Production of Lactic Acid from Cheese Whey by Batch Culture of *Lactobacillus* sp. RKY2

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Abstract

We investigated the fermentative production of lactic acid from cheese whey and corn steep liquor as cheap raw materials using *Lactobacillus* sp. RKY2 to reduce the manufacturing cost of lactic acid. Lactic acid yields were obtained at more than 0.98 g/g from medium containing whey lactose. Lactic aid productivities and yields obtained from whey lactose were slightly higher than those obtained from pure lactose. The final concentration of lactic acid increased with increase, in whey lactose concentration, whereas the lactic acid productivity decreased probably due to substrate inhibition. The fermentation efficiencies were improved by addition of more corn steep liquor to the medium.

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[2)]. Though the biological production of lactic acid has some advantages, it still requires the cheaper substrates for industrial feasibility and lactic acid producers are focused mainly on producing lactic acid using dextrose-based media. The economics of fermentation can be improved by using cheap raw materials. Whey, a major by-product of the dairy industry, contains approximately 60~65 % (w/v) of lactose, protein, fat, and minerals[3)]. This nutrient-rich whey could be fermented to lactic acid by bacteria. Since lactic acid bacteria has a complex nutrient requirement, for complete conversion of lactose to lactic acid, supplementation of nitrogen sources such as yeast extract and/or corn steep liquor (CSL) is needed. Corn steep liquor has been used as an inexpensive source of essential microbial nutrients for a variety of purposes. In the production of lactic acid, CSL can negatively affect the separation and purification of produced lactic acid. However, the utilization of CSL seems to be available for reducing the costs of raw material.

This study was focused on the utilization of whey lactose as substrate for the production of lactic acid by batch culture of *Lactobacillus* sp. RKY2. We also investigated the effect of CSL as a nitrogen source for lactic acid fermentation.

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℃ 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℃ for 15 min.

Fermentation

The fermentations were carried out on 2.5 L jar-fermentor (KF-2.5L, Kobiotech, Daejeon, Korea) with 1 L working volume. The culture temperature was controlled at 36℃ and the agitation speed was adjusted to 300 rpm. The culture pH value was maintained at 6.0 automatically by the addition of 10 N NaOH.

Analyses

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℃; mobile phase, 0.5 mM H_2SO_4; flow rate, 0.6 mL/min; detection, 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.

Result and discussion

To investigate the effect of initial whey lactose concentration 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. As shown in Fig. 1, the final lactic acid produced increased with the increase of 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 obtained to 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). When the medium was supplemented with 125 g/L of whey lactose, approximately 10 g/L of whey lactose remained even after 126 h of fermentation without utilization, which might be due to substrate inhibition(data not shown). 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 decrease in maximal dry cell weight. Dry cell weight increased with the increase of initial whey lactose concentration up to 75 g/L, but then decreased beyond this value.

The fermentation of pure lactose showed similar profiles of lactic acid production and cell growth faster (Fig. 2). When whey lactose was used as a substrate, it was utilized faster than pure lactose and the lactic acid yields were slightly higher than those of pure lactose (Table 1).

In all experiments, however, a nutrient supplement is required for complete conversion of lactose to lactic acid. Otherwise, it may result in the incomplete utilization of lactose and prolonged fermentation.

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. Lactic acid productivities increased with the increase of CSL supplementations (data not shown).

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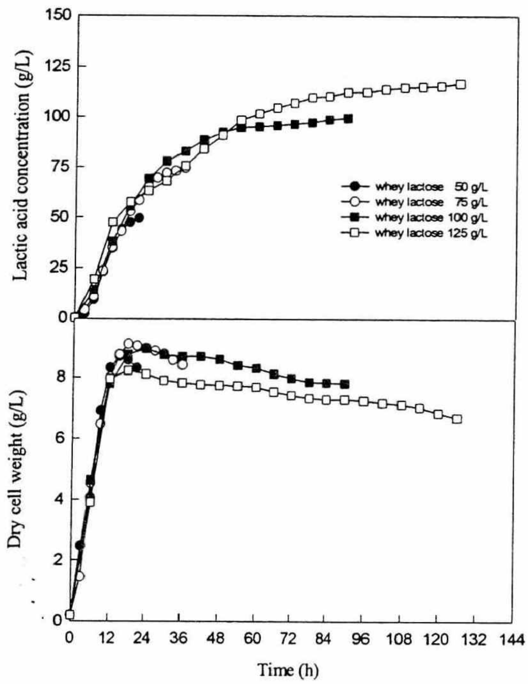

Fig. 1. Time profiles of lactic acid fermentation with various concentrations of whey lactose.

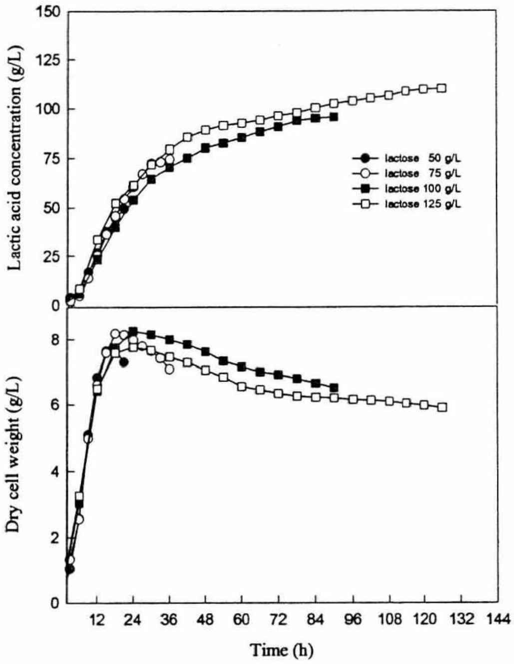

Fig. 2. Time profiles of lactic acid fermentation with various concentrations of pure lactose.

Table 1. Effect of substrate concentration on lactic acid produced, residual substrate, 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)	Productivity (g/L·hr)
Whey lactose	50	0	49.45	8.69	0.989	2.355
	75	0	74:51	9.09	0.988	2.070
	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	49.40	7.71	0.987	2.352
	75	0	74.32	8.18	0.985	2.064
	100	3.2	95.50	8.25	0.978	1.061
	125	9.9	109.96	7.76	0.955	0.873