0273-2289/96/5758—0503$8.25

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

ERIC N. KAUFMAN,* STANLEY P. COOPER, MARIA K. BUDNER, AND GERALD R. RICHARDSON

Bioprocessing Research and Development Center, Chemical Technology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6226

ABSTRACT

A continuous biparticle fluidized-bed reactor (BFBR) is developed for the simultaneous fermentation and recovery of lactic acid. In this processing scheme, bacteria are immobilized in gelatin beads and are fluidized in a columnar reactor. Solid particles (weak-base resin IRA-35) with sorbent capacity for the product are introduced at the top of the reactor and fall countercurrently to the biocatalyst, effecting *in situ* removal of the inhibitory lactic acid while also controlling reactor pH at optimal levels. One-week-long fermentation trials using immobilized *Lactobacillus delbreuckii* with sorbent addition demonstrated a volumetric productivity (6.9 g/L·h) at least 16-fold higher than that of a free-cell batch fermentation with base pH control and identical biomass concentration and medium composition. Regeneration of the loaded sorbent from the BFBR has effected a 35-fold concentration of lactic acid compared with original levels in the fermentation broth (70 vs 2 g/L). Lactic acid concentrations as high as 610 g/L have been observed when the loading solution contained 50 g/L lactic acid. Rich medium formulations did not seem to increase BFBR performance. The benefits of this reactor system, as opposed to conventional batch fermentation, are discussed in terms of productivity and process economics.

Index Entries: Lactic acid fermentation; resin; adsorbent; biparticle fluidized bed; continuous.

INTRODUCTION

Lactic acid is a specialty chemical used in the food industry for the manufacture of cheeses, pickles, and yogurt, and also as a preservative *(1)*. It may also be used as feed in plastics production and in the synthesis of other organic acids,

*Author to whom all correspondence and reprint requests should be addressed.

acrylic acids, acetaldehyde, and ethanol *(2)*, with a current price of $1.15/lb for technical (88%) or food-grade lactic acid *(3)*. Annual US consumption of lactic acid totals over 30 million pounds *(4)*. Biological production of lactic acid accounts for approx 50% of the world's production *(5)*. Biological production is complicated primarily owing to economic considerations arising from product inhibition and the required downstream processing of dilute aqueous product streams. It is estimated that lactic acid purification accounts for 50% of production cost *(6)*. The standard method of biological lactic acid production is the anaerobic fermentation of glucose or sucrose by *Lactobacillus* in batch reactors. The conventional process requires that the base be added to the reactor to control pH *(4,7)*, and/or that calcium carbonate be added to buffer the reactor and to precipitate the lactate *(8,9)*. These processes produce the lactate salt, which must be reacidified (usually by sulfuric acid *[8–10]*), and which yields sulfates, further adding to process chemical costs and waste streams. The lactic acid productivity of these conventional processes are reported in the open literature to be ~1.6 g/L·h *(8)*.

It is postulated that it is the protonated form of lactic acid that is inhibitory to the fermentation *(5,11–13)*. Based on the kinetic modeling of *L. delbreuckii* by Yeh et al. *(11)*, Kaufman et al. *(13)* demonstrated how pH control alone is insufficient to ensure maximal productivity, owing to the small but inhibitory amount of protonated lactic acid that exists even with pH control to 6.0. For instance, according to the kinetic constants of Yeh et al., the production rate of lactic acid is reduced by 50% when the broth concentration of lactic acid reaches 40 g/L at pH 6.0. At a broth concentration of 90 g/L, the fermentation is operating at only 10% of its maximum production rate. It is evident that bioreactors employing *in situ* product removal have the potential to realize great improvements in reactor productivity compared to conventional batch production of lactic acid with pH control alone.

Improvements in volumetric productivity for the lactic acid fermentation have been reported by increasing biomass loading and by reducing product inhibition. High cell densities in reactors have been achieved by the use of cell aggregates *(14)*, growth of biofilm on activated carbon *(15)*, cell immobilization in gelatin beads (for example, *16–19*), cell growth in hollow fibers *(20)*, and cell recycle using hollow fiber membranes *(21,22)*. Product inhibition has been reduced by moving toward a plug flow reactor system through the use of staged CSTR reactors *(23,24)*, such that the product concentration is reduced in the initial stages and inhibition becomes relevant in a smaller portion of the reactor volume. Removal of inhibitory product has been achieved using both liquid extractants *(10,19,25–27)* and solid adsorbents *(25,28)*, either in a product-stripping side stream *(29,30)* or by adding directly to the constantly stirred tank reactor (CSTR) *(31)*. Product removal using ultrafiltration *(22)* and electrodialysis have also been investigated (*see* ref. *27* for a review).

Combining the benefits of cell immobilization and *in situ* product separation, Davison and Thompson *(32)* demonstrated the simultaneous fermentation and separation of lactic acid in a BFBR. In this process, *L. delbreuckii* was immobilized in alginate beads and fluidized by the upflowing liquid media in a tubular reactor. Such fluidized beds have been shown to increase the productivity of fermentations for a variety of processes *(33,34)*. In the demonstration by Davison and Thompson *(32)*, the polyvinyl pyridine resin Reillex 425, which possesses affinity for the lactic acid, was added batchwise to the top of the reactor, fell through the biocatalyst bed, and was found to moderate reactor pH, adsorb the lactic acid product, and increase lactic acid production nearly fourfold over a controlled fluidized-bed reac-

tor without resin addition or pH control. In this proof-of-concept experiment, the resin was added batchwise for a short period of time (7 h) and was added at a constant schedule. Although resin addition moderated the decrease in reactor pH, it did not control the pH at the optimal level. Recently, Kaufman et al. *(35)* screened a series of resins for use in a lactic acid BFBR and demonstrated the potential economic benefits of BFBR as opposed to batch production *(13)*. Two-day-long fermentations in the BFBR were conducted with manual resin addition and were compared to similar runs without pH control or resin addition *(13)*. A 12-fold increase in productivity was reported in BFBR as opposed to immobilized cell fermentations without pH control. In this article, we describe the further demonstration of the BFBR by conducting longer term fermentation trials and providing more relevant control runs in which pH control is utilized in free and immobilized cell reactors with the same media and cell concentrations as the BFBR. This enables the assessment of the impact of *in situ* product removal and cell immobilization on reactor productivity. We also compare the use of rich vs lean media recipes in the BFBR.

METHODS

L. delbreuckii NRRL B445, now being reported as *Lactobacillus rhamnosus (22)* and *Lactobacillus casei* subsp. *rhamnosus (27)*, was used as the biocatalyst in all experiments. Medium for both large-scale biomass growth and cell passaging contained 10 g/L Sheftone T, 5 g/L KH_2PO_4, 5 g/L KH_2PO_4, 0.5 g/L $(NH_4)_2SO_4$, 0.3 g/L $MgSO_4$, 0.002 g/L $FeSO_4$, and 0.10 g/L NaCl. Sheftone T (Sheffield Products, Norwich, NY) is a low-cost alternative nitrogen source to yeast extract. Sheftone T has a 9.8% total nitrogen content with 1.3% amino nitrogen compared to 10.9 and 6.0% for the total and amino nitrogen content of yeast extract. Glucose concentrations varied between 10 and 100 g/L, depending on the expected duration of growth in the given vessel. The media were sterilized by autoclaving and had an initial pH of 6.0. For biomass harvesting in bead production, inocula were grown in Fernbach flasks at 40°C for 2–4 d, after which the cells were used to inoculate a New Brunswick 75-L fermentation system. The system was allowed to ferment for a period of about 3 d, after which the cells were harvested and concentrated using a continuous centrifuge. The resulting paste was washed with sterile water and recentrifuged before adding to the gelling solution below. This step decreased gel viscosity and improved the production of the beads. The bead production method has been described in detail elsewhere *(36)*. Briefly, the bacteria were immobilized into uniform gel beads, 0.7 mm in diameter, using 1% low-viscosity alginic acid (A-2158, Sigma, St. Louis, MO) and stabilized in a 0.2*M* $CaCl_2$ solution. To find the average number of cells per volume of beads, approx 1 mL of beads was placed in 3 mL of water to determine the volume of beads by displacement. Sodium citrate (0.5 g) was then added to dissolve the beads. Cells were counted microscopically using a Petroff-Hausser counting chamber. Cell counts were typically 4×10^{10} cells/mL of bead, or 0.067 g dry wt/mL.

The reactor utilized in the fluidized-bed fermentations has been described previously *(13)* and is shown schematically in Fig. 1. Briefly, the water-jacketed reactor tapered from an inner diameter of 1/2 in. at its base to 1 in. at 23 cm above the liquid entrance. This tapered region at the bottom allowed for efficient disengagement of the denser adsorbent from the biocatalyst beads. A reservoir was added at the reactor base for collecting adsorbent particles. The working volume of

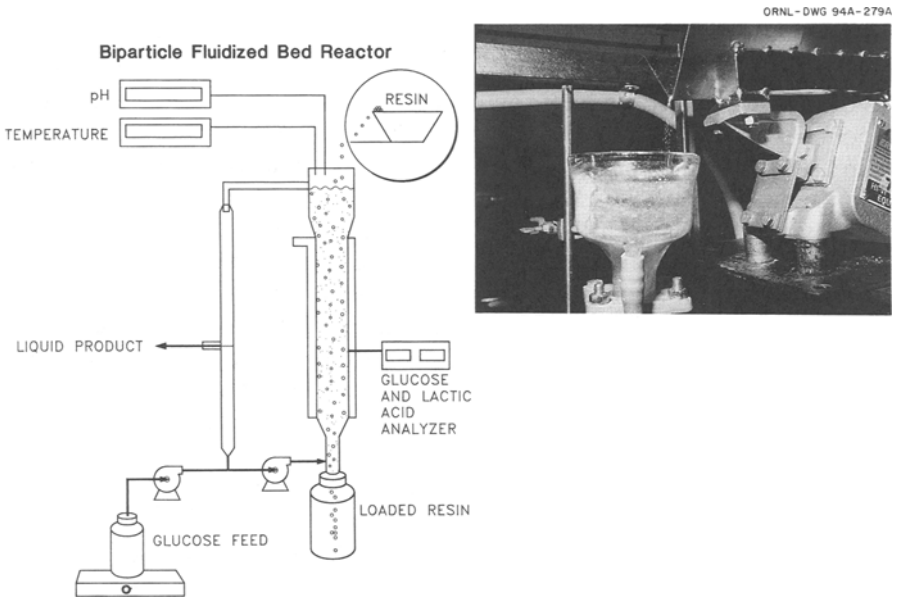

Fig. 1. Schematic of the BFBR. Inset: Resin delivery system in the BFBR. A pH controller located at the reactor apex actuated a vibratory hopper that delivered resin into the BFBR at a controlled rate to affect pH control and *in situ* product removal.

the reactor was 0.7 L, of which 0.2 L was occupied by the biocatalyst. However, surge and resin collection flasks made the total volume of the reactor 2.6 L. A peristaltic pump fluidized the biocatalyst by the upflow of recirculating media at a rate of 50 mL/min. A nitrogen sparge was added to the reactor base with a flow rate of 15 mL/min to improve resin/biocatalyst disengagement. In the envisioned larger scale use of the BFBR, the glucose will be converted in a single pass, and medium recycle will not take place. However, owing to the prohibitively large volume of medium that would be needed to operate this laboratory-scale reactor in a once past medium mode (72 L/d) and the low conversion rate achieved in a single pass, we chose to operate the present reactor in a recycle mode with a small "make-up" feed. The medium that was originally loaded into the BFBR with the biocatalyst beads and recycled during the course of a given experiment consisted of 5 g/L glucose, 10 g/L Sheftone T, 0.5 g/L $(NH_4)_2SO_4$, 0.3 g/L $MgSO_4$, 0.1 g/L $CaCl_2$, 0.002 g/L $FeSO_4$, and 0.1 g/L NaCl. Prior to re-entering the reactor, the media were replenished with glucose via a peristaltic pump drawing from a reservoir containing 250 g/L glucose, 5 g/L Sheftone T, 0.5 g/L $(NH_4)_2SO_4$, 0.3 g/L $MgSO_4$, 0.1 g/L $CaCl_2$, 0.002 g/L $FeSO_4$, and 0.1 g/L NaCl. The glucose level in the reactor was controlled by manipulating the rate of this pump (when the pump was on, the flow rate was typically <0.25 mL/min). The reactor was monitored for pH and temperature by probes placed at the reactor apex. Glucose and lactic acid levels were monitored through the automated sampling allowed by a model 2700 Dual Channel Biochemistry Analyzer (Yellow Springs Instrument Co., Yellow Springs, OH). Bead cell counts as well as the bed height were monitored on a daily basis.

In one set of reactor trials, the columnar reactor with immobilized cells was operated in the "biparticle mode" with continuous, automated addition of sorbent particles added to maintain the reactor pH at 6.0 while simultaneously removing lactic acid. For this operation, a pH probe at the reactor apex was attached to a pH controller (Chem Cadet, Cole Parmer, Niles, IL), which, in turn, actuated a vibrating tray/hopper delivery system (*see* Fig. 1 inset). This tray and hopper were custom designed and manufactured by Eriez Magnetics (Erie, PA) to meet the particularly challenging specifications of low volumetric feed of a self-agglomerating material. When acutated, the hopper delivered resin at a rate of 2.2 dry g/min. The selection of a resin sorbent for this application has been discussed previously *(13,35)*. The resin sorbent used in these studies was Amberlite IRA-35 (Rohm & Haas, Philadelphia, PA), a macroreticular weak-base anion exchanger with tertiary amine functionality in an acrylic-divinylbenzene matrix. This resin possessed a capacity of ~0.2 g/g under the conditions described. The resin was dried under a heat lamp prior to use in the hopper system in order to convey the material in a controlled, reproducible manner. The resin quickly hydrated on addition to the reactor, and this drying process did not affect the resin's capacity. The residence time of a given resin particle under reactor conditions was typically 30 min. As described previously *(13)*, resin removed from the BFBR was regenerated by successive washings with 2*M* sulfuric acid. The volume of eluent and its concentration were recorded on each wash, and the total lactic acid bound to the resin was thus calculated. The resin regenerated in this manner was for the purpose of quickly tabulating the total amount of lactic acid produced and not necessarily for achieving the highest eluent concentration possible. Regeneration experiments designed to achieve high eluent concentration are described in the Results section. In order to assess how the selection of media components would affect lactic acid production in the BFBR, one fermentation trial was conducted with a "rich" media formulation as opposed to the rather lean mixture listed previously. Reactor operation was as described above, except that the reactor media contained 10 g/L pancreatic digest of casein, 10 g/L glucose, 5 g/L yeast extract, 3 g/L tryptose, 1 g/L sodium acetate, 0.2 g/L L-cysteine, 1 mL/L Tween-80, 0.6 g/L $MgSO_4$, 0.2 g/L $MnSO_4$, 0.3 g/L $FeSO_4$, and 0.1 g/L $CaCl_2$. Since the reactor was operated with rich media for a period of about 24 h, no make-up glucose feed was used in this trial.

The columnar reactor was also operated with pH control, but with no *in situ* product removal in order to assess better how product removal can augment productivity in addition to the augmentation afforded by pH control alone. This reactor run utilized the same biocatalyst and normal or "baseline" media recipe used in the BFBR experiment. The pH controller, however, rather than actuating the resin delivery system, actuated a peristaltic pump, which delivered 4*M* ammonium hydroxide.

A control fermentation was performed in a batch, 75-L free-cell reactor (New Brunswick Scientific, Edison, NJ), with pH control via the addition of 4*M* ammonium hydroxide. With the, exception of 100 g/L of glucose, the media were identical to that loaded into the BFBR. In order to operate this reactor with a biocatalyst density similar to that used in the immobilized cell reactors, the batch reactor was operated for a week, and cells were harvested and reintroduced into fresh media in the 75-L reactor. In this manner, the initial cell concentration for the batch reactor run was 5×10^9 cells/mL. Liquid samples were removed daily to allow for cell counts as well as glucose and lactic acid measurements.

Table 1
Media Formulations Used in Screening Experiments[a]

Component, g/L	#1	#2	#3	#4	Baseline
Glucose	20	20	20	20	20
Sheftone T	10	10	10	10	10
$(NH_4)_2SO_4$	0.5	0	0.5	0	0.5
$MgSO_4$	0.3	0.2	0.3	0.3	0.3
$CaCl_2$	0.1	0.1	0.1	0.1	0.1
Peptone	0	10	0	10	0
Sodium acetate	0	5	0	5	0
$MnSO_4$	0	0.1	0.1	0	0
$FeSO_4$	0	0.002	0.002	0	0.002
NaCl	0	0.1	0.1	0	0.1

[a]Four different media formulations, varied according to their salt, protein, and buffer content, were assayed along with the baseline media formulation used in the BFBR. Concentrations of the various components are given in grams/L.

An experiment was also performed with immobilized biocatalyst in serum bottles with various media recipes in order to screen these recipes for use in the BFBR. In this experiment, beads were prepared as described, and 8 g of beads were placed in duplicate 125-mL serum bottles containing 75 mL of the medium formulations shown in Table 1. Flasks were incubated in a rotary shaker at 40°C and 80 rpm, and were assayed daily for glucose and lactic acid concentrations.

RESULTS

Plots of broth lactic acid concentration and cumulative lactic acid produced for the three fermentation runs in different reactor arrangements with the baseline media recipe are shown in Fig. 2 (A and B). The data in Fig. 2B have been normalized to reflect the lactic acid that would be produced in a reactor with an active volume of 0.7 L in order to compare the productivities of various sized reactors. In the batch free-cell bioreactor, the high initial cell loading of 5×10^9 cells/mL plateaued at 1×10^{10} cells/mL after 1 d. The lactic acid concentrations in the broth approached a level of 80 g/L after nearly 10 d of operation. The reactor's productivity was 0.55 g/L·h after 66 h, 0.30 at 162 h, and 0.23 after 234 h of fermentation.

The immobilized-cell reactor with chemical pH control and no lactic acid removal was operated for a period of 62 h. During this time, a lactic acid concentration of 15 g/L was reached, with a reactor productivity of 1.30 g/L·h. The fact that the broth concentration was lower in this case than the free-cell reactor is attributable to the low ratio of active reactor volume to total reactor volume. This allows dilution of the lactic acid produced in the active volume. The cell density remained constant at 4×10^{10} cells/mL biocatalyst, and there were 200 mL of biocatalyst in the reactor; thus, the immobilized- and free-cell reactors had nearly the same amount of biomass per working reactor volume (7×10^{12} and 8×10^{12} cells/700 mL working volume for the immobilized and free-cell reactors, respectively).

The BFBR was operated with continuous resin addition for a period of 1 wk. During this time, the broth lactic acid concentration was kept below 2 g/L by adsorption onto the resin. This simultaneous fermentation and separation minimized the

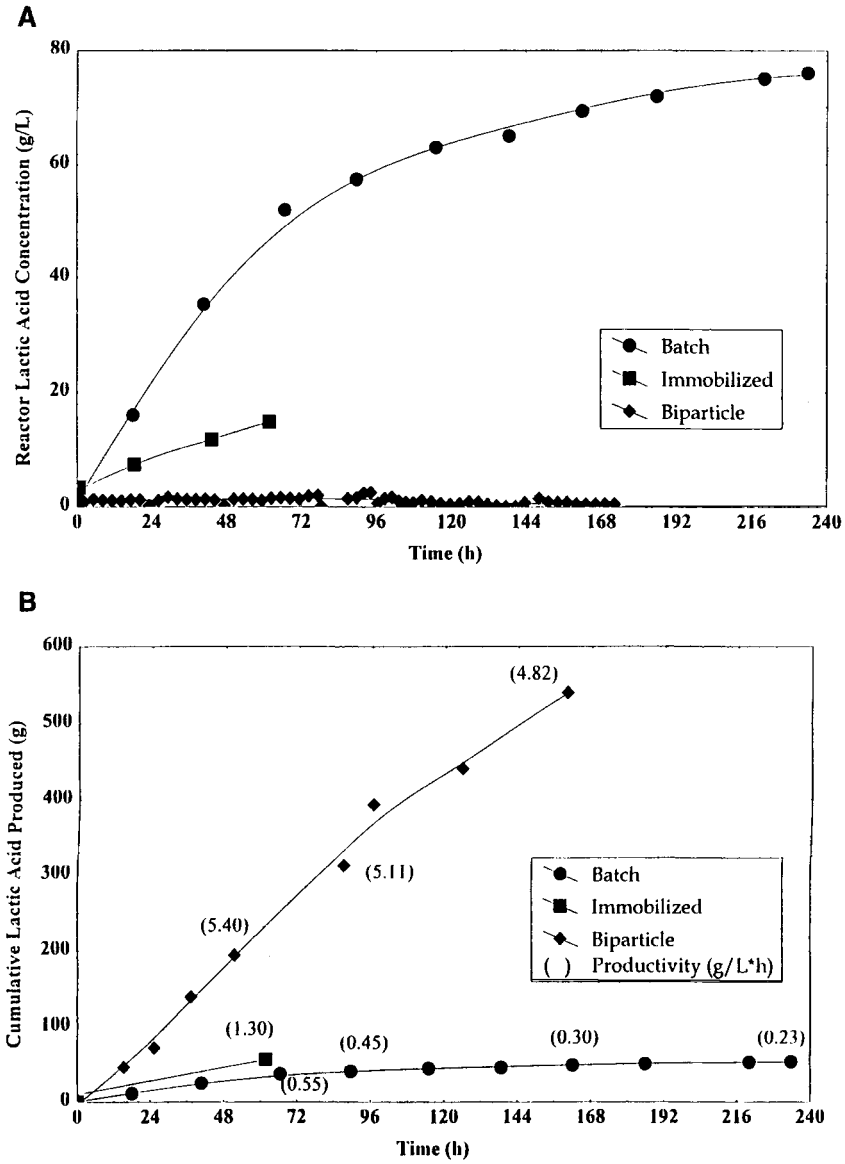

Fig. 2. Fermentation trials in free-cell batch, immobilized-cell fluidized-bed, and BFBRs for the production of lactic acid. **(A)** Broth concentrations of lactic acid as a function of time. The BFBR maintains reactor pH and low-broth concentrations to decrease product inhibition. **(B)** Cumulative lactic acid produced as a function of time. The BFBR outperforms the free-cell fermentation by a factor of 16. Reactor productivities (g/L·h), are shown in parentheses at various times during the fermentation.

product inhibition that occurred in the above fermentation schemes even when the pH of the reactor was controlled to pH 6.0. The cell density in the biocatalyst remained constant during the reactor run at 4×10^{10} cells/mL biocatalyst, and there were 200 mL of biocatalyst in the reactor. Typically, 19 dry g/h of resin were added to the reactor to maintain the pH at 6.0. On regeneration of the resin with 2*M*

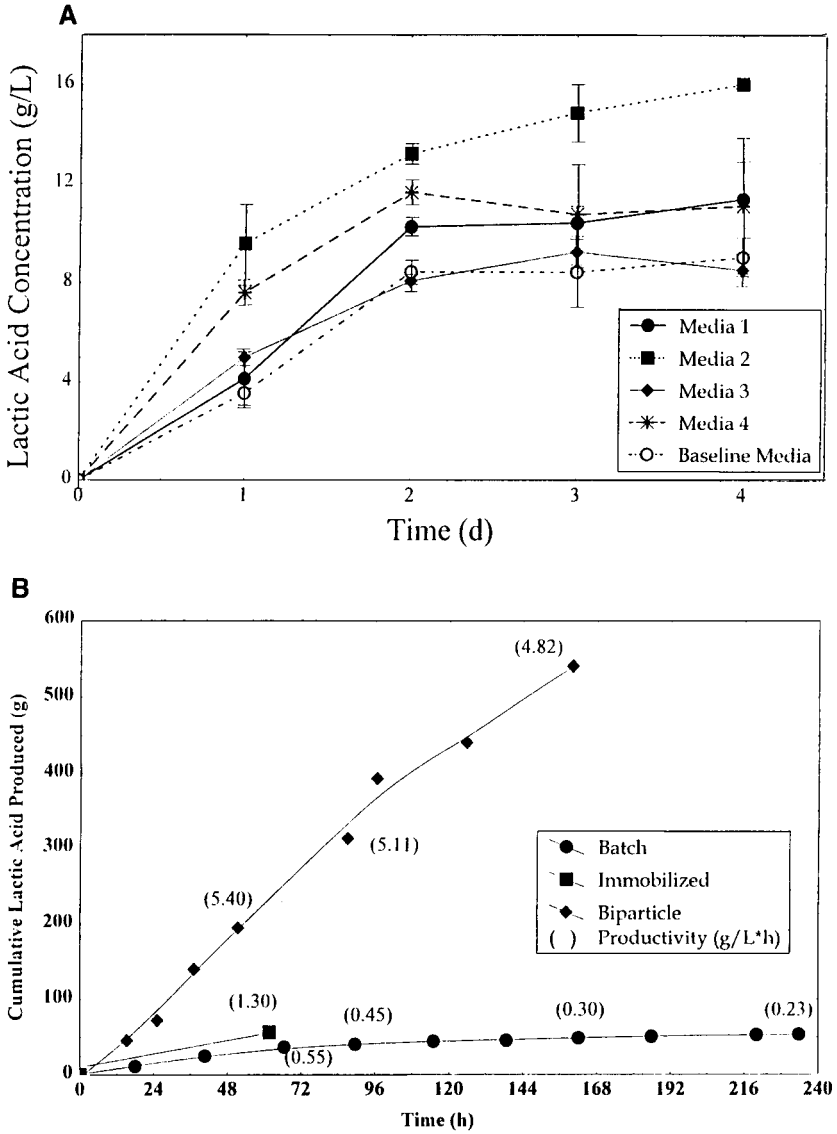

Fig. 3. Lactic acid production by biocatalyst beads in serum bottles as a function of media composition. In conditions in which the pH is not controlled and the inhibitory product is not removed, media formulations with increased buffer and amino acid content yield improved lactic acid production when compared to our baseline medium.

sulfuric acid, product concentrations as high as 70 g/L were achieved, comparable to those obtained in batch fermentations. When the cumulative lactic acid produced as a function of time is studied for this reactor, it is seen that the BFBR far outperforms the conventional bioprocessing schemes. The BFBR exhibited nearly constant volumetric productivity with 5.4 g/L·h after 51 h, and 4.82 after 160 h of operation.

As seen in Fig. 3, "rich" media formulations containing additional buffers and protein extracts resulted in improved lactic acid production when immobilized biocatalyst was incubated in serum bottles without pH control or *in situ* product

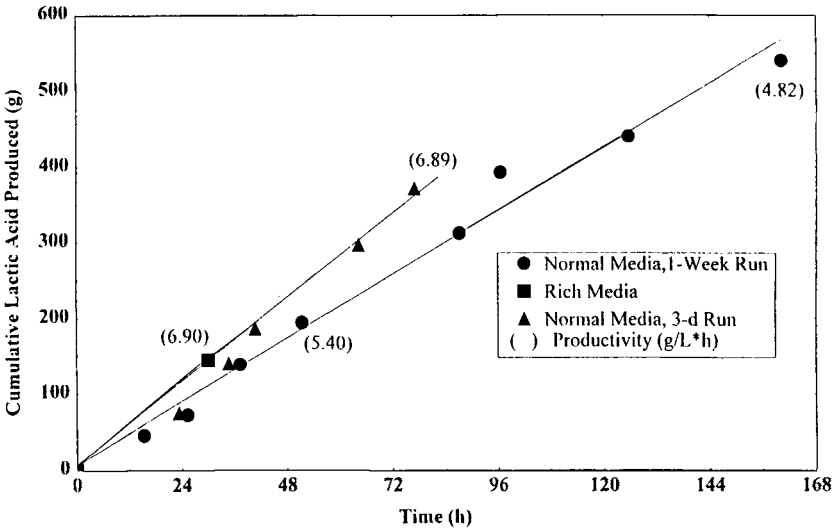

Fig. 4. Cumulative lactic acid produced as a function of time in the BFBR when operated with rich, as opposed to baseline, media formulations. Media recipes are described in the text. Although rich media recipes containing additional buffers and amino acids augment production in a system that is strongly inhibited, they do not seem to impact production in a BFBR in which the pH is controlled and the inhibitory product is continuously removed.

removal. One reason for this improvement is obviously the increased buffering capacity of the media, which allows the biocatalyst to exist at the optimal pH for a longer period of time. It also appears that constituents, such as peptone and tryptose, serve to protect the biocatalyst from the inhibitory effects of lactic acid, which continue to persist even when the pH of the reactor is controlled. Figure 4 demonstrates the cumulative lactic acid produced and the volumetric productivity achieved when the BFBR was operated on rich, as opposed to normal, medium formulations. The three fermentation trials were all performed with identical biocatalyst concentrations. Although the productivity of the BFBR for the 30-h run on rich medium (6.9 g/L·h) was higher than that achieved in the week-long run with normal medium (4.8 g/L·h), this augmentation is believed to be insignificant, since additional fermentation trials with normal medium also exhibited productivities of 6.9 g/L·h (*see* Fig. 4).

Although the BFBR does offer several advantages over batch fermentation in terms of product separation and purification, more research on the selection of an optimal adsorbent and adsorbent regeneration processes is needed in order to realize the full potential of the BFBR system. Owing to the *in situ* product removal, the BFBR does not require separation of the fermentation broth from the biomass as required by batch fermentations. Using a packed column of IRA-35, we have previously demonstrated a 68-fold increase in product-to-substrate ratio over the inlet solution, and a 1734-fold increase over the ratio found in the column effluent *(13)*. In the current study, we have attempted to minimize the volume of eluent in order to achieve higher product concentrations.

To simulate regeneration of resin that is loaded with BFBR fermentation broth, 220 g of IRA-35, as supplied by the manufacturer, were loaded into a 1-in. id packed column and contacted with a single pass (1 L at 5 mL/min) of medium at 40°C.

The baseline media as described was "spiked" with 5 g/L glucose and 5 g/L lactic acid to approximate the concentrations that may be present in the broth from a BFBR. The column was washed with a single volume of water at 40°C followed by 40 mL (at 5 mL/min) of 2*M* H_2SO_4 at 90°C. These 40 mL represent 80% of the column void volume. Eluent was collected in 2-mL fractions and reached a peak concentration of 70 g/L lactic acid, a 14-fold concentration over the loading solution. This single pass of sulfuric acid eluted 75% of the lactic acid loaded onto the column. In order to demonstrate more fully the resin's ability to concentrate lactic acid, this above experiment was repeated using media that contained 10 g/L glucose and 50 g/L lactic acid. On regeneration, lactic acid concentrations reached as high as 610 g/L (~50% [w/v]), a 12-fold concentration of the loading solution. This experiment yielded 72% of the lactic acid originally loaded onto the column. Clearly more research is needed in the area of resin selection and regeneration. Resins with high capacity and specificity are needed in order to decrease the volume of resin required in the reactor at a given time. Improved chromatographic-like techniques need to be developed in order to effect increased product concentrations to decrease downstream processing costs.

DISCUSSION

This study has demonstrated that the BFBR is a viable long-term, continuous method of lactic acid production with several advantages over conventional batch fermentation. The BFBR exhibited volumetric productivities at least 16-fold higher than that of a free-cell reactor with identical biomass concentration and medium composition. On resin regeneration, the BFBR yielded identical product concentrations as the free-cell reactor and has the potential to achieve much higher concentrations with reduced processing cost. Rich media formulations are not needed in the BFBR system, for the cells are already protected from the inhibitory product owing to *in situ* product removal. The BFBR offers considerable economic advantage over batch processing owing to the increased reactor productivity and continuous nature of the process.

The BFBR was operated continuously for a period of 1 wk and demonstrated a productivity that was 16-fold higher than that of a free-cell, pH-controlled reactor with identical media composition and biomass concentration operated for the same period of time. The BFBR exhibited a productivity that was fourfold higher than a pH-controlled, immobilized-cell reactor, again with identical biomass concentration and media composition, but without *in situ* product removal. Although the immobilized-cell reactor appears to have a higher productivity than the free-cell reactor, we believe that this is owing to the "buffering capacity" afforded by the dead volume of the immobilized-cell reactor. The columnar reactor had a total volume of 2.6 L with only 0.2 L of biocatalyst. This dead volume served to dilute the lactic acid produced (15 g/L as opposed to 80 g/L for the free-cell reactor) and minimize product inhibition in this manner. It appears that immobilization of the biocatalyst does not have an adverse effect on activity, in that the biocatalysts in the immobilized-cell reactor are at least as productive as their counterparts in the free-cell reactor. Yield measurements were difficult to obtain in the immobilized-cell systems owing to the aseptic production of the beads and eventual reactor contamination owing to the open configuration of the resin addition system. Product yields on glucose for *L. delbreuckii* have been reported between 74 and 90% *(16,30,37)*. The batch free-cell fermentation reported here exhibited a product yield on glucose of 85%.

Rich media formulations may be considered to assuage product inhibition that exists even when the pH of the reactor is controlled. These rich media contribute significant additional chemical cost to the process, but may pay for themselves in terms of increased productivity. Although rich media were demonstrated to cause increased lactic acid production in serum bottles with no pH control or product removal, these constituents did not increase productivity in the BFBR, presumably because product inhibition was already alleviated owing to the *in situ* product removal afforded by the resin. Since the low-cost medium recipes provide competitive reactor productivities, the BFBR is seen to possess a potential economic advantage in terms of decreased chemical costs.

Since product separation and purification accounts for 50% of lactic acid production costs, the output product concentration of a given fermentation process has tremendous impact on the economic viability of the process. In this article, we demonstrated that the product stream from the BFBR is as concentrated as that from the free-cell batch fermentation (70 g/L). We have also demonstrated that concentrations as high as 610 g/L can be obtained, depending on how the resin is loaded and regenerated. We believe that continued efforts in resin investigation *(6,25,28,30, 35,38–40)* for increased capacity, specificity, and regenerability will lead to great strides in reduced production cost.

Although formal economic assessment of the BFBR process (as opposed to conventional batch fermentation of lactic acid) has yet to be performed, we have previously published a rough comparison of the two processes *(13)*. In this assessment, we demonstrated how increased productivity owing to cell immobilization and *in situ* product removal, increased on-line production time because of the continuous nature of the process, decreased capital costs, and equal raw material, operating, and overhead costs should render the BFBR an attractive alternative to conventional batch fermentation. For a given lactic acid output, the BFBR will be smaller, have increased on-line time, and have equal or decreased chemical costs compared to batch fermentations. Improved resin regeneration will provide further economic advantage. Formal collaboration with a lactic acid producing industrial partner should enable a more complete economic evaluation of this technology.

CONCLUSIONS

A BFBR for the fermentation and simultaneous separation of lactic acid has been demonstrated at the laboratory scale. This reactor has demonstrated a 16-fold increase in volumetric productivity over control experiments in a free-cell, batch reactor. This increased productivity is achieved by providing high cell density and *in situ* removal of the inhibitory product. Rich media formulations are not needed to achieve higher reactor productivity owing to the decreased product inhibition with low broth lactic acid concentrations. Initial economic assessment of this bioprocessing scheme has revealed the potential for cost reduction owing to decreased reactor size, increased on-line production time, and equal chemical, operating, utility, and overhead costs. Further improvements to resin regeneration are expected to reveal additional economic advantages of the BFBR over conventional batch fermentation of lactic acid. The BFBR is a generalized processing scheme that may be applied to any fermentation. It will have the greatest impact on rapid fermentations that yield a strongly inhibitory product.

ACKNOWLEDGMENTS

Research was supported by the Advanced Industrial Concepts Division—Biological and Chemical Technologies Research Program, US Department of Energy under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc. The authors thank Brian H. Davison for his valuable technical discussions and assistance.

REFERENCES

1. Atkinson, B. and Mavituna, F. (1991), *Biochemical Engineering and Biotechnology Handbook*, 2nd ed. Stockton, New York.
2. Lipinsky, E. S. and Sinclair, R. G. (1986),*Chem. Eng. Prog.* **82,** 26–32.
3. *Chemical Marketing Reporter* (1994), March 14, pp. 28–36.
4. Millis, J. (1993), *Am. Chem. Soc.* **38,** 297–299.
5. Ohleyer, E., Blanch, H. W., and Wilke, C. R. (1985), *Appl. Biochem. Biotechnol.* **11,** 317–331.
6. Evangelista, R. L., Mangold, A. J., and Nikolov, Z. L. (1994), *Appl. Biochem. Biotechnol.* **45,** 131–144.
7. Mercier, P., Yerushalmi, L., Rouleau, D., and Dochain, D. (1992), *J. Chem. Tech. Biotechnol.* **55,** 111–121.
8. Buchta, K. (1983), in *Biotechnology: A Comprehensive Treatise Biomass, Microorganisms for Special Applications, Microbial Products I, Energy from Renwable Resources*, Dellweg, H., ed., Verlag Chemie, Weinheim, Germany, 410–417.
9. Mark, H. F., Othmer, D. F., Overberger, C. G., and Seaborg, G. T., eds. (1981), *Kirk-Othmer Encyclopedia of Chemical Technology*, 3rd ed., vol. 13, Wiley, New York.
10. King, C. J. (1992), *Chem. Tech.* **22,** 285–291.
11. Yeh, P. L. H., Bajpai, R. K., and Iannotti, E. L. (1991), *J. Fermentation Bioeng.* **71,** 75–77.
12. Kuhn, R., Peretti, S., and Ollis, D. (1993), *Appl. Biochem. Biotechnol.* **39/40,** 401–413.
13. Kaufman, E. N., Cooper, S. P., Clement, S. L., and Little, M. H. (1995), *Appl. Biochem. Biotechnol.* **51,** 605–620.
14. Melzoch, K. and Konopaskova, L. (1993), *Biotechnol. Lett.* **15,** 517–520.
15. Andrews, G. F. and Fonta, J. P. (1989), *Appl. Biochem. Biotechnol.* **20,** 375–390.
16. Stenroos, S. L., Linko, Y. Y., and Linko, P. (1982), *Biotechnol. Lett.* **4,** 159–164.
17. Guoqiang, D., Kaul, R., and Mattiasson, B. (1991), *Appl. Microbiol. Biotechnol.* **36,** 309–314.
18. Hang, Y. D., Hamamci, H., and Woodams, E. E. (1989), *Biotechnol. Lett.* **11,** 119,120.
19. Yabannavar, V. M. and Wang, D. I. C. (1991), *Biotechnol. Bioeng.* **37,** 1095–1100.
20. Vick Roy, T. B., Blanch, H. W., and Wilke, C. R. (1982), *Biotechnol. Lett.* **4,** 483–488.
21. Shi, Z., Shimizu, K., Iijima, S., Morisue, T., and Kobayashi, T. (1990), *J. Fermentation Bioeng.* **70,** 415–419.
22. Xavier, A. M. R. B., Goncalves, L. M. D., Moreira, J. L., and Carrondo, M. J. T. (1995), *Biotechnol. Bioeng.* **45,** 320–327.
23. Kulozik, U., Hammelehle, B., Pfeifer, J., and Kessler, H. G. (1992), *J. Biotechnol.* **22,** 107–116.
24. Venkatesh, K. V., Okos, M. R., and Wankat, P. C. (1993), *Process Biochem.* **28,** 231–241.
25. Seevaratnam, S., Holst, J. O., Hjorleifsdottir, S., and Mattiasson, B. (1991), *Bioprocess Eng.* **6,** 35–41.
26. Martin, M. S., Pazos, C., and Coca, J. (1992), *J. Chem. Tech. Biotechnol.* **54,** 1–6.
27. Siebold, M., Frieling, P. V., Joppien, R., Rindfleisch, D., Schugerl, K., and Roper, H. (1995), *Process Biochem.* **30,** 81–95.
28. Garcia, A. A. (1991), *Biotechnol. Prog.* **7,** 33–42.
29. Galliot, F. P., Gleason, C., Wilson, J. J., and Zwarick, J. (1990), *Biotechnol. Prog.* **6,** 370–375.
30. Srivastava, A., Roychoudhury, P. K., and Sahai, V. (1992), *Biotechnol. Bioeng.* **39,** 607–613.
31. Davison, B. H. and Scott, C. D. (1992), *Biotechnol. Bioeng.* **39,** 365–368.
32. Davison, B. H. and Thompson, J. E. (1992), *Appl. Biochem. Biotechnol.* **34,** 431–439.
33. Godia, F., Casas, C., and Sola, C. (1987), *Process Biochem.* **22,** 43–48.
34. Davison, B. H. and Scott, C. D. (1988), *Appl. Biochem. Biotechnol.* **18,** 19–34.

35. Kaufman, E. N., Cooper, S. P., and Davison, B. H. (1994), *Appl. Biochem. Biotechnol.* **45,** 545–554.
36. Scott, C. D. (1987), *Ann. NY Acad. Sci.* **501,** 487–493.
37. Major, N. C. and Bull, A. T. (1989), *Biotechnol. Bioeng.* **34,** 592–599.
38. Ernst, E. E. and McQuigg, D. W. (1992) in AICHE 1992 National Meeting, Miami Beach, FL.
39. Nakagawa, M., Nakamura, I., and Kobayashi, T. (1975), *J. Fermentation Technol.* **53,** 127–134.
40. Yang, C. W., Lu, Z., and Tsao, G. T. (1995), *Appl. Biochem. Biotechnol.* **51,** 57–71.