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Lactic Acid Fermentation in Cell-Recycle Membrane Bioreactor

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Abstract

Traditional lactic acid fermentation suffers from low productivity and low product purity. Cell-recycle fermentation has become one of the methods to obtain high cell density, which results in higher productivity. Lactic acid fermentation was investigated in a cell-recycle membrane bioreactor at higher substrate concentrations of 100 and 120 g/dm^3. A maximum cell density of 145 g/dm^3 and a maximum productivity of 34 g/(dm^3·h) were achieved in cell-recycle fermentation. In spite of complete consumption of substrate, there was a continuous increase in cell density in cell-recycle fermentation. Control of cell density in cell-recycle fermentation was attempted by cell bleeding and reduction in yeast extract concentration.

Index Entries: Lactic acid fermentation; cell-recycle reactor; membrane bioreactor; cell bleeding; continuous fermentation; high cell density.

Introduction

There has been a renewed interest in recent years in the production of lactic acid by fermentation as new applications of lactic acid, such as biodegradable polymers, have evolved that require optically pure lactic acid at a low price, which can be possible through the fermentation process. However, the conventional fermentation process suffers from various limitations such as low cell density, low substrate conversion, and low product concentration, besides cell washout at higher dilution rates *(1)*.

One of the areas needing improvement in continuous fermentation is increasing the cell density in the bioreactor, which, in turn, enhances the substrate-to-product conversion rate, resulting in higher productivity *(2–16)*. Higher cell densities have been achieved in the fermentation process

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either by the use of cell immobilization or by cell-recycle using membrane. In addition to the operation of continuous fermentation at a higher dilution rate without cell washout, cell recycle with membranes yields a cell-free fermentation broth that can be directly used in downstream processing *(1)*.

The earliest report on the use of membrane recycle in lactic acid fermentation was by Freidman and Gaden in 1970 *(17)*. Subsequent publications on cell-recycle lactic acid fermentation have concentrated on various aspects, such as the effect of dilution rate on productivity *(2–4,6,14)*, the effect of cell bleeding *(3,4,13)*, the effect of nitrogen concentration *(6)*, the effect on microbial physiology owing to high cell density *(12,13)*, the effect of membrane materials and fouling *(5)*, byproduct formation and substrate limitation conditions *(12,13,18)*, and the effect of cell recycle along with electrodialysis or ion exchange *(7,11,15)*. A two-stage continuous fermentation with membrane recycle has been studied recently that enhanced lactic productivity from 21.6 g/(dm^3·h) in single stage to 57 g/(dm^3·h) in two stages *(10)*. Nishiwaki and Dunn *(19)* numerically studied continuous production of lactic acid in two-stage fermentation with two different configurations.

In this article, we present the results of a study on the effects of some operational parameters on the productivity of continuous lactic acid fermentation with a membrane cell-recycle system.

Materials and Methods

Micro-organism

Lactobacillus rhamnosus NRRL B445 was obtained from the TISTR Culture Collection Center, Bangkok. It was maintained as a stab culture in a solid agar mineral medium with glucose. The pH of the medium was maintained at 6.0. The organism was subcultured every month at 42°C for 24 h and stored at 4°C.

Media

Table 1 presents the compositions of the media used for development of the inoculum and main production. For cell-recycle studies, the glucose concentration was maintained at 100 or 120 g/dm^3, keeping the composition of the other components the same.

Development of Inoculum

The inoculum for the fermentation was developed in two stages. In the first stage, sterile inoculum medium (Table 1) was inoculated with a loopful of cells from a freshly cultured slant in 50-mL rubber-stoppered bottles and incubated at 42°C for 12 h (static culture). The medium used for the second-stage inoculum was the same as the production medium but with the suc-

Table 1
Composition of Medium for Inoculum and Production

Component	Medium for first-stage inoculum (g/dm^3)	Production medium (g/dm^3)
Glucose	50	100–120
Yeast extract	15	16 and 3.2
$MnSO_4 \cdot H_2O$	0.03	0.03
$MgSO_4 \cdot 7H_2O$	0.1	0.1
NaOH	6.2	1.25
KH_2PO_4	0.2	0.2
K_2HPO_4	0.2	0.2
Succinic acid	11.5	2

cinic acid concentration at 11.5 g/dm^3. It was inoculated with 3% (v/v) cells from the first stage and incubated at 42°C for 12 h under static conditions.

Continuous Cell-Recycle Fermentation

Continuous fermentation was carried out in a 2-L fermentor (Virtis Omni) with 3% (v/v) inoculum from the second stage. The pH and temperature of the medium were controlled at 6.3 and 42°C, respectively. The pH was controlled by adding 12% ammonia solution. Nitrogen gas was sparged initially in the medium for 1 to 2 min, and mixing was provided by an agitator (400 rpm).

For cell recycle, a tangential microfiltration system (Sartocon®-Mini) consisting of a polypropylene membrane (0.1-μm pore size, 0.1-m^2 area) in a stainless steel housing was connected to the fermentor through a peristaltic pump (Cole Parmer, Vernon Hills, IL). Figure 1 presents a schematic flow diagram of the experimental setup.

Initially, the reactor was operated in batch mode for 10–12 h. When sufficient biomass was grown, continuous fermentation was started with simultaneous recirculation of the broth through the membrane. The recirculation rate through the membrane was maintained between 60 and 90 $dm^3 \cdot h^{-1}$. Samples were collected at regular intervals up to 80–90 h and analyzed for biomass, glucose, and lactic acid. The biomass was separated from the fermentation broth by centrifuging at 6 to 7°C, and then it was appropriately diluted with distilled water to have an optical density within a value of 0.1–0.9. The cell density was estimated by measuring the optical density of the suspension at 610 nm using a UV-VIS spectrophotometer (UV-1601PC; Shimadzu) and was expressed as dry cell weight per unit volume through a standard calibration curve. Glucose and lactic acid were analyzed by high-performance liquid chromatography (LC10-AD; Shimadzu) using an ion-exchange resin column (SCR101H; Shimadzu) and an RID detector (RID6A; Shimadzu, Kyoto, Japan). Perchloric acid (10 m*M*) was used as the mobile phase at a flow rate of 0.9 mL/min. During analysis, the column temperature was maintained at 50°C.

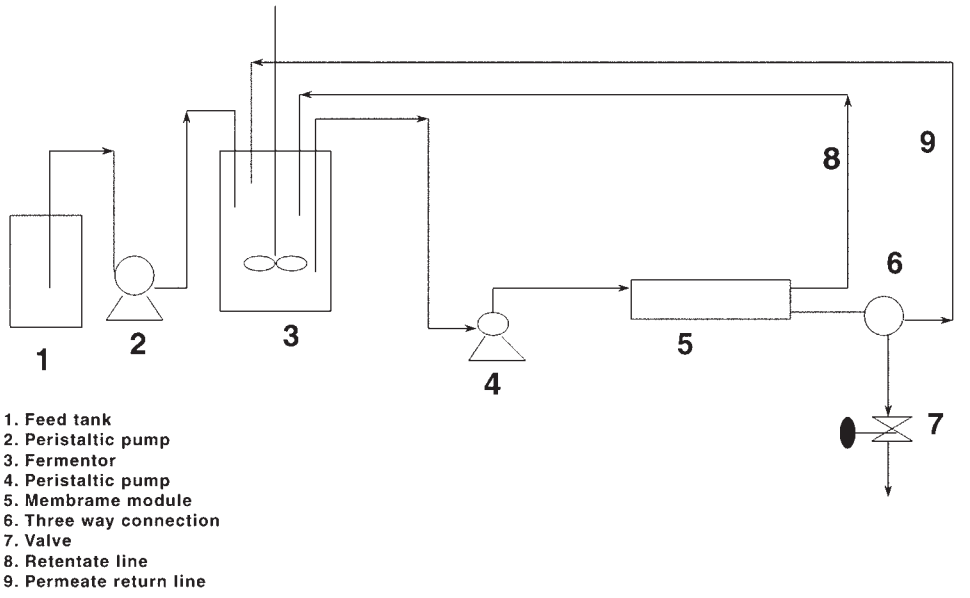

Fig. 1. Schematic of experimental system.

Results and Discussion

The performance of a cell-recycle fermentation is usually affected by various operational parameters such as dilution rate, substrate concentration, and cell concentration. In the present study, we investigated the effects of these parameters on cell-recycle lactic acid fermentation.

Effect of Dilution Rate

Dilution rate is one of the critical parameters for continuous fermentation. Cell-recycle fermentation was carried out with a total working volume of 2 L (including the membrane module volume) at two dilution rates (0.2 and 0.409 h^{-1}). The results (Fig. 2) show that the cell growth increased significantly with increasing dilution rate as the fermentation progressed. At both dilution rates, the feed glucose was completely utilized after 40–45 h of fermentation (Fig. 3). The product concentrations achieved at both dilution rates were nearly similar. The productivity for the dilution rate of 0.409 h^{-1} (34.7 g/[dm^3·h]) was double the productivity for the dilution rate of 0.2 h^{-1} (16.4 g/[dm^3·h]) Product yield coefficients based on substrate consumption were about 0.85 at both dilution rates. Because the product concentrations and substrate conversions were similar at both dilution rates, it can be concluded that the higher productivity and biomass growth observed at the dilution rate of 0.409 h^{-1} was owing to higher substrate feeding rate. It should, however, be noted that at both dilution rates, a steady-state cell concentration was not attained even though the substrate was totally consumed after 40 h. Xavier et al. *(14)* and Yoo et al. *(13)* reported

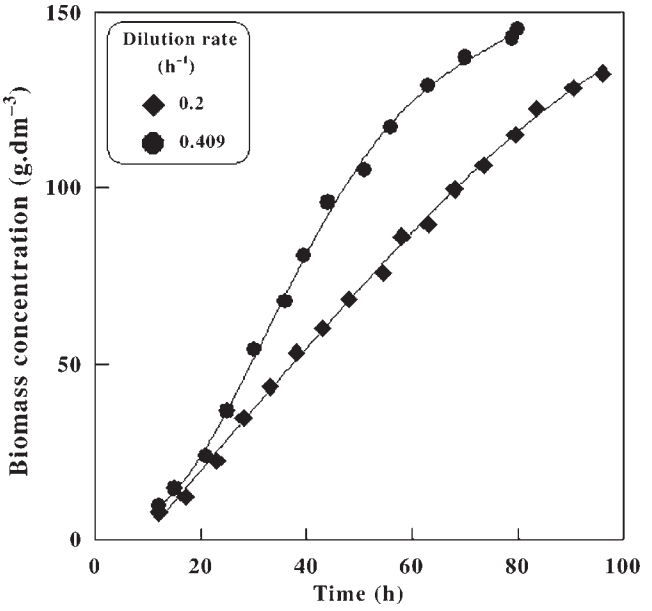

Fig. 2. Biomass growth profiles in cell-recycle fermentation at two dilution rates with 100 g/dm^3 of feed glucose concentration.

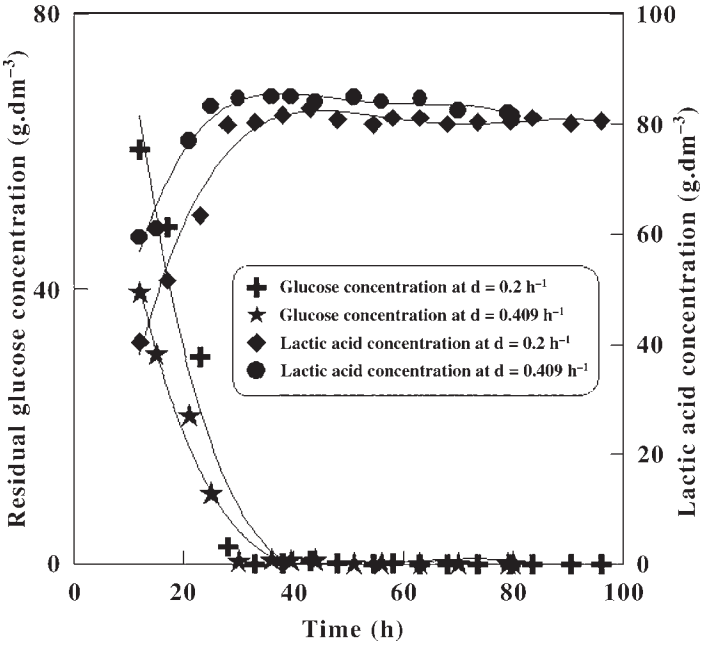

Fig. 3. Lactic acid production and substrate uptake profiles in cell-recycle fermentation at two dilution rates with 100 g/dm^3 of feed glucose concentration.

a similar phenomenon. Xavier et al. *(14)* observed that although the residual substrate was available in the fermentation medium the lactic acid concentration did not increase and biomass growth continued even after 120 h of fermentation. Yoo et al. *(13)* also reported continued biomass growth even after all the glucose was consumed in cell-recycle lactic acid fermentation with *Lactobacillus casei*. In another study on cell-recycle lactic acid fermentation by Hjorleifsdottir et al. *(12)*, it was observed that at a feed glucose concentration of 25 g/dm^3 cell concentration appeared to approach a constant value and glucose was completely consumed, whereas at 50 g/dm^3 cell growth continued even after 150 h of fermentation with unutilized glucose in the broth.

A theoretical analysis was carried out to determine the maximum cell concentration for which the maintenance requirements of the cells can be met from the glucose feed. Using an experimentally determined maintenance coefficient of 0.33 g/(g·h) at dilution rates of 0.2 and 0.409 h^{-1} with 100 g/dm^3 of glucose feed, maintenance requirements of cells can be met up to cell concentrations of 60.6 and 124 g/dm^3, respectively. In other words, no substrate will be available for growth of cell biomass after these concentrations. However, at both dilution rates the cell concentration had increased much higher than these values. Hence, it can be concluded that either a large fraction of cells was in inactive form or some other nutrient source (yeast) was used for cell growth. Inactivation of cells can be owing to various stress factors, such as high shear or high levels of product or substrate concentrations. Theoretically, it has also been shown that various stress factors need to be accounted for during modeling of cell-recycle lactic acid fermentation for better fitting of experimental data *(20)*. However, this could not be confirmed in the present study, because the viable and inactive cell proportions were not measured. Richter and Nottelmann *(6)* experimentally confirmed that a certain part of biomass was inactive in continuous lactic acid fermentation with total cell retention. Xavier et al. *(14)* observed an increase in volumetric productivity with an increase in dilution rate up to 0.4 h^{-1} in their study on lactic acid fermentation using *L. rhamnosus*. However, on further increase in dilution rate to 0.58 h^{-1}, they did not obtain any increase in the productivity owing to lower product concentration. Further, they experienced difficulties in maintaining membrane flux at the higher dilution rate (0.58 h^{-1}). An increase in the viscosity of the broth and severe membrane fouling led to an early termination of fermentation. Mehia and Cheryan *(4)* found that an increase in dilution rate at a constant cell density decreased the substrate conversion and lactic acid concentration. Operation of cell-recycle fermentation at a higher dilution rate requires higher permeate flux, which can be achieved only with a higher energy input and membrane area and concomitant increase in cost.

Effect of Feed Glucose Concentration

Since an increase in productivity was restricted by the limiting dilution rate, an attempt was made to improve lactic acid productivity by

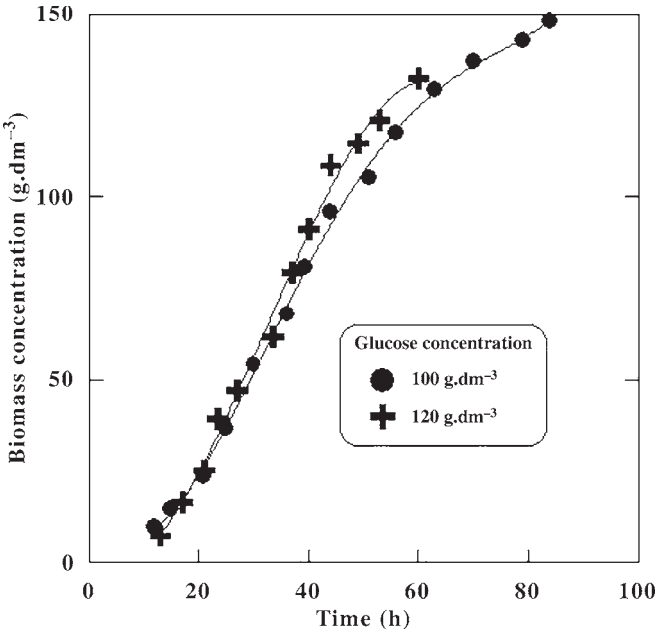

Fig. 4. Biomass growth profiles in cell-recycle fermentation at two feed substrate concentrations and dilution rate of 0.409 h^{-1}.

increasing the feed glucose concentration at the same dilution rate (0.409 h^{-1}). The biomass growth profiles at the two glucose concentrations of 100 and 120 g/dm^3 were almost the same (Fig. 4) but still did not reach a steady-state value. Figure 5 shows that lactic acid concentration at higher glucose concentration picked up slowly and reached a slightly higher value (92 g/L) than achieved at lower glucose concentration (80 g/L). The feed glucose was completely consumed and the lactic acid concentration reached a steady state after 40 h of fermentation at lower glucose concentration. At higher glucose concentration, however, the glucose conversion was not complete, but the lactic acid concentration approached a steady value after 60 h. In batch studies with the same culture, complete substrate conversion was noticed even at a glucose concentration of 150 g/L. Xavier et al. *(14)* also observed incomplete substrate conversion at 120 g/dm^3 at a dilution rate of 0.4 h^{-1}. Increasing the feed glucose concentration resulted in a marginally higher product yield coefficient based on substrate consumed, 0.862 g/g, with a corresponding productivity of 36.88 g/(dm^3·h). The lactic acid productivity achieved in the present study was less than the Kwon et al. *(10)* study with two-stage cell-recycle fermentation, but final lactic acid concentration achieved in the present study was comparable.

Effect of Cell Bleeding

In the previous section, it was stated that although the steady-state glucose and product concentrations were achieved, the growth of biomass

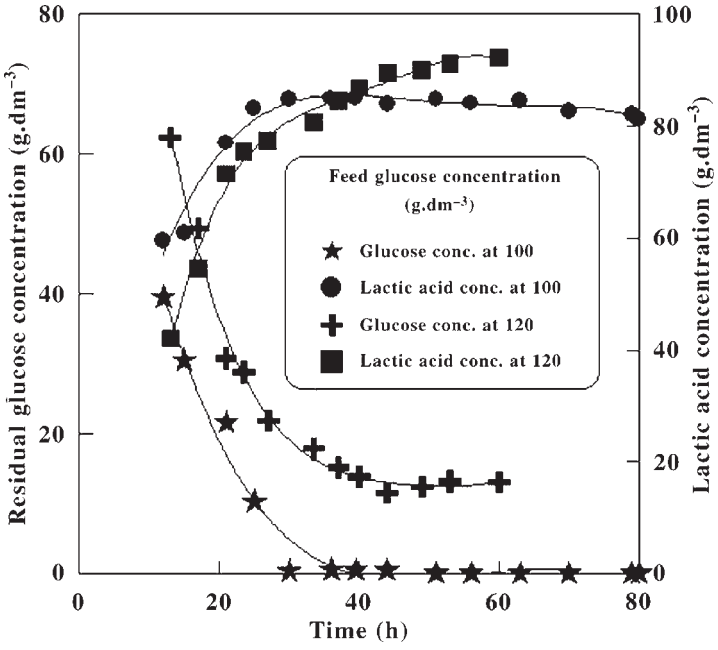

Fig. 5. Lactic acid production and substrate uptake profiles in cell recycle fermentation at two feed substrate concentrations and dilution rate of 0.409 h^{-1}.

never ceased. Biological processes are usually operated in the active state of cells by maintaining a constant cell density in the bioreactor. Bleeding has been used as a control strategy to obtain constant cell density in many cell-recycle fermentation studies *(3,4,13)*. In the present study, cell bleeding was done either continuously or intermittently from the fermentor. In one case, continuous cell bleeding was started after 90 h of fermentation at a rate of 50 mL/h using a peristaltic pump. In another case, the cell bleeding was started when the residual glucose concentration approached zero (40 h) and was done intermittently at a flow rate of 40 mL/h. The results for continuous and intermittent cell bleeding (Figs. 6–8) show that the fermentation performances were quite different in both cases. In the case of continuous cell bleeding, the biomass concentration declined rapidly immediately after the initiation of bleeding (Fig. 6). It continued to decline and a steady state was not attained even after 6 to 7 reactor volume had passed through. Simultaneously, there was a sharp increase in the residual substrate concentration, indicating poor conversion and the corresponding decrease in lactic acid concentration. With intermittent cell bleeding, the cell density continued to increase and reached a constant value after some time (Fig. 7). The corresponding residual glucose and lactic acid profiles in Fig. 8 show that a steady state was reached in both these parameters, with the complete recycle as well as the intermittent-bleeding systems. However, in the case of intermittent cell bleeding, the substrate conversion was incomplete, resulting in a higher level of steady-state substrate concentra-

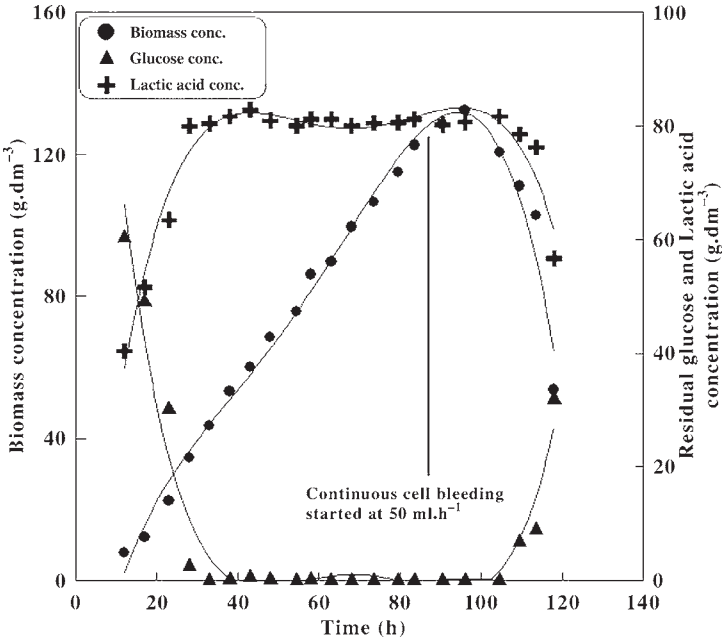

Fig. 6. Biomass growth, substrate uptake, and lactic acid production profiles in cell-recycle fermentation with continuous cell bleeding (50 mL/h) at dilution rate of 0.2 h^{-1} and feed glucose concentration of 100 g/dm^3.

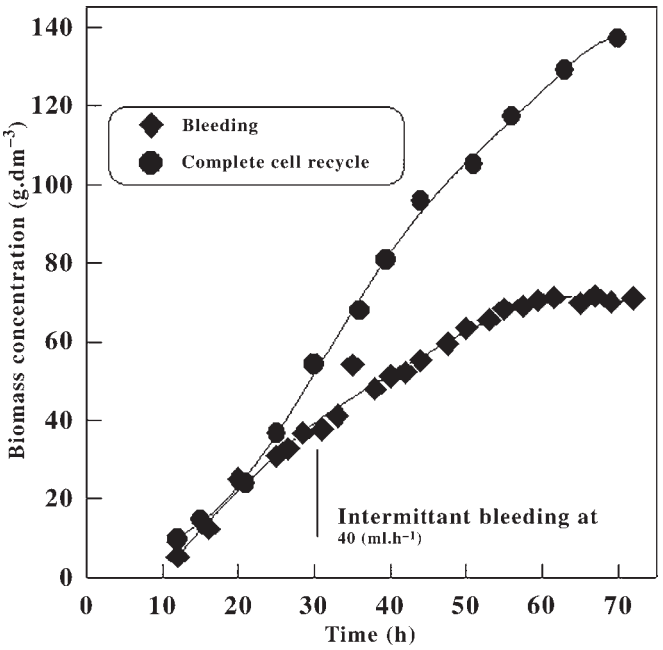

Fig. 7. Biomass growth profiles in cell-recycle fermentation with and without intermittent cell bleeding (40 mL/h) at dilution rate of 0.2 h^{-1} and feed glucose concentration of 100 g/dm^3.

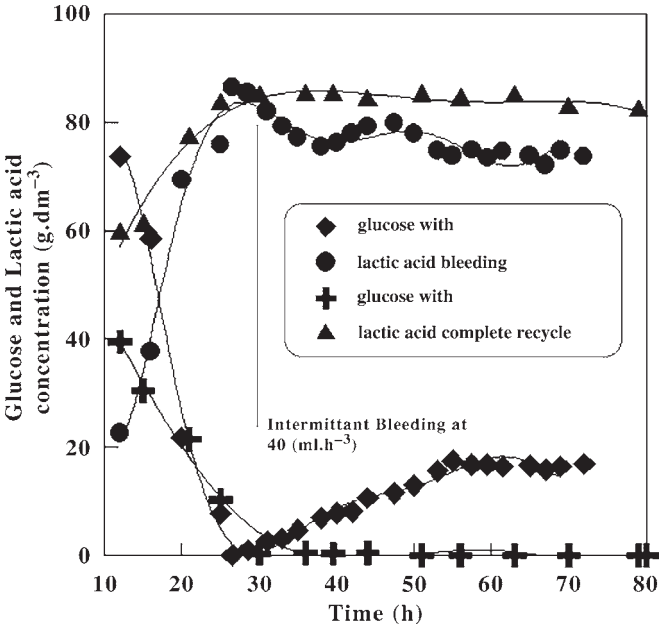

Fig. 8. Lactic acid production and substrate uptake profiles in cell-recycle fermentation with and without intermittent cell bleeding (40 mL/h) at dilution rate of 0.2 h^{-1} and feed glucose concentration of 100 g/dm^3.

tion and corresponding decrease in product concentration. By controlling the rate of bleeding, it might have been possible to get complete substrate conversion and a higher product concentration. However, Yoo et al. *(13)* and Hjorleifsdottir et al. *(12)* reported that a small level of substrate concentration in the broth may be advantageous in avoiding the formation of byproducts. Ohleyer et al. *(3)* reported that with an increase in bleeding ratio, steady-state biomass concentration decreased in cell-recycle lactic acid fermentation with *Lactobacillus delbrueckii.* These results, however, prove that cell bleeding at the early phase of growth can be useful as an effective control parameter to achieve a constant cell density.

Effect of Yeast Extract Concentration in Cell-Recycle Fermentation

Since our batch fermentation studies showed that the yeast extract concentration has a significant effect on biomass growth, we thought that it may be used to control the cell density in the cell-recycle system. Oh et al. *(16)* also reported that yeast extract concentration plays an important role in lactic acid fermentation with *Enterococcus faecalis* RKY1. Their results showed that lactic acid productivity is linearly correlated with yeast extract concentration up to 25 g/dm^3. In the case of a cell-recycle membrane bioreactor with *Lactobacillus paracasei* ATB 160111, an increase in peptone and yeast extract concentration beyond 15.9 g/dm^3 did not improve lactate productivity *(6)*. In the present study, cell-recycle fermentation was started with 16 g/dm^3 of yeast extract at a feed glucose concentration of 100 g/dm^3,

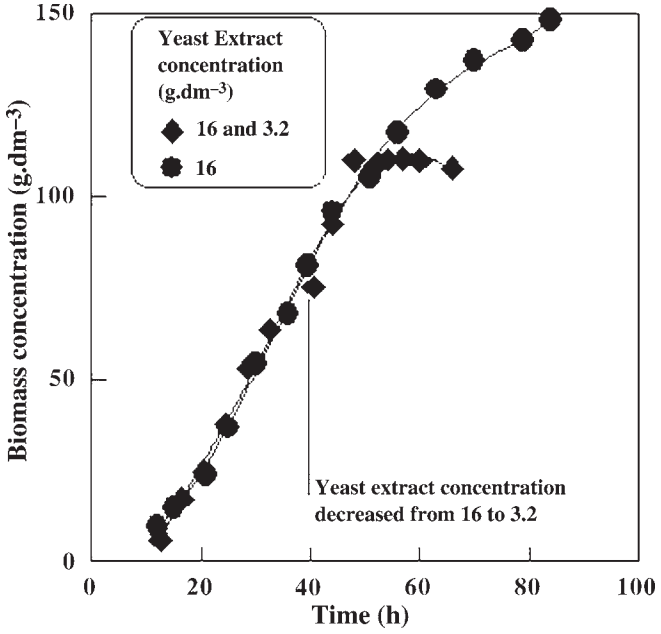

Fig. 9. Biomass growth profiles in cell-recycle fermentation with normal and reduced yeast extract concentration at dilution rate of 0.2 h^{-1} and feed glucose concentration of 100 g/dm^3.

and after 40 h of fermentation, yeast extract concentration was decreased to 3.2 g/dm^3. If this strategy becomes successful, it will also help to reduce the cost of production.

From the results (see Fig. 9), it was observed that the cell growth continued after the reduction in yeast extract concentration but appeared to stabilize after a short time. The steady-state cell density, 110 g/dm^3, was higher than the steady-state value obtained with intermittent bleeding. Surprisingly, however, the residual glucose concentration increased and the lactic acid concentration decreased sharply immediately after decreasing the yeast extract concentration (Fig. 10). This resulted in a corresponding reduction in productivity. Furthermore, the residual glucose and lactic acid concentrations did not reach a steady state even after 20 h. This behavior is quite contrary to the observation of Ohleyer et al. *(3)*, who reported that decreasing the concentration of yeast extract to one-tenth of its initial value did not affect the steady-state performance of the cell-recycle fermentation. In the present study, a decrease in yeast extract concentration seemed to result in arresting the biomass growth rate, but there was a concomitant decrease in final lactic acid concentration and substrate conversion. It is therefore possible that the yeast extract not only served as a source of building blocks for biomass growth but also probably supplied metabolic energy for macromolecular synthesis. The possibility of yeast extract being involved in the catabolic pathway at a higher cell density cannot be ruled out. Thus, by decreasing the yeast extract, this extra source of metabolic

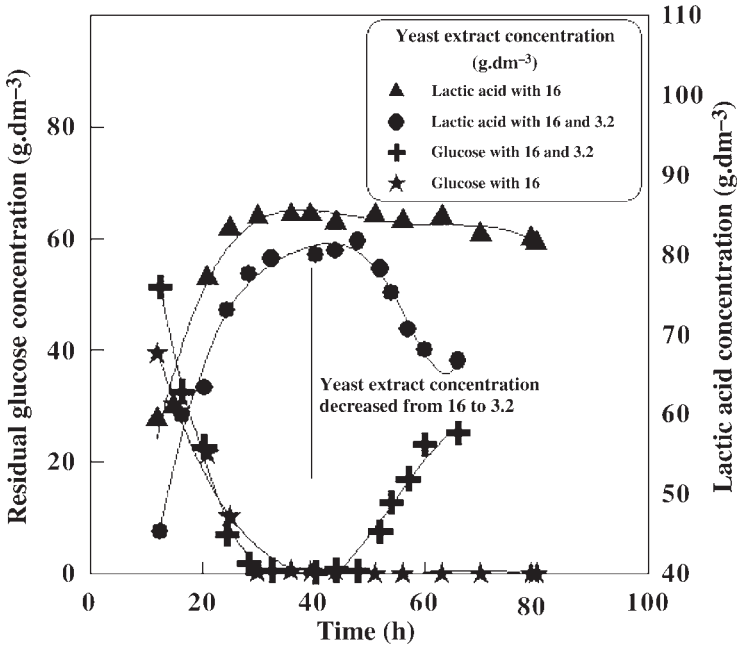

Fig. 10. Lactic acid production and substrate uptake profiles in cell-recycle fermentation with normal and reduced yeast extract concentration at dilution rate of 0.2 h^{-1} and feed glucose concentration of 100 g/dm^3.

energy was affected, resulting in a decrease in biomass growth and simultaneous product formation rate.

Conclusion

Cell recycle using membranes can help enhance the productivity of lactic acid fermentation by providing higher cell densities. Although a steady-state lactic acid concentration may be reached in the complete recycle process, the cell density never reached a constant value. Intermittent cell bleeding can be used to control cell density but at a reduced lactic acid concentration. Reducing the nitrogen source (yeast extract) concentration also controlled the cell density but severely affected productivity.

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