Production of Lactic Acid from Cheese Whey by Repeated Batch and Continuous Cultures

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Abstract

This study is concerned with development of efficient culture methods for lactic acid fermentation of *Lactobacillus* sp. RKY2. The cell-recycle repeated batch fermentation using cheese whey and corn steep liquor as raw materials was tried in order to further enhance the productivity of lactic acid. In addition, fermentation efficiencies could be considerably enhanced by cell-recycle continuous culture. Through the cell-recycle repeated batch fermentation, lactic acid productivity was maximized to 6.34 g/L·h, which corresponded to 6.2 times higher value than that of the batch fermentation. During the cell-recycle continuous fermentation, the last dry cell weight at the end of fermentation could be increased to 25.3 g/L.

Introduction

Lactic acid has numerous applications in the food, chemical, textile, pharmaceutical, and other industries[1)]. Currently, there have been much interests in biologically derived lactic acid as a monomer of polylactic acid that can be used for biodegradable plastics. However, as 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[2)]. This study is concerned with development of efficient culture methods. In order to achieve this, high cell density culture was applied by using a hollow fiber membrane. To improve

the economics of fermentation cost, the cost of fermentation medium as well as the productivity should be considered each other. From this point of view, we investigated the cell-recycle system with repeated batch and continuous culture of *Lactobacillus* sp. RKY2 to obtain a reasonable fermentative efficiency. Moreover reduction of the manufacturing cost of lactic acid was tried by using cheese whey and CSL as raw materials.

Materials and methods

Microorganism

Lactobacillus sp. RKY2 was used throughout this study. The strain was maintained in culture medium consisted of 30 g glucose, 10 g yeast extract, 2.0 g $(NH_4)_2HPO_4$, and 0.1 g $MnSO_4$ per 1 L of deionized water and transferred to fresh medium every 24h[4].

Medium and growth conditions

Whey powder containing 60~65% (w/v) lactose was obtained from Samick Dairy Industry, Korea. It was dissolved to attain the desired lactose concentration and the pH was adjusted to 4 using 10 M HCl, then heated to 100°C for 10 min, followed by cooling to room temperature. Unless indicated otherwise, whey broths were supplemented with 30 g/L CSL, 1.0 g/L yeast extract, 2.0 g/L $(NH_4)_2HPO_4$, and 0.1 g/L $MnSO_4$. The medium was autoclaved at 121°C for 15 min.

Repeated batch fermentation and Continuous fermentations with Cell-recycle

The fermentation was performed on a 2.5 L jar-fermentor (KF-2.5L; Kobiotech, Daejeon, Korea). Working volume of the repeated batch and continuous fermentations was 1 L and 800 mL, respectively. A hollow fiber unit (HUF 1010-BPN30; Chemicore, 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 mm and 300 mm, respectively. A nominal molecular weight cut-off (MWCO) 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. During cell-recycle repeated batch fermentation, 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 equal volume of fresh medium was fed into the fermenter. In cell-recycle continuous fermentation, fresh medium was supplied into the reactor at dilution rate of 0.04-0.12 h^{-1}, while an equal volume of the spent medium was removed from the reactor.

Analysis

The samples obtained at different time intervals were centrifuged at 15,000 rpm. The resulting supernatants were used for analysis of lactic acid and lactose. Lactose concentration was measured by DNSA methods[5]. Lactic acid was analyzed by using a high performance liquid chromatography equipped with an Aminex HPX-87H ion-exclusion column (300×7.8 mm, Bio-Rad, CA, USA) under the following conditions: column temperature, 35°C; mobile phase, 5 mM H_2SO_4; flow rate, 0.6 mL/min; detector, UV 210 nm. Cell concentration was determined turbidimetrically by absorbance readings at 660 nm with spectrophotometer (UV-Vis 1700, Shimadzu, Kyoto, Japan). The absorbance readings were converted to dry cell weight through an appropriate calibration curve.

Results and discussion

The cell-recycle repeated batch fermentation by *Lactobacillus* sp. RKY2 was conducted to improve volumetric productivity. The medium for the first batch run contained 100 g/L of whey lactose, 30 g/L of CSL, 1.0 g/L of yeast extract, 2.0 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, 1.0 g/L of yeast extract, and 2.0 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 ten repeated batch runs, the volumetric productivities were ranged

from 1.02 to 6.34 g/L·h. In other words, lactic acid productivity of the tenth batch run was improved by 6.2-fold higher value compared with that of the first batch run. The cell growth was maximized to 26.98 g/L at the tenth batch run, which corresponded to 3.53 times higher value than that of the first batch run (Figure 1.). During the cell-recycle repeated batch operations, lactic acid productivity and cell growth increased proportionally to the increase in dilution rate. As the dilution rate increased from 0.04 to 0.12 h^{-1}, volumetric productivity of lactic acid and dry cell weight increased from 3.44 to 8.17 g/L·h and 9.9 to 25.3 g/L, respectively. These values were about 3.97-fold and 3.22-fold higher than that of the first batch run (Figure 2). 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.

Reference

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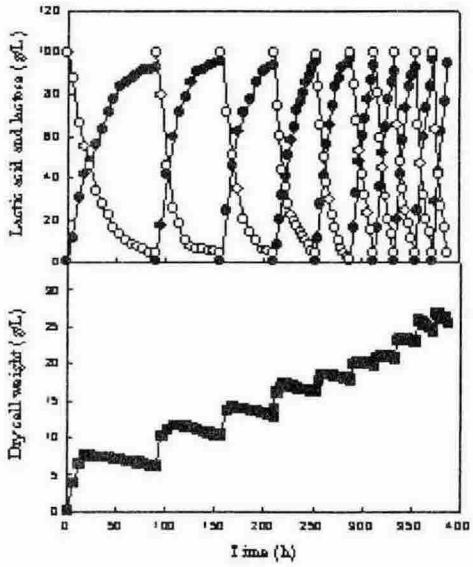

Fig. 1. Lactic acid production and cell growth during cell-recycle repeated batch culture of Lactobacillus sp. RKY2 using whey lactose and corn steep liquor. Symbols: -□-, lactic acid; -□-, lactose; -□-, dry cell weight.

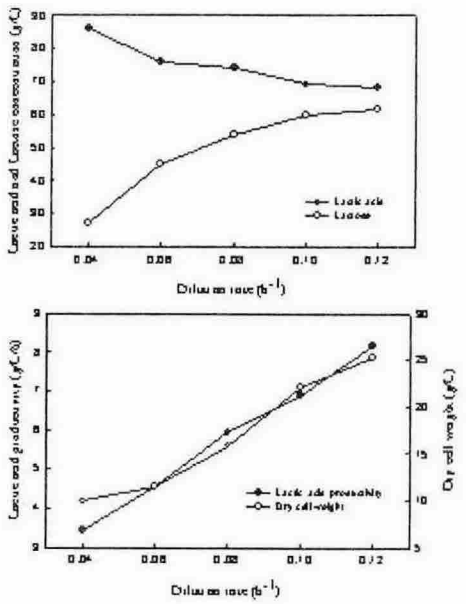

Fig. 2. Effect of dilution rates on lactic acid production, residual lactose, volumetric productivity, and cell growth during cell-recycle continuous culture using whey lactose and corn steep liquor.