0273-2289/92/3435—0431$02.00

Simultaneous Fermentation and Separation of Lactic Acid in a Biparticle Fluidized-Bed Bioreactor

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ABSTRACT

A bioreactor configuration has been tested for simultaneous fermentation and separation of the desired inhibitory product, lactic acid. The bioreactor is a fluidized bed of immobilized *Lactobacillus delbreuckii*. Another solid phase of denser sorbent particles (a polyvinyl pyridine resin) was added to this fluidized bed. These sorbent particles fell through the bed, absorbed the product, and were removed. In test fermentations, the addition of the sorbent enhanced the fermentation and moderated the fall of the pH. The biparticle fluidized-bed bioreactor utilizing immobilized microorganisms and adsorbent particles has been shown to enhance the production of lactic acid fourfold in this nonoptimized system.

Index Entries: Fermentation; adsorption; lactic acid; fluidized bed.

INTRODUCTION

Fluidized-bed bioreactors (FBRs) with immobilized cells have significant advantages over other conventional designs in that the productivity of fermentations for useful products is enhanced (*1*). Previous research at Oak Ridge National Laboratory (ORNL) has successfully demonstrated 10-fold increases in productivity in these FBRs over free-cell, fed-batch reactors for ethanol fermentation (*2*). These multiphase reactors are typically comprised of a solid biocatalyst, a liquid containing the substrate and products, and, often, a gas that can be a reactant and/or product. An unexploited advantage of the multiphase FBR is its potential to increase

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further the number of phases present. If an additional phase with extractive capabilities for the desired product were added to this system, then productivity might be increased as well as a simultaneous product separation achieved.

This extractive phase could be an immiscible liquid or a solid. Some work has been performed in various bioreactors with direct liquid extraction (*3–7*) and with liquid extraction through a hollow-fiber membrane (*8,9*). Busche and Allen have proposed liquid extraction in a fluidized bed (*6*). Solid adsorbents have been considered for subsequent product recovery (*10–12*). Garcia (*13*) has reviewed the use of some polymeric adsorbents. Adsorbents have also been used in a fluidized-bed contactor for product recovery from fermentation broths (*14*). Solid adsorbents have also been added directly to batch fermentations (*15,16*). This system uses a solid adsorbent for *in situ* product removal in a fluidized-bed fermentor.

An essential consideration of the use of another solid phase is the selective removal of this phase from the FBR. This can be accomplished by exploiting a well-known property of fluidized beds, namely, their stratification on the basis of size and density. This particle separation is related to the Stokes' settling velocity. These two solid particles (the biocatalyst and the sorbent) form the biparticle FBR. If the adsorbents are denser or larger than the immobilized cells, they can be introduced at the top and removed from the bottom of the FBR. This would result in a countercurrent flow of the adsorbent with respect to the upward-moving liquid phase (*see* Fig. 1). On the contrary, if the adsorbent is smaller or lighter, it could be added to the base of the column, resulting in cocurrent flow. A biparticle fluidized-bed system has been demonstrated to separate while continuously adding the slightly heavier particles as a slurry to the top of the column with a pump and removing them from the base of the column below the liquid feed inlets using a star-valve (*17*).

The production of lactic acid by *Lactobacillus delbreuckii* was chosen to test this new approach. Lactic acid, a commodity organic chemical that is used in foods and pharmaceuticals, can be produced either by fermentation or by organic synthesis (*18*). Removal of the product is one of two desired effects of sorbent addition for the lactic acid fermentation. The other is the alleviation of inhibition. Lactic acid is inhibitory to *Lactobacillus*, as is the low pH caused by acid production. *L. delbreuckii* prefers a pH of 5 or greater. In general, the undissociated form of organic acids is the most inhibitory, and the amount of the undissociated acid can be controlled by the pH. Therefore, the ability of the sorbent to neutralize the lactic acid solution is important. Seevaratnam et al. (*19*) have tested a sorbent in the solvent phase to enhance recovery of lactic acid from free-cell batch culture. A fluidized-bed reactor has also been operated with a flocculent *Lactobacillus* and conventional pH control at pH 5.5 (*20*).

The separation of the different particles in the system was confirmed (*17*), and now the production of the organic acid, lactic acid, is being studied. As the lactic acid is removed by the sorbents, pH is moderated, and the

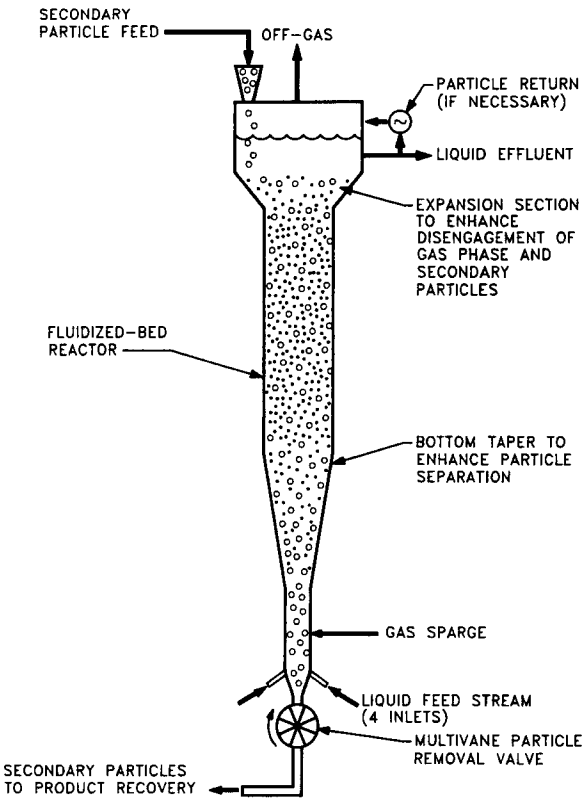

Fig. 1. Schematic of experimental biparticle fluidized-bed bioreactor operating in the countercurrent mode. • are biocatalyst particles; ○ are added sorbent particles.

fermentation is enhanced. One aim is to control pH without the addition of a base, thus, producing the organic acid rather than its salt. Another aim is to enhance the fermentation by the removal of an inhibitory product.

MATERIALS AND METHODS

Organism and Media

L. delbreuckii NRRL B-455 was grown in media containing glucose, yeast extract (5 g/L), $(NH_4)_2SO_4$ (0.5 g/L), $MgSO_4$ (0.3 g/L), KH_2PO_4 (0.2 g/L), and K_2HPO_4 (0.2 g/L), which was sterilized by autoclaving. The initial pH was pH 6. Inocula were grown in Fernbach flasks at 40°C for 2 d on 10 g/L glucose and centrifuged to concentrate the cells. The cells were washed with sterile distilled water and recentrifuged before adding them to the gelling solution below. This step decreased the gel viscosity and improved the production of the beads.

Adsorbents

Reillex 425 (R425), a polyvinyl pyridine (PVP) resin (Reilly Industries, Indianapolis, IN), was used. Other resins screened included Filtrasorb 100®, an activated carbon (Calgon Carbon Corp., Pittsburgh, PA), Reillex HPQ, a partially quarternized PVP resin, a cation-exchange resin (Bio-Rad AG-1, Richmond, CA), and an anion-exchnge resin (Bio-Rad AG-50). Equilibrium capacity tests were performed under varying lactic acid concentrations and with other chemicals. A known amount of sorbent was placed in 100 mL of a determined concentration of lactic acid and allowed to equilibrate for > 1 h. Samples had been deter mined to reach equilibrium within 30 min. Capacities were then calculated by difference using the true dry weight. The native resins, as provided, were dried overnight at 90°C to determine the true dry weight. The R425 was 0.47 g dry wt/g native.

Regeneration was tested by removing the bulk liquid from the lactic acid equilibrated resin by filtration. The resin (~ 1 g) was then placed in 20 mL of either methanol or 1*N* NaOH. After equilibration, the liquid concentration was sampled.

Analysis

Lactic acid was measured by enzymatic assay (Sigma #826-UV) on a spectrophotometer (Shimadzu UV-160, Kyoto, Japan). The assay was confirmed to be unaltered when the lactate was measured in methanol or in 1*N* NaOH. Glucose was measured on a YSI glucose analyzer (Yellow Springs Instruments Company, Inc., Yellow Springs, OH).

Production of Gel Beads

Small uniform gel beads were made of 1% alginate and stabilized in a 0.1*M* $CaCL_2$ solution. The bead production technique has been described in detail elsewhere (*21*). The concentrated washed cells were added to the gel solution before solidification. The bead diameters were measured visually to be ~ 0.8 mm with a calibrated micrometer under a low-power microscope. The bead contents were determined by drying a known volume of beads, washed with distilled water. The bead biomass loading was determined from the difference in density of the biocatalyst beads and unloaded nominal 1% alginate beads. The measured dry weight content of the unloaded washed beads was 18 g/L, which accounts for the bound calcium and shrinkage. The biocatalyst beads could be stored for several weeks at 5°C, and then reactivated with minimal difficulty by simply increasing the temperature and adding nutrients.

Fluidized-Bed Fermentor

This column has a 1.27-cm-id inlet section of 23 cm in length, which tapers over 30 cm to a straight 2.54-cm-id section, which is 61 cm in length

(*see* Fig. 1). At the bottom, the liquid enters through four symmetric radial ports around a 0.6-cm-id central tube. This central tube connects to a star-valve to allow continuous removal of falling particles from the bottom of the column. At the top, the column expands to 7.62 cm id to decrease the superficial liquid velocity and thus retain the biocatalyst solids. A recycle stream was pumped through a UV-sterilizer (Aquafine, Valencia, CA) to the inlet of the column. The total volume of the system was 0.84 L. Peristaltic pumps (Masterflex, Cole-Parmer, Chicago, IL) were used. The column was jacketed and maintained at 40°C by a recirculating water bath.

Two FBR fermentations were performed using complete recycle at 40 mL/min and no pH control. The media was as above with ~5 g/L initial glucose. In all experiments, the pH was monitored with an *in situ* pH probe. Samples were removed, and rapidly filtered and frozen at −4°C for analysis of glucose and lactic acid. The first experiment was a control with no resin added. The added biocatalyst beads were 128 mL in volume and had a cell density of 17 g dry wt/L. The fermenter was allowed to operate in complete recycle until all activity halted. In the second experiment, 73 mL of beads were added with a cell density of 12 g dry wt/L. When the pH fell below 5, manual resin addition began with 10 g of the native resin (4.7 g dry) being added every 12 min. This continued for 7 h. The experiment was allowed to continue without further resin addition overnight. All of the resin accumulated from the base of the column was then back-extracted with 1*N* NaOH to determine the total amount of lactic acid removed in the resin.

RESULTS AND DISCUSSION

Adsorbents

Several adsorbents were screened. Some of these extraction results are presented in Table 1. Both the carbon and the amine resin had significant detrimental pH effects. These effects are owing to the preferential adsorption of the protonated acid over the ion (*12*). Lactic acid has a pK_a of 3.8. The resin, HPQ, was less affected by the pH. Various conditioning pretreatments also were used to increase the capacities of the resins, in particular for the anion (AG-50) and cation (AG-1) exchangers. In the first pretreatment, the resins were washed sequentially with 1*N* NaOH, an acetate solution, methanol and then oven-dried. In the second conditioning pretreatment, the resins were washed in base, HCl, methanol and then dried. As shown in Table 1, the pretreatments increased the capacities of the ion exchangers, but not beyond the capacity of the R425 polyvinyl pyridine. The R425 was apparently not affected by the pretreatments. The best sorbents of those tested were the activated carbon (F100) and the R425. R425 also has no measurable capacity for glucose, unlike the activated carbon (*17*). *Lactobacillus* prefers a pH of 5 or greater.

Table 1
Capacity of Selected Adsorbents for Lactic Acid pH 2 to 3 Except Where Noted

Adsorbent	Treatment	Equilibrium concentration g/L	Capacity g/g sorbent
Act. carbon	None	3.4	0.12
RHPQ	None	4.9	0.07
RHPQ (pH 6)	None	4.6	0.04
AG-1	None	5.1	0.01
AG-1	*a*	4.7	0.03
AG-50	*a*	5.6	0.00
AG-50	*b*	4.6	0.01
R425	*a*	3.4	0.10
R425	None	4.9	0.12
R425 (pH 7)	None	4.6	0.01

[a]Washed 3X w/base, acetate, MeOH, dried.
[b]Washed 3X w/base, HCl, MeOH, dried.

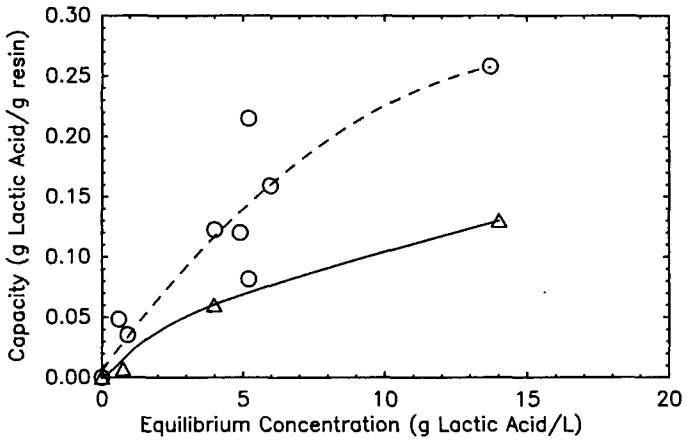

Fig. 2. Capacity of Reillex 425 resin for lactic acid at 25°C (○) and 40°C (△).

Figure 2 shows an equilibrium adsorption isotherm for lactic acid on Reillex 425 at pH 3 at 25 and at 40°C. The capacity increases at higher concentrations and appears to fall to zero as the bulk lactic acid concentration decreases. The capacity decreases with a temperature increase to the fermentation temperature of 40°C. This resin's capacity was measured to be minimally affected by the presence of a phosphate buffer. Reillex 425 had no measurable capacity for glucose.

Initial tests have also been performed on the regeneration of the sorbent. Reillex 425 was loaded with lactic acid. The resin was washed with methanol, which removed 60% of the loaded lactic acid. A second methanol wash removed under 4% of the original lactic acid. No more could be removed. A final wash with 1*N* NaOH recovered the remainder of the

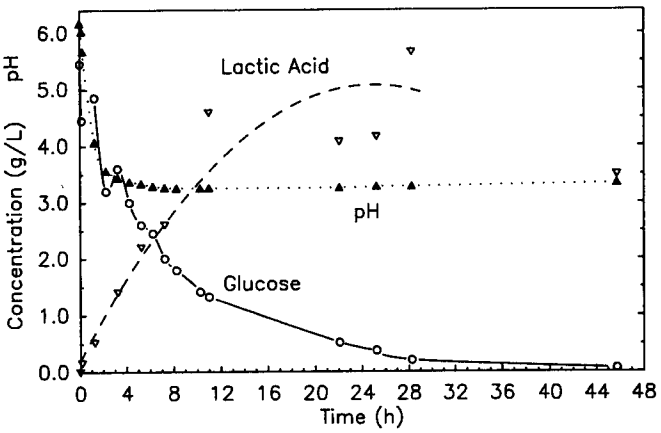

Fig. 3. Lactic acid production in an immobilized cell FBR operated in infinite recycle with no pH control and no sorbents added.

lactate. The methanol-regenerated resin was later tested to have a capacity that was about 60% of its original capacity. Regeneration of the resin with NaOH approaches 100%. However, regeneration with a base will result in a lactic salt that will be more difficult to purify further. Therefore, if the capacity of the partially regenerated resin is adequate, regeneration with solvent would be preferred in order to allow product recovery of the acid by distillation.

FERMENTATION

In an initial batch fermentation with free cells of *L. delbreuckii*, an initial glucose concentration of 10 g/L and no buffering or pH control, the pH quickly dropped to near pH 4 after the conversion of only 2 g/L glucose into lactic acid. The *L. delbreuckii* do not grow below pH 4. Ordinarily, the production of lactic acid will lower the pH and halt the fermentation. The addition of the sorbent particles removed some of the lactic acid product resulting in a decrease in acid concentration and moderating the pH, thus, allowing additional glucose conversion.

Two similar experiments were performed to test the biparticle FBR with the Reillex 425 resin particles as the adsorbent. The systems were operated at infinite recycle and an initial glucose feed addition of 5 g/L. In the control test, without the addition of the resin adsorbent, the pH rapidly reduced to a value of 3.3, and the rate of glucose conversion significantly decreased (*see* Fig. 3). Ultimately, most but not all of the glucose was consumed after more than 28 h with essentially complete conversion to lactic acid at an average rate of about 0.11 g lactate/h/g dry wt biomass. In a second test, similar conditions were used, except that Reillex 425 resin beads were added to the top of the column and recovered at the bottom of the column at a rate of about 0.4 g dry wt/min. Glucose was again

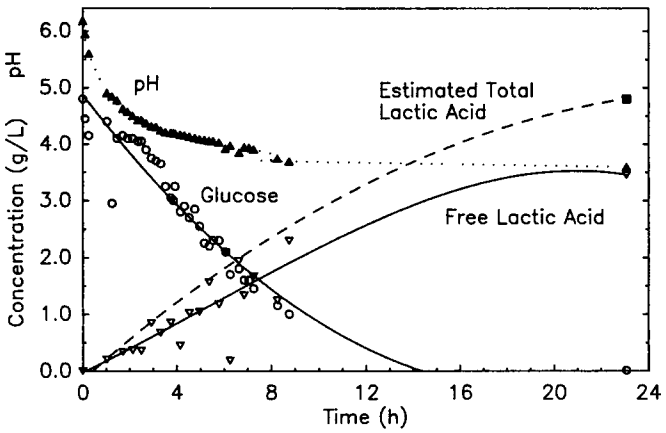

Fig. 4. Lactic acid production in the biparticle-fluidized-bed operated in infinite recycle. Sorbent was added and removed as described in text.

completely consumed, but in less than 14 h, and the pH was maintained at higher levels throughout the test (*see* Fig. 4). Please note that the time scale in Fig. 4 is half that of Fig. 3 and, from the Methods section, that the bead volume and the cell concentration were lower in the second run. There was essentially complete conversion to lactic acid at a rate of 0.37 g/h/g biomass, which is substantially greater than that in the test without the adsorbent. The rates were calculated from the time for the glucose to fall to 1 g/L. The biomass was assured to be constant.

The resin collected from this run (Fig. 4) was separated from the liquid. The lactic acid was extracted with 1*N* NaOH for maximum recovery. A total of 0.3 g lactic was recovered in two washes. This amount was used to estimate the total produced lactic acid in the figure. The dashed line of the estimated total lactic acid was estimated from the ratio of the overall adsorbed lactic acid with the free lactic acid. The overall yield of this fermentation was measured as 1.0 g lactate/g glucose consumed. This is the stoichiometric limit. A surprising observation from these tests is that the fermentation in an FBR continued below pH 5 while, according to the literature and earlier experiments, growth had stopped. The fermentation pathways may be much less sensitive to lactic acid and pH effects than is the growth rate. This could be beneficial for long-term operation of this system.

CONCLUSION

The biparticle FBR concept for simultaneous fermentation and separation has now been shown to be feasible and advantageous in an unoptimized system. With proper design of the system and particles, the continuous countercurrent addition and removal of desired particles to a stable continuous fluidized bed of other particles can be achieved. The particle sizes and densities can be adjusted to change the system hydro-

dynamics and residence times. Improved adsorbents for lactic acid may still be needed. This system could be applied to other fermentation systems and is being extended to the production of acetic acid.

ACKNOWLEDGMENTS

The technical assistance of R. P. Guillory is appreciated. Discussions with C. J. King assisted the selection of an adsorbent. This research was supported by the Advanced Industrial Concepts Division of the US Department of Energy, under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc. The partial support of a NATO collaborative grant is appreciated.

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