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RECOVERY OF L-LACTIC ACID FROM FERMENTATION BROTH BY USING STRONG ANIONIC RESIN

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ABSTRACT

The technique of ion exchange for separation of lactic acid from fermentation broth is used in our study. The ion exchange resin used is a strong anionic resin, a product of Jacobi Carbons Company, Italy (trade name is Resinex A-4). The process at laboratory scale is realized in a chromatographic column with dimensions d x h = 10mm x 110mm which contains 6 g of anionic resin. Two solvents used are distilled water and methanol. The HPLC analysis shows a good efficacy of distilled water.

I - INTRODUCTION

Lactic acid (or 2-hydroxy acid) discovered in 1970 by Scheele, Swedish chemist, is a metabolic intermediate found in many organisms ranging from anaerobic prokaryotes to man and is one of the organic acids the most important. They exist in two optical forms, the D-and L-lactic acid [1].

Currently, the lactic acid is mainly used in food as an excellent natural preservative in food. This acid is also used for non-food applications such as in textile, cosmetic, pharmaceutical, leather industries or as a herbicide. It is also used in the production of basic chemicals such as lactate ester, acetaldehyde, acrylic acid, propanoic acid ... in the chemical industry or used in the production of biodegradable polymer. Vietnam is an agricultural country which has a large resource of starch and cellulose. This is a good place to produce and implement the lactic acid.

In fact, the economic aim of lactic acid production is mostly belong to separation step of lactic acid from fermentation broth. The recovery of lactic acid is complex because it shows a strong affinity with water and has low volatility (boiling point is 122°C at 1661.73 Pa). There are various techniques used for separation process of lactic acid: membrane filtration, distillation, extraction, electrodialysis, the ion exchange resins [2]. The ion exchange resins are widely used for separation of organic products [3-6]. Our study is then concentrated to use the technique of ion exchange for separation of lactic acid from fermentation broth.

II - EXPERIMENT

1. Materials

Lactic acid fermentation broth used is a product of Institute of Micro-organisms and Biotechnology, Vietnam National University in Hanoi) and is already clarified.

The ion exchange resin used is a strong anionic resin, a product of Jacobi Carbons company, Italy (trade name is Resinex A-4). It is in the form of polystyrene divinylbenzene reticulated particles (average particle diameter

is 0.42 - 1.25 mm). The functional group of resin is N^+-$(R)_3$ and the exchanged ion is Cl^- with CEC value is 1.30 equivalents/liter.

Standard L-lactic acid is a solution of 85-90% L-lactic acid in water, product of Fluka BioChemika, reference 69773.

The chromatographic column with dimensions d x h = 10mm x 110mm contains 6 g of anionic resin. Firstly, the functional groups of the resin is regenerated by 3 bed volumes of 1N NaOH solution and then the column is washed with distilled water until the pH of the output is equal to 7. The fluid flow is 1 ml/ min.

2. Protocol of ion exchange

The ion exchange process is essentially discontinuous and includes four stages: saturation, recovery, regeneration and rinsing.

a) Saturation

The ion exchange column is saturated with 25ml of L-lactic acid fermentation broth. The fluid flow is 1 ml/min. Lactic acid is then adsorbed by exchange resin. During the process of saturation, the resin is slowly changing its color from brown to yellow. At starting, the pH output is about 11 ~ 12 and when the column is saturated (the resin completely changes its color), the pH output is equal to the pH input (pH_{input} = 2).

b) Recovery of L-lactic acid

The L-lactic acid solution is obtained by washing the column with distilled water or methanol. The total volume of solvent is 25 ml. The fluid flow is maintained as previous stage 1ml/min. Then, a solution with pH equal to 2 is recovered.

c) Regeneration

After lactic acid is desorbed, the resin is regenerated with 3 bed volumes of 1N NaOH solution. Fluid flow in this stage is also 1ml/min. During the process of regeneration, the resin changed its color from yellow to brown and pH output is equal to

d) Rinsing

After being regenerated by NaOH solution, the anion resin is rinsed by a stream of distilled water until pH output is equal to 7. The column is then ready for the next cycle.

3. Analytical methods

a) Determination of total acid concentration in fermentation broth

The total acid concentration in fermentation broth is determined by titration with 0.1 N NaOH solution and phenolphthalein as indicator.

b) Determination of L-lactic acid concentration by using high performance liquid chromatography (HPLC)

HPLC technique allows determining not only a concentration of L-lactic acid but also a purity of L-lactic acid solution. The sample analyses are realized by HPLC Shimadzu 10A series and analytical conditions as followed:

+) Temperature is 30°C

+) Column is a C18 reverse phase with dimensions: length L = 250 mm, diameter d = 4.6 mm

+) Size of the stationary phase is 5 micrometres.

+) Mobile phase speed is 1ml/min.

+) Composition of mobile phase: acetonitrile/buffer = 3/99.

+) Buffer $(NH_4)_2HPO_4$ has a concentration of PO_4^{3-} of 30 mM and it is adjusted to pH 2.2 by H_3PO_4 solution. Gas bubbles are eliminated by using ultrasound before use.

+) PDA detector, wavelength used is 210 nm.

+) Linearity between concentrations (range 0.4 - 40 mg/ml) and peak area of L-lactic acid is presented by the following equation:

$$y = 97{,}119\ x + 17100 \quad (1)$$

In which:
x: concentration of L-lactic acid (mg/ml).
y: peak area of L-lactic acid.

III - RESULTS AND DISCUSSION

1. Determination of total acid concentration

Fermentation process gives not only lactic acid but also some by-products as acetic acid [7]. The titration of total acid concentration can not give us an exact concentration of L-lactic acid, but this analysis allows us to quickly have an approximate concentration of L-lactic in fermentation broth. The total acid concentration obtained by titration is 0.69 N. This concentration is very small, so an exchanged resin technique for recovering L-lactic acid is a good choice before applying a technique more expensive.

2. Recovery of L-lactic acid

Two types of solvent were used for this stage: distilled water and methanol. The analysis results of high performance liquid chromatography allows us to compare the effectiveness of these solvents.

a) Results with distilled water

The chromatogram of L-lactic acid solution recovered by distilled water shows a significant peak of L-lactic acid at the retention time of 4.069 minutes (figure 1).

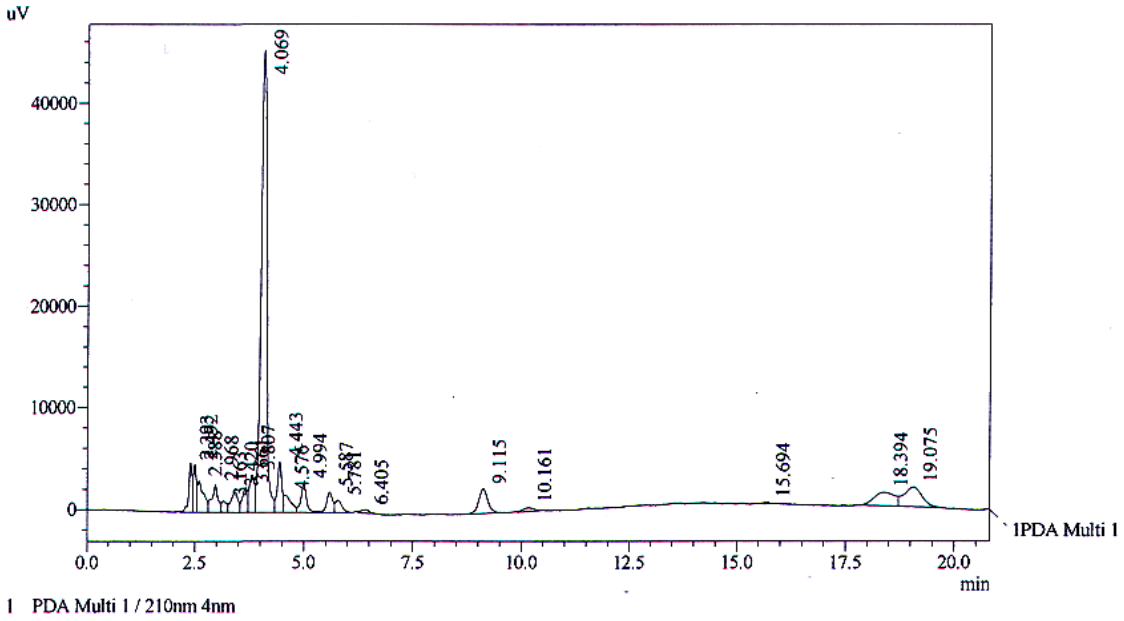

Figure 1: Chromatogram of of L-lactic acid solution recovered by distilled water

The value of L-lactic acid peak surface in chromatogram is 426,428. From this value, L-lactic acid concentration calculated by linearity equation (1) is x = 4.21 mg/ml. Therefore, the amount of lactic acid recovered by 25 ml of distilled water is: 4.21 x 25 = 105.25 mg. And the amount of L-lactic acid obtained by one gram of anion resin in this process is equal to 17.54 (mg/g).

b) Results with methanol

Figure 12 shows us the chromatogram of L-lactic acid solution recovered by methanol. Peak of L-lactic acid is corresponded to retention time of 4.215 minutes and position of this peak is slightly shifted. However, when adding a standard L-lactic acid solution, the same L-lactic acid peak position is showed in the chromatogram (see figure 2).

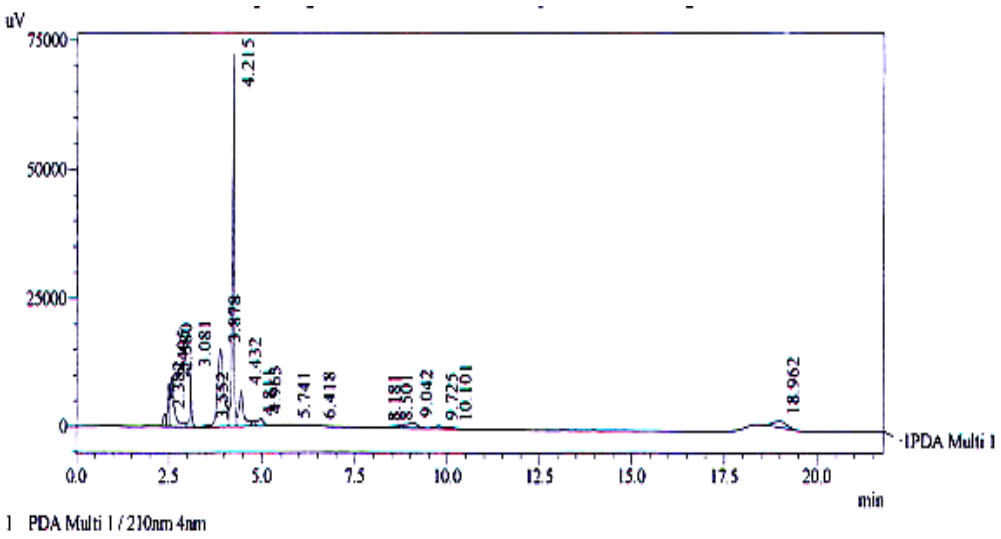

Figure 2: Chromatogram of L-lactic acid solution recovered by methanol

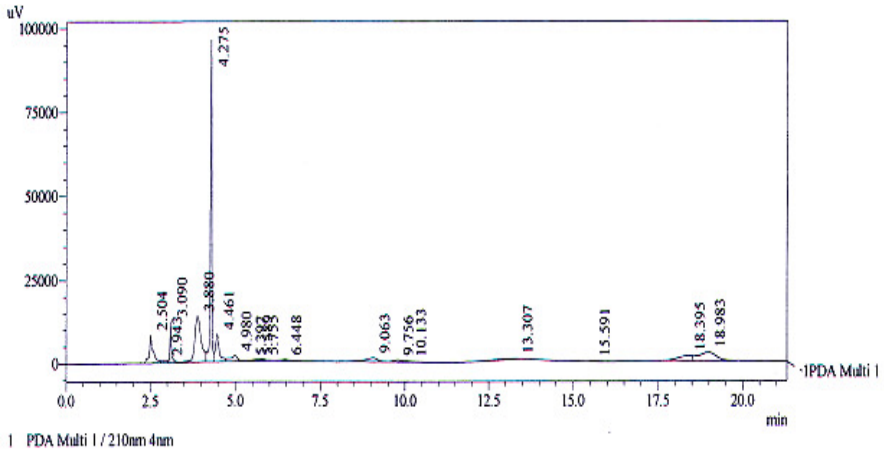

Figure 3: Chromatogram of L-lactic acid solution recovered by methanol when adding a standard L-lactic acid solution

The L-lactic acid peak area is 225,619, so the concentration of L-lactic acid is 2.15 mg/ml. The amount of lactic acid recovered by 25 ml of methanol is: 2.15 x 25 = 53.75 mg. Therefore, the amount of L-lactic acid obtained by a gram of anion resin in this process is 8.95 (mg/g).

IV - CONCLUSIONS

The recovery process of L-lactic acid from fermentation broth was carried out using strong anionic resin named A-4 Resinex (product of Jacobi Carbons Company, Italy). Two solvents used are distilled water and methanol. The HPLC analysis shows a good efficacy of distilled water (the concentration of L-lactic acid in distilled water is 4.21 mg/ml - two times higher than that in methanol).

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