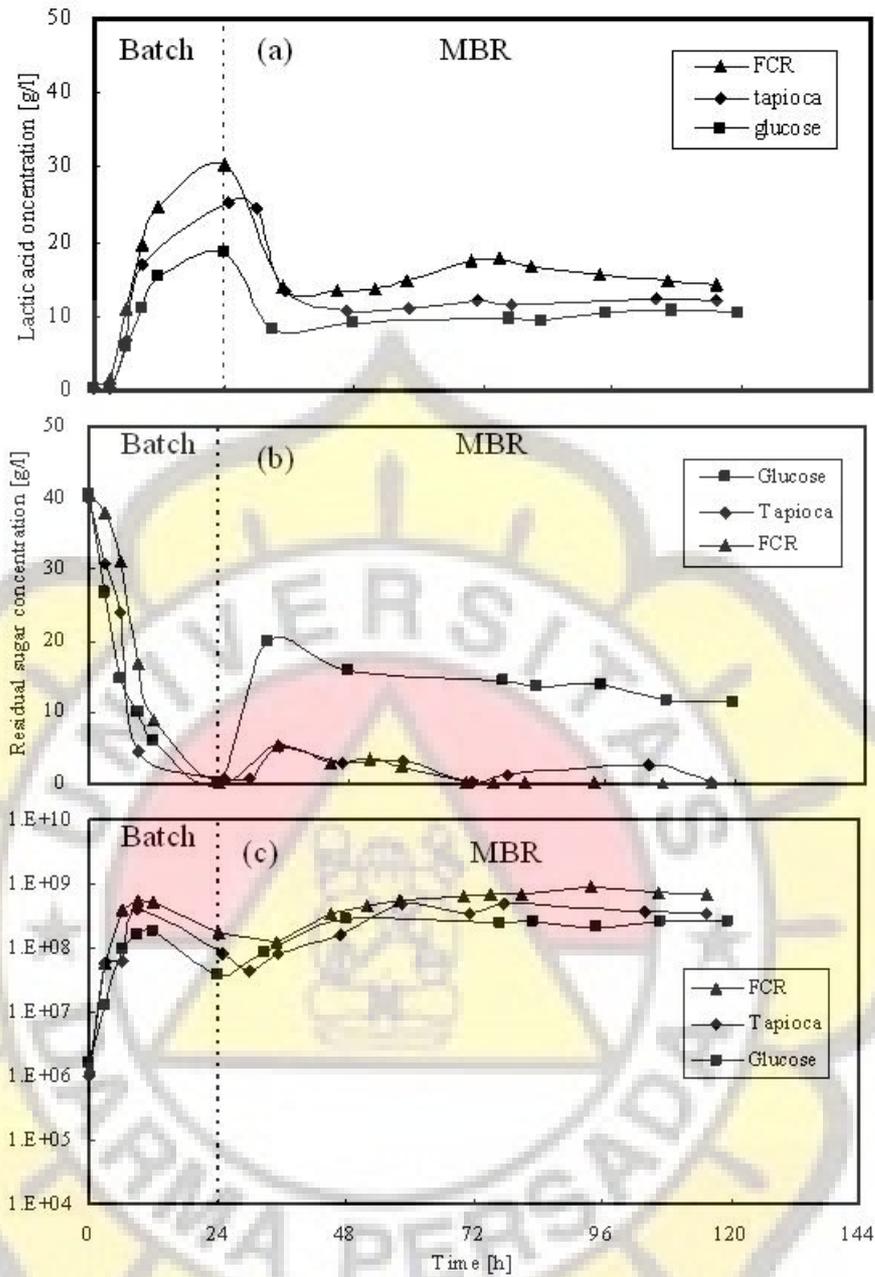


Figure 2. Fermentation of different raw materials using membrane bioreactor at $C_{So} = 40 \text{ g/L}$, $C_{Sf} = 30 \text{ g/L}$, $D = 0.12 \text{ h}^{-1}$: lactic acid concentration (a), residual sugar concentration (b) and viable cells (c)

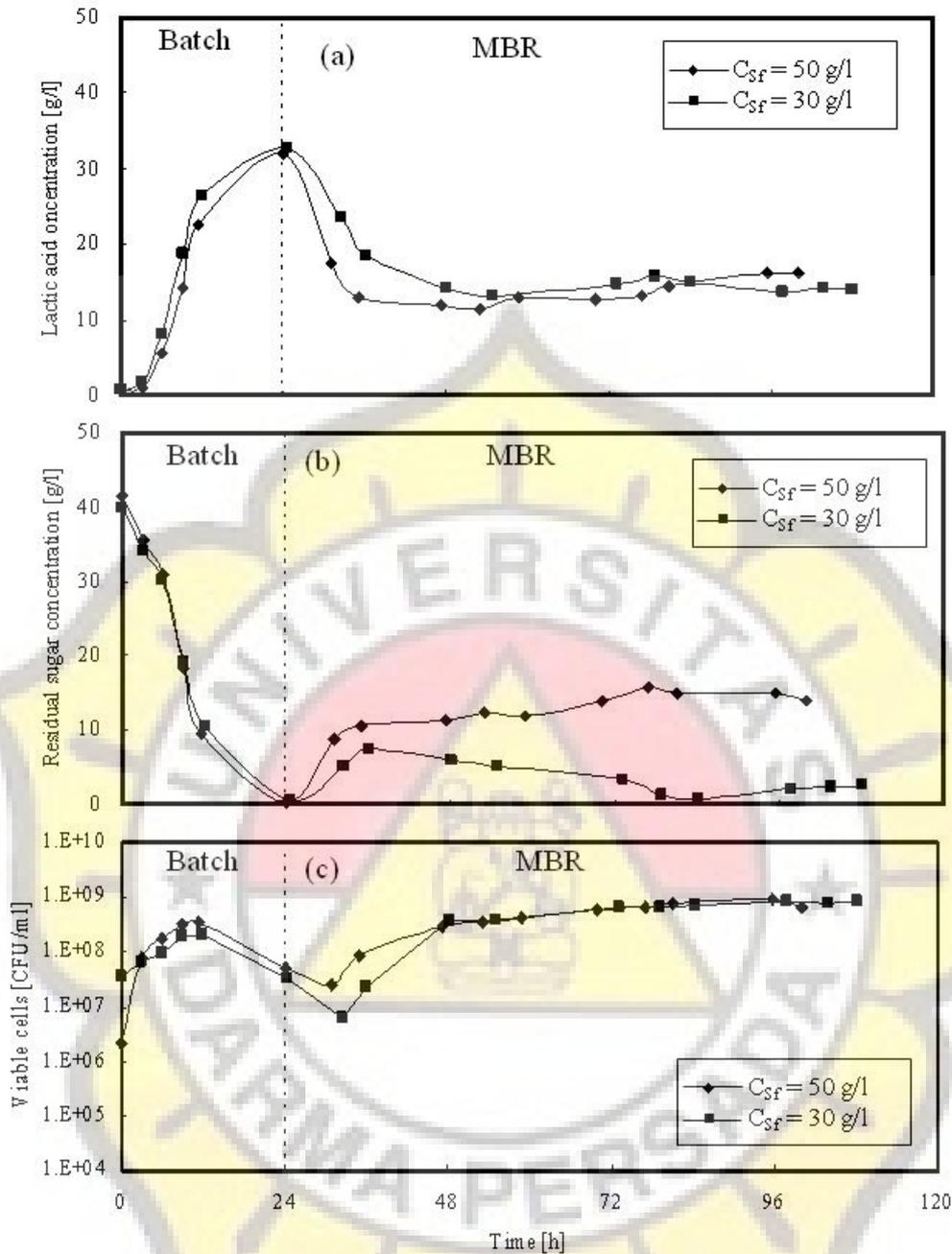


Figure 3. Effect of feed concentration on lactic acid concentration (a), residual sugar concentration (b) and viable cells (c) at $D = 0.07 \text{ h}^{-1}$

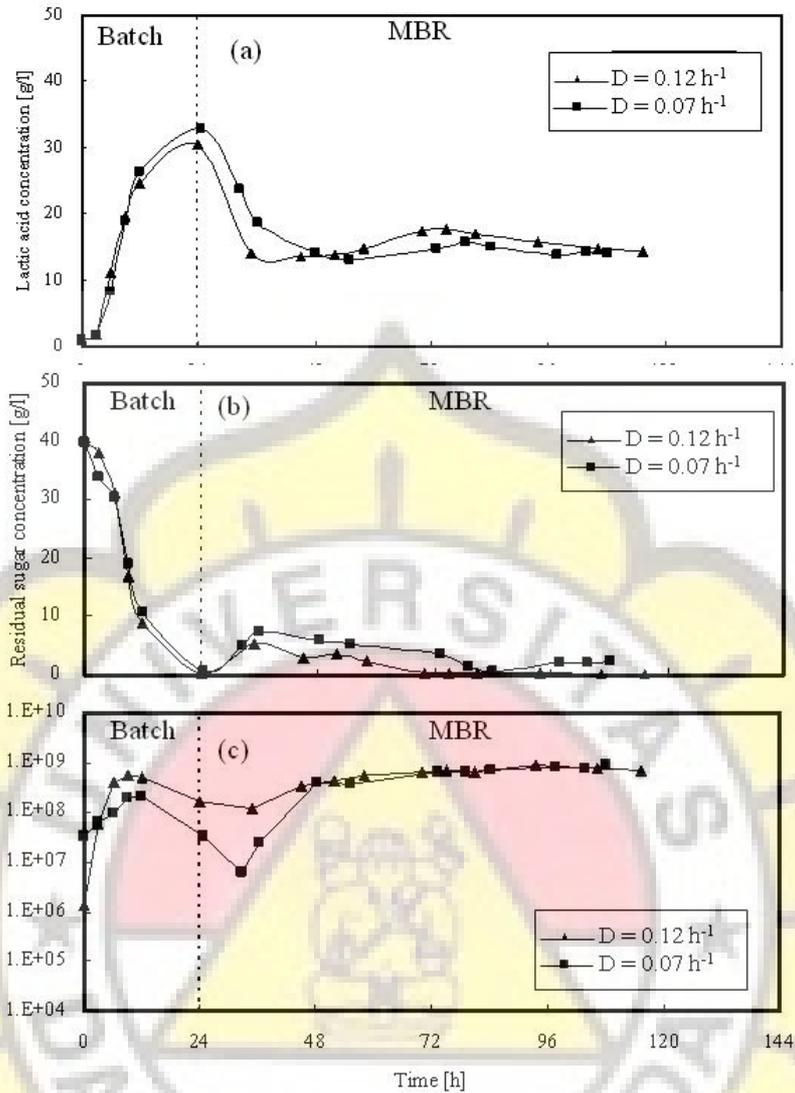


Figure 4. Effect of dilution rate on lactic acid concentration (a), residual sugar concentration (b) and viable cells (c) at $C_{Sf} = 30 \text{ g/L}$

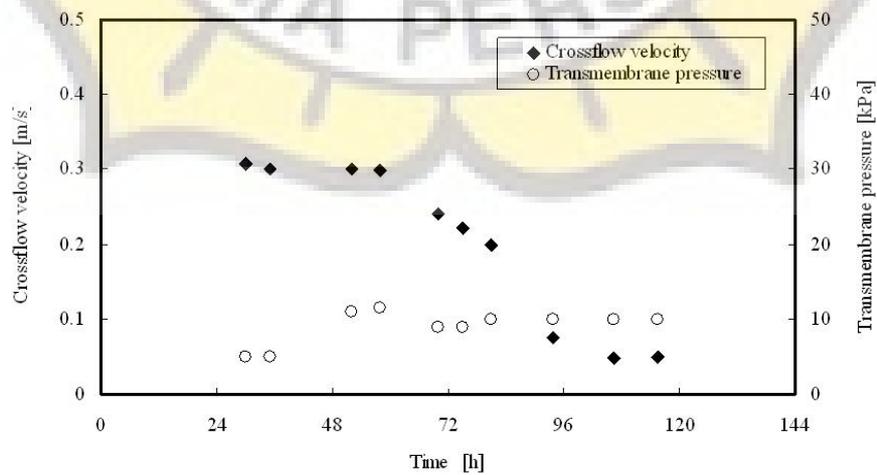


Figure 5. Membrane performance during membrane bioreactor operation at $C_{Sf} = 30 \text{ g/L}$ and $D = 0.12 \text{ h}^{-1}$