Use of a Biparticle Fluidized-Bed Bioreactor for the Continuous and Simultaneous Fermentation and Purification of Lactic Acid

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Abstract

A continuous biparticle fluidized bed reactor is developed for the simultaneous fermentation and purification of lactic acid. In this processing scheme, bacteria are immobilized in gelatin beads and are fluidized in a columnar reactor. Solid particles with sorbent capacity for the product are introduced at the top of the reactor, and fall counter currently to the biocatalyst, effecting *in situ* removal of the inhibitory product, while also controlling reactor pH at optimal levels. Initial long-term fermentation trials using immobilized *Lactobacillus delbreuckii* have demonstrated a 12 fold increase in volumetric productivity during adsorbent addition as opposed to control fermentations in the same reactor. Unoptimized regeneration of the loaded sorbent has effected at least an 8 fold concentration of lactic acid, and a 68 fold enhancement in separation from glucose compared to original levels in the fermentation broth. The benefits of this reactor system as opposed to conventional batch fermentation are discussed in terms of productivity and process economics.

Introduction

Lactic acid is a specialty chemical utilized 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, acrylic acids, acetaldehyde, and ethanol [2], with a current price of $1.15/lb for technical (88%) or food grade lactic acid [3]. Annual U.S. consumption of lactic acid totals over 30 million pounds [4]. Biological production of lactic acid accounts for approximately 50% of the world's production [5]. Biological production is complicated primarily due to economic considerations arising from product inhibition and the required downstream processing of dilute aqueous product. 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 base be added to the reactor to control pH [4, 7] and / or that calcium carbonate be added as a buffer and to precipitate the lactate [8, 9]. These processes produce the lactate salt which must be reacidified (usually by sulfuric acid [8-10]), 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 (see Table 1). Certainly industry has achieved higher productivities through the use of strain selection and genetic engineering, but these numbers are proprietary and are not available.

It is postulated that it is the protonated form of lactic acid which is inhibitory to the fermentation [5, 11, 12] The concentration of undissociated product as a function of total lactic acid concentration and pH may be calculated given that the pKa of lactic acid is 3.8 [11]. With such a calculation, it can be seen that reactor pH control does much to reduce the concentration of undissociated lactic acid in solution. However, kinetic modeling of *Lactobacillus delbreuckii* by Yeh *et al* [11] indicates that pH control alone is insufficient to ensure maximal productivity. According to the kinetic constants of Yeh et al, the production rate of lactic acid is reduced by 50% if the concentration of undissociated lactic acid is just 0.25 g/L at pH 6.0. If the protonated lactic acid level reaches 0.5 g/L (which would occur if one wished to reach > 50 g/L broth concentration in the fermentor) the bacterial productivity drops to just 15% of its optimal value. This decrease in productivity as a function of broth lactic acid concentration at pH 6.0 is shown in Figure 1. Also shown in this graph are the operating ranges for both batch fermentation and the biparticle fluidized bed reactor discussed in this work. It is evident that reactor pH adjustment alone is insufficient to ensure maximal productivity. In situ product removal should also be employed.

Improvements in volumetric productivity for the lactic acid fermentation have been realized by increasing biomass loading and by reducing product inhibition. A sampling of the methods employed and their resulting productivities is shown in Table 1. High cell densities in reactors has been achieved by the use of cell aggregates [13], growth of biofilm on activated carbon [14], cell immobilization in gelatin beads (see for example [15-18]), cell growth in hollow fibers [19], and cell recycle using hollow fiber membranes [20]. Product inhibition has been reduced by moving toward a plug flow reactor system through the use of staged CSTR reactors [21, 22] 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, 18, 23, 24] and solid adsorbents [23, 25] either in a product stripping side stream [26, 27] or added directly to the CSTR reactor [28]. *In situ* product removal during the fermentation has the potential to minimize process waste streams by obviating the need for reactor pH control and lactic acid precipitation.

Combining the benefits of cell immobilization and *in situ* product separation, Davison and Thompson [29] recently demonstrated the simultaneous fermentation and separation of lactic acid in a biparticle fluidized bed reactor (BFBR). In this process, *Lactobacillus delbreuckii* were immobilized in alginate beads and fluidized by the up flowing liquid media in a tubular reactor. Such fluidized beds have been shown to increase the productivity of fermentations for a variety of processes [30, 31]. In the demonstration by Davison and Thompson [29], 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 four-fold over a control fluidized bed reactor without resin addition. In this proof of concept experiment, the resin was added batchwise for a short period of time (7 hrs), in small quantities, and was added at a constant schedule. While resin addition moderated the decrease in reactor pH, it did not control the pH at the optimal level. Further improvements should be achievable using this reactor configuration as the biocatalyst, resin, and media are optimized.

In this paper, we describe the further development and demonstration of the BFBR first proposed by Davison and Thompson. Longer operation and improved control of the reactor have demonstrated further increases in volumetric productivity. The use of a weak base ion exchange resin has effected both improved pH control and increased product concentration and separation. Finally, we briefly discuss the possible economic advantage of utilizing the BFBR technology for lactic acid fermentation as opposed to conventional batch fermentation.

Methods

Lactobacillus delbreuckii NRRL B445 was grown in media containing glucose at 30 g/L, yeast extract, KH_2PO_4, and KH_2PO_4 at 5 g/L each, $(NH_4)_2SO_4$ at 0.5 g/L, and $MgSO_4$ at 0.3 g/L. The media was sterilized by autoclaving and had an initial pH of 6. Inocula were grown in Fernbach flasks at 40 ^oC for 2-4 days after which the cells were used to inoculate a New Brunswick 75 liter fermentation system. The fermentation system was operated using 56 liters of the above media that was sterilized within the fermentation vessel. Amphotericin B (A-9528, Sigma Chemical Company, St. Louis, MO) was added at 5 mg/L to prevent yeast contamination. The system was allowed to ferment for a period of 4-5 days 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 [32]. Briefly, the bacteria were immobilized into uniform gel beads 0.7 mm in diameter using 1% low viscosity alginic acid (A-2158, Sigma) and stabilized in a 0.2 M $CaCl_2$ solution. To find the average number of cells per volume of beads, approximately 1 mL of beads were 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 10^{11} cells/ml of bead

The media that is originally loaded into the reactor with the biocatalyst beads and recycled during the course of a given experiment contained glucose (5 g/L), Sheftone T (20 g/L), $(NH_4)_2SO_4$ (0.5 g/L), $MgSO_4$ (0.3 g/L), and CaCl2 (0.1 g/L). Sheftone T (Sheffield Products, Norwich, NY) is a low cost alternative nitrogen source to yeast extract. Amphotericin B (A 9528, Sigma Chemical Co. St. Louis, MO) was added to the reactor at 5 mg/L to reduce yeast contamination which was present due to the aseptic production and loading of biocatalyst beads and resin addition. This antibiotic should not be necessary in larger scale BFBR use due to the fact that the rapid passage of the media will wash out contaminants which are free in solution.

A schematic of the reactor utilized in these fluidized bed fermentations is shown in Figure 2. The water jacketed reactor tapers 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 allows for efficient disengagement of the denser adsorbent from the biocatalyst beads. A reservoir is added at the reactor base for collection of adsorbent particles. A peristaltic pump fluidizes the biocatalyst by the up flow of recirculating media at a rate of 50 ml/min. In the envisioned larger scale use of the BFBR, the glucose will be converted in a single pass, and media recycle will not take place. A nitrogen sparge is added to the reactor base with a flow rate of 15 ml/min to improve resin/biocatalyst disengagement in some studies. The reactor tapers at its apex from 1 in. to 3 in. diameter in order to contain the fluidized biocatalyst and allow for unimpeded flow of the recycled media stream. The recycled media falls from the top of the reactor, past an outflow line which maintains constant liquid volume and into a bank of UV sterilization lamps. Prior to reentering the reactor,

the media is replenished with glucose via a peristaltic pump drawing from a reservoir containing glucose (250 g/L), Sheftone T (5 g/L), $(NH_4)_2SO_4$ (0.5 g/L), $MgSO_4$ (0.3 g/L) and $CaCl_2$ (0.1 g/L). The glucose level in the reactor is controlled by manipulating the rate of this pump. The reactor is monitored for pH and temperature by probes placed at the reactor apex. Glucose and lactic acid levels are monitored through the automated sampling allowed by a model 2700 Dual Channel Biochemistry Analyzer (Yellow Springs Instrument Co., Yellow Springs, OH). This system utilizes immobilized enzyme technology for substrate and product analysis. While automated sampling is taken from the midline of the reactor, septa placed along the length of the reactor enable manual sampling at any location. Bead and liquid cell counts as well as the bed height were monitored on a daily basis. The reactor system is 1.6 L in total volume. Typically the fluidized biocatalyst occupied approximately half of this volume.

Our previous work has investigated the screening of a variety of resins for their capacity, specificity, and ease of regeneration and focused upon the use of the polyvinyl pyridine resin Reillex 425 [33]. While this resin could be regenerated using only hot water, its capacity for lactic acid at low equilibrium concentrations (~0.02 g/g at 3 g/L lactic acid) would require resin material to occupy a prohibitive amount of volume in the biparticle reactor. Further experimentation has focused upon weak base ion exchange resins. In this study, we utilized Amberlite IRA-35 (Rohm & Haas Philadelphia, PA), a macro reticular weak-base anion exchanger with tertiary amine functionality. This resin possessed a capacity of ~0.2 g/g under the conditions described above. Resin was used directly as supplied by the manufacturer without rinsing or pretreatment.

As suggested by Nakagawa *et al.* [34], the IRA-35 resin can be regenerated (to strip the lactic acid and regenerate the hydroxyl sites on the resin) with either 4M ammonium hydroxide alone or with 2M sulfuric acid followed by 4 M ammonium hydroxide. While the ammonium hydroxide regeneration is seemingly less expensive, it produces ammonium lactate which requires acidification if protonated lactic acid is the desired product. The sulfuric acid / ammonium hydroxide cycle both yields the protonated lactic acid, and regenerates the hydroxyl sites on the resin. In this study we investigated both regeneration processes. Some experiments reported here regenerated the resin in packed columns by recirculating the minimum amount of liquid necessary to wet the resin. These experiments were performed to demonstrate the degree of lactic acid concentration and purification realizable using the resin adsorbent. Other experiments

stripped the resin directly in the resin collection bottle which was attached to the base of the BFBR. In these experiments excess liquid was used to strip the resin, and maximal concentration of the lactic acid product was not achieved. This resin regeneration method was utilized to quickly determine the total amount of lactic acid adsorbed to the resin.

Three different types of fermentations were performed in the fluidized bed reactor to assess the benefits of the biparticle fluidized bed reactor process. In the first configuration, the control fermentation, the reactor was operated as stated above without the addition of resin particles at any time or manner during the course of the fermentation. To begin the experiment, the reactor was sparged with several volumes of fresh media until lactic acid concentrations fell below 0.1 g/L. At this point (denoted time 0) the reactor was placed on recycle operation and the fermentation commenced. The only stream entering the reactor during the control fermentation was the glucose input, the pH of the reactor was not controlled or moderated in any form.

Two types of experiments were conducted to demonstrate how the treatment of the media recycle stream with a weak base ion exchange resin could enhance fermentation productivity by moderating reactor pH, and by removing the inhibitory lactic acid as it was produced. Both packed columns of resin and continuous resin addition (the biparticle fluidized bed reactor) were investigated. In packed column experiments, ~200 gms of Amberlite IRA-35 were packed into 1 inch glass chromatography columns and placed in the reactor's media recycle line so that, prior to re-entering the rector, the broth passed over the ion exchange resin. All other conditions were the same as in the control fermentations. This fermentation configuration was operated twice, each for a period of about 20 hours. Following the fermentation run, the resin columns were removed from the media recycle stream and stripped to concentrate, purify and quantify the lactic acid produced during the fermentation.

Finally, the reactor has been operated with continuous, long term resin addition i.e. as a biparticle fluidized bed reactor (BFBR). We have operated the reactor with manual resin addition, while means of automated resin addition are under investigation. In these experiments, IRA-35 was added to the top of the reactor as needed to keep the pH of the recirculating broth at 6.0. Typically, this required the addition of ~6 grams every 10 min. This process was maintained continuously for periods as longs as 72

hours. Resin was collected at the base of the reactor and was stripped in a plastic bottle with several cycles of sulfuric acid and ammonium hydroxide in order to determine the total amount of lactic acid produced.

Results

Four "control" fermentations were performed without the addition of resin to the reactor, and without pH control. A representative graph of one of these control runs is shown in Figure 3. It can be seen that in these control experiments that the reactor pH quickly drops below the optimal value of 6. As the pH falls, the fermentation productivity rapidly deteriorates. All four of the control fermentations performed exhibited volumetric productivities between 0.4 and 0.5 grams of lactic acid per hour per liter of reactor containing biocatalyst. To demonstrate the long term operation of the fluidized-bed reactor, a single charge of biocatalyst was kept in the reactor for a period of two and a half weeks as various reactor configurations were analyzed. Occasional media replenishment ensured active biocatalyst, and the reactor was purged with fresh media prior to the initiation of each experimental run.

Two fermentation runs were performed using packed columns of resin. For the first run of this type, the resin was stripped with cycles of 2 M sulfuric acid followed by 4 M ammonium hydroxide. The concentration of lactic acid desorbed in the initial acid wash was 34.6 gm/L, an 8 fold increase in concentration over that found in the fermentation broth at the endpoint of the fermentation. In the second packed column run, the resin was regenerated using only ammonium hydroxide and not acid. In the initial base wash, the product lactic acid desorbed at a concentration of 27.4 gm/L a 7 fold increase in concentration over that found in the fermentation broth at the endpoint of the fermentation. These fermentation trials were characterized by an initial increase in broth pH due either to the adsorption of anions or the leaching of basic components form the resin (under further investigation). The decrease in reactor pH was not as rapid as in the control experiments, although the resin columns eventually reached their capacity, and the fermentation slowed as the lactic pH dropped within the reactor. The volumetric productivity in the two packed column experiments was 1.5 and 1.3 g/l*hr, a three to four fold increase over the control fermentations.

Three separate experiments were conducted with continuous resin addition for periods ranging from 1 to 3 days. A graph depicting the pH and lactic acid concentrations

in the media recycle stream during one of these experiments is shown in Figure 4. Continuous resin addition maintained the pH of the recycled broth between 5.8 and 6.2 while also maintaining the lactic acid level below 3.5 g/L throughout the course of the fermentation. This ensured maximal productivity of the biocatalyst as shown in Figure 1. In the fermentation run depicted in Figure 4, when the resin was stripped to determine the total lactic acid produced during the fermentation, we calculated a volumetric productivity of 4.6 gm/l*hr for the first 22 hour period and 4.7 gm/l*hr for the next 26 hours. This represents a 12 fold improvement over the control fermentation.

As mentioned above, we have demonstrated an 8 fold increase in concentration of lactic acid over that occurring in the fermentation broth by regenerating the ion exchange resin in a packed column in an unoptimized manner. In order to demonstrate that this method may also increase product purity, we have demonstrated that, through the use of various stripping cycles, the product lactic acid may be separated from the feed substrate, glucose. In one experiment, 224 gms of IRA-35 in a packed column was contacted with 215 ml of water containing 40 g/L glucose and 10 g/L lactic acid. The resin contacting decreased the glucose concentration in the liquid to 34.3 gm/L and the lactic acid concentration to 0.338 g/L. The packed column of resin was then contacted with ~ 200 ml of water which yielded a effluent rich in glucose (13.3 g/l) but poor in lactic acid (0.151 g/l). This represents a 22 fold purification of the ratio of glucose to lactic acid over that in the original feed solution. Glucose was enriched in this water rinse apparently because it is contained in the liquid filled pores of the resin and is not bound to the resin through charge interactions. A subsequent strip with sulfuric acid yielded an effluent at 0.679 g/L glucose and 11.6 g/l lactic acid, a 68 fold increase in product to substrate ratio over the feed solution, and a 1,734 fold increase over the ratio found in the column effluent. Thus, not only can the lactic acid be concentrated over that found in the fermentation broth, it may also be purified. This separation of glucose from lactic acid may have implications in process economics as it may be advantageous to operate the reactor so that there is incomplete glucose conversion. In this case, glucose not utilized can be separated and recycled back into the reactor feed, rather than discarded.

Discussion/ Economic Assessment

While formal economic assessment of the BFBR process as opposed to conventional batch fermentation of lactic acid has yet to be performed, several key

advantages should be analogous to previous assessments of immobilized cell versus batch fermentations. Increased productivity due to cell immobilization and in situ product removal, increased online production time due to 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.

The general processes for batch and BFBR production of lactic acid are shown in Figure 5. The raw materials and supporting chemicals necessary to yield 50 - 100 g/L protonated lactic acid are identical with the exception of the ion exchange resin and the required cell immobilization. In the batch fermentation, nutrient raw materials are combined with biocatalyst and allowed to ferment for 2 - 3 days with the addition of base (NaOH, CaOH, or NH_4OH) to control reactor pH. This fermentation yields a lactate salt at a concentration of 50 - 100 g/ml whose composition depends upon the base used to control the reactor pH. This salt is then reacidified using sulfuric acid to form 50 - 100 g/L lactic acid and a sulfate salt. In the BFBR, raw material is continuously added along with ion exchange resin to immobilized biocatalyst. The loaded resin is removed from the reactor on a continuous or semi-continuous basis and is treated with sulfuric acid and then a base in order to release the protonated lactic acid and regenerate the adsorptive sites. The BFBR also yields 50 - 100 g/L lactic acid and a sulfate salt. Down-stream processing of the resulting lactic acid is then independent of the fermentation process.

As shown above, preliminary experimentation with the BFBR for lactic acid production has demonstrated a 12 fold increase in volumetric productivity compared to control fermentations in the same FBR, and a 3 fold increase in productivity when compared to reported values from batch reactors with pH control. In addition, the continuous nature of the BFBR should yield 20 - 25% more production time than a 2-3 day batch fermentation followed by a day to empty, clean, autoclave and inoculate for the next batch. Given this increase productivity and online percentage, a plant utilizing BFBR technology will require smaller reactor vessels than a plant utilizing batch fermentation for the same lactic acid production basis. Of course this savings is non-linear due to equipment pricing. In our economic assessment of immobilized cell technology for ethanol fermentation, a 20-fold increase in volumetric productivity reduced reactor costs by 50% compared to batch fermentation [35]. This reduction in reactor cost would be expected to reduce the total plant cost by 15 - 20% [35], lowering capital and thus production costs. While such increases in volumetric productivity have

yet to be realized in the lactic acid fermentation, the potential for such savings is readily apparent with expected improvements in biocatalysts. Further, optimization of the means of resin regeneration (i.e. zonal elution chromatography) should yield product lactic acid concentrations in excess of 200 g/L, decreasing downstream processing costs compared to batch fermentation.

To further demonstrate the potential advantages of the BFBR process, the estimated chemical costs and fermentation time for the production of 1 kg of lactic acid based upon the productivity and chemicals used in our BFBR are shown in Table 2. For comparison purposes, the chemical costs and production time of a batch fermentation utilizing the same media and NH_4OH to control reactor pH is also shown in this table. Note that the BFBR would complete the fermentation in 2.2 days as compared to 7.5 days for a batch reactor of the same working volume. Chemical costs would be nearly identical. While the experiments presented here have utilized glucose as the carbon source, there is no reason why the BFBR could not utilize molasses as is done industrially. The reusable resin will present an initial capital expense, however, these resins are routinely used in water treatment facilities with hundreds to thousands of cycles [36], and should not contribute significantly to the overall chemical costs. Previous economic analysis has demonstrated that overall process economics are insensitive to biocatalyst cost and cell immobilization [35]. Further, the utilities, operating costs, and overhead have been estimated to be equal regardless of fermentation technology in three separate economic studies comparing FBR to batch fermentation of acetone/butanol [37], citric acid [38], and ethanol [35]. It is thus seen that the benefits of BFBR technology do not bear additional expense and have the potential for significant savings in plant capital and downstream processing cost.

Conclusions

A biparticle fluidized bed reactor for the fermentation and simultaneous separation of lactic acid has been demonstrated at the laboratory scale. In unoptimized operation, this reactor has demonstrated a 12 fold increase in volumetric productivity over control experiments in the same reactor (without pH control), and a 3 fold increase over reported values for conventional batch fermentation. This increased productivity is achieved by providing high cell density and in situ removal of the inhibitory product. Initial economic assessment of this bioprocessing scheme has revealed the potential for

cost reduction due to decreased reactor size, increased on-line production time, and equal chemical, operating, utility and overhead costs. Further improvements in biocatalyst, media and resin regeneration are expected to reveal further economic advantages of the biparticle fluidized bed reactor over conventional batch fermentation of lactic acid.

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Bibliography

1. Atkinson, B. and F. Mavituna, *Biochemical Engineering and Biotechnology Handbook.* 2 ed. 1991, New York: Stockton Press.

2. Lipinsky, E.S. and R.G. Sinclair, Chem. Eng. Prog., 1986. **82**: p. 26-32.

3. *Chemical Marketing Reporter.* March 14, 1994, p. 28-36.

4. Millis, J. *American Chemical Society Annual Meeting.* 1993. Denver, CO: American Chemical Society. **38**: p. 297-299

5. Ohleyer, E., H.W. Blanch, and C.R. Wilke, Applied Biochemistry and Biotechnology, 1985. **11**: p. 317-331.

6. Evangelista, R.L., A.J. Mangold, and Z.L. Nikolov, Applied Biochemistry and Biotechnology, 1994. **45**: p. 131-144.

7. Mercier, P., L. Yerushalmi, D. Rouleau, and D. Dochain, J. Chem. Tech. Biotechnol., 1992. **55**: p. 111-121.

8. Buchta, K., *Lactic Acid,* in *Biotechnology: A Comprehensive Treatise Biomass, Microorganisms for Special Applications, Microbial Products I, Energy from Renwable Resources,* H. Dellweg, Editor. 1983, Verlag Chemie: Weinheim.

9. Kirk-Othmer, *Encyclopedia of Chemical Technology.* 3 ed. Vol. 13. 1981, New York: John Wiley & Sons.

10. King, C.J., ChemTech, 1992. **22**(5): p. 285-291.

11. Yeh, P.L.H., R.K. Bajpai, and E.L. Iannotti, Journal of Fermentation and Bioengineering, 1991. **71**(1): p. 75-77.

12. Kuhn, R., S. Peretti, and D. Ollis, Applied Biochemistry and Biotechnology, 1993. **39/40**: p. 401-413.

13. Melzoch, K. and L. Konopaskova, Biotechnology Letters, 1993. **15**(5): p. 517-520.

14. Andrews, G.F. and J.P. Fonta, Applied Biochemistry and Biotechnology, 1989. **20**: p. 375-390.

15. Stenroos, S.L., Y.Y. Linko, and P. Linko, Biotechnology Letters, 1982. **4**(3): p. 159-164.

16. Guoqiang, D., R. Kaul, and B. Mattiasson, Appl. Microbiol. Biotechnol., 1991. **36**: p. 309-314.

17. Hang, Y.D., H. Hamamci, and E.E. Woodams, Biotechnology Letters, 1989. **11**(2): p. 119-120.

18. Yabannavar, V.M. and D.I.C. Wang, Biotechnology and Bioengineering, 1991. **37**: p. 1095-1100.

19. Vick Roy, T.B., H.W. Blanch, and C.R. Wilke, Biotechnology Letters, 1982. **4**(8): p. 483-488.

20. Shi, Z., K. Shimizu, S. Iijima, T. Morisue, and T. Kobayashi, Journal of Fermentation and Bioengineering, 1990. **70**(6): p. 415-419.

21. Kulozik, U., B. Hammelehle, J. Pfeifer, and H.G. Kessler, Journal of Biotechnology, 1992. **22**: p. 107-116.

22. Venkatesh, K.V., M.R. Okos, and P.C. Wankat, Process Biochemistry, 1993. **28**: p. 231-241.

23. Seevaratnam, S., J.O. Holst, S. Hjorleifsdottir, and B. Mattiasson, Bioprocess Engineering, 1991. **6**: p. 35-41.

24. Martin, M.S., C. Pazos, and J. Coca, J. Chem. Tech. Biotechnol., 1992. **54**: p. 1-6.

25. Garcia, A.A., Biotechnology Progress, 1991. **7**: p. 33-42.

26. Galliot, F.P., C. Gleason, J.J. Wilson, and J. Zwarick, Biotechnology Progress, 1990. **6**: p. 370-375.

27. Srivastava, A., P.K. Roychoudhury, and V. Sahai, Biotechnology and Bioengineering, 1992. **39**: p. 607-613.

28. Davison, B.H. and C.D. Scott, Biotechnology and Bioengineering, 1992. **39**: p. 365-368.

29. Davison, B.H. and J.E. Thompson, Applied Biochemistry and Biotechnology, 1992. **34**: p. 431-439.

30. Godia, F., C. Casas, and C. Sola, Process Biochem., 1987. **22**: p. 43-48.

31. Davison, B.H. and C.D. Scott, Applied Biochemistry and Biotechnology, 1988. **18**: p. 19-34.

32. Scott, C.D., Annals of the New York Academy of Sciences, 1987. **501**: p. 487-493.

33. Kaufman, E.N., S.P. Cooper, and B.H. Davison, Applied Biochemistry and Biotechnology, 1994. **45**: p. 545-554.

34. Nakagawa, M., I. Nakamura, and T. Kobayashi, J. Ferment. Technol., 1975. **53**(3): p. 127-134.

35. Fluor Daniel Inc., *Economic Assessment of Ethanol Production Comparing Traditional and Fluidized Bed Bioreactors*. 1994, Oak Ridge National Laboratory, DOE P.O. No. 32X-SM954V.

36. Rohm and Haas Company, *Amberlite IRA-35*. 1975.

37. Chem Systems Inc., *Technical and Econimic Assessment of Processes for the Production of Butanol and Acetone*. 1984, The Jet Propulsion Laboratory.

38. Chem Systems Inc., *Implications of ORNL Immobilization Technology on Citric Acid Production*. 1986, Jet Propulsion Laboratory.

39. Hanson, T.P. and G.T. Tsao, Biotechnology and Bioengineering, 1972. **14**: p. 233-252.

40. Guoqiang, D., R. Kaul, and B. Mattiasson, Applied Microbiology and Biotechnology, 1992. **37**: p. 305-310.

Figure Captions

Table 1: A survey of methods to increase volumetric productivity in the lactic acid fermentation. Listed are the authors, the bacterial strain, major media components, and reactor configuration used in the study, and the resulting productivity. For the purposes of this paper, productivity is the "overall productivity" i.e. total amount of lactic acid produced divided by the total time and the volume of the reactor containing biocatalyst. Improvements in productivity have been realized through cell immobilization and reduction of product inhibition.

Table 2: Simulated chemical costs and required production time for the fermentation of 1 kg of lactic acid (at 100 g/L concentration) in a 4 L BFBR as opposed to a batch fermentor of the same size. For purposes of comparison we have assumed a productivity of 4.7 g/l*h for the BFBR and 1.6 g/l*h for the batch fermentor. The media composition is that used in this study. This comparison assumes a 90% lactic acid yield on glucose and that 100 gms of IRA-35 resin are utilized repeatedly with a loss of 5% resin during the entire course of the resin regeneration. It further assumes that equimolar amounts of sulfuric acid and ammonium hydroxide (relative to the lactic acid) are needed to strip and regenerate the resin (for the BFBR) or pH the reactor and reacidify the lactate salt in the case of the batch fermentor. The selling price of lactic acid is $1.12 per lb. The batch fermentation typically reaches completion in 2 to 3 days. Since the batch fermentation would require at least two runs with assumed reactor size, one day has been added to the production time to account for reactor cleaning, sterilization and reinoculation. Chemical costs are taken from [3].

Figure 1: Reduction in maximum lactic acid production rate as a function of total lactic acid concentration at pH 6.0. This curve was constructed using the inhibition constants of Yeh *et al.* [11]. Batch fermentations typically operate until broth concentrations are 100 - 150 g/L lactic acid and thus most of the fermentation time is spent at less than 20% of maximum productivity. The biparticle fluidized bed reactor utilizes in situ product removal so that the entire production times is spent at maximal productivity.

Figure 2: Schematic of the biparticle fluidized bed reactor.

Figure 3: Control fermentation run without pH control or resin addition. The reactor pH quickly drops below the optimal fermentation level of 6.0, and lactic acid productivity decreases to a value of about 0.4 g/L*hr.

Figure 4: Fermentation with continuous resin addition. IRA-35 resin was added as needed to keep the pH of the reactor between 5.8 and 6.2, and to remove the inhibitory lactic acid from the fermentation broth. Note that the measured lactic acid concentration

as a function of time was that in the fermentation broth. The total lactic acid in the broth was determined by stripping the lactic acid adsorbed to the resin at the conclusion of the fermentation and calculating what the concentration of lactic acid in the reactor would be if this lactic acid were also in the broth. The estimated line is a prediction of this concentration as a function of time assuming a constant productivity. A volumetric productivity of 4.7 g/l*hr was achieved, representing a 12 fold increase over control fermentations in the same reactor.

Figure 5: Comparison of batch vs. BFBR fermentation processes.

Table #1

Reference	Strain	Media	Configuration	Productivity
Butcha [8]	L. delbreuckii	sucrose, corn steep liquor	batch w/ pH control	1.6 g/l*h
Hanson [39]	L. delbreuckii	glucose, yeast extract	batch w/ pH control	1.5 g/l*h
Melzoch [13]	L. acidophilus	glucose, yeast extract	cell aggregates in gas lift w/ pH control	9 g/l*h
Guoqiang [40]	L. casei	glucose, yeast extract	alginate beads in batch w/ pH control	1.6 g/l*h
Srivastava [27]	L. delbreuckii	sucrose, yeast extract	batch w/ recycle through ion exchange resin	1.7 g/l*h
Shi [20]	S. inulinus	glucose, yeast extract, polypeptone	fed batch with hollow fiber cell recycle and acid removal, pH control	20 g/l*h
Kulozik [21]	L. helveticus	whey	cascade of 7 CSTR's w/ pH control	50 g/l*h

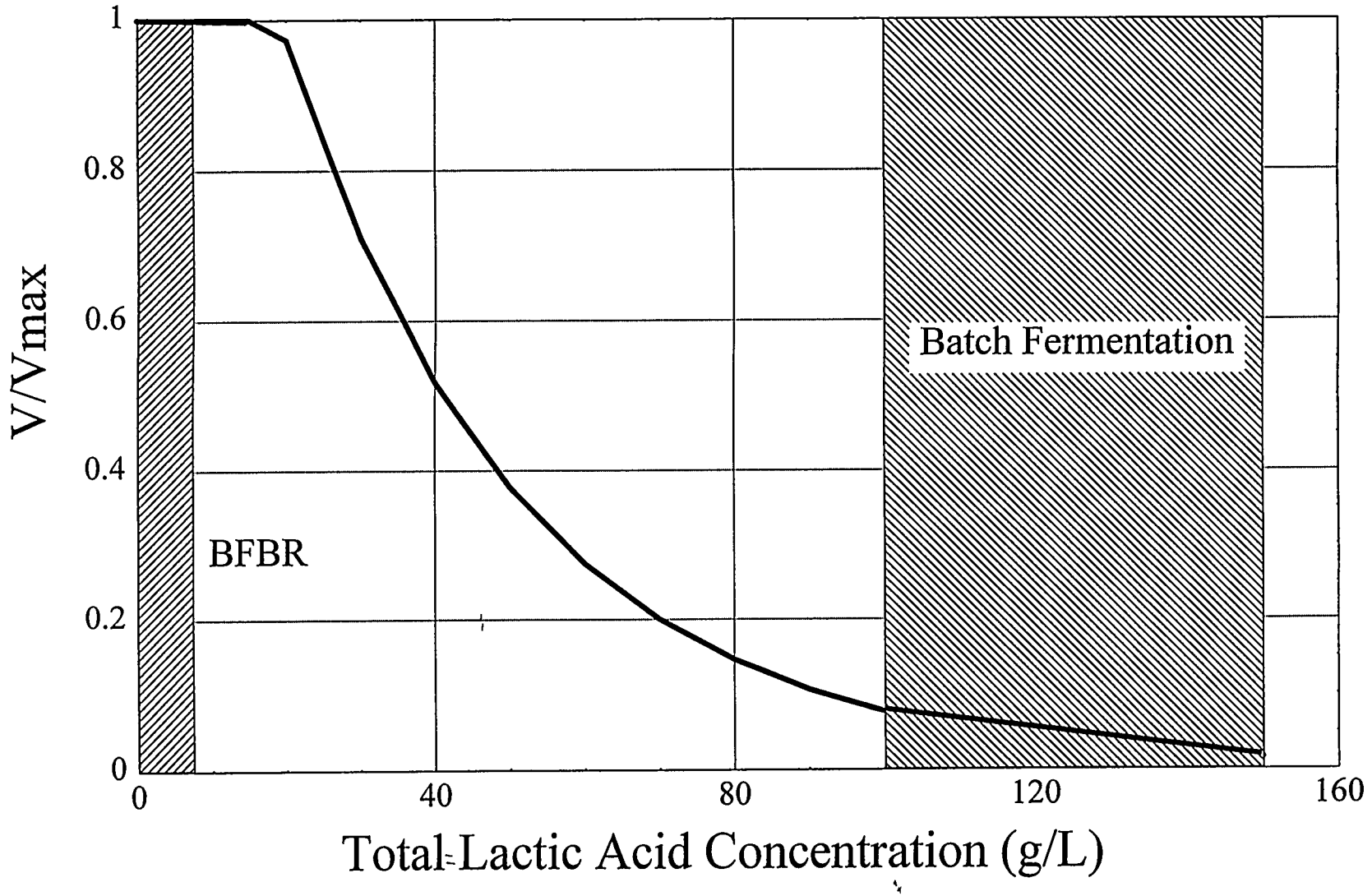
Table #2

Biparticle FBR

	Cost ($/g)	Concentration (g/L)	Volume (L)	Mass (g)	Cost ($)
Glucose	0.0006			1111	0.6666
Sheftone	0.0011	20	4	80	0.088
Ammonium Sulfate	6.61E-05	0.5	4	2	0.0001
Magnesium Sulfate	0.0004	0.3	4	1.2	0.0005
Calcium Chloride	0.0004	0.1	4	0.4	0.0002
IRA-35	0.01			5	0.05
Sulfuric Acid	9.00E-05			1090	0.0981
Ammonium Hydroxide	1.00E-04			390	0.039
Total Chemical Cost					0.9425
Selling Cost					2.5
Production Time	2.2 days				

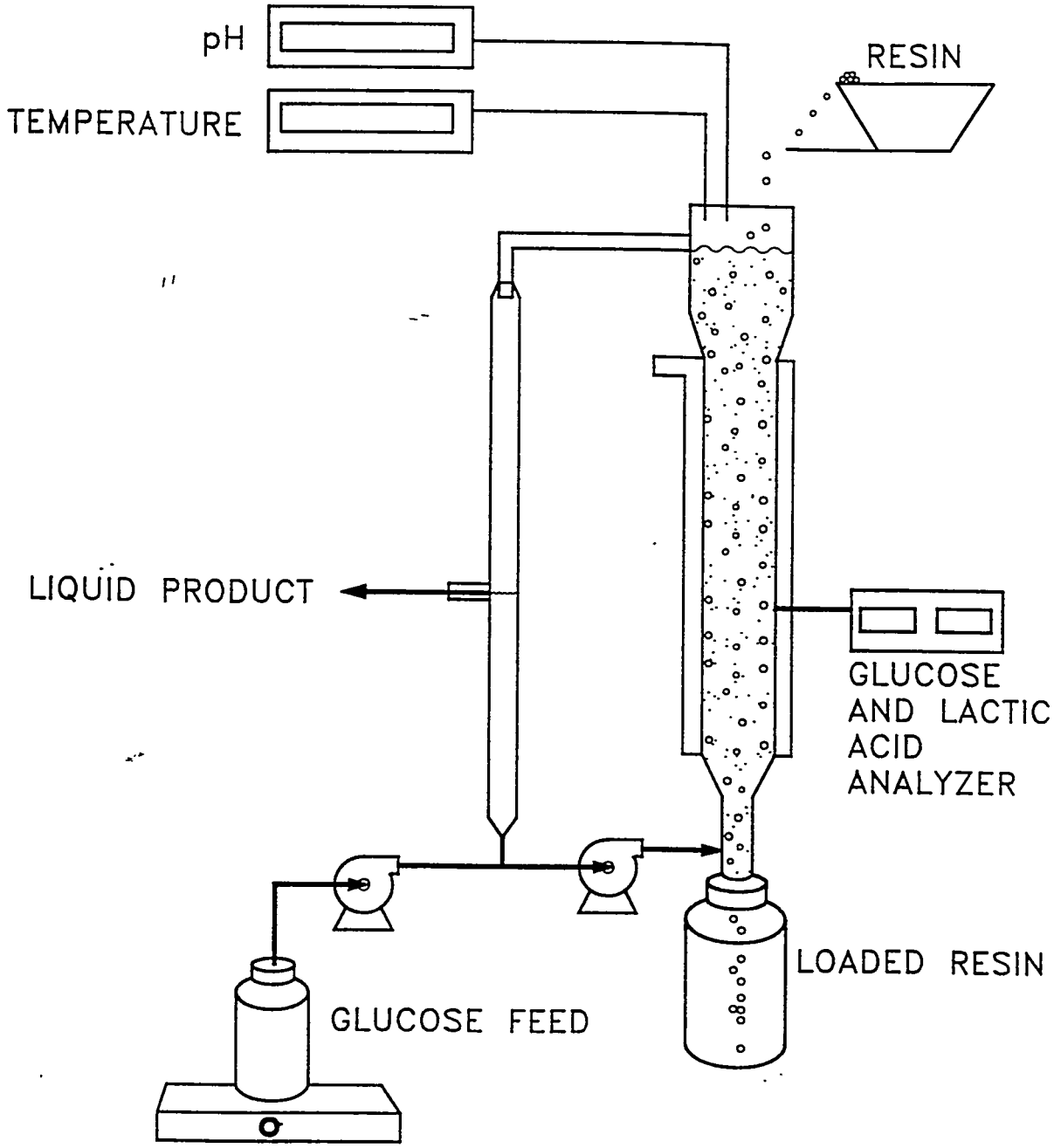
Conventional

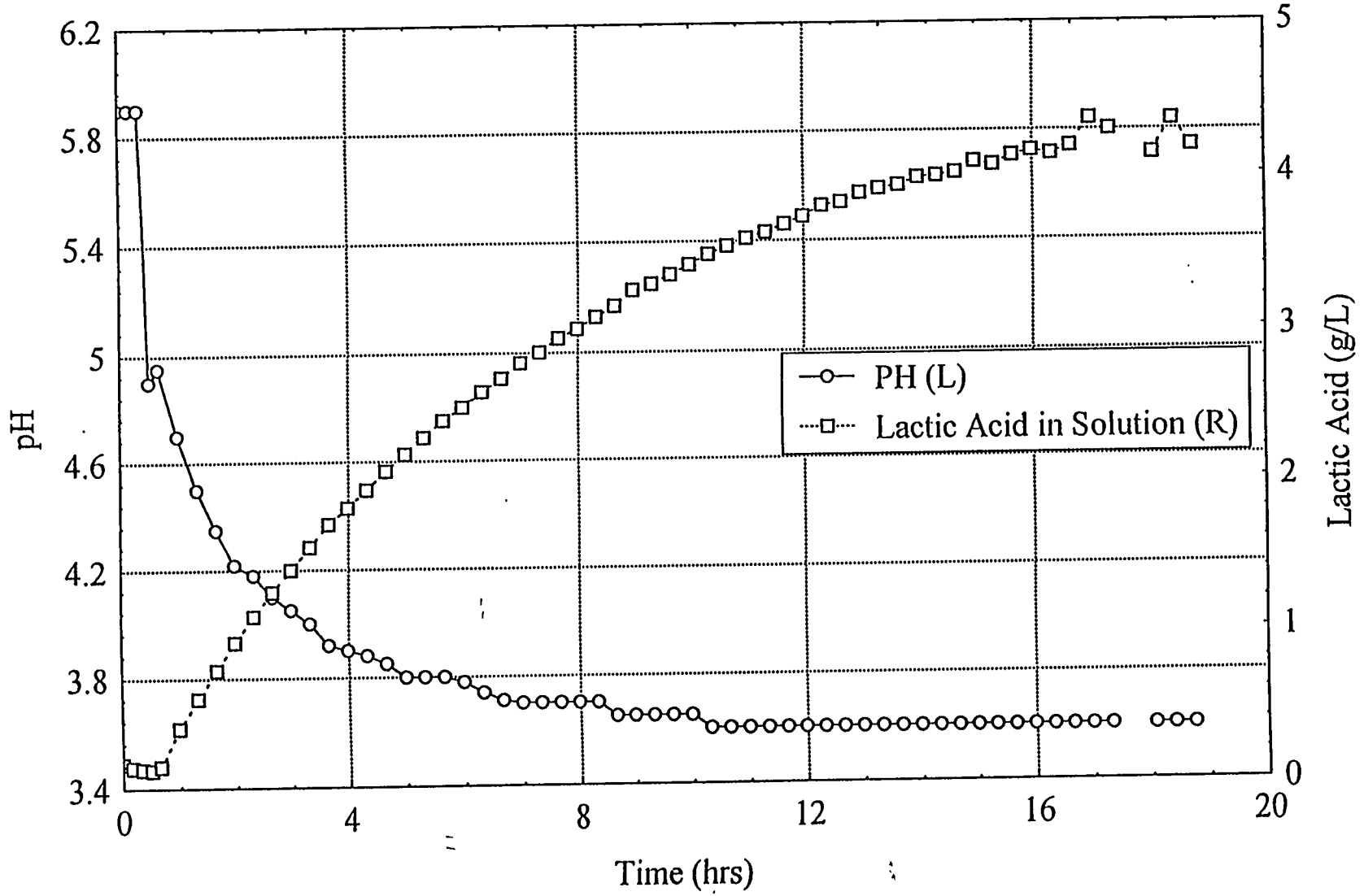
	Cost ($/g)	Concentration (g/L)	Volume (L)	Mass (g)	Cost ($)
Glucose	0.0006			1111	0.6666
Sheftone	0.0011	20	4	80	0.088
Ammonium Sulfate	6.61E-05	0.5	4	2	0.0001
Magnesium Sulfate	0.0004	0.3	4	1.2	0.0005
Ammonium Hydroxide	1.00E-04			390	0.039
Sulfuric Acid	9.00E-05			1090	0.0981
Total Chemical Cost					0.8923
Selling Cost					2.5
Production Time	7.5 days				

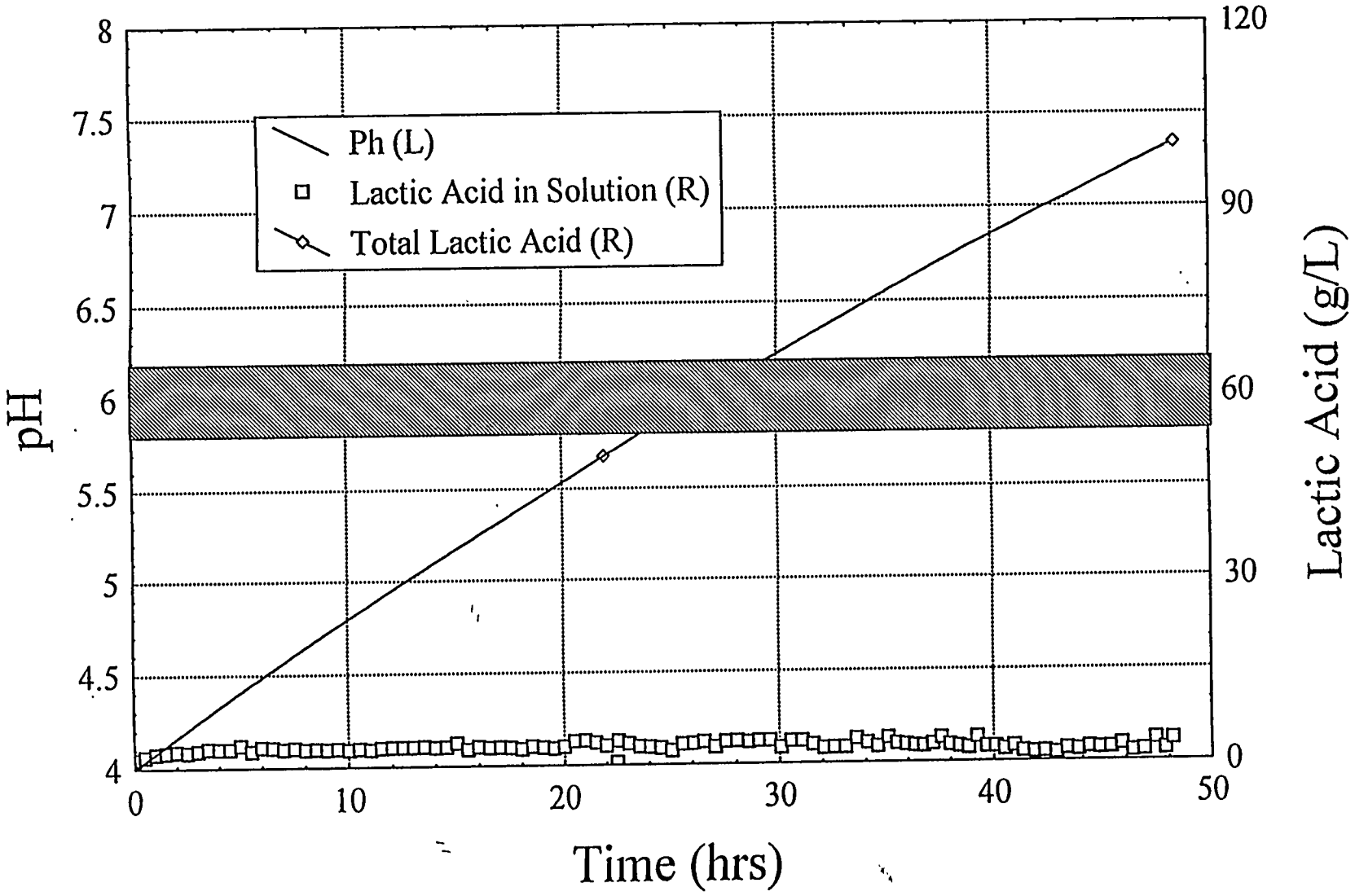

1
0.8
0.6
0.4
0.2
0
V/Vmax
BFBR
Batch Fermentation
0
40
80
120
160
Total Lactic Acid Concentration (g/L)

ORNL DWG 94H-279

Biparticle Fluidized Bed Reactor




6.2
5.8
5.4
5
4.6
4.2
3.8
3.4
pH
0
4
8
12
16
20
Time (hrs)
5
4
3
2
1
0
Lactic Acid (g/L)
PH (L)
Lactic Acid in Solution (R)


Ph (L)
Lactic Acid in Solution (R)
Total Lactic Acid (R)
pH
Lactic Acid (g/L)
Time (hrs)
8
7.5
7
6.5
6
5.5
5
4.5
4
120
90
60
30
0
0
10
20
30
40
50

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Batch vs. BFBR Fermentation