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Screening of Resins for Use in a Biparticle Fluidized-Bed Bioreactor for the Continuous Fermentation and Separation of Lactic Acid†

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ABSTRACT

A continuous particle fluidized-bed reactor is being developed for the simultaneous fermentation and purification of lactic acid. Unlike conventional fermentation schemes, the biparticle fluidized bed does not require the addition of salts for reactor pH control and product separation as the inhibitory product is removed directly from the reactor. This minimizes process waste and enhances reactor efficiency. This work has screened a series of adsorbents for possible utilization in the biparticle fluidized-bed fermentation of lactic acid by immobilized *Lactobacillus delbreuckii*. Capacity, specificity, regenerability, and kinetics were investigated. Although a resin exhibiting all of the desired properties has yet to be found, Reillex 425 appears satisfactory and will be utilized in initial process demonstration as resin screening continues.

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

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INTRODUCTION

Lactic acid is a commodity chemical utilized in the food industry for the manufacture of cheese, pickles, and yogurt, and also as a preservative (*1*). It is used as feed in plastics production and in the synthesis of other organic acids, acetaldehyde, and ethanol (*2*). Annual US consumption of lactic acid totals over 30 million pounds (*3*). Biological production of lactic acid accounts for approx 50% of the world's production (*4*). Biological production is complicated primarily because of economic considerations arising from product inhibition and the required downstream processing required of dilute aqueous product streams. The standard method of biological lactic acid production is the anaerobic fermentation by *Lactobacillus* in batch reactors. The conventional process requires that base be added to the reactor to control pH (*3*) and/or that calcium carbonate be added to precipitate the lactate. These processes produce the lactate salt, which must be reacidified (usually by sulfuric acid [*5*]), which yields calcium sulfate, further adding to process chemical costs and waste streams.

To increase volumetric production in lactic acid fermentations, high cell density has been achieved through growth of biofilm on activated carbon (*6*) or cell immobilization in gelatin beads (*see*, for example (*7–10*]). Removal of inhibitory product has been achieved using both liquid extractants (*5,10–12*) and solid adsorbents (*11,13*) either in a product stripping side stream (*14,15*) or added directly to the CSTR reactor (*16*). *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 (*17*) 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 were fluidized by the liquid media. Such fluidized beds have been shown to increase the productivity of fermentations for a variety of processes (*18,19*). In the BFBR, the polyvinyl pyridine resin Reillex 425 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 control fluidized-bed reactor. In this proof of concept experiment, the product lactic acid was not continuously recovered from the resin, and resin addition was performed batchwise, manually, for a period of 7 h.

Ongoing research has focused upon the demonstration of long-term, continuous lactic acid fermentation and separation in a BFBR. Meeting this objective requires the screening of resins for their chemical properties, such as capacity, specificity, ease of regeneration, ability to withstand repetitive regeneration, and regenerant product concentration. Because

of the nature of the process, consideration must also be given to adsorption and desorption kinetics, and hydrodynamic behavior in a liquid fluidized bed of biocatalysts. This article undertakes the screening of a series of solid adsorbents for lactic acid. Mild conditions and chemicals are tested for repetitive resin regeneration with minimal waste stream production and with consideration to bacterial toxicity. Adsorption and desorption kinetics are investigated, and resin specificity is addressed. Although a resin exhibiting all of the desired properties has yet to be found, Reillex 425 appears satisfactory and will be utilized in initial process demonstration as resin screening continues.

METHODS

Resin regeneration was investigated using hot water, methanol, acetone, and hot vacuum stripping procedures. These solvents were selected for their mild conditions and ease of downstream processing. Lactic acid loading/recycle experiments were performed using the following resins: Reillex 402 (Reilly Industries, Indianapolis, IN, polyvinyl pyridine in a powder form), Reillex 402I (a nonpharmaceutical grade form of Reillex 402), Reillex 425 (Reilly Industries polyvinyl pyridine in a bead form), Amberlite IRA-35 (Rohm & Haas Philadelphia, PA, macroreticular weak-base anion exchanger with tertiary amine functionality), and Amberlite IRA-93 (Rohm & Haas macroreticular weak-base anion exchanger with tertiary amine functionality). Initial research prewashing resins in methanol and acetone or methanol and hydrochloric acid all demonstrated decreased capacity for lactic acid. In the experiments reported here, resins were used directly as supplied by the manufacturer. Resin dry weight was determined on separate resin samples and resin capacity is reported on a dry-weight basis.[1] Approximately 5 g of resin were added to 100 mL of 5.0 g/L lactic acid (L+ lactic acid #L-1875, Sigma Chemical Company, St. Louis, MO), pH 2.5, at room temperature and allowed to reach equilibrium in 125-mL sealed serum bottles. After 24 h, the supernatant was sampled, additional liquid removed using vacuum, and the adsorbed lactic acid stripped from the resin using one of the following precedures:

1. Ten milliliters of water at 90°C;
2. Ten milliliters of methanol at room temperature;
3. Ten milliliters of acetone at room temperature; or
4. Vacuum at −23 in Hg and 90 or 175°C followed by condensation in liquid nitrogen.

[1] Resin capacity was calculated as:

$$\text{capacity (g/g dry resin)} = \{ \text{vol} \times (c_i - c_f) / \text{g resin} \times [(\text{g dry resin} / \text{g resin})] \}$$

Following the desorption procedure (conducted for 24 h for liquid regenerants and until the resin was noticeably dry in the case of vacuum stripping), the resin was reloaded with lactic acid, and the process was repeated for 5 loading/regeneration cycles. Lactic acid samples were analyzed using an enzyme-based assay, YSI lactate analyzer model 27 (Yellow Springs Instruments Company, Inc., Yellow Springs, OH).

Experiments were performed to assess resin specificity for the fermentation product lactic acid over the substrate glucose. In addition to the IRA resins 35 and 93 and the Reillex 425, Silicilate S-115 (Union Carbide), which demonstrated promising characteristics in acetic acid screening (*20*) in both powder and pellet form, were screened for competitive adsorption and specificity. Fermentation media (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]) was sterilized by autoclaving, with lactic acid added to achieve a concentration of 3 g/L served as the loading solution. To avoid issues of water adsorption and subsequent substrate concentration, resins were preequilibrated in 40°C water prior to adsorption experiments at 40°C. Lactic acid capacity was measured and calculated as described previously. Glucose concentration was measured on the YSI model 27 analyzer with a glucose membrane substituted for the lactate membrane. Lactic acid and glucose were desorbed from the resins using 10 mL of water at 90°C.

Adsorption isotherms of R425 for lactic acid at 40 and 90°C were constructed to aid in process design. Most experiments at 40°C incubated 100 mL of fermentation media with lactic acid added at various concentrations with 5 g of R425 in serum bottles. Additional experiments at 40°C used lactic acid in water with a loading volume of 25 or 100 mL. All experiments at 90°C used 100 mL lactic acid in water with 5 g of resin.

The residence time of the solid adsorbent in the fluidized-bed reactor will be controlled by the wetting characteristics of the resin, the opposing superficial velocity of the liquid feed, and the solids fraction of both the sorbent and biocatalyst. It is probable in laboratory-scale bioreactors that this residence time will be shorter than the time required to reach equilibrium adsorption of the lactic acid. In the original study by Davison and Thompson (*17*) using R425 (275–1000 μm in diameter), a portion of the added resin did not settle in the reactor, but built up into a layer at the top of the reactor. A larger form of the Reillex 425 (denoted R425L), 1–1.5 mm in diameter, was screened to see if it would exhibit similar capacity and kinetics with preferable reactor hydrodynamics. An adsorption/desorption time-course experiment was performed adding 100 mL of 5 g/L lactic acid to 5 g of R425 or R425L at 40°C and sampling (100 μL/sample) the well-mixed container at various time intervals. Subsequent desorption kinetics were performed by removing the remaining liquid, bringing the system to 90°C, and adding 10 mL of 90°C water. To screen resin hydrodynamics, resin was added to the 45 in. tall reactor (*17*) and fluidized at an identical superficial velocity (0.13 cm/s) as used in the Davison and Thompson study, without biocatalysts in the bed.

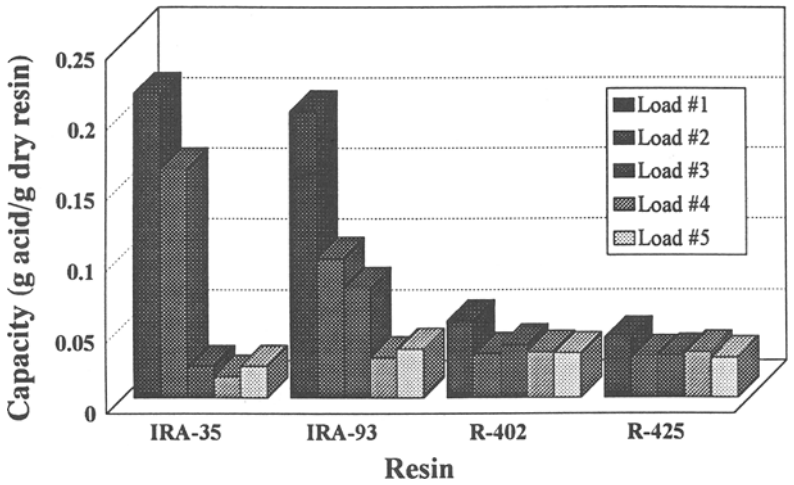

Fig. 1. Resin capacity for lactic acid as a function of recyle number when regenerated with methanol. The IRA resins exhibit the highest initial capacity for lactic acid. However, this capacity is unstable with repetitive use. The Reillex resins exhibit lower, but stable capacity. Results for hot water and acetone regeneration are identical and are not shown for the sake of brevity.

RESULTS

Figure 1 demonstrates the capacities of the four resins for lactic acid as a function of recycle number when they were regenerated with methanol. Results were nearly identical when regenerated with water or acetone, and are not shown. It is seen that although the IRA resins exhibit larger initial capacities than the Reillex resins, their capacities quickly diminish in subsequent loadings because of the inability to regenerate the resins in the tested solvents and the resins' instability at elevated temperature.[2] The Reillex resins have much lower initial capacity for lactic acid, but after an initial decrease in capacity owing to irreversibly bound material (~50% of the lactic acid bound in the initial loading appeared to be bound irreversibly, with ~100% regeneration in subsequent cycles), are able to maintain stable performance. Following the initial loading/regeneration cycle, the Reillex resins consistently produced "product streams" in the 10-mL stripping solution of ~4.6 g/L lactic acid.

The regeneration of resin using water is attractive since it would not require additional chemical species in the process stream, and unlike other solvents, would not be toxic to the biocatalyst. Resin regeneration by vacuum would have the potential to achieve a more significant product concentration since it would not require a liquid make-up stream to achieve solids transport in an operating process. Initial results indicate, however,

[2] The maximum recommended temperature for IRA-35 is 60°C (*21*). That for IRA-93 is 100°C (*22*).

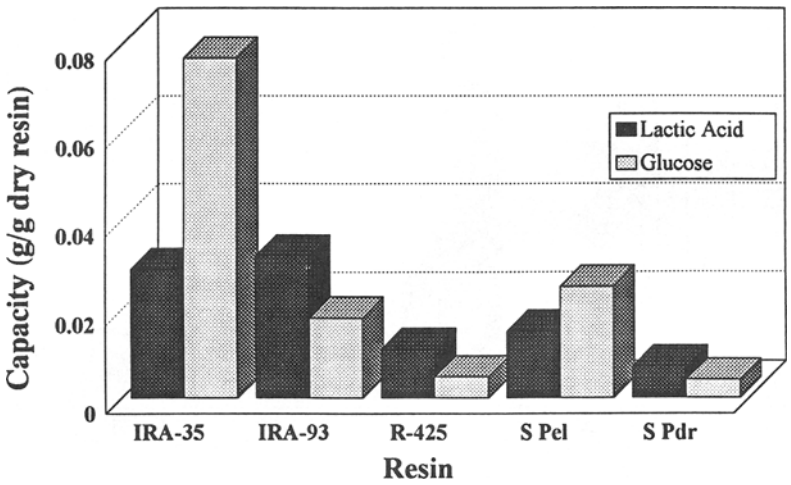

Fig. 2. Resin capacity for both lactic acid and glucose when present in fermentation media. The IRA-35 and Silicalite pellets exhibit a higher capacity for the substrate glucose than for the product lactic acid, clearly an unacceptable characteristic for use in the proposed process. Reillex 425 and Silicalite powder exhibited lower, but still appreciable capacities for glucose.

that 23-in. Hg of vacuum were unable to desorb the loaded lactic acid even when the resin was heated to 175°C. This indicates that liquid/solid contact is a requirement for acid desorption and that the product cannot be sublimed from the resin surfaces.

The capacities of five adsorbents for both lactic acid and glucose when contacted with fermentation media are shown in Fig. 2. For all the adsorbents, the glucose and media constituent adsorption appeared to be competitive with lactic acid uptake because of the fact that lactic acid capacity was lower in the presence of glucose and media constituents than it was with lactic acid in water. The IRA-35 resin exhibited higher capacity for the substrate glucose than the product lactic acid, obviously an unacceptable behavior for use in the BFBR, but perhaps useful in the biofilm process scheme suggested by Andrews and Fonta (*6*). Sixteen percent of the lactic acid desorbed from the IRA-35 when treated with hot water, whereas only 1% of the glucose desorbed. The Silicilate powder exhibited the lowest glucose capacity with favorable ability for lactic acid desorption. However, in the presence of binder to form pellets in order to make the Silicilate useful in a BFBR, the alumina binder appeared to have appreciable glucose capacity. Both glucose and lactic acid adsorption by the pellets were nearly irreversible. As demonstrated earlier, IRA-93 did not desorb lactic acid appreciably in the solvents tested. The adsorption of both glucose and lactic acid on Reillex 425 appears fully reversible after an initial adsorption/desorption cycle. It would be preferred if either the resin exhibited no glucose capacity, or the glucose bound irreversibly to the resin so that

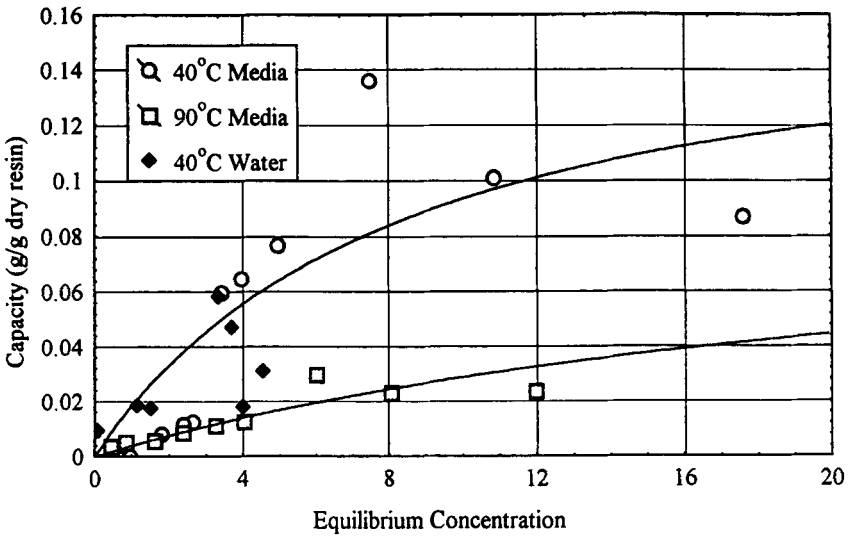

Fig. 3. Isotherms of Reillex 425 for lactic acid in water and media. Resin capacity is plotted vs the equilibrium concentration of lactic acid in solution.

after presaturation the resin would exhibit negligible capacity for the substrate and the substrate would not contaminate the product stream. None of the screened resins exhibited this desired behavior. Initial screening identified Reillex 425 as the most promising adsorbent for use in the BFBR.

Reillex R425 capacity for lactic acid as a function of equilibrium concentration and temperature is shown in Fig. 3. Such information regarding resin capacity as a function of product equilibrium concentration is necessary for process design. This allows the estimation of the solids holdup required to maintain product concentration in the reactor at the desired level. Obviously one would desire to operate at high acid concentrations to effect greater resin capacity and the potential for increased lactic acid concentration in the product stream. However, fermentations with immobilized *Lactobacillus delbreuckii* in a liquid fluidized bed (*17*) have demonstrated greatly diminished production when the pH fell below 4 (corresponding to a lactic acid concentration in the media of ~1 g/L). This corresponds to that observed in the classic experiments by Luedeking and Piret (*see* ref. *1* for a review). Preliminary process design, thus, specifies a target lactic acid concentration in the reactor of ~1 g/L. At this concentration, and at the desired fermentation temperature of 40°C, R425 exhibits a capacity for lactic acid of ~0.01 g/dry g resin in fermentation media. The decreased capacity of the resin in water at 90°C may also be observed in Fig. 4.

Experiments comparing Reillex 425 and 425L indicated that both forms of the resin exhibited similar capacities and adsorption/desorption kinetics. As shown in Fig. 4, the vast majority of lactic acid uptake occurred during the first 10 min of contact, with lower accumulation occurring during the

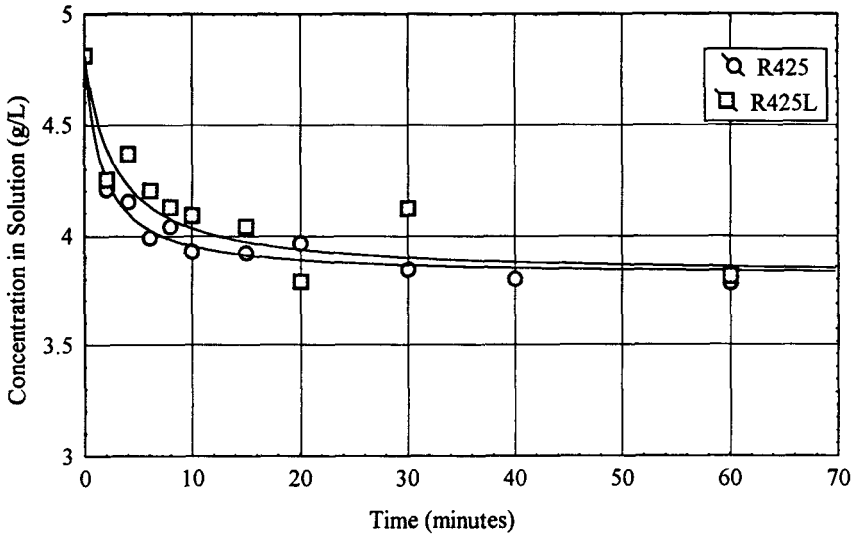

Fig. 4. Adsorption time-course of Reillex 425 and 425L for lactic acid at 40°C. Most of the change in solution concentration takes place in the first 10 min of contact.

next 50 min. The final lactic acid concentration in this uptake experiment after 24 h was 3.5 g/L. Regeneration kinetics were also fairly rapid, with the concentration in the supernatant increasing from 0 to 4 g/L in the first 10 min and leveling off to 4.5 g/L after 1 h. In reactor-settling experiments, Reillex 425 had a residence time of ~2.5 min, whereas the 425L had a residence time of ~1.5 min (for a 45 in. long reactor). The R425 contained fines that never settled. Residence times are expected to be two to four times longer in the presence of 0.8-mm alginate beads (the biocatalyst) at high-volume fractions. In light of adsorption kinetics and reactor residence times, the Reillex 425 appears preferable to 425L. This is because of the fact that it will have a slightly longer residence time in the reactor. However, the Reillex 425 will require presieving to remove fines that may not sediment in the BFBR. The residence time will be even less of a concern as the process is scaled up and column height is increased.

DISCUSSION

A variety of adsorbents have been screened for the multiple of chemcal and physical properties required for use in the continuous fermentation and separation of lactic acid in a BFBR. These properties included capacity, specificity, ease of regeneration, ability to withstand repetitive regeneration, regenerant product concentration, adsorption/desorption kinetics, and residence time in the fluidized-bed reactor. No absorbent exhibited all of the desired properties, and resin screening will continue since initial BFBR experiments utilized the most promising resin to date: Reillex 425.

The Amberlite resins tested exhibited the highest initial lactic acid capacity, but this capacity fell dramatically as the resin was repetitively loaded and recycled. In addition, the IRA-35 possessed a capacity for the substrate glucose, which was larger than its capacity for the product lactic acid. Silicalite powder exhibited favorable specificity, but when bound into pellets for use in a BFBR, the binder effected a large capacity for glucose. Of the adsorbents tested to date, only the Reillex resins possess stable capacity with repetitive use. However, at the desired reactor media conditions, Reillex 425 exhibits low capacity for lactic acid (0.01 g/g) and slight (0.004 g/g) capacity for glucose. It is not known at this point if this lack of strong specificity will be a problem in the BFBR, which will have high lactic acid concentrations at the top of the reactor (where the resin will enter) and high glucose concentrations at the base (where the resin will exit). The adsorption and desorption process is quite rapid for the R425 with the majority of uptake or release occurring in 10 min. At the lactic acid production rates expected in a BFBR (0.32 g/h [*17*] in an unoptimized system, perhaps 10-fold larger production rates will be achieved with continuous resin addition), only 32 g of Reillex 425 would have to be added continuously each hour in order to meet the target reactor concentrations. This is clearly an insignificant solids holdup. Thus, specificity and regenerability are more important characteristics than product capacity under these conditions. Future experimentation will continue to screen resins for the desired physical and chemical characteristics for use in a BFBR for lactic acid production and separation. Initial demonstration will utilize the Reillex 425 in a system with continuous resin addition and regeneration, producing a continuous product stream.

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REFERENCES

1. Atkinson, B. and Mavituna, F. (1991), *Biochemical Engineering and Biotechnology Handbook*, 2nd ed. Stockton Press, New York.
2. Demirci, A. and Pometto, A. L. (1992), *J. Ind. Microbiol.* **11,** 23–28.
3. Millis, J. (1993), *American Chemical Society Annual Meeting*. American Chemical Society, Denver, CO.
4. Ohleyer, E., Blanch, H. W., and Wilke, C. R. (1985), *Appl. Biochem. Biotechnol.* **11,** 317–331.
5. King, C. J. (1992), *ChemTech* **22,** 285–291.

6. Andrews, G. F. and Fonta, J. P. (1989), *Appl. Biochem. Biotechnol.* **20,** 375–390.
7. Stenroos, S. L., Linko, Y. Y., and Linko, P. (1982), *Biotechnol. Lett.* **4(3),** 159–164.
8. Guoqiang, D., Kaul, R., and Mattiasson, B. (1991), *Appl. Microbiol. Biotechnol.* **36,** 309–314.
9. Hang, Y. D., Hamamci, H., and Woodams, E. E. (1989), *Biotechnol. Lett.* **11(2),** 119,120.
10. Yabannavar, V. M. and Wang, D. I. C. (1991), *Biotech. Bioeng.* **37,** 1095–1100.
11. Seevaratnam, S., Holst, J. O., Hjorleifsdottir, S., and Mattiasson, B. (1991), *Bioproc. Eng.* **6,** 35–41.
12. Martin, M. S., Pazos, C., and Coca, J. (1992), *J. Chem. Tech. Biotechnol.* **54,** 1–6.
13. Garcia, A. A. (1991), *Biotechnol. Prog.* **7,** 33–42.
14. Galliot, F. P., Gleason, C., Wilson, J. J., and Zwarick, J. (1990), *Biotechnol. Prog.* **6,** 370–375.
15. Srivastava, A., Roychoudhury, P. K., and Sahai, V. (1992), *Biotechnol. Bioeng.* **39,** 607–613.
16. Davison, B. H. and Scott, C. D. (1992), *Biotechnol. Bioeng.* **39,** 365–368.
17. Davison, B. H. and Thompson, J. E. (1992), *Appl. Biochem. Biotechnol.* **34,** 431–439.
18. Godia, F., Casas, C., and Sola, C. (1987), *Process Biochem.* **22,** 43–48.
19. Davison, B. H. and Scott, C. D. (1988), *Appl. Biochem. Biotechnol.* **18,** 19–34.
20. Davison, B. H. and Thompson, J. E. (1993), *J. Chem Tech. Biotechnol.* submitted.
21. Rohm and Hass Company, (1975).
22. Rohm and Haas Company, (1978).