Purification of Lactic Acid from Fermentation Broths by Ion-Exchange Resins

ARTICLE *in* INDUSTRIAL & ENGINEERING CHEMISTRY RESEARCH · APRIL 2006

Impact Factor: 2.59 · DOI: 10.1021/ie051263a

4 AUTHORS, INCLUDING:

Silvia Álvarez
Universitat Politècnica de València
60 PUBLICATIONS **890** CITATIONS

Francisco Riera
University of Oviedo
72 PUBLICATIONS **887** CITATIONS

Purification of Lactic Acid from Fermentation Broths by Ion-Exchange Resins

M. Isabel González,† Silvia Álvarez,‡ Francisco A. Riera,*,† and Ricardo Álvarez†

Department of Chemical Engineering and Environmental Technology, University of Oviedo, Julián Clavería, 8, 33006 Oviedo, Spain, and Department of Chemical and Nuclear Engineering, Polytechnic University of Valencia, Camino de Vera s/n, 46022 Valencia, Spain

In this work, a novel process for lactic acid purification based on ion exchange is proposed. It consists of two stages: first, a strong cation resin was used to reduce the broth pH below the pK_a of lactic acid (3.86) and remove the cations present in the solution; then lactic acid was separated from the other anions in the broth using a weak anion exchanger. The ion exchangers selected were Lewatit S2568H and Lewatit S3428 (Bayer, Germany). The Lewatit S2568H resin retained the cations in the broth with an apparent capacity of 1.7 equiv/L, reducing the broth pH below 3.86. It was able to decrease the pH of the broth to as low as 1.5, generating 4 bed volumes (BV) of acidified and partial demineralized broth. In the second step, the acidified broth was purified by the Lewatit S3428 resin, which retained the other anions present in the broth, producing an aqueous solution of lactic acid. This resin was able to produce 25 BV of purified lactic acid with selectivity higher than 99.9%. The final purity of lactic acid was higher than 99%.

1. Introduction

Lactic acid is a versatile chemical used in the food and chemical industries. It may be manufactured either by chemical synthesis or by carbohydrate fermentation. The conventional process for fermentative production of lactic acid is a discontinuous process with low productivity and high capital and operating costs. Therefore, alternatives to this manufacturing process are being studied.[1] To reduce these costs, many studies on lactic acid separation have been conducted using different separation techniques, such as reactive extraction, membrane technology, ion exchange, electrodialysis, and distillation.[2−7] The recovery of lactic acid is rather difficult due to its chemical behavior, as it shows strong affinity to water and low volatility. Therefore its purification is the most cost-intensive processing step.

Ion exchange is widely used in bioseparations, and different processes for lactic acid recovery based on ion exchange have been reported. Kulprathipanja patented a process for lactic acid recovery from a fermentation broth using anionic polymeric adsorbents;[3] adsorbing lactic acid below its pK_a, Evangelista and Nikolov recovered lactic acid from fermentation broth using weak base anion exchangers (MWA-1, IRA-35, VI-15).[8] The pH was maintained below lactic acid pK_a for its adsorption, with the fermentation broth being acidified using a cation-exchange resin. Methanol and 5% NaOH were employed as eluants. Using 1.5 times the bed volume (BV) of 5% NaOH, all the lactic acid adsorbed on a MWA-1 column could be recovered; but the purity of the product was not high. However, using 6.8 times the bed volume of methanol, lactic acid was completely desorbed from a VI-15 anion-exchange resin with higher purity. Srivastava et al. studied extractive lactic acid fermentation using an ion-exchange resin.[9] This study focused on improving the fermentation yield; the separation performance of the anionic resin (Amberlite IRA-400, Rohm and Haas, USA) was not considered. Ye et al. also proposed a process for lactic acid production combining a membrane bioreactor and membrane filtration.[10] Lactate was recovered from clarified fermentation broth using an anion exchanger (Amberlite IRA-400). The operating procedure was similar to that described by Srivastava et al.,[9] as they used the same ion exchanger. Lactic acid was eluted from the column with hydrochloric acid. Vaccari et al. recovered lactic acid from clarified fermentation broth using an anionic resin (Amberlite IRA-420, Rohm and Haas, USA).[11,12] This resin retained lactic acid and other inorganic anions present as nutrients in the broth. Once the column was saturated with lactic acid, the column was washed with water and the lactic acid was recovered from the resin as its ammonium salt using 5% ammonium carbonate. At the same time, the resin was regenerated back to the carbonate form. The eluate from the anionic resin contained ammonium lactate, which was treated with a strong cation-exchange resin (Amberlite IR-120, Rohm and Haas, USA), yielding a lactic acid solution that was subsequently concentrated. Cao et al. studied lactic acid recovery using a strongly basic ion exchanger (Amberlite IRA-400).[13] They worked with lactic acid solutions and fermentation broths at different pH values (2 and 5). Lactic acid was retained in the column, and various eluants were used.

The present work, in contrast to what occurs in the above references, does not focus on the retention of every anion present in the broth and the selection of the eluant that gives the highest lactate recovery and purity. What is proposed is an ion-exchange process that makes it possible to remove undesired ions from the fermentation broth, while lactic acid remains in solution. In the proposed process, no chemical reagent is added and lactic acid is obtained directly in the effluent in aqueous solution with a high purity. It can then be concentrated to obtain commercial food grade lactic acid (50 wt %). Other desalination technologies such as nanofiltration and reverse osmosis were previously studied, but low selectivity for lactate over other ions present in the broth was obtained. Another technique that could be used to separate the lactic acid is electrodialysis with bipolar membranes. This technology could be useful to remove anions and cations simultaneously from a solution of nondissociated lactic acid. Electrodialysis with bipolar membranes is being investigated by the authors of this work to produce salicylic acid. However, fouling of the membranes was observed to be

* To whom correspondence should be addressed. Tel.: +34985103436. Fax: +3498510 3434. E-mail: far@uniovi.es.
† University of Oviedo.
‡ Polytechnic University of Valencia.

10.1021/ie051263a CCC: $33.50
Published on Web 03/24/2006

an important drawback. Moreover, energy consumption was observed to be very high.

2. Materials and Methods

2.1. Feed Preparation. Two types of experiments were conducted. The first set of experiments was performed with artificial mixtures of lactic acid, hydrochloric acid, and orthophosphoric acid at different pH values, adjusted with sodium hydroxide. The solutions were prepared with 80% lactic acid (Purac, Spain), 37% hydrochloric acid (Panreac, Spain), 85% orthophosphoric acid (Panreac), and 40% sodium hydroxide (Panreac). Second, the fermentation broth was treated under the selected conditions. The fermentation broth was prepared by fermentation of sweet whey ultrafiltration permeate supplemented with 2.5 g/L yeast extract at 42 °C and pH 5.6. To maintain the pH at the required value, a solution of 40% (w/v) sodium hydroxide was used. The microorganism used was *Lactobacillus helveticus* (Rhodia, France). The fermentation broth was then clarified by ultrafiltration using an inorganic membrane with a molecular weight cutoff (MWCO) of 15 kDa (KERASEP 482/1T Model, Orelis, France).

2.2. Ion Exchangers. The anion exchanger selected was Lewatit S3428 (Bayer, Germany). This is a weakly basic macroporous anion-exchange resin based on polystyrene, which contains only tertiary amino groups. It has a high total capacity and good chemical stability. The cation exchanger selected was Lewatit S2568H (Bayer, Germany). This is a strongly acidic macroporous cation-exchange resin with beads of uniform size based on a styrene−divinylbenzene copolymer. Both resins comply with the current legislation on food and food-contact uses.

2.3. Operating Procedure. The experiments were performed in a jacketed glass column of 1.5 cm diameter and 25 cm length, where 25 g of resin was placed. The free space at the top of the column allowed the expansion of the resin. The column temperature was adjusted using a thermostatic system.

The feed was pumped by a peristaltic pump (Masterflex 133-7554-95, Cole Parmer Instrument Co., USA) from the bottom to the top of the column. The effluent was collected in fractions of 18 mL. The pH and conductivity of each sample were measured. The experiment was concluded when the processed volume was high enough to saturate the column.

Countercurrent regeneration was chosen to reduce the consumption of reagents. Backwashing, regeneration, and rinsing stages were conducted from top to bottom of the column. Before regeneration, the column was backwashed with 2 bed volumes (BV) of distilled water. The regeneration of the cation exchanger was carried out with 2.5 BV of 1 M HCl at 5 m/h and room temperature. The anion exchanger was regenerated with 6 BV of 0.75 M NaOH under the same operating conditions. After regeneration, the column was rinsed with distilled water at 5 m/h and room temperature. The correspondence between flow rate (BV/h) and velocity (m/s) depended on the resin used. For the anion exchanger Lewatit S3428, 1 m/s was approximately equal to 6 BV/h, and for the cation exchanger Lewatit S2568H it was equal to 5.3 BV/h.

The experimental work was carried out in three steps:

1. Artificial mixtures of lactate and the main anions present in the broth (phosphate and chloride) were treated with the anion exchanger Lewatit S3428 to determine the selectivity of this anion exchanger for the separation of lactic acid from undesired anions present in the broth. Thus the viability of the process was tested. The effect of temperature and flow rate on the separation was also studied. The experiments were conducted at two different flow rates (14 and 36 BV/h) and temperatures (25 and 40 °C).

2. The fermentation broth clarified by ultrafiltration was demineralized by the cation exchanger Lewatit S2568H to eliminate the cations present in the broth and convert the lactate anion into lactic acid. The broth pH was reduced as well. The effect of operating parameters, such as temperature and flow rate, on the separation was also studied. The experiments were performed at three different flow rates (13, 24, and 33 BV/h) and two different temperatures (25 and 40 °C).

3. The effluent from the cation-exchanger column was then passed through the anion exchanger Lewatit S3428 under the operating conditions selected in the experiments carried out with artificial mixtures.

Table 1. Ionic Composition of the Clarified Fermentation Broth

component	concn, g/L	concn, mequiv/L
sodium	12.07	524.68
potassium	2.22	56.77
magnesium	0.17	7.08
calcium	0.49	24.75
lactate	55.55	624.18
chloride	2.90	81.77
phosphate	1.78	56.23

2.4. Analytical Methods. The amount of sodium, potassium, calcium, and magnesium was measured by atomic absorption spectroscopy (PU9200X spectrophotometer, Philips, The Netherlands). The concentration of lactic acid/lactate was determined by ionic chromatography (Dionex-120, Dionex, USA) and using an enzymatic method (Test 1112821, Boehringer Mannheim, Germany, ISO 8069:2005). Chloride was analyzed by potentiometric titration (Crison Compact Titrator, Crison Instruments S.A., Spain). Phosphate content was analyzed by determination of total phosphorus using a colorimetric method (AOAC-986.249).

2.5. Expression of Results. Breakthrough curves represent the concentration in the effluent as a function of the volume of treated solution expressed in bed volumes. The volume of treated solution was calculated as the sum of the volume fractions collected at the outlet of the column. The apparent capacity was calculated by integration of the breakthrough curves using the DPLOT Version 1.2 computer program (HydeSoft Computing, USA).

3. Results and Discussion

The clarified fermentation broth used as feed in this work was mainly composed of lactate and inorganic salts. The main cations were sodium, magnesium, calcium, and potassium. Other anions present in the broth were basically phosphate and chloride. By means of ultrafiltration proteins and lipids were entirely removed, while the amount of lactose and nonprotein nitrogen (NPN) in the clarified fermentation broth was about 0.02% and 0.05%, respectively. It had a total solids content of 77.75 g/L and a density of 1035.6 g/L. The ionic composition of the ultrafiltered fermentation broth used as feed is shown in Table 1.

In the first part of the work, the affinity of the weak anion exchanger for the main acids in the solution was studied. Figure 1 shows the breakthrough curves when an artificial mixture of the three acids was treated. The concentration of the three acids in the feed solution was the same (0.26 equiv/L each acid). It can be seen that the affinity of the resin for the three acids follows the order chloride > phosphate > lactate. As the resin reached saturation, lactate and phosphate were displaced by chloride. These results show that it can be possible to separate

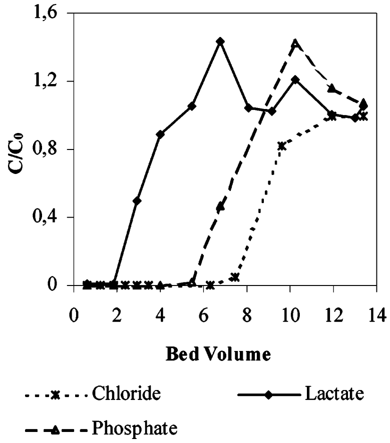

Figure 1. Breakthrough curves for the Lewatit S3428 column when an artificial mixture of lactic, phosphoric, and hydrochloric acids at the same concentration (0.26 equiv/L each) was treated at 25 °C, flow rate 14 BV/h, and pH 1.07 (C, effluent concentration; C_0, feed concentration).

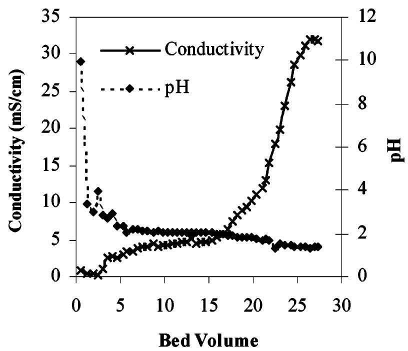

Figure 2. Conductivity and pH of the effluent when a mixture of lactic, phosphoric, and hydrochloric acids at the concentration of the fermentation broth and pH 1.2 was treated with the resin Lewatit S3428 (temperature, 25 °C; flow rate, 14 BV/h).

the three main anions in the broth by means of the Lewatit S3428 resin.

Separation depends on operating conditions. When weak base ion exchangers are used, pH plays an important role. When pH values are greater than the ion exchanger pK_a, the resin is un-ionized and it does not accept anions.[14,15] Mixtures of lactic acid, phosphoric acid, and chloride at the same concentration as the fermentation broth were treated by Lewatit S3428. The experiments were carried out at two different pH values: (a) the original pH of the mixture (pH 1.2); (b) pH adjusted to the pH of the fermentation broth (pH 5.6) by addition of sodium hydroxide.

Figures 2 and 3 show the evolution of pH and conductivity during the experiments. Several differences can be observed between these two figures. In the first case (acid mixture), the pH dropped quickly from a pH value of 10, whereas the conductivity increased slightly at the beginning, and then very rapidly when the bed volume was around 20. In the second case, the conductivity reached soon the feed value (8 BV).

In Figures 4 and 5, the concentrations of lactate, phosphate, and chloride are plotted against the treated volume for both pH values. The ion exchanger presented good selectivity for phosphate and chloride over lactate at the most acidic pH value. However, at the pH of the fermentation broth (pH 5.6), the ion exchanger presented no selectivity for those anions and had a very low retention capacity. It is obvious that acidic conditions must be considered for the separation of phosphate and chloride from lactate with the weak base anion exchanger Lewatit S3428.

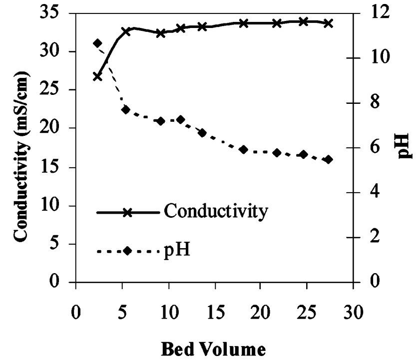

Figure 3. Conductivity and pH of the effluent when a mixture of lactic, phosphoric, and hydrochloric acids at the concentration of the fermentation broth and pH 5.6 was treated with the resin Lewatit S3428 (temperature, 25 °C; flow rate, 14 BV/h).

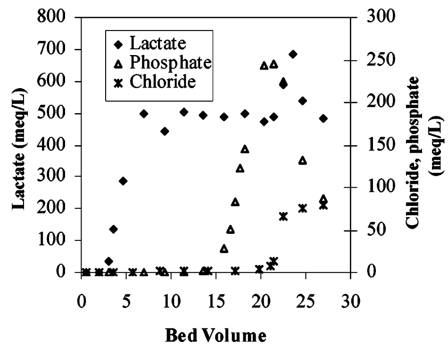

Figure 4. Concentration profile in the effluent when a mixture of lactic, phosphoric, and hydrochloric acids at the concentration of the fermentation broth and pH 1.2 was treated with the resin Lewatit S3428 (temperature, 25 °C; flow rate, 14 BV/h).

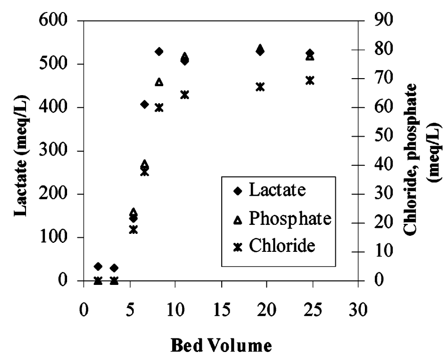

Figure 5. Concentration profile in the effluent when a mixture of lactic, phosphoric, and hydrochloric acids at the concentration of the fermentation broth and pH 5.6 was treated with the resin Lewatit S3428 (temperature, 25 °C; flow rate, 14 BV/h).

The effect of temperature on the ion-exchange process was also studied. The experiments were performed at two different temperatures: at room temperature (25 °C) and at the temperature at which the fermentation broth is recovered after fermentation and ultrafiltration steps, i.e., 40 °C. As can be seen in Figure 6, the selectivity at 40 °C was slightly higher than the selectivity of the anion exchanger at 25 °C. At this temperature, lactate concentration in the effluent soon reached the feed concentration and a higher volume was treated before chloride appeared in the effluent. The phosphate breakthrough curve was not significantly affected by temperature.

Additionally, the effect of flow rate on the process was studied. The experiments were conducted at two different flow rates: 14 and 36 BV/h. Figure 7 shows that the increase in the flow rate resulted in more spread out breakthrough curves.

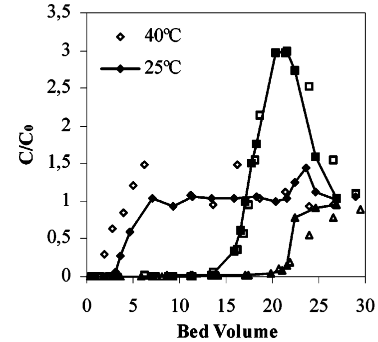

Figure 6. Breakthrough curves for the Lewatit S3428 column at different operating temperatures, flow rate 14 BV/h, and feed solution pH 1.2 (◇, lactate; □, phosphate; △, chloride).

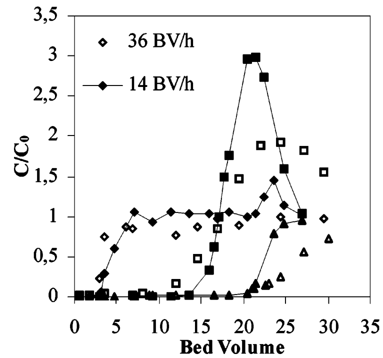

Figure 7. Breakthrough curves for the Lewatit S3428 column at different feed flow rates, 25 °C, and feed solution pH 1.2 (◇, lactate; □, phosphate; △, chloride).

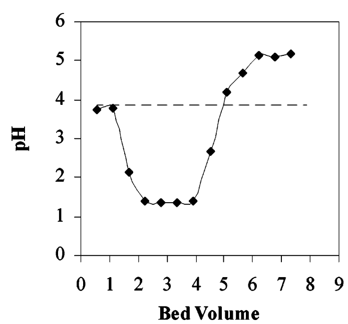

Figure 8. Evolution of pH of the clarified fermentation broth treated with the resin Lewatit S2568H at 25 °C and flow rate 13 BV/h.

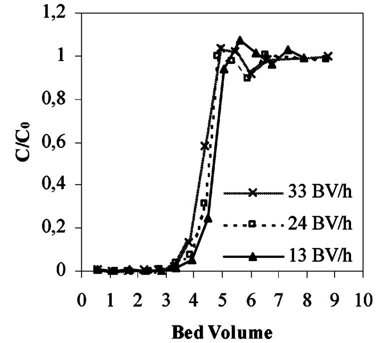

Figure 9. Cation breakthrough curves for the Lewatit S2568H column when the clarified fermentation broth was treated at different feed flow rates and 25 °C.

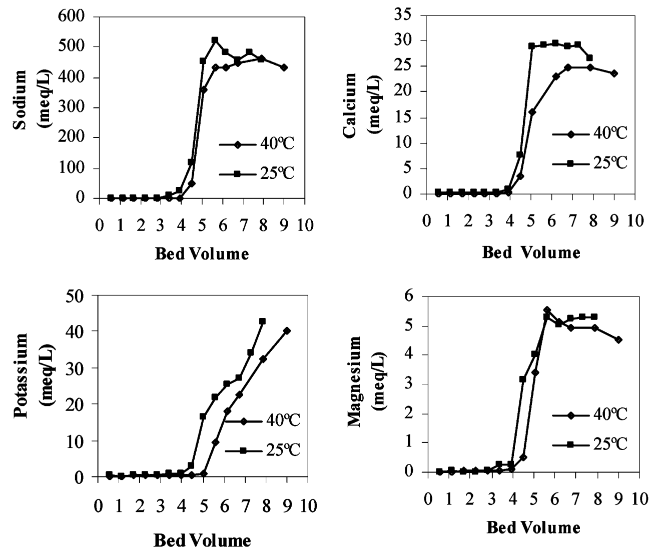

Figure 10. Breakthrough curves of the main cations in the clarified fermentation broth treated with the resin Lewatit S2568H at different operating temperatures and flow rate 13 BV/h.

Therefore, high flow rates are not recommended as selectivity decreases and lactate losses increase with this variable.

In the next step of this work, the clarified fermentation broth was passed through the Lewatit S2568H resin in order to (a) acidify the clarified fermentation broth to convert lactate into undissociated lactic acid and to obtain the optimum conditions for anion separation using the resin Lewatit S3428, as discussed above, and (b) purify the lactic acid by removing from the broth cations such as sodium, potassium, magnesium, and calcium.

Similar uses of cation exchangers have been reported in recent years. Lian and Kiang proposed the conversion of sodium dichromate to chromic acid by means of a strong acid cation exchanger (Ambersep 132, Rohm and Haas Co., USA).[16] Evangelista and Nikolov used the cationic resin Duolite C-464 (Rohm and Haas Co., USA) in hydrogen form to acidify a fermentation broth to pH values lower than 3, obtaining 0.5 BV of acidified broth.[8]

The resin Lewatit S2568H was able to decrease the pH of the broth to as low as 1.5 (Figure 8). Moreover, it produced 4 BV of demineralized broth (Figure 9) at a pH value lower than 3.86 (lactic acid pK_a). The apparent capacity was 1.7 equiv/L.

The experiments were carried out at three different flow rates: 33, 24, and 13 BV/h. Retention was improved by decreasing the flow rate, as shown in Figure 9, though differences were not relevant.

Another important operating parameter in an ion-exchange process is the temperature. The behavior of the cation resin bed at two different temperatures was studied as well.

In Figure 10, the breakthrough curves of the main cations present in the broth are plotted. It was observed that at lower temperatures some components (calcium and sodium) were desorbed from the column when it was close to saturation. The same effect was observed for magnesium at both temperatures. Nevertheless, operating at either 25 or 40 °C resulted in 4 BV of acidified and cation-free broth.

The fractions obtained when 2−4 BV was passed through the column had a sodium content less than 100 ppm. These fractions were collected and fed to the anion exchanger. The sodium content of the sample was found to be 30 ppm. The amount of the other cations was negligible. The composition of the solution that was fed to the anion column is shown in Table 2.

The effluent collected from the cation-exchanger column was treated with the Lewatit S3428 resin at a feed flow rate of 14 BV/h and 25 °C. The results obtained are shown in Figure 11.

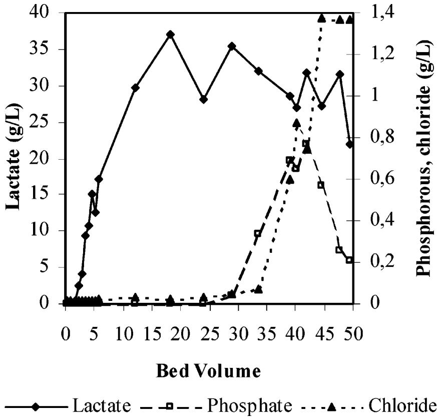

Figure 11. Concentration profile for the Lewatit S3428 column when demineralized fermentation broth was treated at 25 °C and feed flow rate 14 BV/h.

Table 2. Characteristics of the Solution Fed to the Anion Exchanger

feed	value
pH	1.81
conductivity (mS/cm)	14.3
lactic acid (g/L)	32.7
phosphorus (g/L)	0.21
chloride (g/L)	1.37

Lactate was barely retained by the anion exchanger, while phosphate and chloride were highly retained. Thus 25 BV of purified lactic acid was obtained with good selectivity (greater than 99.9%).

The volume of the ion exchanger needed in this process was much less than those previously reported.[8] Moreover, no inorganic or organic solvent was added along with the lactic acid solution, obtaining a dilute lactic acid solution with high purity (higher than 99% on a dry basis) that could be easily concentrated by vacuum evaporation to obtain food grade lactic acid. The remaining 1% of the purified solution was mainly composed of residual lactose and NPN (about 0.9%) and inorganic salts not retained by the resin (about 0.1%). Only a small amount of lactate was retained by the resin at the beginning of the process. The dilution due to the water used to rinse the resin after the regeneration step (dead volume) has to be taken into account as well. Lactate losses were estimated to be less than 15%.

However, the main drawback of ion-exchange processes is the need to use chemicals to regenerate the resins. After the regeneration process, highly acidic and basic solutions are obtained, which should be treated. In this work the cation exchanger was regenerated with 2.5 BV of 1 M HCl and the anion exchanger was regenerated with 6 BV of 0.75 M NaOH. After the regeneration, the solutions will also contain sodium, potassium, magnesium, and calcium phosphates and chlorides. The posttreatment of these solutions was not studied in this work. However, they could be mixed in order to be neutralized and/or they could be further concentrated by means of different techniques or the salts could be precipitated.

4. Conclusions

Ion exchange is an adequate separation method for the acidification and purification of lactic acid from fermentation broths. The Lewatit S3428 resin showed greater affinity for phosphate and chloride anions than for lactate at acidic pH values. The Lewatit S2568H resin was able to remove the main cations in the broth, as well as to acidify the broth producing lactic acid in undissociated form and also creating the adequate operating conditions for the second purification step. It generated 4 BV of acidified broth, which was fed to the anion exchanger. The Lewatit S3428 resin was able to remove the undesired anions present as nutrients in the fermentation broth with high selectivity (greater than 99.9%). Moreover, this resin was able to produce 25 BV of purified lactic acid. The proposed purification process is simple and feasible for the dairy industry to produce food grade lactic acid. The final purity of the lactic acid obtained was greater than 99%, and no chemical additive was necessary.

Acknowledgment

This research work was supported by the dairy industry ILAS S.A. (Reny Picot) and the Spanish Ministry of Science and Technology.

Literature Cited

(1) Saha, B. C.; Woodward, J. *Fuels and chemicals from biomass*; ACS Symposium Series 666; American Chemical Society: Washington, DC, 1997.

(2) Datta, R. Recovery and purification of lactate salts from whole fermentation broth by electrodialysis. U.S. Patent 4,885,257, 1989.

(3) Kulprathipanja, S. Separation of lactic acid from fermentation broth with an anionic polymeric absorbent. U.S. Patent 5,068,418, 1991.

(4) Van Nispen, J. G. M.; Jonker, R. Process for the fermentative preparation of organic acids. U.S. Patent 5,002,881, 1999.

(5) Russo, L. J.; Kim, H. Membrane-based process for the recovery of lactic acid by fermentation of carbohydrate substrates containing sugars. U.S. Patent 5,503,750, 1996.

(6) Baniel, A. M.; Eyal, A. M.; Mizarahi, J.; Hazan, B.; Fisher, R. Lactic Acid Production, Separation and Recovery Process. U.S. Patent 6,187,951, 2001.

(7) Eyal, A. M.; Starr, J. N.; Fisher, R.; Hazan, B.; Canari, R.; Witze, D. R.; Gruber, P. R.; Kolstad, J. J. Lactic acid processing methods arrangements and products. U.S. Patent 6,229,046, 2001.

(8) Evangelista, R. L.; Nikolov, Z. L. Recovery and purification of lactic acid from fermentation broth by adsorption. *Appl. Biochem. Biotechnol.* **1996**, *57*, 471.

(9) Srivastava, A.; Roychoudhury, K.; Sahai, V. Extractive lactic acid fermentation using ion exchange resin. *Biotechnol. Bioeng.* **1992**, *39*, 607.

(10) Ye, K.; Jin, S.; Shimizu, K. Cell recycle and broth reuse fermentation with cross-flow filtration and ion exchange resin. *J. Chem. Technol. Biotechnol.* **1996**, *66*, 223.

(11) Vaccari, G.; González-Vara, A.; Campi, A. L.; Dosi, E.; Brigidi, P. Fermentative production of L-Lactic acid by Lactobacillus casei DSM 20 011 and product recovery using ion exchange resins. *Appl. Microbiol. Biotechnol.* **1993**, *40*, 23.

(12) Vaccari, G.; Mantovani, G.; Campi, A. L. Process for the production of purified lactic acid aqueous solutions starting from fermentation broths. U.S. Patent 5,210,294, 1993.

(13) Cao, X.; Yun, H. S.; Koo, Y. Recovery of L(+)-lactic acid by anion exchange resin Amberlite IRA-400. *Biochem. Eng. J.* **2000**, *11*, 189.

(14) Calmon, C.; Kressman, T. R. E. *Ion Exchangers in Organic and Biochemistry*; Interscience Publishers: New York, 1957.

(15) Helffreich, F. *Ion Exchange*; McGraw-Hill: New York, 1962.

(16) Lian, S. H.; Kiang, C. D. Chromic acid recovery from waste acid solution by an ion exchange process: equilibrium and column ion exchange modeling. *Chem. Eng. J.* **2000**, *4107*, 1.

Received for review November 15, 2005
Revised manuscript received February 15, 2006
Accepted February 28, 2006

IE051263A