0273-2289/03/105-108/0603/$20.00

Lactic Acid Production Through Cell-Recycle Repeated-Batch Bioreactor

Hurok Oh, Young-Jung Wee, Jong-Sun Yun, and Hwa-Won Ryu*

Faculty of Applied Chemical Engineering, Institute of Bioindustrial Technology, Chonnam National University, Gwangju 500-757, Korea, E-mail: hwryu@chonnam.ac.kr

Abstract

The effect of various nitrogen sources on cell growth and lactic acid production was investigated. The most effective nitrogen source was yeast extract; more yeast extract gave higher cell growth and lactic acid productivity. Yeast extract dosage and cell growth were proportional up to a yeast extract concentration of 30 g/L, and lactic acid productivity was linearly correlated up to a yeast extract dosage of 25 g/L. However, increasing the yeast extract content raises the total production cost of lactic acid. Therefore, we attempted to find the optimum yeast extract dosage for a repeated-batch operation with cell recycling. The results show that when using *Enterococcus faecalis* RKY1 only 26% of the yeast extract dosage for a conventional batch fermentation was sufficient to produce the same amount of lactic acid, whereas the lactic acid concentration in the product stream (92–94 g/L) and lactic acid productivity (6.03–6.20 g/[L·h]) were similar to those of a batch operation. Furthermore, long-term stability was established.

Index Entry: Lactic acid; repeated-batch; cell-recycle; hollow-fiber module; nitrogen source; yeast extract.

Introduction

Lactic acid ($CH_3CHOHCOOH$) is an organic hydroxy acid with widespread industrial applications. Recently, much attention has been paid to lactic acid as a monomer for biodegradable plastic. Between the two methods for producing lactic acid, fermentation is reported to be more favorable than the chemical synthetic method, because of its environmental friendliness and selectable production of a single enantiomer *(1–4)*.

However, there are two major problems that make the biological production of lactic acid economically unfavorable. One is the fastidious

*Author to whom all correspondence and reprint requests should be addressed.

nutrient requirements of lactic acid bacteria, because of their inability to biosynthesize some essential amino acids and vitamins. The other is end-product inhibition by the accumulated lactic acid *(5–8)*.

Most lactic acid bacteria require complex nutrient sources, such as yeast extract and peptone, which are usually expensive. According to Tejayadi and Cheryan *(9)*, the nitrogen source costs about 38% of the total production cost.

To solve these problems some investigators studied inexpensive nutrient sources, such as barley, wheat, cell autolysate, and soybeans *(10–12)*. Others tried to develop a more efficient process, mainly by improving the lactic acid productivity with high-cell-density cultures using cell recycling with or without an *in situ* separation apparatus *(13,14)*.

In the present study, we characterized a cell-recycle repeated-batch operation using a hollow-fiber filtration module to simultaneously reuse the produced biomass and reduce the yeast extract dosage. The study focused on the effects of various nitrogen sources, followed by characterizing the effects of the yeast extract dosage during batch operation. The minimum yeast extract dosage during a repeated-batch operation with cell recycling giving high cell growth and lactic acid productivity was also determined.

Materials and Methods

Strains and Culture Media

The homofermentative L (+)-lactic acid producer, *Enterococcus faecalis* RKY1 *(15–18)* was used. Stock cultures were stored in the culture medium with 50% glycerol at –20°C. The composition of the growth medium or inoculum was 30 g/L of glucose, 10 g/L of yeast extract, and 10 g/L of K_2HPO_4. The fermentation medium was composed of 30–150 g/L of glucose, 0–40 g/L of yeast extract, and no additional nutrients.

Batch Fermentation

In preparation for experiments, 1 mL of a stock culture was inoculated into a 20-mL vial containing 15 mL of growth medium, and cultivated at 38°C and 200 rpm for 10 h. After three consecutive propagation steps, 2 mL of the culture broth was transferred to a 50-mL vial containing 40 mL of sterilized growth medium for vial cultivation or inoculum preparation for 2.5-L jar fermentor experiments. In the vial experiments, the pH was adjusted by manually adding 2 *M* Na_2CO_3. Temperature and agitation speed were 38°C and 200 rpm, respectively.

Fermentations were performed using a 2.5-L jar fermentor (KF-2.5L; Korea Fermentor, Inchon, Korea) with automatic temperature, pH, and agitation speed controls. During the batch fermentations, 4% (v/v) inoculum was transferred to a 2.5-L jar fermentor containing 1 L of sterilized medium. The temperature was maintained at 38°C, with a constant agitation speed of 200 rpm, and pH of 7.0, controlled by 10 *M* NaOH.

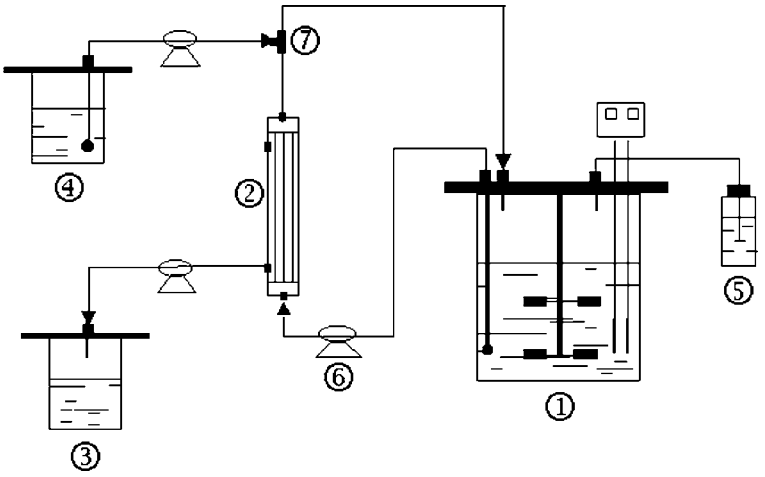

Fig. 1. The schematic diagram of cell-recycle membrane bioreactor. 1, Bioreactor; 2, hollow-fiber membrane filtration module; 3, product storage tank; 4, fresh medium storage tank; 5, neutralizer reservoir; 6, peristaltic pump; and 7, three-way valve.

Cell-Recycle Repeated-Batch Operation

Repeated-batch operation with cell recycling was performed in the same fermentor as the batch operation, but with a hollow-fiber module (SKUF-103-0830; SK Chemical, Suwon, Korea). Figure 1 shows a schematic diagram of the membrane cell-recycle bioreactor. The filtration module contains about 100 fibers, with a 25-mm id and 300-mm length. The nominal molecular weight cutoff of the membrane was 30 kDa and the total surface area was 0.06 m^2.

Nutrient-rich medium (containing 15 g/L of yeast extract) was used during the first batch operation. After depletion of glucose of the first batch run, 90% (v/v) of the culture broth was withdrawn through the hollow-fiber module and the same volume of fresh medium was fed into the reactor. Subsequent batch cultures were performed in the same manner.

Analytical Methods

To determine cell growth the optical density was measured using a spectrophotometer (UV-160A; Shimadzu, Tokyo, Japan) and converted to a dry cell weight with a precalculated standard curve.

Glucose concentration was enzymatically measured by the glucose oxidase–peroxidase method using a glucose E-kit (Young-Dong Pharmaceuticals, Seoul, Korea).Lactic acid concentration was quantified using a high-performance liquid chromatograph (Waters486; Millipore, Bedford, MA) with an Aminex HPX-87H ion-exclusion column (Bio-Rad, Hercules, CA) and a UV detector set at 210 nm. The mobile phase was 5 m*M* H_2SO_4, at a flow rate of 0.6 mL/min. The temperature of the column was maintained at 35°C.

Table 1
Effect of Various Nitrogen Sources on Cell Growth and Lactic Acid Productivity During Batch Cultivation of *E. faecalis* RKY1[a]

Nitrogen sources	Cell growth (g/L)	Lactic acid (g/L)
None	0.20	3.42
Yeast extract	6.71	23.87
Bactopeptone	4.69	18.35
Polypeptone	4.62	16.89
Corn steep liquor	3.55	16.82
Corn steep solid	1.07	9.14
Beef extract	3.17	16.80
Pharmamedia	0.74	8.36
Soybean flour	0.68	7.22
Cottonseed flour	0.56	6.87
Malt extract	0.48	5.22
Sodium-L-glutamate	0.33	4.37
Ammonium sulfate	0.43	5.24
Ammonium chloride	0.35	5.23
Urea	0.30	4.18

[a]Culture conditions: 50-mL serum bottle containing 40 mL of medium (with 10 g/L of each nitrogen source) at 38°C, 200 rpm, for 10 h.

Results

Effect of Nitrogen Sources

To investigate the effect of various nitrogen sources on lactic acid fermentation, organic, inorganic, and several cereal-derived nitrogenous materials were tested. Table 1 presents the results of 50-mL serum bottle experiments containing 10 g/L of each nitrogen source.

The highest dry cell weight (6.7 g/L) and overall lactic acid productivity (2.4 g/[L·h]) were recorded when yeast extract was used as the nitrogen source. Bactopeptone, polypeptone, corn steep liquor and beef extract showed comparable results. Next to those nitrogen sources were corn steep solid and some cereal-based materials, which showed some possibilities as substitutive nitrogen sources. However, adding single organic or inorganic substrates, such as sodium L-glutamate, ammonium sulfate, ammonium chloride, and urea, promoted little in both the cell growth and lactic acid production.

Effect of Yeast Extract Dosage on Batch Cultivation

Because yeast extract was proven to be the most effective nitrogen source, we investigated the influence of yeast extract dosage during batch cultivation with the 2.5-L fermentor experiments. Figure 2 presents cell growth and lactic acid production in the medium with 100 g/L of glucose and 0–40 g/L of yeast extract. As the yeast extract dosage was increased, the dry cell weight and lactic acid production rate increased linearly up to 30 and 25 g/L of yeast extract, respectively. For yeast extract dosage >30 g/L, however, both cell growth and lactic acid production rate did not increase.

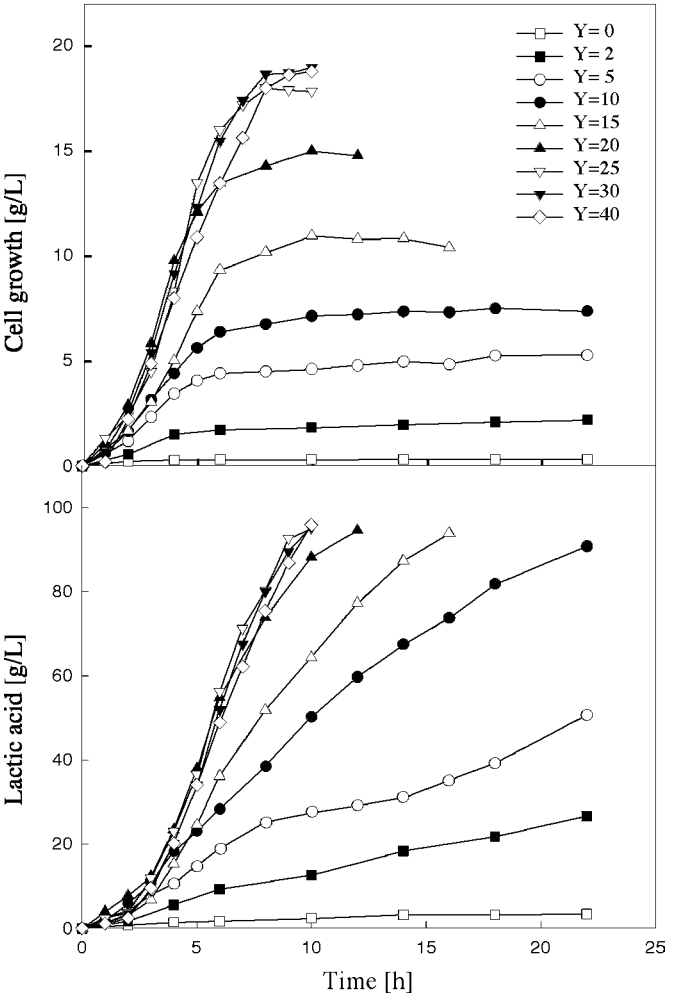

Fig. 2. Effect of yeast extract dosage on cell growth and lactic acid production during batch cultivation of *E. faecalis* RKY1. Medium composition: 100 g/L of glucose, 0–40 g/L of yeast extract.

Cell Recycle Repeated Batch Operation

A study on repeated-batch cultivation with cell recycling was performed to reduce the yeast extract requirement in the medium. Figure 3 shows the results for cell-recycle repeated-batch operation supplemented with 100 g/L of glucose and no yeast extract. Cell growth rate was lower than cell lysis rate from the second batch run owing to limitations in nitrogen sources. Lactic acid productivities of each subsequent batch were 6.19, 3.42, and 1.98 g/(L·h), with a gradual decrease in cell activity.

Figure 4 presents the results for cell-recycle repeated-batch cultivation using fresh medium containing 100 g/L of glucose and 3 g/L of yeast

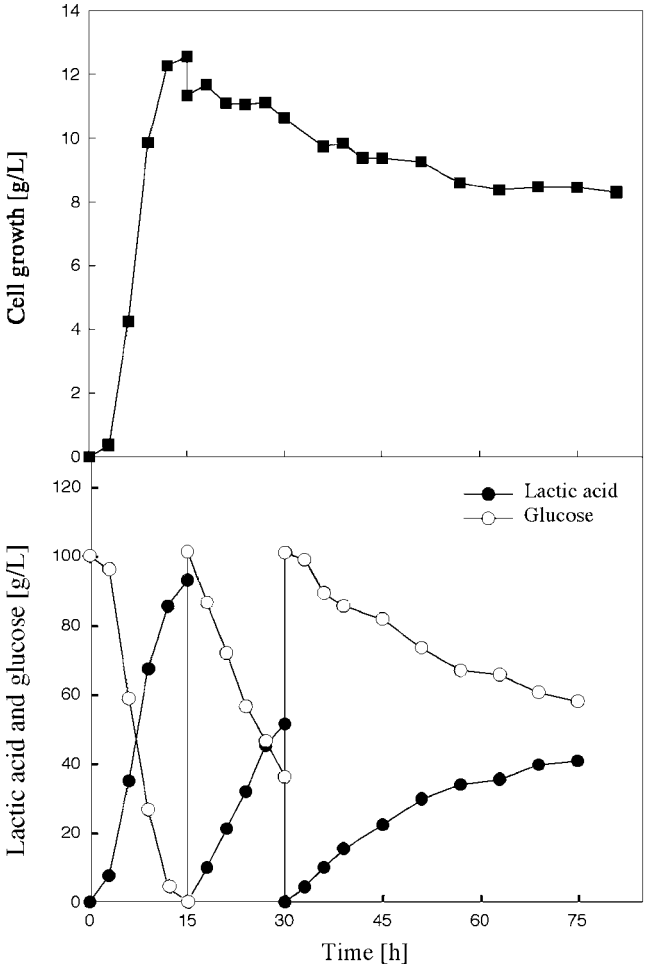

Fig. 3. Time course for cell-recycle repeated-batch operation with no yeast extract. Culture conditions: 2.5-L jar fermentor containing 1 L of medium at 38°C, 200 rpm; composition of fresh medium: 100 g/L of glucose, 0 g/L of yeast extract.

extract. After the fifth batch, the accumulated cell growth was about 17 g/L, but the lactic acid productivity and the substrate consumption rate were lower than those of the first batch by 14 and 13%, respectively.

Figure 5 shows the fermentation profiles of repeated-batch operation with cell-recycle using fresh medium containing 100 g/L of glucose and 4 g/L of yeast extract. A consistent cell growth was seen from the second to tenth batches, with the maximum cell growth of 28.5 g/L. Through the repeated-batch runs, the lactic acid productivities and the substrate consumption rates were stable, in the range of 6.03–6.39 g/(L·h) and 95–100%, respectively.

Figure 6 shows the results of the cell-recycle repeated-batch operation using fresh medium supplemented with 150 g/L of glucose and 7 g/

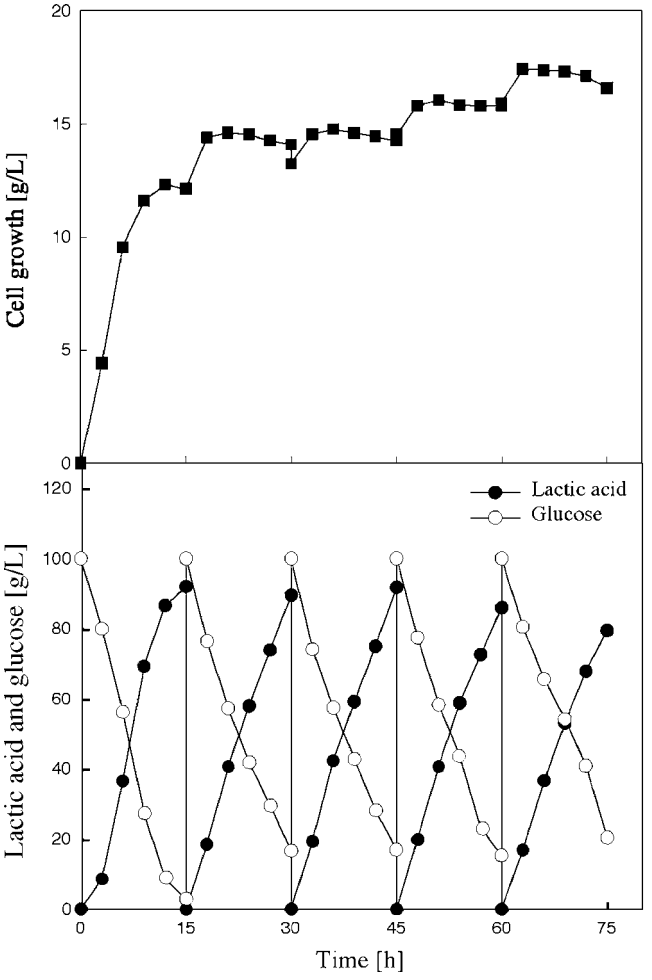

Fig. 4. Time course for cell-recycle repeated-batch operation with 3 g/L of yeast extract. Culture conditions: 2.5-L jar fermentor containing 1 L of medium at 38°C, 200 rpm; composition of fresh medium: 100 g/L of glucose, 3 g/L of yeast extract.

L of yeast extract. The substrate consumption rates were 95–99% throughout the experiment. In other words, the system was stably maintained under these nutritional conditions, as with the operation with 100 g/L of glucose and 4 g/L of yeast extract. The maximum cell growth was 28 g/L, and the lactic acid productivities of each batch were between 5.75 and 5.90 g/(L·h).

Discussion

Both cell growth and lactic acid productivity were low when single organic or inorganic nitrogen source was added to the medium. When cereal-based nutrients were tested as nitrogen sources, cell growth was low, but the specific productivity based on cell mass (1.06–1.12 g of lactic acid/

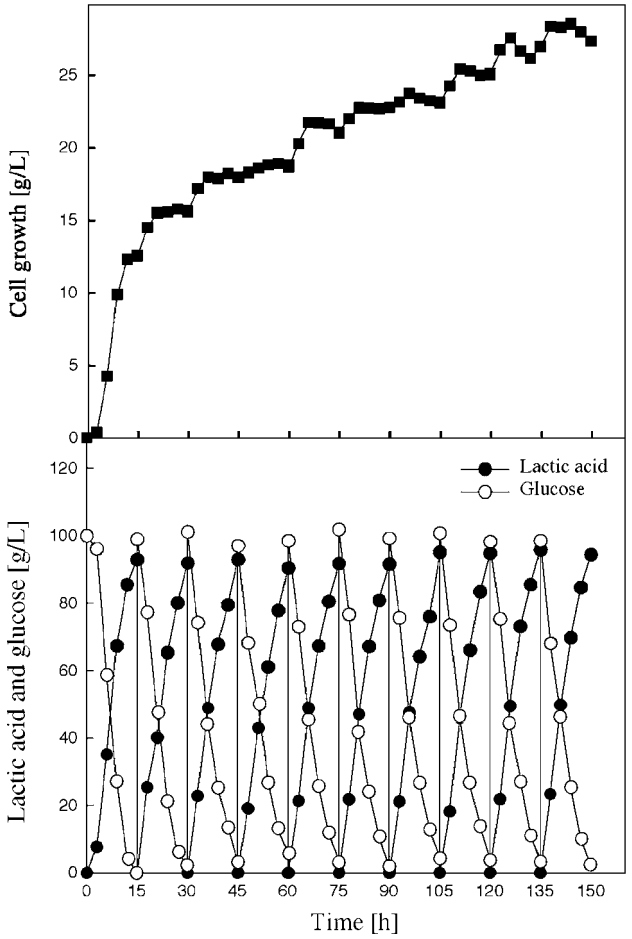

Fig. 5. Time course for cell-recycle repeated-batch operation with 4 g/L of yeast extract. Culture conditions: 2.5-L jar fermentor containing 1 L of medium at 38°C, 200 rpm; composition of fresh medium: 100 g/L of glucose, 4 g/L of yeast extract.

[g of cell·h]) was higher than any other nutrient sources. The highest cell growth and lactic acid productivity were found with undefined organic substrates. Like most studies on lactic acid biosynthesis, the most effective nitrogen source was yeast extract *(1,2,7)*.

During batch operation, when the effects of yeast extract dosage on lactic acid fermentation were tested, it was found that more added yeast extract caused higher cell growth and lactic acid productivity. Figure 7 presents the relationship between the yeast extract dosage and maximum cell growth, and the relationship between yeast extract dosage and overall productivity. Figure 7 also shows that the yeast extract dosage and cell growth were proportional up to a yeast extract dosage of 30 g/L, and that lactic acid productivity was linearly correlated up to a yeast extract dosage of 25 g/L.

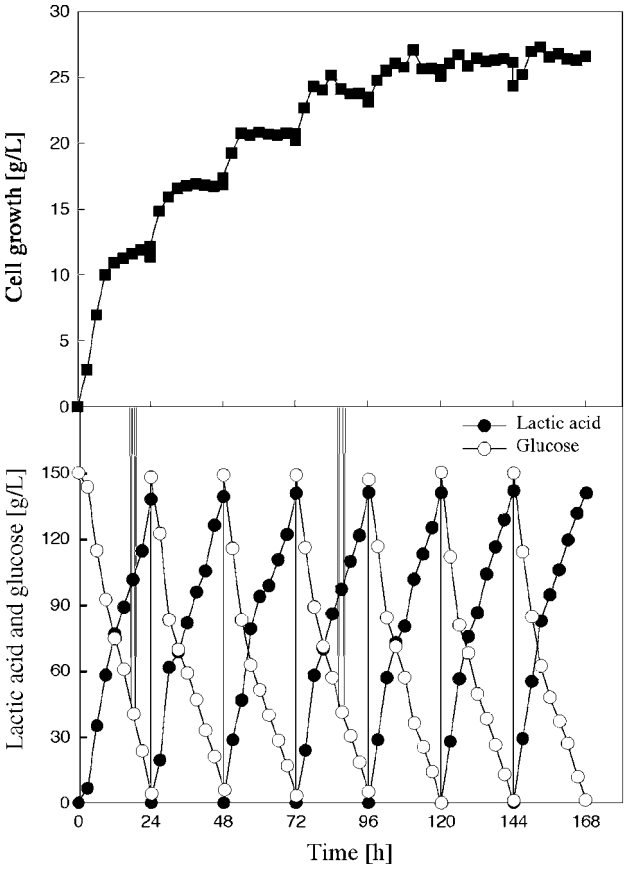

Fig. 6. Time course for cell-recycle repeated-batch operation with 150 g/L of glucose and 7 g/L of yeast extract. Culture conditions: 2.5-L jar fermentor containing 1 L of medium at 38°C, 200 rpm; composition of fresh medium: 150 g/L of glucose, 7 g/L of yeast extract.

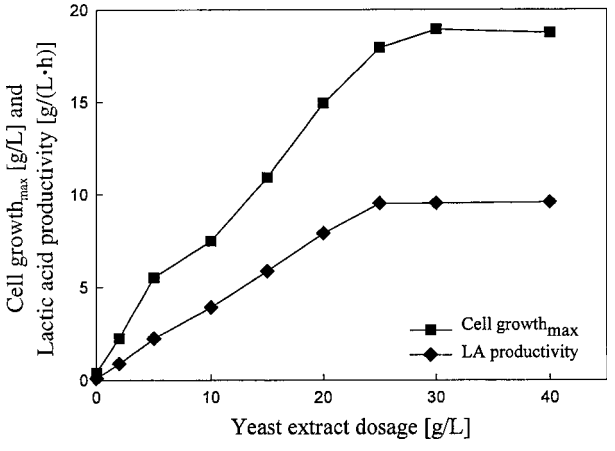

Fig. 7. Effect of yeast extract dosage on cell growth and volumetric productivity of lactic acid during batch cultivation of *E. faecalis* RKY1. LA, lactic acid.

These results are similar to those of Kulozik and Wilde *(6)*. When *Lactobacillus helveticus* was grown on 60 g/L of lactose, the lactic acid production rate increased linearly with increases in the yeast extract concentration up to 10 g/L of yeast extract, whereas the cell growth was maximized at 15 g/L of yeast extract.

However, with the higher yeast extract concentration in the media, the cost of raw material increases. Therefore, cell-recycle repeated-batch operation was characterized, because it was believed that it could reduce the yeast extract requirement while sustaining the productivity of a batch operation.

If the medium contained 100 g/L of glucose and 4 g/L of yeast extract, 43 g of yeast extract was required to produce 1 kg of lactic acid. This is only 26% the yeast extract dosage of batch fermentation, whereas the lactic acid concentration in the product stream (92–94 g/L) and lactic acid productivity (6.03–6.20 g/[L·h]) were similar to those of a batch operation. Furthermore, cell-recycle repeated-batch operation gave long-term stability in the lactic acid production.

When the fresh medium containing 150 g/L of glucose was used, 50 g of yeast extract was required to produce 1 kg of lactic acid. The lactic acid concentration in the product stream was 138 to 142 g/L, and the lactic acid productivity was 5.75–5.90 g/(L·h). The initial concentration of lactic acid of each repeated-batch operation was in the range of 8.6–14.5 g/L owing to retained medium, but inhibition by the initial lactic acid was not observed during fermentation.

Conclusion

Among the various nitrogen sources tested, the most effective nitrogen source was yeast extract. As well, with greater addition of yeast extract, higher cell growth and lactic acid productivity were achieved. When the repeated-batch operation with cell-recycle was performed with glucose and yeast extract concentration of 100 and 4 g/L, respectively, only 26% of the yeast extract dosage required for batch fermentation was needed to produce 1 kg of lactic acid, whereas the lactic acid concentration in the product stream (92–94 g/L) and the lactic acid productivity (6.03–6.20 g/[L·h]) were similar to those of batch operations. Furthermore, long-term stability was established.

Acknowledgments

This work was supported by the Ministry of Commerce, Industry and Energy through the Korea Institute of Industrial Technology Evaluation and Planning.

References

1. Hofvendahl, K. and Hahn-Hägerdahl, B. (2000), *Enzyme Microb. Technol.* **26,** 87–107.
2. VickRoy, T. B. (1985), in *Comprehensive Biotechnology*, vol. 3, Moo-Young, M., ed. Pergamon Press, Tarrytown, NY, pp. 761–776.

3. Amass, W., Amass, A., and Tighe, B. (1998), *Polym. Int.* **47,** 89–144.
4. Chung, E. C., Kim, S. C., So, J. S., and Yun, H. S. (2001), *Biotechnol. Bioprocess Eng.* **6,** 128–132.
5. Olmos-Dichara, A., Ampe, A., Uribelarrea, J.-L., Pareilleux, A., and Goma, G. (1997), *Biotechnol. Lett.* **19(8),** 709–714.
6. Kulozik, W. and Wilde, J. (1999), *Enzyme Microb. Technol.* **24,** 297–302.
7. Hujannen, M. and Linko, Y.-Y. (1996), *Appl. Microbiol. Biotechnol.* **45,** 307–313.
8. Amrane, A. and Pringent, Y. (1997), *J. Biotechnol.* **55,** 1–8.
9. Tejayadi, S. and Cheryan, M. (1995), *Appl. Microbiol. Biotechnol.* **43,** 242–248.
10. Javanainen, P. and Linko, Y.-Y. (1995), *Biotechnol. Technique* **9(8),** 543–548.
11. Hsiesh, C. M., Yang, F.-C., and Iannotti, E. L. (1999), *Process Biochem.* **34,** 173–179.
12. Amrane, A. (2000), *World J. Microbiol. Biotechnol.* **16,** 207–209.
13. Bibal, B., Vayssier, Y., Goma, G., and Pareilleux, A. (1991), *Biotechnol. Bioeng.* **37,** 746–754.
14. Ye, K., Jin, S., and Shimizu, K. (1996), *J. Chem. Technol. Biotechnol.* **66,** 223–226.
15. Yun, J. S. and Ryu, H. W. (2001), *Process Biochem.* **37,** 235–240.
16. Kang, K. H. and Ryu, H. W. (1999), *J. Microbiol. Biotechnol.* **9(2),** 191–195.
17. Kang, K. H., Yun, J. S., and Ryu, H. W. (2000), *J. Microbol. Biotechnol.* **10(1),** 1–7.
18. Ryu, H. W., Kang, K. H., Pan, J. G., and Chang, H. N. (2001), *Biotechnol. Bioeng.* **72(1),** 119–124.