0273-2289/96/5758—0471$7.50

Recovery and Purification of Lactic Acid from Fermentation Broth by Adsorption

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ABSTRACT

Riedel-de-Haen VI-15, Dowex MWA-1 and Amberlite IRA-35 were employed for lactic acid recovery using model fermentation broth. The broth was first acidified by using a cation exchanger before sorption of lactic acid onto the basic sorbents. Lactic acid was completely recovered from the VI-15 column after 7 bed volumes (BV) of methanol, whereas only 64% was recovered from MWA-1 after 4.5 BV and 18% from IRA-35 after 5 BV. The 5% NH_4OH eluted all lactic acid from the MWA-1 column in 1.5 BV with a maximum effluent concentration of 113 mg/mL. The simple recovery scheme employed was not sufficient to produce heat-stable lactic acid. Other broth components were also adsorbed by the basic sorbents and could not be removed during the rinse step, but eluted with lactic acid during the desorption step.

Index Entries: Lactic acid; recovery; sorption; ion exchange.

INTRODUCTION

As the world's crude oil resources diminish and the prices of petroleum products continue to increase, the production of chemicals by biological processes is becoming more competitive. High-purity and heat-stable lactic acid, which was mainly available from synthetic manufacture, is now being produced by fermentation. About 82% of the estimated 36×10^6 kg/yr of lactic acid produced worldwide is used by the food industry. The rest is being used in pharmaceutical, cosmetics, textile, and leather industries. These markets are projected to grow at 2–4%/yr *(1)*. The use of polylactic acid (PLA) for biodegradable plastics and controlled-release drugs and pesticides can transform lactic acid into a commodity chemical *(2)*. The commercial success of PLA, however, hinges on the cost of producing heat-stable lactic acid. Therefore, one of the major challenges in lactic acid production is to reduce the cost of acid recovery and purification.

Adsorption is a process suitable for recovering substances produced in dilute concentrations and in complex solutions, such as fermentation broth. Synthetic polymeric sorbents with varying degrees of basicity have been used in the recovery of carboxylic acids from fermentation broth *(3–10)*. The polymeric sorbents have also been used to extract lactic acid and control pH during fermentation *(10–13)*.

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Batch fermentation of lactic acid is usually conducted at pHs between 5 and 7 to minimize product inhibition by the free acid *(14)*. This is done by adding a base to neutralize the acid as it is produced. The lactic acid in the broth is therefore produced as a salt of the base used. The adsorption of lactate can be achieved by using a strong-base ion exchanger. Recovering lactate from the sorbent, however, requires a stronger desorbent. For example, if NaOH is used, the product is sodium lactate. If the desired product is the undissociated (free) acid form, further processing is necessary. To recover 1 mol of lactic acid, 1 mol each of base and acid will be consumed. One mol of waste salt will also be generated, which creates a disposal problem. Weak-base adsorbents, on the other hand, adsorb only free lactic acid; therefore, they are not effective at pHs above the pK_a of lactic acid (3.86) where mostly lactates are present. Acidifying the broth with mineral acid is not an option because it introduces competing acids *(15)*. The advantage of weak-base sorbents over strong-base sorbents is that the free lactic acid adsorbed is easily recovered by using alcohols (e.g., methanol and ethanol), acetone, and methyl acetate *(4,11,13, 16,17)*, or by temperature-swing desorption using water as eluant *(9)*. A free acid product is produced after evaporating the eluant. Concentrating lactic acid is also cheaper because the low-boiling solvents require less energy to evaporate than water. Furthermore, the solvents can also be recycled, thus significantly reducing waste generation.

The main objective of this research was to investigate the use of weak-base polymeric sorbents in the primary recovery and/or purification of lactic acid from fermentation broth. In the preliminary evaluation of selected basic sorbents using pure lactic acid *(18)*, it was recommended that when weak-base (pyridine and imidazole functional group) and moderate-base sorbents (tertiary amine functional group) were to be used in lactic acid recovery, the broth pH must be below the pK_a of lactic acid for an efficient utilization of the sorption capacity and to minimize leaching of lactates. Of the two weak-base sorbents, Riedel-de-Haen VI-15 was selected over Reillex 425 for further evaluation because of its higher sorption capacity even at low (<70 mg/mL) lactic acid concentrations. The capacity of VI-15 is comparable to that of the moderate-base sorbents evaluated. Dowex MWA-1 and Amberlite IRA-35 were included for comparison. In this article, the results of lactic acid recovery from model fermentation broth will be presented.

MATERIALS AND METHODS

Resin Preparation

The three basic polymeric sorbents selected for this study (Table 1) were conditioned following the procedures outlined in the *Rohm and Haas Ion Exchange Resin Laboratory Guide (21)*. The hydrated sorbents were transferred into a 1-L (35 × 6 cm) column. The sorbent bed was first backwashed with deionized water to remove the fine particles. This was followed by washing (downflow) of the sorbent with 3 BV of 5% HCl, 5 BV of deionized water, 3 BV of 4% NaOH, and another 5 BV of deionized water. The flow rate was adjusted to allow at least 30 min of contact time between the sorbent and HCl or NaOH solutions. These steps were repeated one more time, and the final water rinse was performed until the effluent pH was <8. The cation exchange resin was also conditioned in a similar manner to the basic sorbents, except that the order of NaOH and HCl rinse was reversed to obtain a

Table 1
Properties of Selected Basic Adsorbents

Properties	Resin/manufacturer		
	VI-15	MWA-1	IRA-35
	Riedel-de-Haen, Seelze, Germany	Dow Chemical, Midland, MI	Rohm and Haas, Philadelphia, PA
Type	Gel	Macroporous	Macroporous
Matrix	Methylene-bis-acrylamide	Styrene-divinylbenzene	Acrylic-divinylbenzene
Functional group	Imidazole	3°-amine	3°-amine
pK_a	6.9[a]	8.8[b]	11.1[c]
Capacity for chloride	7.9[d]	3.8[d]	4.9[c]
Capacity for lactic acid (mg/g)	3.1[d]	4.0[d]	4.4[c]
Association constant for lactic acid, K (g/g)	2200[d]	8400[d]	28,160[c]

[a]Weast (1987) *(19)*.
[b]Kuo et al. (1987) *(20)*.
[c]Tung (1993) *(15)*.
[d]Evangelista et al. (1994) *(18)*.

sorbent in H^+ form. The moisture contents of the conditioned sorbents were determined by using the oven method.

Broth Preparation

The model lactic acid broth used in this study contained 10 g yeast extract (Difco Laboratories, Detroit, MI), 100 g lactic acid (Certified ACS, Fisher Scientific, Pittsburgh, PA), 10 g glucose (Sigma, St. Louis, MO), 0.6 g $MgSO_4 \cdot 7H_2O$, 0.5 g K_2HPO_4, and 0.03 $MnSO_4$/L. The pH of the broth was adjusted to 4.5 by using ammonium hydroxide. This starting broth pH was chosen because the final pH of the broth from batch lactic acid fermentation would be around this value.

Analytical Methods

Lactic acid and glucose concentrations were analyzed by using high-performance liquid chromatography (HPLC) (Maxima 820, Waters, Milford, MA) equipped with a refractive index detector and Bio-Rad Aminex HPX-87H column (300 × 7.8 mm) (Bio-Rad, Hercules, CA). The column temperature was maintained at 65°C by using a column oven. The mobile phase was 6 m*M* H_2SO_4 at a flow rate of 0.8 mL/min.

Lactic Acid Recovery Scheme

Lactic acid was recovered from fermentation broth following the scheme shown in Fig. 1. The acidification step was carried out by using a weak-acid cation exchanger (Duolite C-464, Rohm and Haas Co., Philadelphia, PA) prepared in H^+ form. The lactic acid from the acidified broth was then adsorbed on the basic sorbents. The exhausted sorbent was rinsed with water to wash the unbound components of the broth from the bed. The adsorbed lactic acid was eluted from the

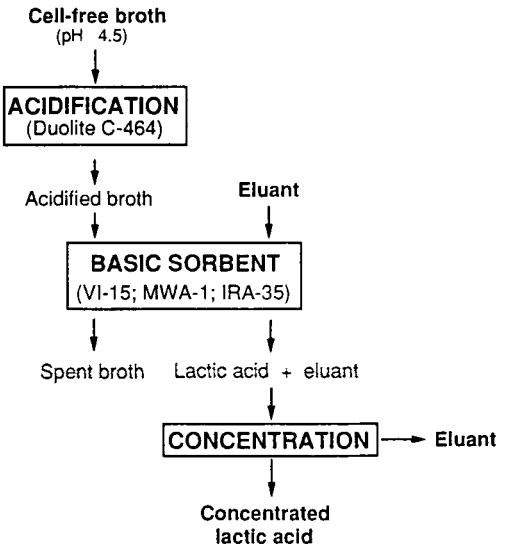

Fig. 1. Scheme for lactic acid recovery and purification.

sorbents by using HPLC-grade methanol (Fisher Scientific) or 5% NH_4OH, depending on the sorbent used.

Broth Acidification

The broth was passed through the Duolite C-464 column in H^+ form at a flow rate of 3 BV/h. Fractions were collected and analyzed for lactic acid and glucose using HPLC. The pH of each fraction was also measured. Fractions containing acidified lactic acid with pH ≤ 3.0 were pooled and set aside for sorption on basic sorbents. The column was regenerated by using sulfuric acid. Several cycles were run to produce enough acidified broth for sorption in the basic sorbents.

Sorption in Weak-Base Sorbents

A jacketed 30 × 1 cm column (Kontes Scientific Instrument, Vineland, NJ) equipped with adjustable plungers was charged with conditioned sorbent to a bed height of at least 10 cm. The air trapped in the column was removed by adding water to the column and stirring the sorbent. The plunger was then lowered to the top of the bed. The acidified broth was introduced to the column upflow at 3 BV/h. The column was rinsed with water to remove the excess broth and other unbound broth components. This was followed by elution of bound lactic acid with methanol or 5% NH_4OH. Fractions were collected and analyzed for lactic acid, glucose, and pH. Methanol was evaporated from the eluate by using a rotary evaporator. Eluants from NH_4OH desorption were not concentrated. The column was regenerated by using 3 BV 4% NaOH and rinsed with 5 BV water.

RESULTS AND DISCUSSION

Broth Acidification

The Duolite C-464 column decreased the pH of the broth to as low as 2.1 (Fig. 2). Fractions with pH ≤ 3 were pooled, producing 0.4 BV of acidified broth. The

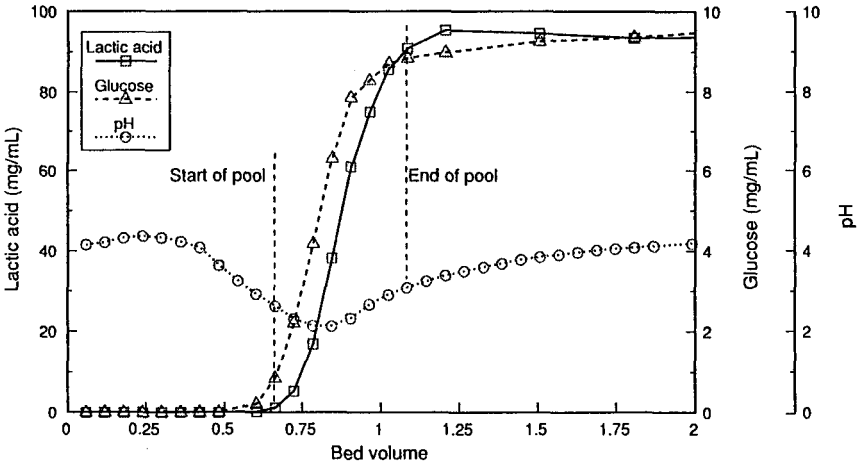

Fig. 2. Effluent profile of model broth on Duolite C-464 column.

acidified broth had a pH of 2.9 and contained 58.2 mg lactic/mL and 6.8 mg glucose/mL. Despite the dilution, the concentration of lactic acid was well above the minimum equilibrium lactic acid concentration (about 10 mg/mL) needed to attain maximum sorption for all three basic sorbents *(15,18)*.

Duolite C-464 also removed colored compounds from the broth, as manifested by the marked color reduction in the broth (from amber to faint yellow). The HPLC chromatograms, however, showed no significant decrease in the number of broth components. The faint yellow color in the pooled acidified broth came from the fractions approaching the cut-off point. The cation exchanger was regenerated back to H^+ form by using sulfuric acid followed by a water rinse.

Recovery of Lactic Acid from Acidified Broth by Using Basic Sorbents

Riedel-de-Haen VI-15

The resin bed required 6 BV of acidified broth to reach saturation (Fig. 3 and Table 2). At this point, the BV increased by 30% owing to the swelling of the resin. No glucose was detected in the effluent after 4 BV of water-rinse effluent. About 55% of the total lactic acid in the column came out with the rinse water and 71 mg lactic acid/mL resin remained adsorbed in the column. In practice, the rinse effluent can be recycled to the adsorption column because the pH is still low—even lower than the pH of the acidified broth. The slightly lower pH in the rinse effluent indicated that, aside from the lactic acid sorbed in the pores, some adsorbed lactic acid was also eluted.

The lactic acid was completely desorbed by using 6.8 BV of methanol. This is about twice the amount of methanol needed to desorb lactic acid from the weaker basic sorbent poly(4-vinylpyridine) *(16)*. The highest lactic acid concentration of the effluent was 31 mg/mL, which would be the expected concentration of lactic acid during desorption in a countercurrent operation. The effluent near the end of the first BV of the methanol eluant was cloudy. Because the micropores are accessible to smaller compounds, but not to lactic acid, the cloudiness may have been caused by salts (like sulfates) or other compounds that are insoluble in methanol. The

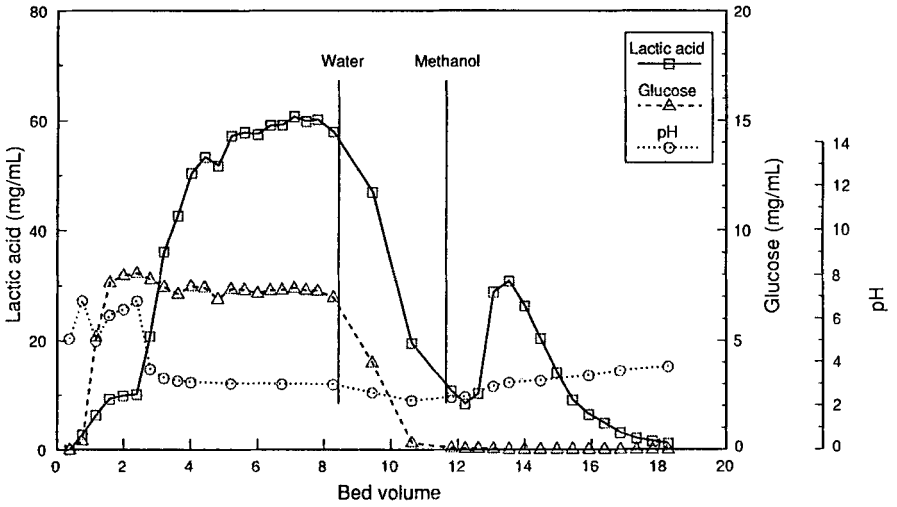

Fig. 3. Effluent profile for VI-15 column.

Table 2
Summary of Lactic Acid Recovery Using Weak-Base Sorbents

Stage of the process	Resin		
	VI-15	MWA-1	IRA-35
Sorption			
Volume of broth to reach saturation, BV	6.0	3.5	3.2
Total lactic acid in the column, mg	1960	2517	1336
Rinse			
Volume when no glucose was detected in the rinse effluent, BV	4	3	3
Fraction of total lactic acid in rinse effluent, %	55	51	48
Lactic acid adsorbed by the resin, mg/mL resin	71	72	75
Desorption			
Methanol			
Adsorbed lactic acid recovered, %	100	64	18
Volume of eluant used, BV	6.8	4.5	5
Highest lactic acid concentration, mg/mL	31	21	5
NH_4OH			
Adsorbed lactic acid recovered, %	nd[a]	100	nd
Volume of eluant, BV	nd	1.5	nd
Highest lactic acid concentration, mg/mL	nd	113	nd

[a]nd, no data.

shrinking of the sorbent as it came in contact with methanol facilitated the exclusion of the liquid from the sorbent, including that in the micropores.

The cloudy fractions contained 29% of the total lactic acid recovered from the column. These fractions were not included in the pool that was subsequently concentrated. The concentrated lactic acid was yellowish, but not turbid. The HPLC chromatogram of the lactic acid product showed that, aside from glucose (0.05%),

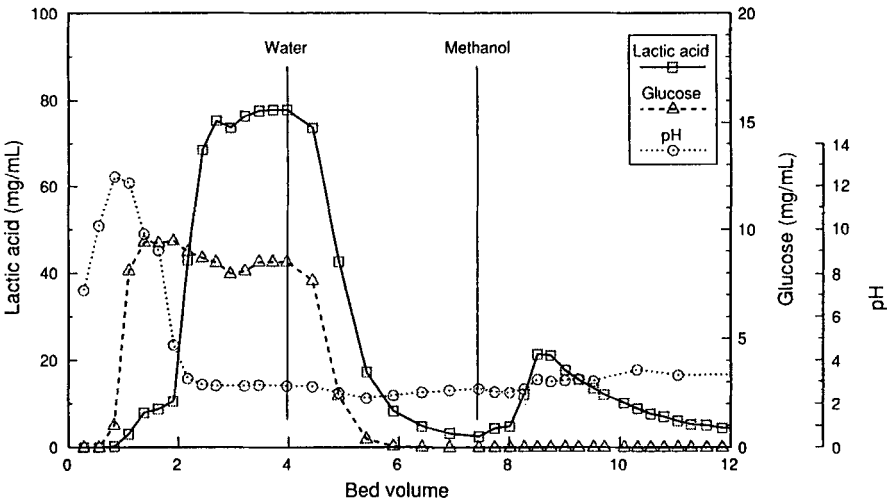

Fig. 4. Effluent profile for MWA-1 column with methanol as desorbent.

small amounts of other impurities were also present. The presence of readily carbonizable residues was verified by the positive result of the sulfuric acid test (formation of brown layer at the interface of sulfuric acid and lactic acid).

The column was regenerated with 1*M* NaOH to remove broth components not desorbed by methanol. The rinse effluent was yellowish, indicating that other compounds were also adsorbed by the resin and could not be eluted readily with methanol. A 30% decrease in column capacity for lactic acid was observed after a 10-cycle run without NaOH rinse. The sorbent, therefore, required periodic regeneration with a strong base to remove these strongly bound broth components and to restore its capacity.

The shrinking of the sorbent during desorption also led to compaction of the sorbent bed, causing a significant flow reduction through the column. The sorbent had to be loosened by stirring the bed and backwashing with water at a high flow rate to prevent compaction of the bed during the next loading cycle. The backwashing step also removed the methanol remaining in the column.

The excessive swelling and shrinking of VI-15 resin would cause attrition of the sorbent and shorten its service life. Swelling of the resin also increases the internal void volume, thus resulting in increased sorption of other broth components because of pore filling. These impurities, if not removed during the rinse step, may be trapped inside or may elute slowly with lactic acid as the sorbent shrinks during desorption with methanol.

Dowex MWA-1

The resin reached saturation after 3.5 BV of acidified broth (Fig. 4 and Table 2), which was about half the volume needed to saturate the VI-15 column. The shorter saturation time for MWA-1 was a combined effect of the matrix structure and the basicity of the functional group. The macroporous structure in MWA-1 allows for a much faster diffusion of lactic acid into the sorbent than in the microporous structure of VI-15. MWA-1 also becomes saturated at a much lower equilibrium concentration because of its higher basicity (pK_a) and, consequently, higher affinity

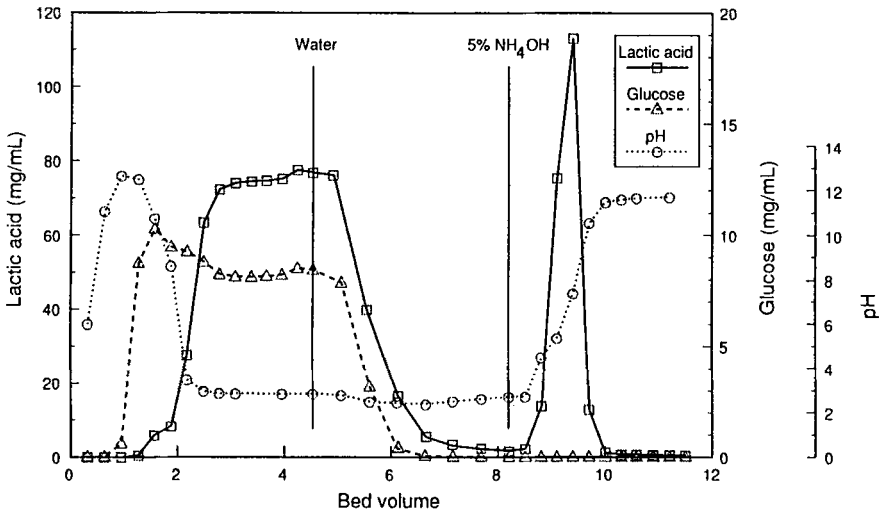

Fig. 5. Effluent profile fore MWA-1 column with NH_4OH as desorbent.

(K) for lactic acid than that of VI-15 (Table 1). The rinse effluent was free of glucose after 3 BV, 1 BV less than was needed by VI-15. Fifty-one percent of the total lactic acid sorbed in the column came out with the rinse water and 72 mg lactic acid/mL resin remained adsorbed by the resin. Methanol was not effective in desorbing lactic acid from the MWA-1 column despite the higher temperature (50°C) used. Only 64% of the adsorbed lactic acid was recovered after 4.5 BV. The highest concentration of lactic acid in the eluate was only 21 mg/mL. The cloudy fractions, which contained 12% of the total lactic acid recovered, were not included in the pool that was concentrated. The concentrated lactic acid was cloudy and yellowish in color. The HPLC chromatogram of the product showed that no glucose was present, but other broth components were detected. The sulfuric acid test for readily carbonizable residues also came out positive.

When 5% NH_4OH was used as desorbent, 100% recovery was achieved in 1.5 BV of eluant (Fig. 5 and Table 2). The highest concentration of lactic acid in the effluent was 113 mg/mL, about 50% greater than the lactic acid concentration in the feed. However, NH_4OH also desorbed other broth components adsorbed by the resin, resulting in a product with more impurities than the one desorbed with methanol. The sulfuric acid test for readily carbonizable residues also turned out positive, despite the absence of glucose.

Amberlite IRA-35

The IRA-35 column required the same amounts of acidified broth (3.2 BV) to reach saturation and the same amount of rinse water to get rid of glucose as in MWA-1 (Fig. 6 and Table 2). About 48% of the lactic acid in the column was washed out with the rinse water, and 75 mg lactic acid/mL resin remained adsorbed by the sorbent. Methanol eluted only 18% of the adsorbed lactic acid after 5 BV. The maximum lactic acid concentration in the effluent was 5 mg/mL. The HPLC chromatogram of the eluted lactic acid revealed that no glucose was present, but other broth components were also eluted. The sulfuric acid test for readily carbonizable residue was also positive despite the dilute concentration of lactic acid used in the test.

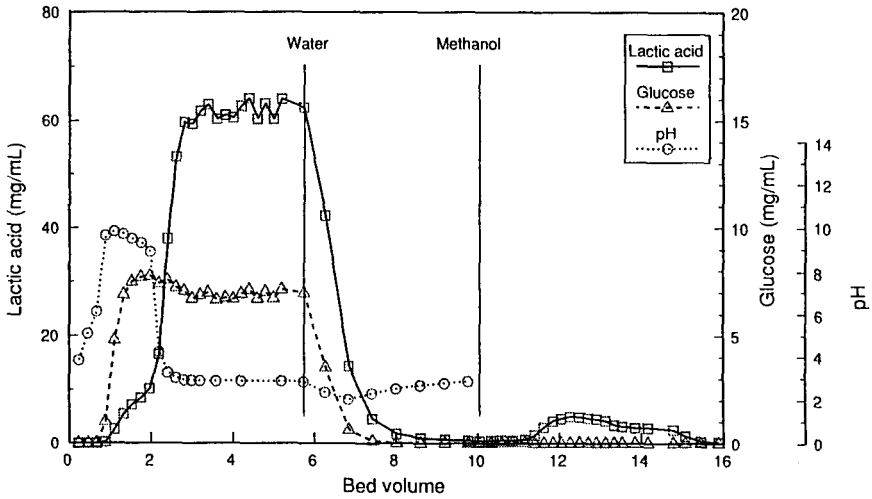

Fig. 6. Effluent profile for IRA-35 column with methanol as desorbent.

The much lower recovery of lactic acid from IRA-35 by methanol was not surprising since IRA-35 has a higher pK_a and higher association constant (K) for lactic acid than MWA-1 (Table 1). This disparity in their basicity is owing to the difference in the environment around tertiary amine groups in each sorbent. The styrene ring and the quaternary ammonium group in MWA-1 have a base-weakening effect on the amine group. In contrast, the aliphatic backbone and the presence of carboxylates in IRA-35 increase the basicity of the amine group *(15)*.

CONCLUSIONS

Duolite C-464 was able to lower the broth pH to an acceptable level, but it generated only 0.4 BV of acidified broth. The amount of acidified broth may be increased by using a strong cation exchanger. This method of broth acidification, however, still produces waste salt during regeneration of sorbent back to H^+ form.

Among the three basic sorbents employed in this study, VI-15 was the most attractive sorbent for lactic acid recovery. Its working capacity compared very well with that of the tertiary amines, and the adsorbed lactic acid can be easily eluted by using methanol. A more physically stable resin, however, is desirable to avoid problems related to excessive swelling and shrinking.

The recovery scheme employed was not sufficient to produce heat-stable lactic acid. Other broth components were also adsorbed by the basic sorbents and could not be removed during the rinse step, but eluted with lactic acid during the desorption step. Therefore, broth pretreatment or polishing steps would be necessary to improve the purity of the product.

ACKNOWLEDGMENTS

The authors thank Rod R. Fisher of Cargill, Inc., for his helpful comments and suggestions. This project was supported by the Iowa Corn Promotion Board and is a contribution of the research program of the ISU Center for Crops Utiliza-

tion Research. This is journal paper No. J-16374 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, Project 3017.

REFERENCES

1. HRA, Inc. (1991), *Lactic Acid and Its Derivatives: A Global Review.* HRA, Inc., Prairie Village, KS.
2. Lipinsky, E. S. and Sinclair, R. G. (1986), *Chem. Eng. Prog.* **82,** 26–32.
3. Yasuda, S., Niwa, T., and Kurohara, T. (1984), Japanese Patent 59-118731.
4. Kiel, K. H., Greiner, U., Engelhardt, F., Kuhlein, K., Heb, G., Reinhold, K., and Schlingmann, M. (1985), German Patent DE 3328093.
5. Obara, H. (1988), Japanese Patent 63188632.
6. Collin, H. and Buresch, E. (1990), European Patent 0377430.
7. Kulprathipanja, S. and Oroskar, A. R. (1991), US Patent 5,068,418.
8. Rossiter, G. J. (1991), in *Preparative and Process-Scale Liquid Chromatography,* G. Subramanian, G., ed., Ellis Horwood, West Sussex, UK.
9. Ernst, E. E. and McQuigg, D. W. (1992), Paper presented at the AIChE Annual National Meeting, November, Miami Beach, FL.
10. King, C. J. and Tung, L. (1992), US Patent 5,132,456.
11. Yates, R. A. (1981), US Patent 4,282,323.
12. Srivastava, A., Roychoudhury, P. K., and Sahai, V. (1992), *Biotechnol. Bioeng.* **39,** 607–613.
13. Tsao, G. T., Lee, S. J., Tsai, G., Seo, J., McQuigg, D. W., Vorhies, S., and Iyer, G. (1993), International Patent Appl. PCT/US92/07738.
14. Vickroy, T. B. (1985), in *Comprehensive Biotechnology: The Principles, Applications and Regulations of Biotechnology in Industry, Agriculture and Medicine,* Moo-Young, M., ed., Pergamon, New York.
15. Tung, L. (1993), Report No. LBL-34669, Lawrence Berkeley Laboratory, University of California, Berkely, CA.
16. Kabawata, N., Yoshida, J., and Tanigawa, Y. (1981), *Ind. Eng. Chem. Prod. Res. Dev.* **20,** 386–390.
17. Frierman, M., Kuo, Y., Joshi, D., Garcia, A. A., and King, C. J. (1987), *Sep. Purif. Methods.* **16,** 91–102.
18. Evangelista, R. L., Nikolov, Z. L., and Mangold, A. J. (1994), *Appl. Biochem. Biotech.* **45/46,** 131–144.
19. Weast, R. C. (1987), *Handbook of Chemistry and Physics,* CRC, Boca Raton, FL, p. D-99.
20. Kuo, Y., Munson, C. L., Rixey, W. G., Garcia, A. A., Frierman, M., and King, C. J., (1987), *Sep. Purif. Methods.* **16,** 31–64.
21. Rohm and Haas (1988), *Rohm and Haas Ion Exchange Resin Laboratory Guide,* Philadelphia, PA.