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EXTRACTIVE FERMENTATION OF LACTIC ACID BY IMMOBILIZED *Lactobacillus casei* USING ION—EXCHANGE RESIN

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SUMMARY

A novel method of lactic acid fermentation by *Lactobacillus casei* immobilized in Ca—alginate gels is described, in which an ion—exchange resin packed column is attached to a fermentor for separation of lactic acid from fermentative broth. The technique successfully alleviated the restriction imposed by lactic acid on bacterial growth and product formation. As compared to the conventional batch fermentation, the new fermentation technique enhanced the lactic acid productivity and sugar conversion rate from 0.328 g/L • h and 88.2% to 0.482 g/L • h and 98.6%, respectively.

INTRODUCTION

Lactic acid is produced commercially by the fermentation of carbohydrates with homofermentative lactic acid bacteria. It is one of the important organic acids and widely used in the food, pharmaceutical and chemical industry (Buchta, 1983).

In the fermentation, the lactic acid produced inhibits further production of lactic acid. To prevent this inhibition, an alkali, such as $CaCO_3$, NaOH or NH_4OH is usually employed. Recently, the electrodialysis and membrane separation technique were also used to alleviate the inhibition (Vickroy et al., 1982; Hongo et al., 1986; Boyaval et al., 1987). Extractive fermentation is a

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technique used to reduce the end product inhibition by removing the fermentation products *in situ* and applitation to lactic acid fermentation has been studied by several authors (Laane, et al. 1985; Yabanavar and Wang 1985). However, the solvent extraction technique causes problems with the activity of the microbial cells. As a results, adsorbents have been considered for subsequent product recovery and used in batch fermentation (Wang et al., 1981). From the viewpoint of industrial production, ion exchange resin is preferred to be used in lactic acid fermentation because it involves low operational and maintenance cost.

In this article, the studies have been performed on extractive lactic acid fermentation by *Lactobacillus casei* immobilized in calcium alginate bead gels, in which an anion—exchange resin D301 was used for the removal of lactic acid from the fermentation broth *in situ*.

MATERIALS AND METHODS

Microorganism: *Lactobacillus casei* was used in all experiments. It was maintained on PDA slant stored at 4℃ and renewed every other month.

Medium: The fermentation medium contained (g/L): glucose 50.0; yeast extract 10.0; polypeptone 10.0; KH_2PO_4 1.0; $MgSO_4 \cdot 7H_2O$ 1.0.; in conventional batch fermentation, 20 g/L of $CaCO_3$ was added.

Cell Immobilization: 100 ml of cell suspension was mixed with 100 ml of 5% sterile Na—alginate solution. The mixture was added into 5% $CaCl_2$ solution and hardened for 2 h. The beads (mean diameter 3mm) were washed with sterile physiological saline to remove excess calcium ions and unentrapped cells.

Assay Methods: Lactic acid was estimated by the colorimetric method of Barker and Summerson (1941). The adsorbed lactic acid was recovered by eluting the column with 2.0 mol/L HCl. The eluted lactic acid was analyzed. Residual sugar was determined as glucose by the phenol sulfuric acid method of Dubois et al. (1956). Calcium lactate was analyzed by the method of EDTA titration (Zhou et al., 1993).

Resin and Its Preparation: An anion—exchange resin D301 was used for lactic acid separation from the fermentation broth. The pretreated resin was filled in a packed column (2 cm in inside diameter and 40 cm in length). The resin packed column was then steam sterilized before it was connected with the fermentor.

Extractive Fermentation: The extractive fermentaion of lactic acid was performed in a 300 ml bioreactor with 200 ml working volume at the temperature of 45℃. The bioreactor was connected to a column packed with anion—ex-

change resin for simultaneous removal of lactic acid from fermentation broth. The conventional batch fermentation was carried out exactly the same system but using saturated ion exchange resin with lactic acid instead of fresh resin. All experiments were conducted in three replicates. The results were the mean value of all tests.

RESULTS AND DISCUSSION

The profiles of conventional batch fermentation of lactic acid production are given in Fig. 1.

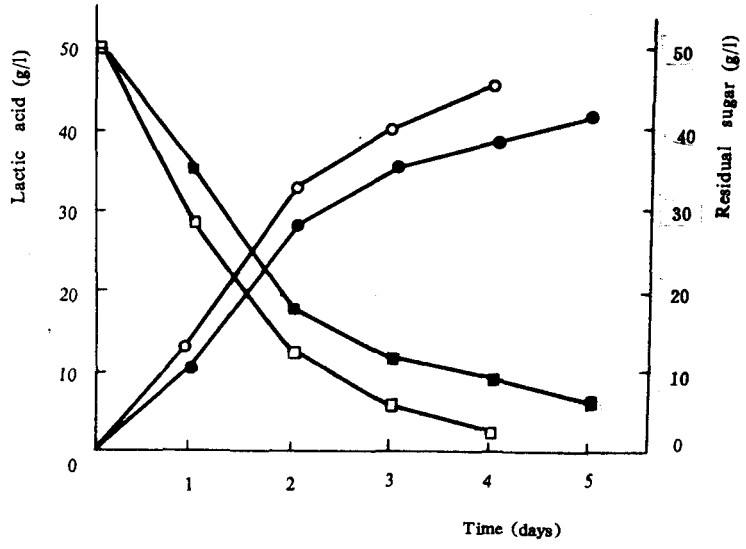

Fig. 1 Profiles of extractive and conventional batch fermentation of lactic acid production by immobilized cells.
(○). (□). extractive fermentation, (●). (■). conventional batch fementation
(○). (●). lactic acid (□). (■). residual glucose

It can be seen that, in conventional batch fermentation, the lactic acid concentration reached its maximum level (39. 4g/L) after 120 h fermentation. The overall sugar conversion is observed to be 88. 2%. Further increase in the sugar conversion as well as lactic acid productivity can only be possible through the simultaneous removal of lactic acid from fermentation broth. The results of extractive lactic acid fermentation were also shown in Fig. 1. The lactic acid concentration attained the highest amount of ca. 46. 2g/L after 96 h fermentation, the sugar conversion was 98. 6%. It had been shown that the removal of lactic acid from the broth decreased its inhibition on fermentation. The comparative study of the conventional batch and extractive fermentation process was summarized in Table 1.

Table 1. Comparison of conventional batch and extractive fermentation of lactic acid

Parameters	batch fermentation	extractive fermentation
Time (h)	120	96
Sugar conversion rate (%)	88.2	98.6
Product yield (g/g)	0.924	0.788
Productivity (g/L • h)	0.328	0.482

It is evident that extractive fermentation reduced the fermentation time by 20% in comparison with the conventional batch process. The direct consequence of this time reduction was indicated by a 1.47—fold increase in the overall lactic acid productivity, simultaneous removal of lactic acid from fermentation broth increased the lactic acid yield, and resultcd in more substrate diverting for lactic acid formation. This can be explained by the fact of that, through the removal of lactic acid from fermentation broth by resin adsorption, the pH value was maintained at a constant without the use of traditional pH control. This successfully alleviated the inhibitory effect caused by the lactic acid produced in the broth. The results from these preliminary experiments suggest the possibility of combination of simultaneous fermentation and separation by resin adsorption for lactic acid production.

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