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Continuous Production of Lactic Acid from Glucose and Lactose in a Cell-Recycle Reactor

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ABSTRACT

Growth and lactic acid production of *Lactobacillus delbreuckii* were compared using glucose and lactose as carbon sources. A continuous-flow stirred-tank fermenter was coupled with a cross-flow filtration unit to permit operation at high-cell concentrations. At steady state, yeast extract requirements for lactic-acid production were lower when glucose was used as a substrate than with lactose fermentation. Once steady state was obtained, with glucose feed, it was possible to lower the yeast extract concentration without affecting biomass concentration and lactic acid production. The lactic-acid concentration that inhibited cell growth and lactic acid production was found to depend on the choice of a carbon substrate.

Index Entries: Lactic acid from glucose and lactose; cell-recycle reactor; fermentation; yeast extract; whey permeates, as carbon source in fermentation; biomass; lactose fermentation.

INTRODUCTION

Continuous fermentation processes provide several advantages over batch fermentations (*1–4*), including stability, ease of control, and increased volumetric productivity, thus reducing capital and maintenance

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costs. This work examines the use of a continuous cell recycle system for lactic acid production.

Lactic acid is widely used today in the food industry as an acidulent and preservative. About half of the world's production is made via fermentation. The rest is obtained by synthetic routes (*5*). Fermentation substrates used commercially are principally sucrose, dextrose from hydrolyzed corn starch, cheese whey, molasses, and sulfite waste liquor (*6*). To minimize the recovery costs, only homofermentative bacteria are of industrial importance for lactic acid manufacture. A clean substrate and the absence of residual carbohydrates in the fermentation broth enhance recovery. Cheese whey, a byproduct of cheese manufacture, provides some of these advantages. In 1981, the total amount of whey produced worldwide was nearly 10.4×10^7 t (*7*). This byproduct poses a severe pollution problem because of its high biological oxygen demand (BOD). The high BOD for the whey permeate is because of the presence of lactose concentrations between 4 and 5%. This makes whey permeate an attractive carbon source for fermentation. Comparing the fermentation results obtained with glucose as a substrate (*4*), this lactose concentration appears adequate to produce lactic acid without residual sugar.

In this work, an efficient continuous cell recycle system was used to investigate the fermentation of glucose and lactose to lactic acid. The difference in behavior of Lactobacillus grown on a glucose or a lactose medium is examined.

MATERIALS AND METHODS

Organism

Lactobacillus delbreuckii ATCC 9649 was used in all experiments. The culture was maintained at 4°C on a glucose agar slant (0.2% glucose, 0.15% yeast extract, and 0.07% agar). This strain is a faculative anaerobe, gram-positive, and a homofermentative lactic-acid producer.

Media

Synthetic media were prepared by filter sterilization of glucose, lactose, and mineral solution through a 0.2-mm filter (Nucleopore). Yeast extract was autoclaved separately. The media contained glucose, lactose, yeast extract (concentrations are stated in Figs.), and $(NH_4)_2HPO_4$ (1 g/L) (*3*).

Inoculum Preparation

The inoculum was prepared by growing the bacteria in culture tubes (20 mL) for 24 h. The culture was grown batchwise with pH control for 24 h before starting the cell-recycle operation.

Continuous Cell Recycle System

The experimental apparatus is shown in Fig. 1. A glass fermenter with baffles and impellers was connected to a Minipore Pellicon tangential-flow filtration unit. The working volume of the fermentation system was maintained at 0.6 L. The balance between the feed- and the permeate-flow rates was monitored by an electronic level controller (Dyan Sense 7186) coupled to an electrosolenoid valve. The speed of the diaphragm cycling pump (AMF-CUNO) was set at 1.45 L/min. A peristaltic pump (Sigma Motor AL2E), at constant speed, was used for the feed. The filtration unit was a stainless-steel Pellicon Cassette System (Millipore), containing 0.04 m^2 of membranes (Millipore HVLP OH20). Experiments of more than 2-wk duration were conducted without any major technical problems. All fermentations were performed with pH maintained between 5.9 and 6.10 with addition of 8*N* ammonium hydroxide.

Analytical Methods

Glucose, Lactose, and Lactic Acid Determination

Samples were taken from the cell-free effluent. Glucose, lactose, and lactic-acid concentrations were measured by HPLC. An Aminex HPX-87X Column (Bio-Rad) was used, with 0.1*N* sulfuric acid as eluant, at a flow rate of 0.6 mL/min. Detection was performed with a refractive index detector (Waters Associates).

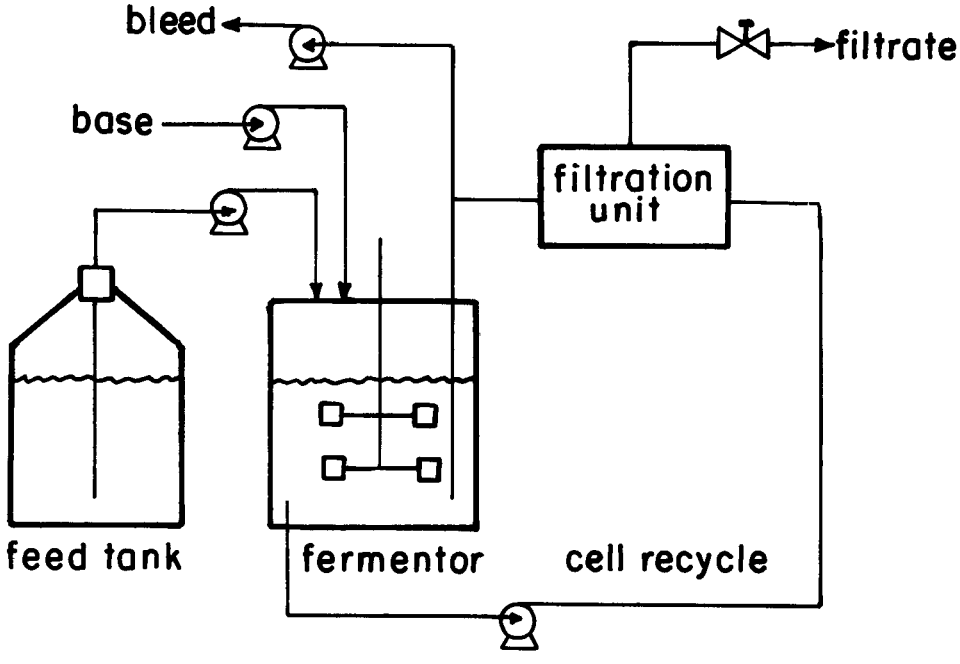

Fig. 1. Cell-recycle fermentation system.

Biomass Determination

Cell concentration was determined optically at 610 nm and the optical density was calibrated with dry weight. Dry cell weight estimations were made by filtering aliquots of the fermentation broth through tared 0.4 mm filters (Millipore), drying for 48 h at 85°C, and reweighing the filters plus the dried biomass.

RESULTS AND DISCUSSION

It has been established in several studies that continuous cell-recycle fermentation systems improve volumetric productivity by providing a high-viable biomass concentration and a possible decrease in product inhibition (*1–4*).

In this study, glucose and lactose fermentations to lactic acid, using *L. delbreuckii*, were conducted at a constant dilution rate in a CSTR with cell recycle. The effect of yeast extract, providing essential amino acids and vitamins for growth, and lactose concentration were investigated once a steady state was obtained.

Figure 2 shows the result of glucose fermentation. From previous work, it is known that with this cell-recycle system operation, above 60 g/L of lactic acid results in complete inhibition of lactic acid production (*4*). For all the different feeds employed, the glucose concentration lay between 66 and 74 g/L, and the dilution was fixed at 1.1/h. Feed-1 was used to quickly obtain a steady state in lactic acid and biomass concentra-

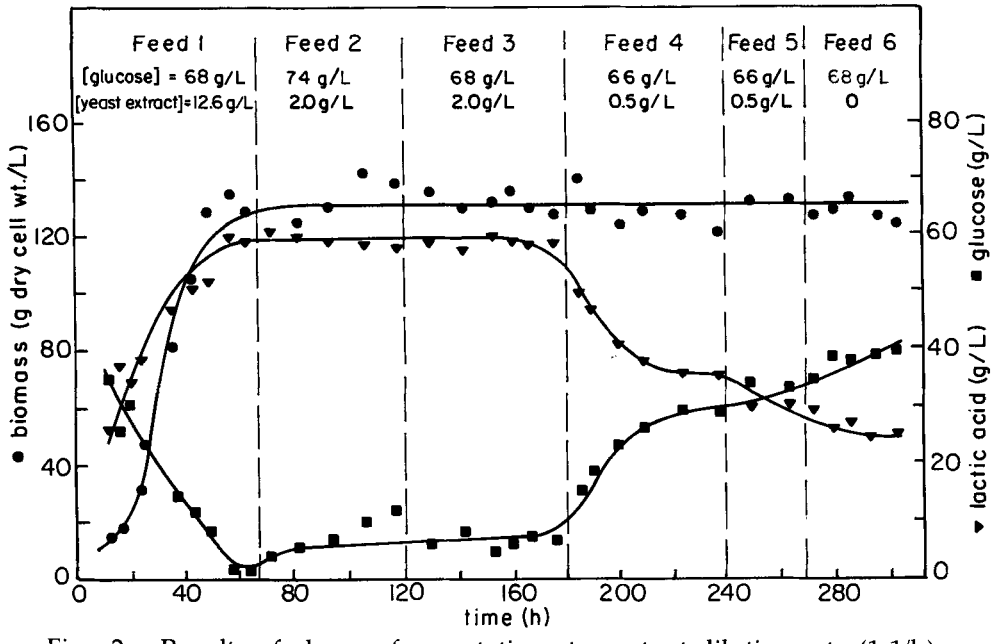

Fig. 2. Results of glucose fermentation at constant dilution rate (1.1/h).

tion. Then the feed was switched to a medium with a similar glucose concentration, but a lower yeast extract concentration (2 g/L). Such conditions did not affect the performance of the fermentation; biomass concentration and lactic acid productivity remained constant. With feeds-5 and -6, the glucose concentration remained the same, but the yeast extract concentration was reduced to 0.5 g/L. Under these conditions, a steady state was not obtained, although the cell concentration remained constant, the lactic acid productivity decreased from 66 to 33 g/L/h after 90 h. Feed-6, with no yeast extract, was then employed. After 60 h with this medium, the lactic-acid productivity decreased to 25 g/L/h and continued to decrease further. The biomass concentration, however, remained constant. From these observations, it is clear that a certain amount of yeast extract is required for production of lactic acid. Yeast extract is a complex mixture providing nitrogen, vitamins, and cofactors required for cell growth, cell maintenance, and lactic acid production. Consequently, it is difficult to identify exactly the rate-limiting factors for growth and acid production. These results show that once a steady state is obtained, the yeast-extract concentration can be decreased without affecting biomass concentration significantly.

Experiments using lactose as a carbon source were conducted analogously to those employing the glucose medium. Taxonomically, *L. delbreuckii* is not expected to ferment lactose. In fact, in batch fermentation on lactose medium, Lactobacillus shows little growth. For example, with the same size inoculum, after a 24-h fermentation, the cell concentration obtained on lactose is only 0.9 g cells DW/L, whereas the biomass concentration is 8.4 g cells DW/L on glucose medium. But when the cell recycle commences, Lactobacillus grows well, and a high cell concentration can be obtained. Before operating a continuous cell recycle fermentation, a batch fermentation with a medium composed of 15 g/L lactose and 25 g/L glucose concentration was used in order to obtain a significant cell mass before commencing the continuous feed.

Figure 3 shows the results of lactose fermentation. This experiment was run at a constant dilution rate (0.95/h) for 260 h. Feed-1 yielded a steady state after 40 h, at this point the cell concentration was 80 g cells DW/L and the lactic acid concentration 40 g/L. Then feed-2, with only 2 g/L yeast-extract concentration, was used. The lactic acid concentration subsequently decreased to 18 g/L, but the biomass concentration remained constant. Feed-3, with a lower lactose concentration, did not affect this trend. At 180 h, feed-4, with a higher lactose level (74 g/L) and yeast extract concentration (20 g/L), was used. The biomass concentration remained constant, but the lactic acid concentration increased to 42 g/L. This demonstrates that, although a low yeast-extract concentration affects lactic acid production, the bacteria retain their ability to produce lactic acid when all the nutrients required are resupplied.

These results show that the inhibitory lactic-acid concentration is around 40 g/L in the case of lactose fermentation: 20 g/L less than for glu-

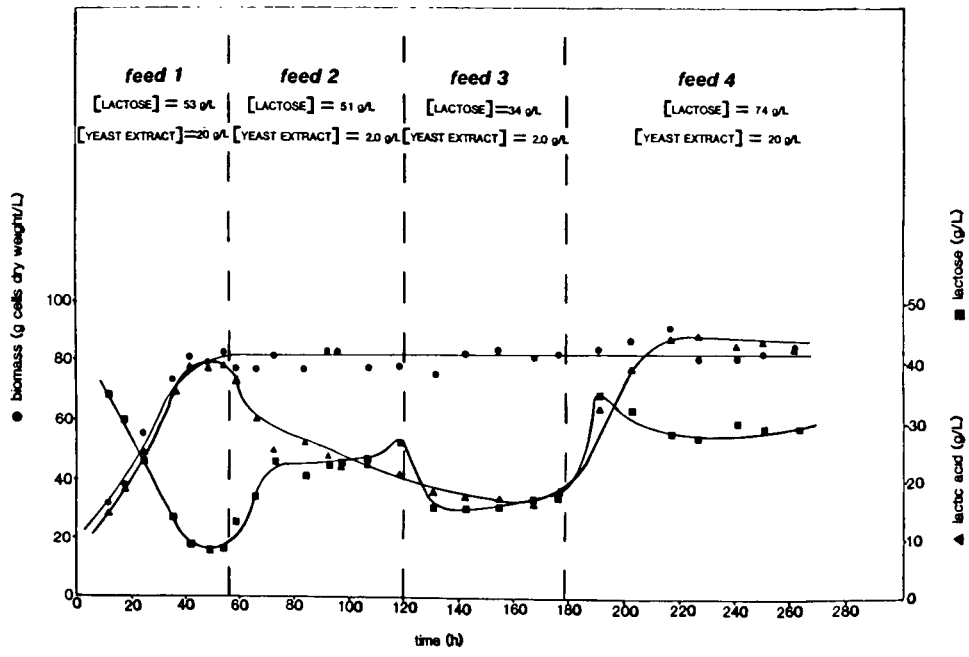

Fig. 3. Results of lactose fermentation at constant dilution rate (0.95/h).

cose fermentation. With almost an identical dilution rate, the biomass concentration at steady state is one-third less with lactose than with glucose. Yeast extract requirements at steady state are higher for lactose metabolism than for glucose. With a glucose medium, biomass and lactic acid production can be maintained with 2 g/L yeast extract. For lactose fermentation, this yeast extract concentration is not sufficient, and lactic acid production decreases fairly quickly.

From these experimental results, it is also clear that lactose fermentation requires more growth factors than glucose. A simple hypothesis is to consider the difference resulting from lactose and glucose transport through the cell membrane. Glucose enters the cells solely by facilitated diffusion. In this process, no energy is required, and only a concentration gradient is the driving force. For lactose transport, an active transport is involved. Lactose induces the biosynthesis of the three enzymes required for its entry into the cell and subsequent catabolism.

In order to check whether the residence time could be a limiting factor for the reactions involved in lactose fermentation, a fermentation was run at a low dilution rate (0.3/h). Figure 4 shows that with a residence time of 3 h, no more lactic acid was produced than when a residence time of 1 h was used. Experiments shown on Figs. 3 and 4 confirm that for lactose fermentation, a maximum lactic-acid concentration of 40 g/L was found. Beyond this limit, product inhibition was severe.

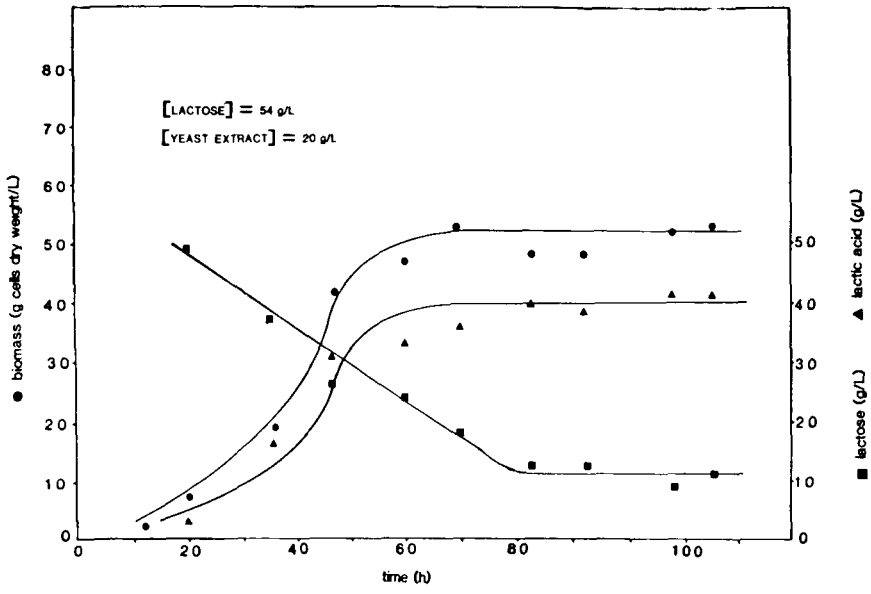

Fig. 4. Fermentation results of lactose at low dilution rate (0.3/h).

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