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# Production of Lactic Acid From Cheese Whey by Batch and Repeated Batch Cultures of *Lactobacillus* sp. RKY2

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## Abstract

The fermentative production of lactic acid from cheese whey and corn steep liquor (CSL) as cheap raw materials was investigated by using *Lactobacillus* sp. RKY2 in order to develop a cost-effective fermentation medium. Lactic acid yields based on consumed lactose were obtained at more than 0.98 g/g from the medium containing whey lactose. Lactic acid productivities and yields obtained from whey lactose medium were slightly higher than those obtained from pure lactose medium. The lactic acid productivity gradually decreased with increase in substrate concentration owing to substrate and product inhibitions. The fermentation efficiencies were improved by the addition of more CSL to the medium. Moreover, through the cell-recycle repeated batch fermentation, lactic acid productivity was maximized to 6.34 g/L/h, which was 6.2 times higher than that of the batch fermentation.

**Index Entries:** Corn steep liquor; lactic acid; *Lactobacillus*; lactose; whey.

## Introduction

Lactic acid has numerous applications in food, chemical, textile, pharmaceutical, and other industries *(1)*. Recently, there has been a great demand for lactic acid, because it can be used as a monomer for the production of biodegradable polymer, polylactic acid (PLA), which can be alternative to synthetic polymers derived from petroleum resources *(2)*. In 1999, the annual world production of lactic acid was estimated to be approx 80,000 t produced both by chemical synthesis and by biological fermentation processes *(1)*. However, a number of applications of lactic acid currently resulted in a significant increase in its demand. While the

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only racemic DL-lactic acid is produced through a chemical synthesis, a desired stereoisomer, i.e., an optically pure L(+)- or D(-)-lactic acid, could be produced through a fermentative production from renewable resources, if the proper microorganisms would be chosen for lactic acid fermentation *(3)*. The optical purity of lactic acid is important to the physical properties of PLA. Especially, L(+)-lactic acid of high purity is polymerized to a high crystal polymer, that is suitable for fiber and oriented film. Also as an optically active material, L(+)-lactic acid is expected to be useful for the production of liquid crystals *(4)*. However, lactic acid fermentation process generally requires a complex basal medium, which may result in an increased production cost. Nevertheless, many studies on the production of lactic acid by the lactic acid bacteria were mainly focused on producing from the dextrose-based media and/or expensive nutrients such as yeast extract *(3,5)*. Therefore, the studies on alternative and low-cost media for lactic acid fermentation will be needed owing to its industrial feasibility and an economic consideration. The economics of lactic acid fermentation would be typically improved by using cheap raw materials.

Whey is a major byproduct of dairy industry, and it contains approx 60–65% (w/v) of lactose and some moieties of protein, fat, and mineral salts. The worldwide production of whey is approx $120 \times 10^6$ t/yr and its greater portion remains unutilized, which causes an environmental pollution as a result of high biochemical oxygen demand (BOD, 40,000–60,000 ppm) and chemical oxygen demand (COD, 50,000–80,000 ppm). More than 90% of whey BOD is caused by lactose moiety in the whey. In order to reduce the BOD level and to acquire some useful compounds, this nutrient-rich whey can be utilized for the production of lactic acid by bacteria as a cheap carbohydrate source *(6–12)*. However, lactic acid bacteria have a complex nutrient requirement because they have a limited capacity to synthesize B-vitamin and amino acids *(1)*. Therefore, for complete conversion of lactose to lactic acid, the supplementation of nitrogen sources such as yeast extract or corn steep liquor (CSL) is needed *(13)*.

CSL is a byproduct from corn milling industry, which has been used as an inexpensive nutrient source for several fermentations. In the production of lactic acid, though CSL seems to negatively affect the separation and purification of the produced lactic acid and to reduce the productivity, it should be an attractive source for the economical production of lactic acid as mentioned before *(14,15)*. This study was mainly focused on the utilization of whey lactose as a substrate for the production of lactic acid by batch culture of *Lactobacillus* sp. RKY2. In addition, the effects of CSL on lactic acid fermentation using cheese whey were also investigated. The cell-recycle repeated batch production of lactic acid using cheese whey and CSL as raw materials was tried in order to further enhance the productivity of lactic acid.

## Materials and Methods

### *Microorganism*

*Lactobacillus* sp. RKY2, which is stocked in the Korea Collection for Type Cultures (KCTC) with the accession number KCTC 10353BP *(16–18)*, was used throughout this study. The stock cultures were maintained at –20°C in 5-mL vial containing 50% (v/v) glycerol and culture medium composed of 30 g glucose, 10 g yeast extract, 2 g $(NH_4)_2HPO_4$, and 0.1 g $MnSO_4$ per 1 L of deionized water. After three consecutive transfers to 20- mL vial containing the fresh medium, the final culture was transferred to 50-mL vial with 40 mL of the fresh medium, which was then used for the inoculation after incubation for 12 h.

### *Preparation of Medium*

Whey powder containing 60–65% (w/v) lactose was obtained from Samick Dairy Industry (Gimje, Korea). It was dissolved in deionized water to attain the desired lactose concentration, and the pH was adjusted to 4.0 by adding 10 *M* HCl, which was then heated to 100°C for 10 min and cooled to the room temperature *(19)*. The resulting whey solution was centrifuged at 15,540*g* to remove a solid, and the supernatant was used for the fermentation. Unless otherwise indicated, whey solution was supplemented with 30 g/L CSL, 1 g/L yeast extract, 2 g/L $(NH_4)_2HPO_4$, and 0.1 g/L $MnSO_4$. Whey solution and other components were autoclaved separately at 121°C for 15 min.

### *Batch Fermentation*

The batch fermentations were carried out in a 2.5-L jar-fermenter (KF-2.5 L; Kobiotech, Incheon, Korea) with 1-L working volume. The culture temperature was controlled at 36°C and the agitation speed was adjusted to 300 rpm. The culture pH was maintained at 6.0 by automatically adding 10 *M* NaOH.

### *Cell-Recycle Repeated Batch Fermentation*

The repeated batch fermentation with cell-recycle was performed in the same fermenter stated above in order to improve the lactic acid productivity and nutrient economics. A hollow fiber unit (HUF 1010-BPN30, Chemicore Inc., Daejeon, Korea) was used for cell recycling, and it contained 100 polysulfone hollow fiber membranes. The internal diameter and length of the module were 32 and 300 mm, respectively. A nominal molecular weight cutoff of the membranes was 300 kDa and the total filtration area was 0.07 $m^2$. A peristaltic pump was used for recycling the culture broth through the hollow fiber filtration unit with a flow rate of approx 100 mL/min. The hollow fiber filtration unit was sterilized with 200 ppm sodium hypochlorite for 12 h, and then washed with sterile water

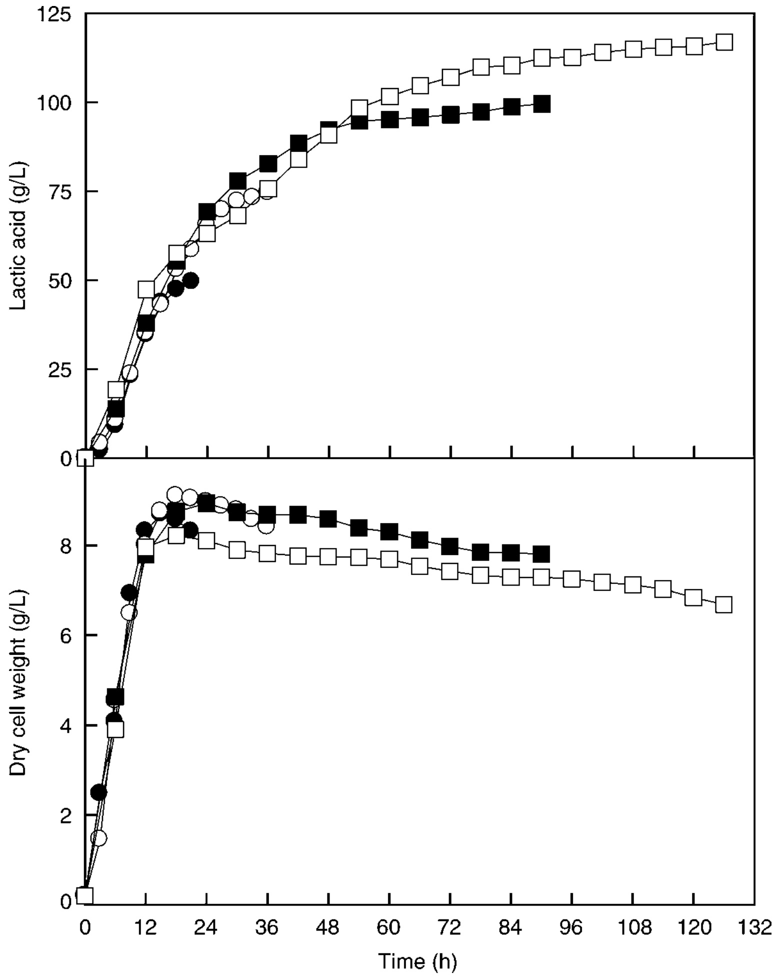


**Fig. 1.** Lactic acid production and cell growth from various concentrations of whey lactose by batch culture of *Lactobacillus* sp. RKY2. Symbols: -●-, whey lactose 50 g/L; -○-, whey lactose 75 g/L; -■-, whey lactose 100 g/L; -□-, whey lactose 125 g/L.

to remove the remainders of sodium hypochlorite before each fermentation experiment. In cell-recycle repeated batch fermentations, once the sugar was completely consumed at the former batch run, 90% (v/v) of the culture broth was taken out of the fermenter through the hollow fiber filtration unit. Then, the same volume of fresh medium was fed into the fermenter. The subsequent batch fermentations were performed by the same manner as described earlier.

### Analyses

Cell concentration was determined turbidimetrically by measuring the optical density at 660 nm using a spectrophotometer (UV-Vis 1700, Shimadzu, Kyoto, Japan). The optical density was converted to dry cell weight through an appropriate calibration curve. The samples obtained at different time intervals were centrifuged at 17,620*g*. The resulting supernatants were used

Table 1
Effect of Lactose Concentrations on Lactic Acid Produced, Residual Lactose, Maximal Dry Cell Weight, Yield, and Productivity in Batch Culture of *Lactobacillus* sp. RKY2

| Substrate | Initial lactose (g/L) | Residual lactose (g/L) | Lactic acid (g/L) | Max. dry cell weight (g/L) | Yield (g/g)[a] | Productivity (g/L/h) |
|---|---|---|---|---|---|---|
| Whey lactose | 50 | 0 | 49.45 | 8.69 | 0.989 | 2.355 |
| | 75 | 0 | 74.51 | 9.09 | 0.988 | 2.07 |
| | 100 | 0 | 99.35 | 8.94 | 0.987 | 1.106 |
| | 125 | 6.3 | 116.92 | 8.22 | 0.985 | 0.928 |
| Pure lactose | 50 | 0 | 49.4 | 7.71 | 0.987 | 2.352 |
| | 75 | 0 | 74.32 | 8.18 | 0.985 | 2.064 |
| | 100 | 3.2 | 95.5 | 8.25 | 0.978 | 1.061 |
| | 125 | 9.9 | 109.96 | 7.76 | 0.955 | 0.873 |

[a]The yield of lactic acid was calculated on the basis of the amount of consumed lactose in whey.

for the analysis of lactic acid and lactose. Lactose concentration was measured by DNSA methods using lactose as a standard *(20)*. Lactic acid was analyzed by a high-performance liquid chromatography equipped with an Aminex HPX-87H ion-exclusion column (300 × 7.8 mm; Bio-Rad, CA) under the following conditions: column temperature, 35°C; mobile phase, 5 m*M* $H_2SO_4$; flow rate, 0.6 mL/min; detection, UV 210 nm.

## Results and Discussion

### *Influence of Initial Whey Lactose Concentration*

In an attempt to evaluate the influence of whey lactose concentrations on lactic acid fermentation by batch culture of *Lactobacillus* sp. RKY2, the medium containing 50, 75, 100, and 125 g/L of whey lactose was tested. The fermentations were conducted in a 2.5-L jar-fermenter with 1-L working volume at 36°C and 200 rpm. As shown in Fig. 1, lactic acid production curves followed the similar pattern and the final lactic acid produced increased with increasing the initial whey lactose concentration. The maximal lactic acid (116.92 g/L) was obtained after 126 h of fermentation at an initial whey lactose concentration of 125 g/L. The lactic acid yields based on consumed lactose in cheese whey were 0.989, 0.988, 0.987, and 0.985 g/g at 50, 75, 100, and 125 g/L of whey lactose, respectively (Table 1). The lactic acid productivities usually decreased with increasing the initial whey lactose concentration. Table 1 shows also the fermentation parameters, such as final lactic acid produced, lactic acid yields, and volumetric productivity, at various concentrations of whey lactose. However, when the medium was supplemented with 125 g/L of whey lactose, more than 10 g/L of whey lactose remained even after 126 h of fermentation without being used, which is probably owing to substrate and product inhibitions. In addition, when the medium was supplemented with 100 g/L of whey lactose, the fermentation time was severely prolonged. This was expected because there was also a rapid decrease in dry cell weight. Dry cell weight usually increased with increasing the initial whey lactose concentration up to 75 g/L, but then decreased beyond this value. Although Büyükkileci and Harsa *(6)* reported that there was no substrate inhibition in lactic acid production from whey by batch culture of *Lactobacillus casei*, *Lactobacillus* sp. RKY2 used in this study was slightly inhibited by whey lactose above 100 g/L.

### *Influence of Initial Pure Lactose Concentration*

To investigate the effect of initial pure lactose concentration on lactic acid fermentation by batch culture of *Lactobacillus* sp. RKY2, the medium containing 50, 75, 100, and 125 g/L of pure lactose was tested. The fermentation of pure lactose showed similar profiles of lactic acid production and cell growth as compared with the fermentation of whey lactose (Fig. 2). In cases of lactic acid yields and productivities, these values were almost similar when 50–75 g/L of whey lactose and pure lactose were used, but lactic acid yields and productivities are slightly higher when more than 100 g/L of whey lactose was used as a substrate (Table 1). As shown in Fig. 2, the final lactic acid produced increased with increasing the initial pure lactose concentration. The maximal lactic acid (109.96 g/L) was obtained after 126 h of fermentation at an initial pure lactose concentration of 125 g/L. The lactic acid yields based on consumed lactose were obtained to 0.987, 0.985, 0.987, and 0.955 g/g at 50, 75, 100, and 125 g/L of pure lactose, respectively (Table 1). In all experiments, however, a nutrient supplement is needed for complete conversion of lactose to lactic acid. Otherwise, it may result in incomplete utilization of lactose and prolonged fermentation.

### *Influence of CSL Concentration*

Lactic acid bacteria are generally fastidious organisms, which require complex nutrients such as amino acids and vitamins for cell growth. To investigate the effect of CSL as a cheaper nitrogen source on lactic acid fermentation, 15–60 g/L of CSL was supplemented to whey lactose medium. Figure 3 shows the time-course of lactic acid production and cell growth during the fermentation at various concentrations of CSL. As can be seen in Fig. 3, both the lactic acid production rate and cell growth increased with increasing the CSL concentrations. The maximal cell growth (10.36 g/L of dry cell weight) was obtained with the medium supplemented with 60 g/L of CSL. Although a little lactose remained unconverted after the end of fermentation, most of the lactose was nearly converted to lactic acid (Table 2).

Table 2 summarizes the fermentation parameters of the earlier experiments at various concentrations of CSL, such as lactic acid produced, lactic acid yields, residual lactose, and productivities. From 30 to 60 g/L

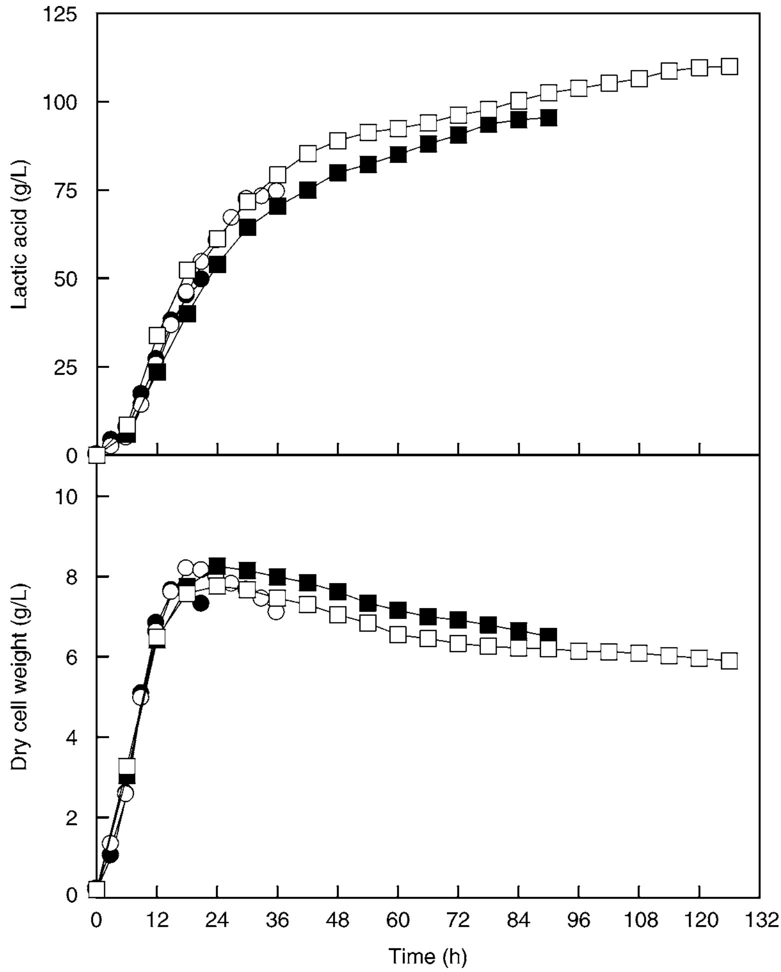


**Fig. 2.** Lactic acid production and cell growth from various concentrations of pure lactose by batch culture of *Lactobacillus* sp. RKY2. Symbols: -●-, lactose 50 g/L; -○-, lactose 75 g/L; -■-, lactose 100 g/L; -□-, lactose 125 g/L.

of CSL concentrations, lactic acid yields based on total lactose consumed was maintained above 0.98 g/g. In general, lactic acid fermentation using *Lactobacillus* sp. RKY2 should be severely affected by CSL concentration as a nitrogen source added to the medium, which is well agreed with the previous reports by Hujanen and Linko *(21)* who investigated the effect of nitrogen sources on lactic acid production by *Lactobacillus casei*.

## *Lactic Acid Production Through Cell-Recycle Repeated Batch Fermentation*

Because lactic acid is a high-volume and low-price chemical, it is necessary to reduce the manufacturing cost. Numerous studies on reducing the manufacturing cost of lactic acid were carried out by several groups. According to Leudeking and Piret *(22)*, lactic acid production rates could

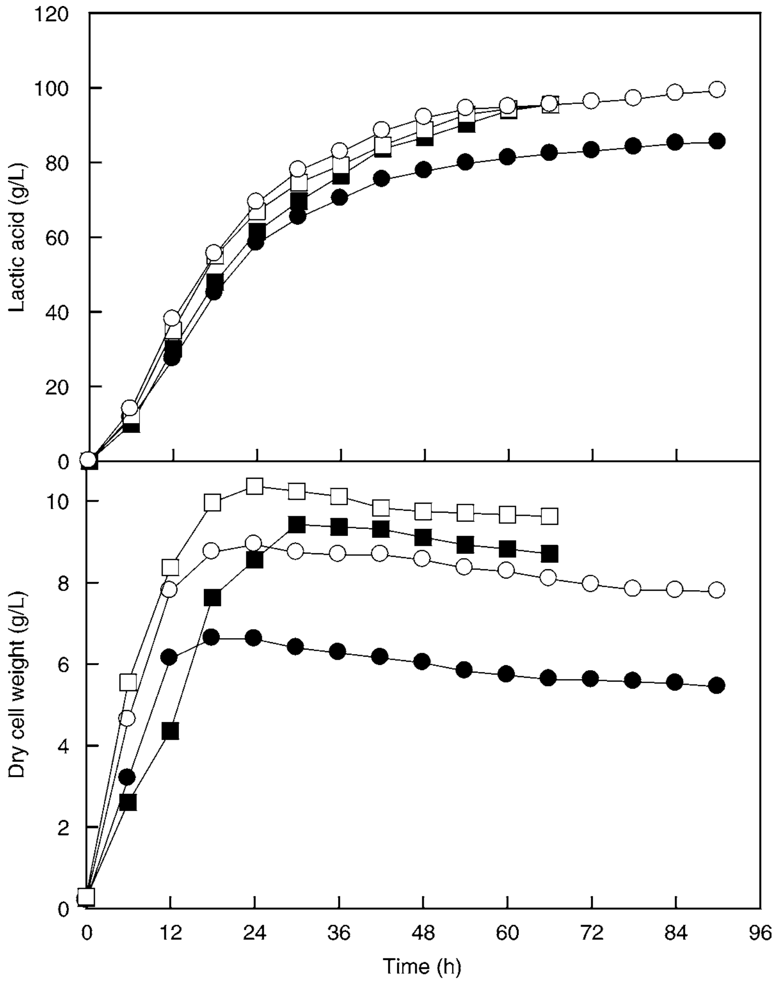


**Fig. 3.** Lactic acid production and cell growth from whey lactose and various concentrations of CSL by batch culture of *Lactobacillus* sp. RKY2. Symbols: -●-, CSL 15 g/L; -○-, CSL 30 g/L; -■-, CSL 45 g/L; -□-, CSL 60 g/L.

be increased by increase of cell densities in the bioreactor. In order to achieve this, the cell-recycle repeated batch fermentation was conducted by using a hollow fiber membrane. For economical fermentation of lactic acid, cost of fermentation medium as well as purification cost should be reduced. In addition, fermentation method such as batch, repeated batch, and continuous fermentations should be considered to improve productivity of final product. From this point of view, the cell-recycle repeated batch system might be a potential process that can maintain a reasonable productivity and reuse of the former culture as a seed.

The cell-recycle repeated batch fermentation by *Lactobacillus* sp. RKY2 was conducted to improve volumetric productivity. Figure 4 shows the results of the cell-recycle repeated batch fermentation. The medium for the first batch run contained 100 g/L of whey lactose, 30 g/L of CSL, 1 g/L of

Table 2
Effect of CSL Concentrations on Lactic Acid Produced, Residual Lactose, Maximal Dry Cell Weight, Yield, and Productivity in Batch Culture of *Lactobacillus* sp. RKY2

| CSL concentration (g/L) | Residual lactose (g/L) | Lactic acid (g/L) | Max. dry cell weight (g/L) | Yield (g/g)[a] | Productivity (g/L/h) |
|---|---|---|---|---|---|
| 15 | 3.4 | 85.32 | 6.63 | 0.88 | 0.95 |
| 30 | 0 | 99.15 | 8.94 | 0.983 | 1.1 |
| 45 | 3.9 | 95.5 | 9.43 | 0.988 | 1.44 |
| 60 | 3.8 | 95.38 | 10.36 | 0.989 | 1.45 |

[a]The yield of lactic acid was calculated on the basis of the amount of consumed lactose in whey.

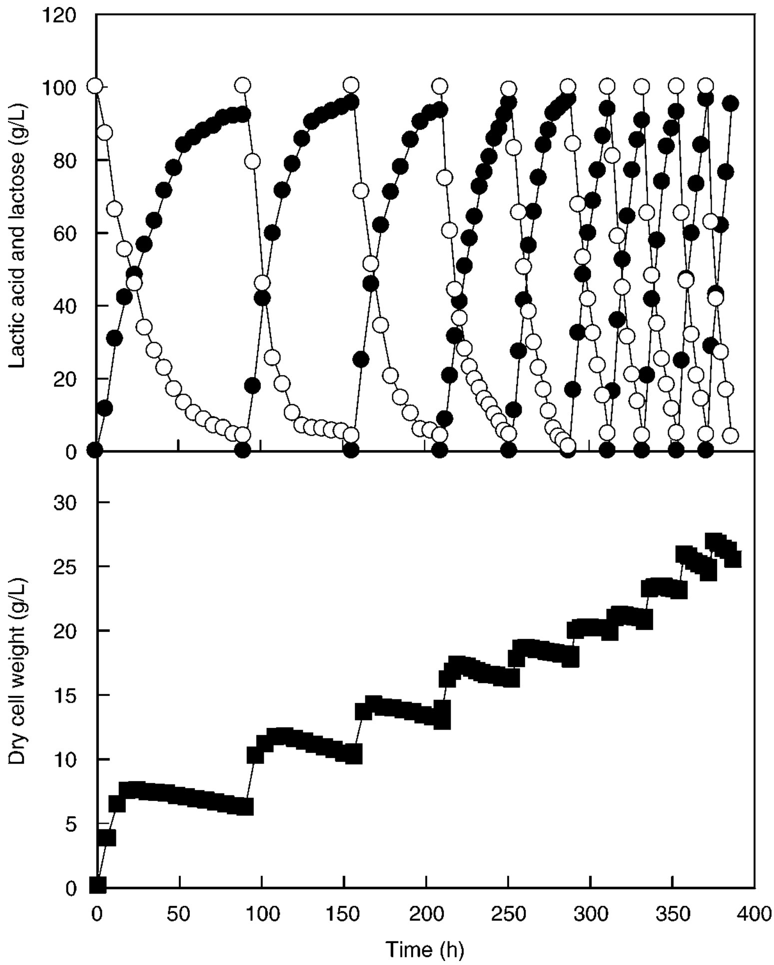


**Fig. 4.** Lactic acid production and cell growth from whey lactose and CSL by cell-recycle repeated batch culture of *Lactobacillus* sp. RKY2. Symbols: -●-, lactic acid; -○-, lactose; -■-, dry cell weight.

Table 3
Fermentation Results of the Cell-Recycle Repeated Batch Culture of *Lactobacillus* sp. RKY2

| Batch number | Lactic acid (g/L) | Max. dry cell weight (g/L) | Yield (g/g)[a] | Productivity (g/L/h) |
|---|---|---|---|---|
| 1 | 94.06 | 7.64 | 0.98 | 1.06 |
| 2 | 95.47 | 11.82 | 0.99 | 1.45 |
| 3 | 93.38 | 14.28 | 0.97 | 1.72 |
| 4 | 94.61 | 17.4 | 0.99 | 2.25 |
| 5 | 96.53 | 18.7 | 0.98 | 2.68 |
| 6 | 93.79 | 20.29 | 0.98 | 3.91 |
| 7 | 90.61 | 21.26 | 0.95 | 4.31 |
| 8 | 93.03 | 23.46 | 0.98 | 4.42 |
| 9 | 94.84 | 25.97 | 0.99 | 5.27 |
| 10 | 95.11 | 26.98 | 0.99 | 6.34 |

[a]The yield of lactic acid was calculated on the basis of the amount of consumed lactose in whey.

yeast extract, 2 g/L of $(NH_4)_2HPO_4$, and 0.1 g/L of $MnSO_4$, and the medium for subsequent batches contained 100 g/L of whey lactose, 30 g/L of CSL, 1 g/L of yeast extract, and 2 g/L of $(NH_4)_2HPO_4$. The second batch run was completed 36 h faster than the first batch, which resulted in the improvement of lactic acid productivity from 1.02 g/L/h to 1.45 g/L/h. During 10 repeated batch runs, the volumetric productivities ranged from 1.02 to 6.34 g/L/h (Table 3). In other words, lactic acid productivity of the 10th batch run was improved by 6.2-fold higher value compared with that of the first batch run. The cell concentration maximized to 26.98 g/L at the 10th batch run, which corresponded to 3.53 times higher value than that of the first batch run. During the cell-recycle repeated batch operations, lactic acid productivity increased proportionally to the increase in cell growth. Therefore, the cell-recycle system seems to be a potential tool for the production of lactic acid from whey and CSL as cheap raw materials.

## Conclusions

The biological production of lactic acid from cheese whey and CSL by *Lactobacillus* sp. RKY2 was investigated. In batch lactic acid fermentation, lactic acid yields based on consumed lactose were obtained at more than 0.98 g/g from the medium containing whey lactose or pure lactose. However, lactic acid productivities and yields obtained from whey lactose were slightly higher than those obtained from the pure lactose. The final concentration of lactic acid increased with increasing whey lactose concentration. Lactic acid productivity and cell growth in cheese whey and CSL were improved by the addition of more CSL to the medium. When the cell-recycle repeated batch fermentation was performed with 100 g/L of lactose in cheese whey, 30 g/L of CSL, and 1 g/L of yeast extract, lactic acid productivity was maximized to 6.34 g/L/h, which was 6.2 times higher than that of the batch fermentation.

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