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RAPID COMMUNICATION

Effect of operating parameters on precipitation for recovery of lactic acid from calcium lactate fermentation broth

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Abstract−Precipitation is a simple, efficient method for separating and recovering lactic acid in the form of calcium lactate from fermentation broth by adding sulfuric acid. Major operating parameters of the recovery step as well as the temperature of concentration of the recovered lactic acid solution and the type and amount of adsorbent used for pigment (color) removal were optimized. When the molar ratio of calcium lactate to sulfuric acid was 1 : 1 and the pH was increased to a value greater than the pKa (3.86), calcium sulfate was precipitated and could be removed more effectively, allowing for more efficient separation and recovery of supernatant lactic acid. Precipitation could be facilitated by adding calcium lactate solution with mixing (up to 220 rpm) and was completed in over 18 h. The optimal temperature for the concentration of lactic acid recovered from the supernatant after removing the precipitated calcium sulfate was found to be 90 °C in terms of the time required for concentration and the stability of the product. Activated carbon (SX-PLUS, 9 g/L) was most effective as an adsorbent for color removal from the recovered lactic acid. Under the optimized precipitation conditions, an overall yield of 92% of lactic acid from fermentation broth could be achieved.

Key words: Lactic Acid, Recovery, Precipitation, Operation Parameters, Fermentation Broth

INTRODUCTION

Lactic acid (2-hydroxypropanoic acid, $CH_3CHOHCOOH$, CAS 50-21-5) is an organic hydroxycarboxylic acid produced by either microbial fermentation or chemical synthesis, the former method currently being used in over 50% of total production [1]. Traditionally, lactic acid has been used widely in the food industry as an additive as well as in the medical industry in the production of surgical suture. Demand for lactic acid is expected to increase as it has also been used recently as a platform chemical for substances such as lactic acid-base functional (biodegradable and bioactive) macromolecules [2].

To be able to produce a functional, biodegradable macromolecule such as polylactic acid using lactic acid as a platform chemical, an economical means of mass producing high purity lactic acid is essential. Although chemical synthesis offers a product with a relatively low production cost, an optically inactive racemic mixture (DL-lactic acid) is always produced. Conversely, by fermentation, the selectivity of lactic acid can be attained by using an appropriate bacteria species and/or by genetic engineering of the species [3-5]. However, lactic acid produced by fermentation may contain a large amount of impurities unless an efficient recovery process is applied. Also, efficiency of recovery is crucial to the economy of the whole process since the cost of separation and recovery is more than half the entire cost of production [6-10].

Methods of lactic acid separation currently in use include solvent extraction [11], electrodialysis [12], ion exchange [13], nanofiltration [7], and reverse osmosis [5]. These methods are effective in the primary purification of lactic acid from fermentation broth but do not easily offer a high purity product. Traditional methods of separation and purification involve removing microorganisms from the fermentation broth and adding lime to precipitate calcium lactate [$Ca(LA)_2$], which is then subjected to a chemical reaction with sulfuric acid to obtain lactic acid only after the precipitation of calcium sulfate [14]. This method, however, is too complicated and results in a low yield of lactic acid due to the high solubility of calcium lactate [14,15]. Recently, a simple method was developed that can recover lactic acid produced in the form of calcium lactate from fermentation broth by adding sulfuric acid [16]. This method, however, cannot yet be widely applied because information on the effects of various operating parameters on process efficiency is lacking. Therefore, we optimized the major operating parameters of the recovery step as well as conditions for the concentration of recovered lactic acid solution and pigment (color) removal. We believe that the results of this study could be directly applied to mass production of lactic acid of high purity and yield from fermentation broth.

MATERIALS AND METHODS

1. Preparation of Samples

In this study, we used a model calcium lactate solution and actual fermentation broth in developing a lactic acid recovery process and optimizing major process parameters. Purchased calcium lactate solution (98% purity; Fluka Co.) was used as the model solution. The actual fermentation broth was prepared by culturing *Lactobacillus paracasei* on MRS (Difco Co.) at 35 °C and removing the cells from the fermentation broth (purity: 69%) supplied by the Korea

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2. Analysis of Organic Acids

An HPLC system (Waters, USA) was used for the analysis of organic acids (lactic, acetic, propionic and citric acids) and was performed using a C18 column (4.6×150 mm, 5 μm; Beckman, USA). The applied mobile phase was 0.01 M H_2SO_4, and the flow rate and injection volume were 0.7 mL/min and 10 μL, respectively. The effluent was monitored at 210 nm with a UV detector. For standard material, product from Sigma-Aldrich (purity: 98%) was used.

3. Ion Chromatography (IC) and Inductively Coupled Plasma (ICP) Analysis

Sulfuric acid was added to calcium lactate solution to promote precipitation of calcium sulfate. After removing the precipitate, the concentrations of residual sulfate ion (SO_4^{2-}) and calcium ion (Ca^{2+}) in the solution were measured using IC (Metrohm, Switzerland) and ICP (PerkinElmer, USA), respectively. An IC system was performed using a Shodex IC SI-90 4E column (4.0 mm×250 mm). The applied mobile phase was 1.18 mM for $Na_2CO_3^+$ and 1.7 mM for $NaHCO_3$, the flow rate was 1 mL/min and the injection volume was 10 μL. Suppressed CD was used. For analysis with ICP (Perkin Elmer, USA), an RF power of 1,300 W was applied as well as a neb flow rate of 0.65 L/min, an aux flow rate of 0.2 L/min, a plasma flow rate of 15 L/min and a specimen flow rate of 1.5 mL/min.

4. Precipitation Process

To separate and recover lactic acid from calcium lactate solution, sulfuric acid (Samchun Pure Chemical Co.) was added. As shown in Fig. 1, calcium sulfate was precipitated by adding sulfuric acid to the solution and was removed from the solution before recovering supernatant lactic acid. We attempted to achieve high purity and yield of lactic acid by optimizing the precipitation reaction conditions ($Ca(LA)_2/H_2SO_4$ molar ratio, pH, mixing and feeding methods). Reaction tests were carried out at molar ratios of 1 : 0.5, 1 : 0.75, 1 : 1, 1 : 1.25 and 1 : 1.5 by using different molar concentration of sulfuric acid and a constant molar concentration of calcium lactate. To evaluate the effect of changes in the pH of the calcium lactate solution on its reaction with sulfuric acid, tests were performed in a pH range of 2.83-6.83 by adding more HCl dropwise to the original calcium lactate solution at pH 6.83. After the reaction was allowed to proceed for 24 h, it was centrifuged for 30 min before the precipitated calcium sulfate was removed and supernatant lactic acid was recovered. To obtain a high purity of lactic acid, the concentration temperature was optimized and the concentrations of residual SO_4^{2-} and Ca^{2+} ions in the solution were monitored using ICP and IC. Also, an electronic microscope (SV-35 Video Microscope system; Sometech, Korea) was used to determine the shapes and sizes of calcium sulfate particles precipitated by the reaction with sulfuric acid. The video images of these particles were observed at 100× magnification using an IT-Plus system (Sometech).

5. Removal of Pigment

To remove pigment (color) from the lactic acid recovered from the fermentation broth, adsorbent treatment was performed under optimized conditions (type and amount of adsorbent) using sylopute (Fuji Silysia Chemical Ltd., Japan), active clay P-1G (Mizukalife Chemical Co., Japan) or activated carbon CA-1 or SX-PLUS (NORIT, Netherlands). Nine grams per liter of adsorbent was added and the solutions were stirred in a shaking incubator for 30 min at 250 rpm and 25 °C.

RESULTS AND DISCUSSION

1. Effect of $Ca(LA)_2/H_2SO_4$ Molar Ratio

In this study, we attempted to develop a method of separating and recovering lactic acid by adding sulfuric acid to calcium lactate solution to facilitate the precipitation of calcium sulfate and by separating and recovering the supernatant product. To optimize conditions for the reaction of the calcium lactate model solution with sulfuric acid, we first determined the effect of different $Ca(LA)_2/H_2SO_4$ molar ratios on the precipitation process. Fig. 2 shows the amounts of calcium sulfate that precipitated from the reaction between calcium lactate and sulfuric acid. When the $Ca(LA)_2/H_2SO_4$ molar ratio

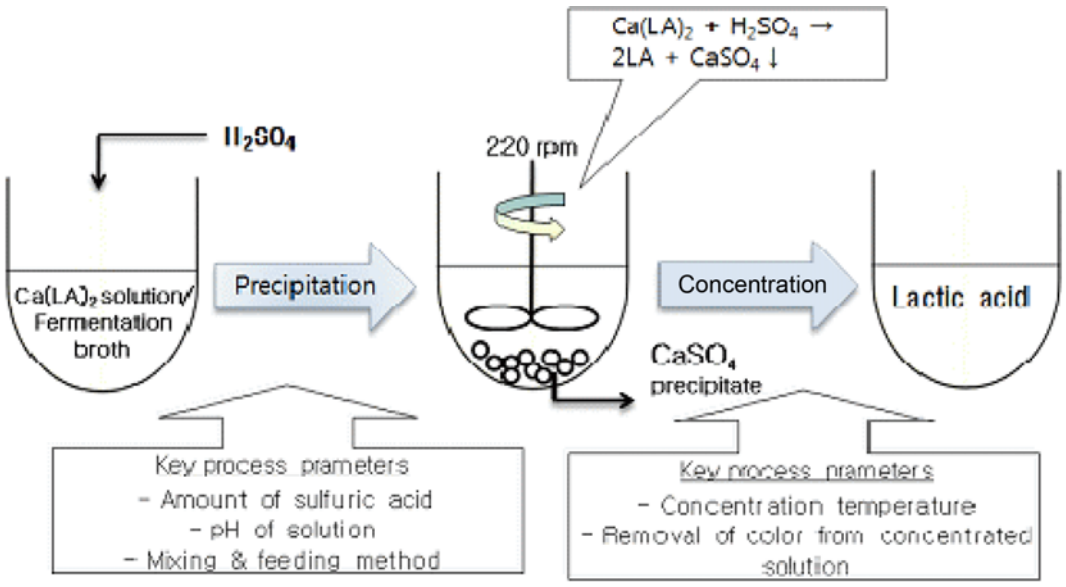

Fig. 1. Schematic diagram of the process for recovery of lactic acid from calcium lactate model solution and fermentation broth.

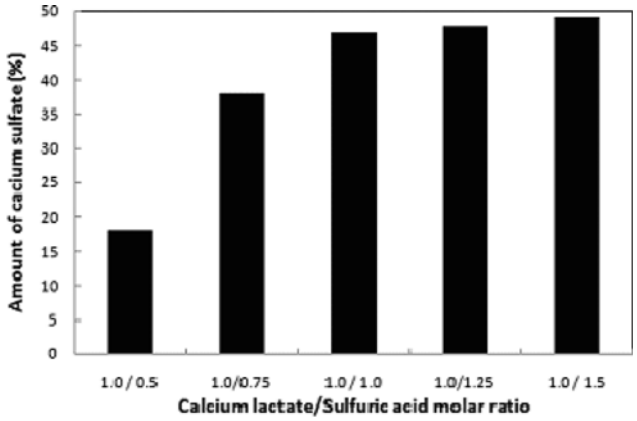

Fig. 2. Effect of calcium lactate/sulfuric acid molar ratio on amount of calcium sulfate precipitated. The initial pH, stirrer speed, and reaction time were 4.8, 240 rpm, and 24 h, respectively.

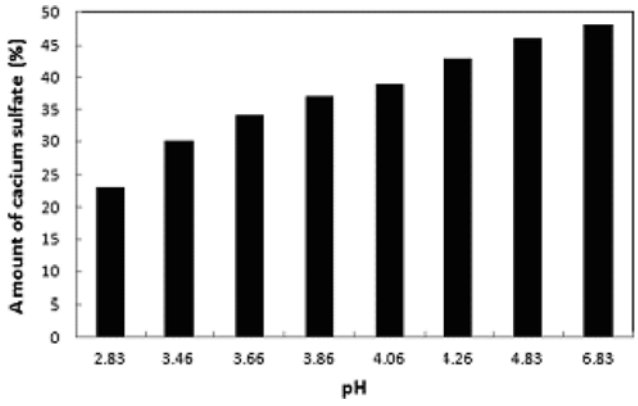

Fig. 4. Effect of pH on the amount of calcium sulfate precipitated. The calcium lactate/sulfuric acid molar ratio, stirrer speed, and reaction time were 1 : 1, 240 rpm, and 24 h, respectively.

was 1 : 1, the amount of calcium sulfate produced was 50 wt% of calcium lactate, which was more than the amount produced at a molar ratio of 1 : 0.5 but similar to the amount produced at 1 : 1.5. We therefore concluded that a $Ca(LA)_2/H_2SO_4$ molar ratio of 1 : 1 is optimal based on the finding that this molar ratio was sufficient for the reaction process. This finding is similar to that of previous studies that considered the stoichiometric ratio of calcium lactate to sulfuric acid [16], which was 0.95-1.02. Using the 1 : 1 molar ratio of calcium lactate to sulfuric acid, the solution was subjected to a sulfuric reaction and centrifugation and the precipitated calcium sulfate was then removed before recovering the supernatant lactic acid. Subsequent analysis showed that most of the lactic acid (98%) had been recovered. Analysis of residual sulfate and calcium ions in the supernatant was then performed using IC and ICP, respectively. As shown in Fig. 3, the amount of sulfuric acid was not sufficient for reaction when the molar ratio was 1 : 0.5, resulting in a relatively greater amount of residual calcium ion. On the other hand, the reaction using a molar ratio of 1 : 1.5, in which the amount of sulfuric acid was more than sufficient, resulted in relatively larger amount of residual sulfate ion. The morphology of the calcium sulfate precipitate was analyzed during precipitation with a video microscopy system. The amount of precipitate using a molar ratio of 1 : 0.5 was less than the amount obtained using ratios of 1 : 1 and 1 : 1.5 since the amount of added sulfuric acid was not sufficient (data not shown).

2. Effect of pH of Calcium Lactate Solution

In the reaction between calcium lactate and sulfuric acid, pH is one of the important process parameters. It is related to the pKa value of calcium lactate (3.86). When the pH of a calcium lactate solution is lower than its pKa value, some of the calcium lactate exists in an undissociated (unionized) form, making reaction with sulfuric acid difficult, whereas when the pH is greater than the pKa, calcium lactate exists in dissociated (ionized) form and reacts easily with sulfuric acid, thus producing calcium sulfate more efficiently [17]. The amount of calcium sulfate produced from reactions between calcium lactate and sulfuric acid at these different pHs is indicated in Fig. 4. The amount of calcium sulfate production indicates the degree of reaction between calcium lactate and sulfuric acid. Less calcium sulfate was produced at pH 2.83, being 23 wt% of the original amount of calcium lactate used, which is only half as much as the amount of calcium sulfate produced when the pH was 4.83

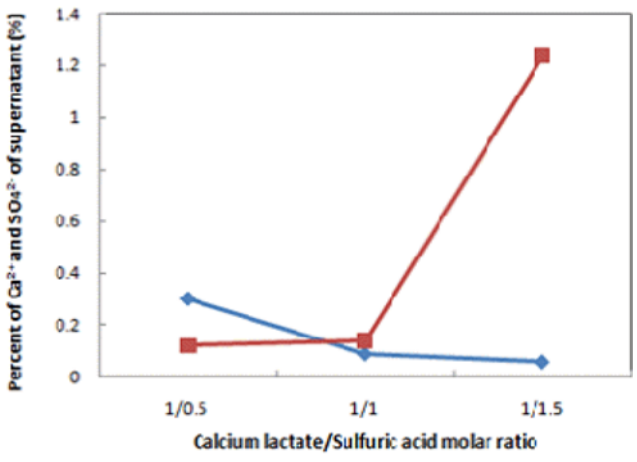

Fig. 3. Effect of calcium lactate/sulfuric acid molar ratio on concentration of residual calcium ion (◆) and sulfate ion (■).

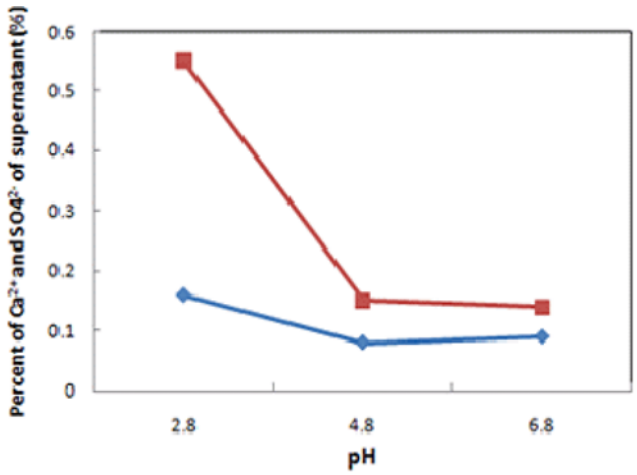

Fig. 5. Effect of pH on the concentration of residual calcium ion (◆) and sulfate ion (■).

and 6.83 (>47 wt%). The amount of residual calcium and sulfate ions in the supernatant solution was measured after the reaction with sulfuric acid at a pH of 2.83, 4.83 and 6.83 using IC and ICP. Although there was no significant difference in the amount of calcium ion, the amount of sulfate ion decreased to less than half as the pH decreased from 4.83 to 2.83, as shown in Fig. 5, indicating that reaction with sulfuric acid was more effective when the pH was higher than the pKa value. The morphology of calcium sulfate precipitate was also analyzed at different pH values with a video microscopy system. The relative amount of precipitation was significantly less when the pH of the calcium lactate solution (2.8) was lower than the pKa (3.86) (data not shown).

3. Effect of Mixing and Feeding Methods

The effect of the method used to mix the calcium lactate solution after the addition of sulfuric acid on the efficiency of precipitation was evaluated. As indicated in Fig. 6, the efficiency of the removal of calcium sulfate increased when the calcium lactate solution was mixed (up to 220 rpm) after the addition of sulfuric acid, and lactic acid could be recovered after 18 h of precipitation, presumably because movement caused by mixing during precipitation causes physical aggregation of particles through collision [18,19]. Fig. 7 shows photographs of precipitated calcium sulfate particles with and without mixing according to precipitation time. Compared with precipitation without mixing, the calcium sulfate mass precipitated with mixing was broken into smaller pieces. To investigate the effect of the injection method used for the addition of sulfuric acid, different amounts of sulfuric acid (100, 90, 70 or 50% of the total) were first injected and calcium sulfate was recovered after 12 h. The rest of the scheduled amount of sulfuric acid (0, 10, 30 or 50% of the total) was added before recovering calcium sulfate after a further 12 h and the total amount of calcium sulfate was measured. There was no significant effect on the efficiency of the removal of calcium sulfate (data not shown).

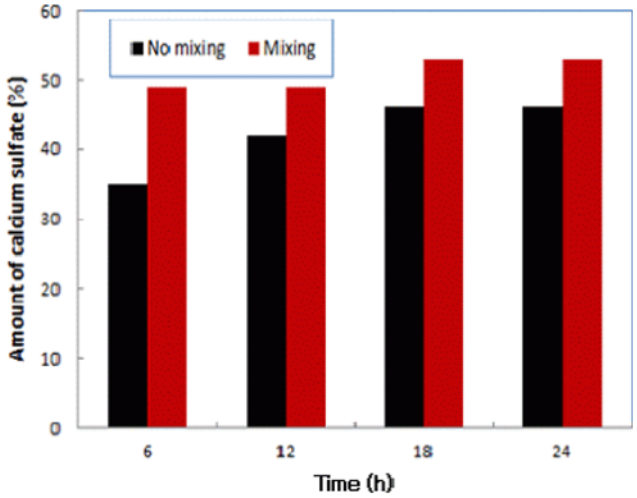

Fig. 6. Effect of mixing on amount of calcium sulfate precipitated.

4. Recovery of Lactic Acid from Calcium Lactate Fermentation Broth

We applied the optimal conditions that were determined using the model calcium lactate solution to the fermentation broth (lactic acid purity: 69%, pH: 7.3). Using the same method as was used with the model solution, the broth was subjected to sulfuric acid reaction at a $Ca(LA)_2/H_2SO_4$ molar ratio of 1 : 1 and centrifugation, then precipitated calcium sulfate was removed. The purity and yield of recovered supernatant lactic acid were 70.6% and 92%, respectively. The concentrations of calcium and sulfate ions in the supernatant were also analyzed using ICP and IC and were found to be 0.1% and 0.16%, respectively, values that are similar to those found in the test using the model calcium lactate solution. Analysis of the fermentation broth using HPLC showed that it contained 69.641 g/L

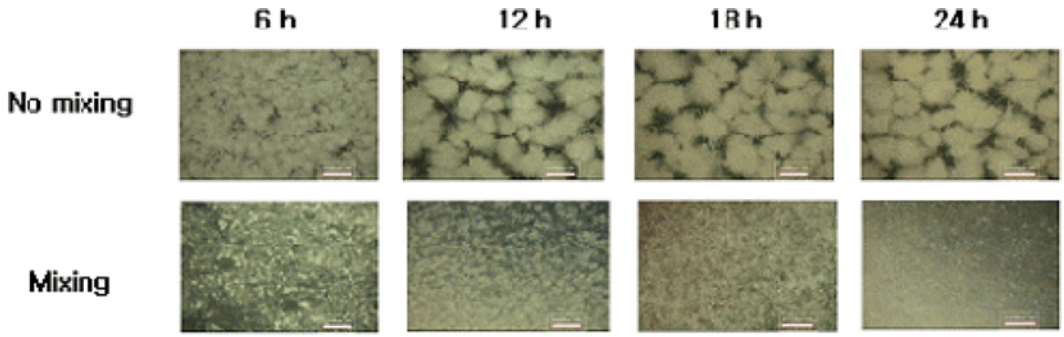

Fig. 7. Effect of mixing on morphology of calcium sulfate precipitate. All bars indicate 10 mm (×100).

Table 1. Recovery of lactic acid and removal of other organic acids (citric acid, acetic acid, and propionic acid) by precipitation process from fermentation broth

Major organic acids in fermentation broth	Concentration of major organic acids (g/L)	Recovery of lactic acid after precipitation (%)	Removal of other organic acids after precipitation (%)
Lactic acid	69.641	92.0	-
Citric acid	3.713	-	26.0
Acetic acid	4.362	-	18.0
Propionic acid	1.935	-	100.0

lactic acid, 4.362 g/L acetic acid, 3.713 g/L citric acid and 1.935 g/L propionic acid (Table 1). The recovery of lactic acid and removal of other organic acids (citric acid, acetic acid, and propionic acid) from the fermentation broth after the reaction with sulfuric acid was investigated, the results of which are shown in Table 1. The recovery of lactic acid from the fermentation broth was 92%. While propionic acid could be removed completely, only 18% of the acetic acid and 26% of the citric acid were removed.

After treatment with sulfuric acid, precipitated calcium sulfate was removed and tests to determine the optimal temperature for the recovery of a high concentration of lactic acid from the supernatant were performed. Five milliliters of recovered lactic acid was first subjected to a concentration process at 760 mmHg at a temperature of 60, 70, 80, 90 and 100 °C. The stability and concentration of lactic acid varied little with temperature, indicating that there was no degradation (data not shown). Also, the time required for concentration was not shortened using a temperature over 90 °C, as shown in Fig. 8. Based on these results, we concluded that 90 °C is the optimal temperature for concentration of lactic acid in terms of both stability and the time required. Such a finding also coincides with that of a previous study, which reported a concentration temperature below 90 °C [16]. To remove the dark brown pigment from the fermentation broth, we evaluated the efficiency of active clay, activated carbon and sylopute as adsorbents. As shown in Fig. 9, the pigment was most effectively removed when activated carbon (SX-Plus) was used, as was reported by Pyo et al. [20]. Compared with active clay, activated carbon not only has a greater capability to remove pigment from the cell broth but speeds up the process since it can be filtered faster after treatment.

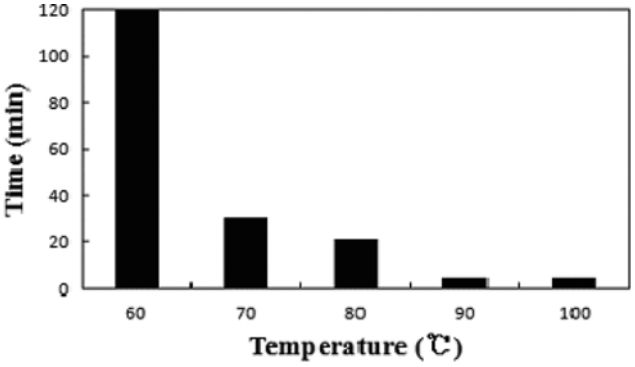

Fig. 8. Effect of temperature on concentration time using fermentation broth. Five milliliters of recovered lactic acid was first subjected to a concentration process at 760 mmHg at a temperature of 60, 70, 80, 90 and 100 °C.

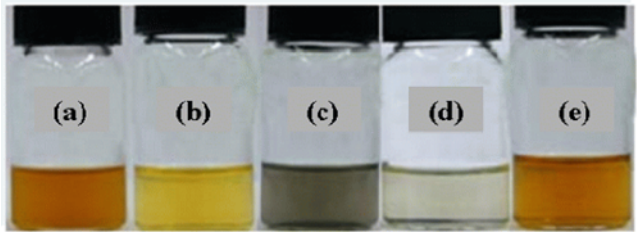

Fig. 9. Effect of adsorbent on the removal of pigment (color). (a) Fermentation broth only, and fermentation broth with (b) active clay P-1G, (c) activated carbon CA-1, (d) activated carbon SX-PLUS, and (E) sylopute, all added at a concentration of 9 g/L.

CONCLUSIONS

Precipitation is a simple, efficient method for separating and recovering lactic acid in the form of calcium lactate from fermentation broth by adding sulfuric acid. In this study, we optimized major process parameters as well as the temperature of concentration and the type and amount of adsorbent used for pigment (color) removal. When the $Ca(LA)_2/H_2SO_4$ molar ratio was 1 : 1 and the pH was higher than the pKa value, calcium sulfate could be precipitated and efficiently removed; thus, lactic acid could be effectively separated and recovered from the supernatant. Mixing of the calcium lactate solution facilitated the precipitation process, which was complete in a minimum of 18 h. Mixing broke up the mass of precipitating calcium sulfate into smaller pieces. The efficiency of calcium sulfate removal was little affected by the injection method used to add sulfuric acid. A temperature of 90 °C was found to be optimal for the concentration of lactic acid recovered after removing precipitated calcium sulfate in terms of both the time required and the stability of the product. Activated carbon was found to be the most effective adsorbent for the removal of pigment from the recovered lactic acid after the precipitation process. Under the optimal precipitation conditions, a high yield (92%) could be achieved in recovering lactic acid from fermentation broth.

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