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Improved lactic acid productivity by simultaneous recovery during fermentation using resin exchanger

Tanapawarin Rampai[1], Sitanan Thitiprasert[2], Wasinee Boonkong[2], Kentaro Kodama[2], Vasana Tolieng[2], Nuttha Thongchul[2]

[1]Program in Biotechnology, Faculty of Science, Chulalongkorn University, Phayathai Road, Pathumwan, Bangkok 10330 Thailand
[2] Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, Phayathai Raod, Pathumwan, Bangkok 10330 Thailand
[*]Corresponding author:nuttha.T@chula.ac.th

Abstract

Lactic acid is a versatile organic acid that can be used in various applications. One of the promising applications of lactic acid is in bioplastic industry. Lactic acid is used as the monomer building block in polylactic acid synthesis. Unlike the existing applications in food and pharmaceutical industries in which lactic acid is used as the additive, it becomes the major raw material in polylactic acid production. This, therefore, raises the demand of lactic acid and eventually increases the market price. In order to expedite the bioplastic industry, cost competitiveness to the existing plastic is a big concern. The cost effectiveness and process robustness are the key success factors in bioplastic industry. Currently, lactic acid is produced via bacterial fermentation using the lime-based process. After fermentation, lactic acid was recovered from the fermentation broth by various techniques including solvent extraction, reactive distillation, ion exchanger, and electrodialysis. To achieve low production cost, not only high fermentation rate is necessary, effective recovery process is also required. In this study, we attempted simultaneous recovery of lactic acid coupled with fermentation to drive the productivity and long-term operation in continuous culture. Anion exchange resin, Amberlite IRA-400, was selected for recovering lactic acid from the bacterial culture. The preliminary results in batch adsorption showed that among other resins studied, Amberlite IRA-400 provided the best separation efficiency. Further study on fixed bed adsorption and simultaneous fermentation and lactic acid recovery using this resin were conducted and the results will be discussed in this presentation.

Keywords : *Lactic acid, Anion exchange, Fermentation, Recovery*

1. Introduction

Lactic acid has long been widely used in food, pharmaceutical, cosmetic, and chemical industries. This acid is currently attracting a great deal of research and development in an emerging application for the production of polylactic acid, a renewable, biodegradable, and biocompatible polymer that provides an environmental friendly alternative to biodegradable plastics derived from petrochemical materials (4), (6), (8). Lactic acid naturally exists in two optical isomers: D(−)-lactic acid and L(+)-lactic acid. Since elevated levels of the D-isomer are harmful to human, L(+)-lactic acid is the preferred isomer for food-related and pharmaceutical industries (9). Lactic acid can be produced using chemical or biological fermentation process. Chemical synthesis of lactic acid by hydrolysis lactonitrile acid, a derivative of petrochemicals. The products are DL-lactic (4) The advantage of being produced by fermentation biological fermentation can be used raw material prices such as molasses, molasses, starch, cellulose and other raw materials rich. Carbohydrates (2), (11), the cost of raw materials is one of the key factors in the production that can be used in determining the production with the best performance. However, in the process, the biological need to check the development of production systems for highly specific and provide products with high purity and (5) The conventional recovery processes of lactic acid from fermentation broth are quite complicated. Separation of lactic acid from dilute wastewater or fermentation broths using evaporation has an economic problem since the vaporization of water consumes much energy. Also distillation is not useful due to non-volatility of lactic acid. In conventional processes, precipitation of calcium lactate using calcium hydroxide has the following steps: precipitation, filtration, addition of sulfuric acid, purification using activated carbon, evaporation and crystallization. Separation and final purification stages account up to 50 % of the production costs. Researchers have conducted studies and develop manufacturing processes to reduce the cost of production.

2. Objectives

The aim of this work was to improve the efficient of separation and purification L(+)-lactic acid from the fermentation broth**.** The research will be studied to enhance concentration of lactic acid using ion exchange column.

3. Materials and Methods

3.1 Resins preparation

Anionic resin, Amberlite IRA-400, was used in this work. Before use, the resins were activated into working forms (OH-). The freshly new resin was treated with 1.0 M NaOH, and then washing the resins by distilled water (until pH 7).

3.2 Fermentation broth preparation

Our in-house *bacillus sp* BC-001 was used to ferment lactic acid in GYP medium consisted of 11 g glucose monohydrate, 5 g yeast extract, 5 g peptone, 4 g NH_4Cl, 0.5 g KH_2PO_4, 0.5 g K_2HPO_4, 1 g $Ca(OH)_2$,20 g ager and 20 mL salt solution per litre. The fermentation was carried out in the stirred fermentor with the automatically pH (pH 6.8) control by the concentrated 10 M NaOH for 18 h when the highest lactate titer was achieved. The fermentation broth was harvested for our lactate recovery processes.

3.3 Batch adsorption

3.3.1 Equilibrium time

One g of wet resin (in 3.1.) was soaked with 25 mL lactic acid solution and incubated at 200 rpm with different temperatures (30-70 °C). Samples were taken every 30 s. for analyzing the remaining lactic acid using HPLC. Sampling was stopped when the concentration of lactic acid remained in the solution became constant. Lactic acid adsorption was calculated according to the initial lactic acid present in the fermentation and the remaining at different times. The equilibrium time was determined from the plot of lactic acid adsorbed on resins versus time.

3.3.2 Adsorption isotherm

One gram of Amberlite IRA-400 wet resin was added to 10 mL lactic acid solution at various concentrations (0-100 mg/mL), pH 6.0. The samples were incubated at 50 °C, 200 rpm for 12 h. Residual lactic acid concentration was measured and the amount of adsorbed lactic acid per gram wet resin was calculated. The adsorption isotherm was obtained from the plot of adsorbed lactic acid on resins versus the remaining lactic acid in the solution. The isotherm equation was predicted from the best suited trend line of the plotted data.

3.3.3 Elution profiles

Eluant was selected in this work. One gram of Amberlite IRA-400 was thoroughly soaked with 100 g/L lactic acid solution at pH 6.0 to ensure equilibrium. After that the solution was discarded and the resins were washed with DI water before soaking with different eluants (10 mL) including HCl, H_2SO_4, H_3PO_4, NH_3, NaCl, and DI water. The concentration effect of all eluants was also studied. Samples were taken for analysis of lactic acid eluted from the resins until the constant concentration was obtained.

3.4 Analytic methods

High performance liquid chromatography (HPLC) was used to analyze the organic compounds (the remaining glucose and end products) present in the fermentation broth and lactic acid in the recovery samples. Samples were centrifuged, filtered through hydrophilic PTFE embrane, and diluted with double distilled water. 20 µL diluted particle-free samples were injected into an organic acid analysis column (Biorad, Aminex HPX-87H ion exclusion organic acid column; 300mmx7.8mm) maintained at 45°C in a column oven. 0.005 M H_2SO_4 was used as an eluent at 0.6 mL/min flow rate. A refractive index detector was used to detect the organic compounds. A standard containing 2 g/L of each component was injected as a reference to determine the sample concentration. The peak area was used for the comparison basis.

4. Results and Discussion

4.1 Determining the equilibrium time

Figure 1 shows the example of lactic acid adsorption on Amberlite IRA-400 OH^- form. From the results, lactic acid rapidly adsorbed onto the resins at the first 5 min. Later on the adsorption reached plateau. It was found that pH and temperature affected the adsorption process. From Table 1, increasing the pH and temperature helped improve adsorption capacity of lactic acid on the resins. The maximum adsorption capacity of 1.8 g lactic acid per g wet resins was obtained at pH 6.0 and 70 °C. Nonetheless, according

to our developed in-house fermentation that was operated at 50 °C and pH 6.8 combined with the obtained results in Table 1, we suggested that adsorption in further study would be conducted at 50 °C and pH 6.8. This finding is similar to the work reported by (3).

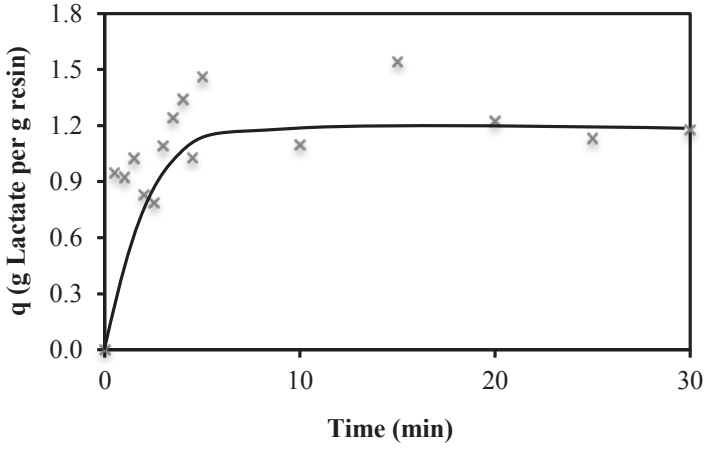

Figure 1. Example of lactic acid adsorbed on Amberlite IRA-400. The resin was soaked with lactic acid solution at the ratio of 0.1 g resin to 2.5 mL lactic acid solution (pH 6.0). The mixture was incubated at 50 °C, 200 rpm.

Table 1. Adsorption capacity[a] of lactic acid on Amberlite IRA-400 at different pH and temperature

Temperature (°C)	pH		
	2.0	4.0	6.0
30	0.25	0.35	0.50
50	0.80	1.0	1.20
70	0.90	1.0	1.80

[a]values in g lactic acid per g wet resin

4.2 Lactic acid adsorption isotherm on Amberlite IRA-400

Figure 2. shows the typical isotherm of lactic acid at 50 °C, pH 6.0.

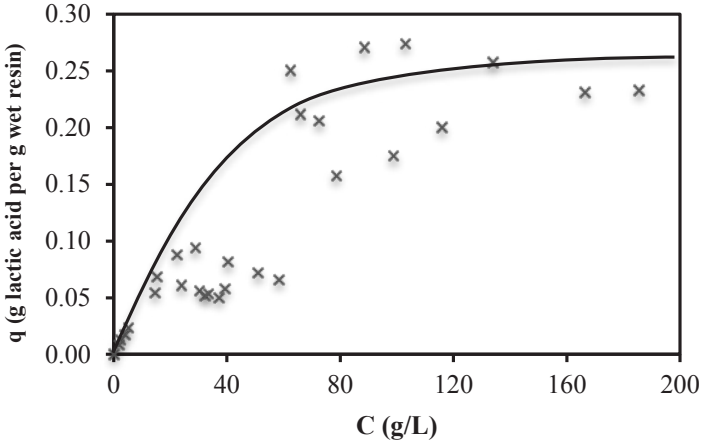

Figure 2. Typical isotherm of lactic acid on Amberlite IRA-400. The resins were soaked with lactic acid solution at various concentrations at 50 °C, pH 6.0, 200 rpm until equilibrium.

Curve fitting was conducted to obtain the best suited isotherm equation as shown in Figure 3. Both Langmuir and Freundlich isotherms were applied. From the curve fitting results, it was found that the adsorption of lactic acid on Amberlite IRA-400 was likely considered as chemisorption; thus, followed Langmuir isotherm (eq.1) where K_d was the dissociation constant, q_m was the maximum adsorption capacity, c was the remaining lactic acid in solution, and q was the amount of lactic acid adsorbed on the resin and the constant q_m and K_d values were 0.14 g lactic acid per g wet resin and 27.78 g/L, respectively.

$$q = \frac{q_m C}{K_d + C} \qquad \text{(eq.1)}$$

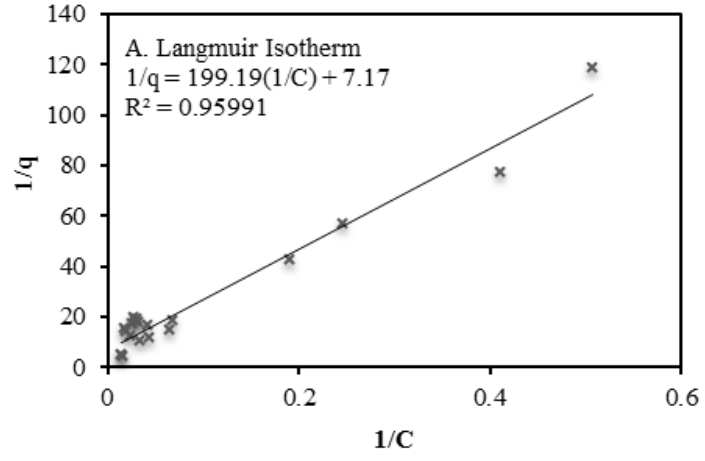

Figure 3. Curve fitting for isotherm prediction on Amberlite IRA-400: (A) Langmuir isotherm, (B) Freundlich isotherms.

4.3 Eluant selection

In this study, we attempted to use different eluants to elute lactic acid from the saturated resins (Figure 4). Among the eluants studied, it was clear that acidic eluants gave the better recovery as observed from the higher concentration of lactic acid eluted from the resins. While some eluant (NaCl) and DI water unsuccessfully eluted lactic acid from the resins. Increasing the concentration of eluants caused the adverse effect on lactic acid recovery from the resins. Therefore, among the 3 concentrations studied, 1.0 M gave the highest lactate recovery. It should be noted that H_2SO_4 seemed to be more effective than other acids studied. This was presumably due to the divalent SO_4^{2-}, which provided the stronger binding ability to the monovalent cation on the resin surface compared to Cl^- or OH^-.

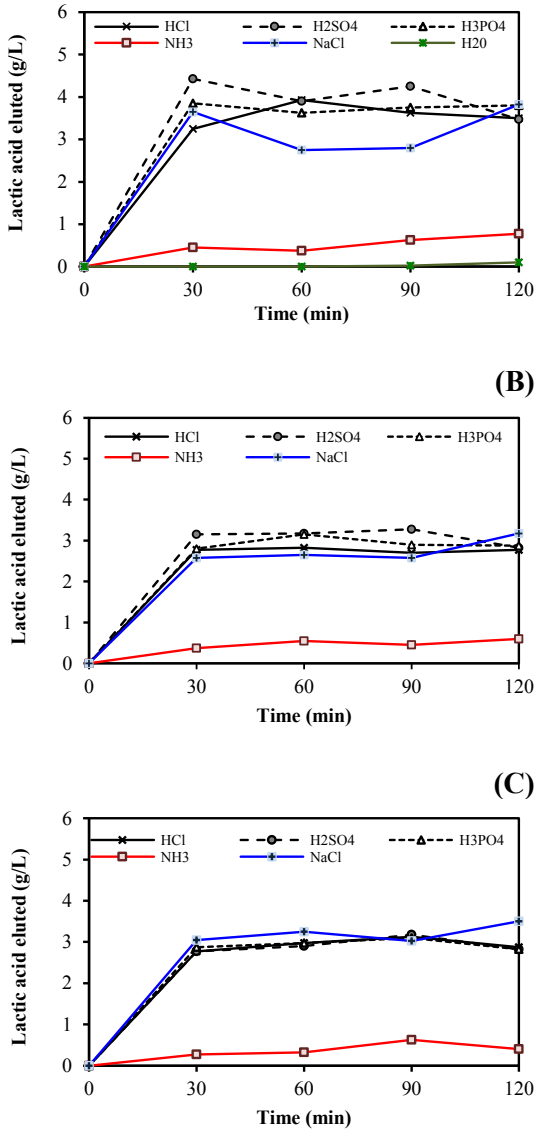

Figure 4. Lactic acid elution from saturated Amberlite IRA-400 using acids, base, salts, and DI water at different concentrations: (A) 1.0 M, (B) 1.5 M, and (C) 2.0 M.

5. Acknowledgments

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