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Immobilization of *Lactobacillus bulgaricus* in a Hollow-Fiber Bioreactor for Production of Lactic Acid from Acid Whey Permeate

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ABSTRACT

Lactobacillus bulgaricus was immobilized in the shell side of an industrial hollow-fiber ultrafiltration module. Acid whey permeate, containing 46 g/L lactose supplemented with 10 g/L yeast extract, was pumped through the tube side at dilution rates of 0.2–2.5/h. At a cell concentration of 100 g/L, productivity was 1.5–5 g lactic acid/L/h.

Index Entries: *Lactobacillus bulgaricus*, immobilization of; hollow-fiber ultrafiltration module, production of lactic acid from whey permeate in; lactic acid, production of from whey permeate; acid whey permeate.

INTRODUCTION

Cheese whey is a byproduct of the cheese manufacturing industry. The present, annual, world-wide production is about 4×10^{10} kg, with about half being produced in the USA alone. Current estimates are that

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only about half this amount is being utilized in a useful manner (*1*). The major hindrance to its utilization is the relatively high lactose content, which makes up 75–88% of the solids, depending on the type of cheese. An obvious solution is fermentation of the lactose to useful products, such as ethanol (*1–4*), or to lactic acid (*5–7*). Conventional methods of fermentations, however, using free cells in batch processes, have several disadvantages (*1,2*), such as long times needed for completion of the fermentation, the need to separate the cells at the end of the fermentation, their inherent inefficiency caused by their start-up and shut-down nature, and batch-to-batch variation in the product stream. The resulting low productivity results in high operating and capital costs.

Continuous-culture type processes, on the other hand, although overcoming the problems associated with the batch processes, are limited by cell "washout." To overcome this limitation, cells can be immobilized onto solid supports or entrapped in a gel matrix. This creates another set of problems, such as diffiusional resistances, steric hindrance, high-pressure drops in packed-bed column reactors, and the expense of the immobilization step.

We have been investigating the use of synthetic semipermeable membranes for the immobilization of cells (*1–5,8*). These membrane bioreactors take advantage of the size differences between microbial cells and the product of the fermentation. In the plug-flow configuration, the bioreactor consists of several tubes of a synthetic, self-supporting membrane housed in a cartridge in a "shell-and-tube" configuration. The microbial cells are loaded into one side of the membrane (usually the shell side), which is then sealed, thus effectively immobilizing the cells, but in their free state. The media, containing the fermentable sugar, is pumped through the tube side. With the appropriate pressure differential across the membrane, substrate will diffuse through the membrane, react with the cells, and product will diffuse back into the tube side and exit the module. In this paper we report on the immobilization of *Lactobacillus bulgaricus* in hollow-fiber membrane modules and its use in the continuous production of lactic acid from deproteinized cottage cheese whey.

MATERIALS AND METHODS

Lactobacillus bulgaricus was obtained from Chr. Hansen's Laboratory, Milwaukee, WI. Figure 1 is a schematic of the continuous bioreactor system. The cheese whey was obtained from a local cottage cheese whey plant. It was pasteurized and clarified before processing by ultrafiltration in a Romicon system, as described earlier (*2,5*). The whey was fractionated into two streams, a protein-rich retentate, which can be marketed as a highly functional food ingredient known as whey protein concentrate (WPC), and the deproteinized fraction, the permeate. The permeate, containing 46 g/L lactose and 0.4 g/L total nitrogen, was supplemented

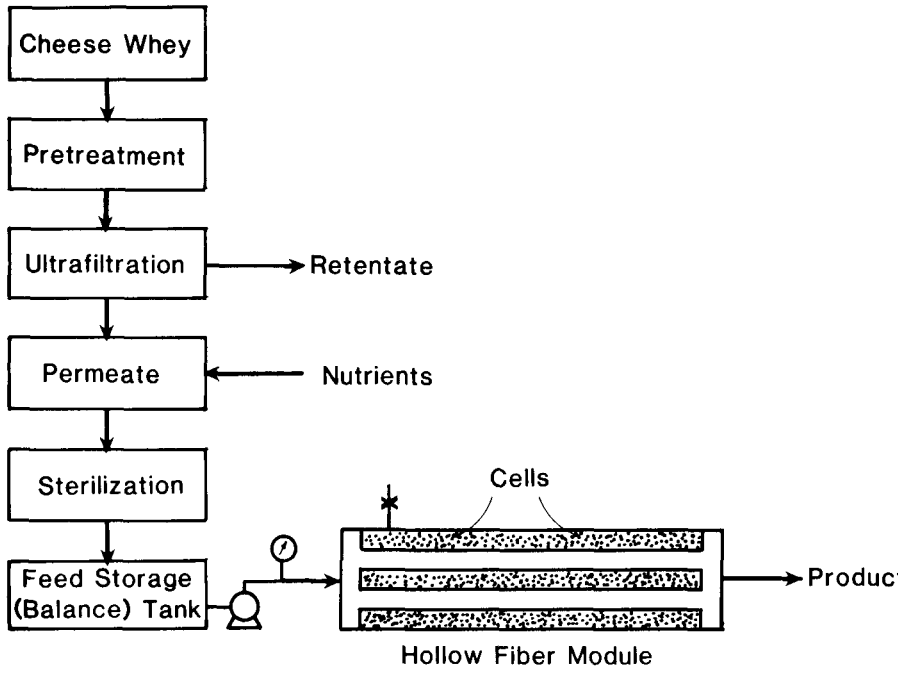

Fig. 1. Schematic of a hollow-fiber bioreactor system for continuous production of lactic acid from acid whey.

with 10 g/L yeast extract (Difco). It was then cold-sterilized by passing it through a 0.2-μm cross-flow microfilter (Acroflux, Gelman Sciences, Ann Arbor, MI, USA). The sterile whey permeate was stored in a sterile tank and used for batch and continuous membrane experiments.

Continuous hollow fiber bioreactor experiments were performed with the *Lactobacillus* cells packed in an industrial PM-50 short–short hollow-fiber cartridge (Romicon Inc., Woburn, MA). The module had a molecule weight cut-off of 50,000 and a surface area of 0.6 m^2. The shell-side volume was 430 mL and the fiber volume was 195 mL. Cells were loaded into the shell side of the cartridge by back-flushing a suspension of the cells through what is normally the permeate port of the cartridge. The permeate ports were then closed off, and the whey permeate was pumped through the tube side with a back pressure of 1.2 atm. Further details on the operation of hollow-fiber bioreactors are available elsewhere (*1–4,8*).

Batch experiments were performed in a New Brunswick Microferm unit, using 2-L volumes. Experiments were conducted at 45°C and the pH maintained at 5.6 with 8*N* ammonium hydroxide.

Analytical Methods

Cell concentration is expressed as cell dry weight per unit volume (g/L). Cell concentration was measured optically at 525 nm. Cell dry

weights were obained by drying washed cells at 105°C until constant weight.

Lactose concentration was measured by the method of Summer and Somero (*9*). Lactic acid concentration was measured using an enzymatic procedure described by Sigma Chemical Co. St. Louis, MO (Method Number 726UV/826UV). The procedure is based on the reaction between lactate dehydrogenase and lactic acid to convert nicotinamide (NAD) to its reduced form, NADH, which is measured spectrophotometrically at 340 nm.

RESULTS

Figure 2 shows typical batch fermentation data. With an initial cell concentration of 1.7 g/L, the fermentation was completed in about 9 h, and the final lactic acid concentration was 43.9 g/L. This corresponds to a productivity of about 4.9 g lactic acid/L/h and a maximum specific growth rate of 0.4/h.

Figure 3 shows data obtained with the hollow-fiber bioreactor operated in a single-pass mode at an initial cell concentration of 100 g/L. The dilution rate (*D*), which is the flow rate divided by the bioreactor volume, is based on the total hollow-fiber bioreactor volume, which is the sum of the shell-side and fiber volumes. As *D* is increased, the residence time decreases and the conversion of substrate to lactic acid decreases. The productivity (*PD*), which is *D* × product concentration (*P*), increases ini-

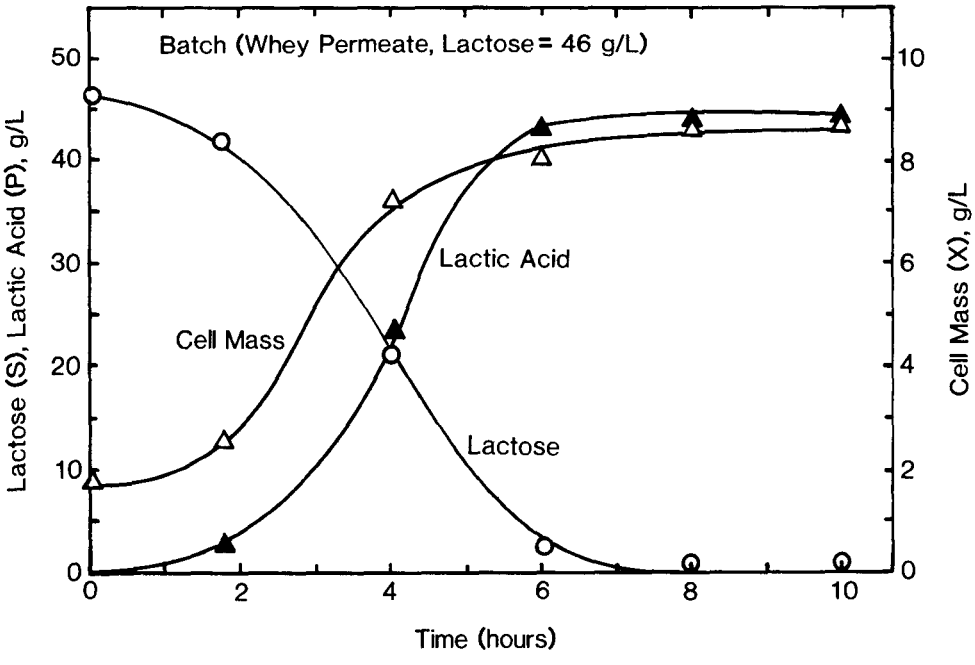

Fig. 2. Batch fermentation of acid whey permeate with *L. bulgaricus*.

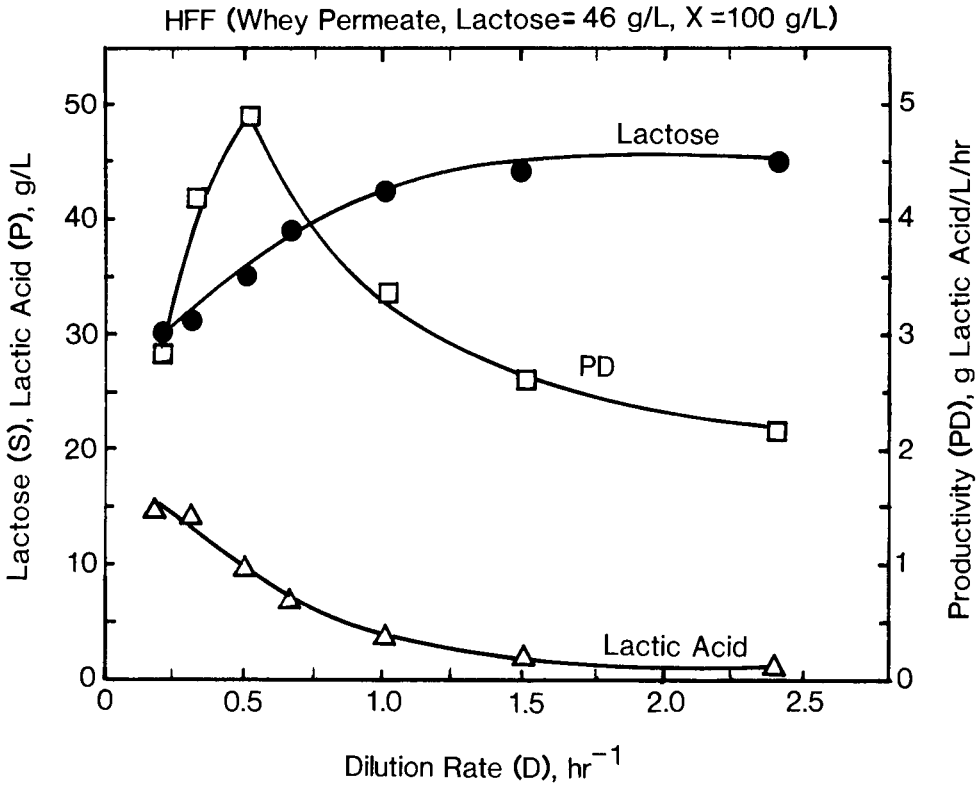

Fig. 3. Fermentation kinetics of a hollow-fiber bioreactor operated in a single-pass continuous mode. Initial cell concentration in the shell side of the bioreactor was 100 g/L.

tially, but decreases at high dilution rates. Maximum productivity was 5 g/L/h, but substrate conversion under these conditions was only 24%.

Figure 4 shows the stability of the hollow-fiber bioreactor. The dilution rate was set at 0.2/h. Lactic acid concentration was 13 g/L after about 8 h, and productivity was a corresponding 2.6 g/L/h. At the end of 12 d, the productivity had dropped to 1.4 g/L/h.

DISCUSSION

The hollow-fiber system is an effective means of immobilizing microbial cells. No cell leakage or grow-through was observed during the entire series of experiments. The performance of the hollow-fiber bioreactor is comparable to conventional immobilized whole cell reactors. Stenroos et al. (*10*) obrtained a productivity of 3 g/L/h with *Lactobacillus delbrueckii* immobilized in calcium alginate, and Tuli et al. (*7*) obtained a productivity of 0.5 g/L/h with 73% conversion of lactose with immobilized *Lactobacillus casei*. However, these productivities and product concentrations are not high enough to justify adopting these bioreactors in place of conven-

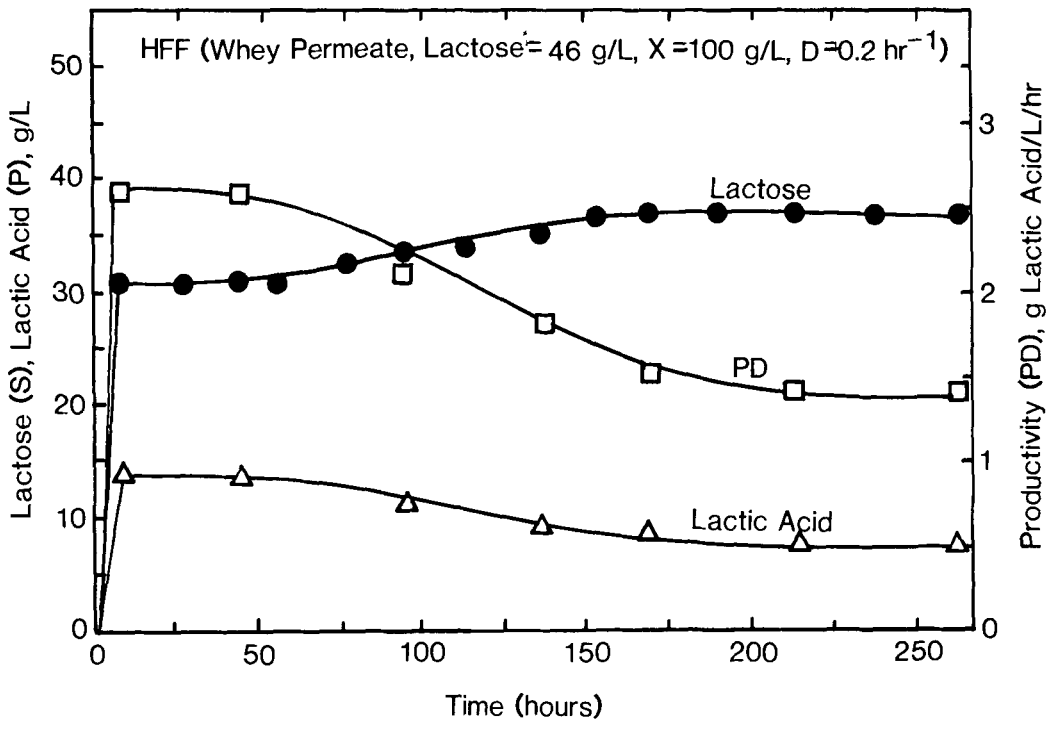

Fig. 4. Stability of the hollow-fiber bioreactor processing whey permeate. Initial cell concentration = 100 g/L. Dilution rate = 0.2/h.

tional batch processes. In this study, the high productivity of the batch fermentor is probably a result of the fairly heavy innoculum used in those experiments.

Maximum substrate utilization is of major importance since a large portion of the final product cost is in the raw material. In other experiments not reported here, even slowing the dilution rate down to 0.05h did not substantially improve the substrate utilization or productivity. Vick Roy et al. (*11*) also reported a relatively poor performance with a similar hollow-fiber bioreactor used for the production of lactic acid from glucose by *L. delbrueckii*.

This is partly the result of the fluid dynamics inherent in this type of bioreactor. Because the substrate and the cells are separated by a physical barrier (the membrane), the limiting step becomes the diffusion of the substrate into, and the diffusion of the product out of, the shell side. The shell side, being completely enclosed, will equilibrate at a pressure that is the average of the inlet and outlet pressures. A certain portion of the substrate will by-pass the shell side altogether and move straight through the tube side unconverted. Thus, there will be poor substrate–cell contact and inadequate mixing of the reactor contents. Control of pH is difficult in such plug-flow designs and none was attempted in this study. For these reasons, the viability of the cells will be affected, which is reflected in the decline in productivity shown in Fig. 4. Flow distribution within

the fiber bed will have to be modified to improve the performance of hollow-fiber reactors. In contrast, much better performance was obtained with membrane recycle bioreactors operated under completely mixed conditions (*1,2,5,8*).

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