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pH Neutralization while Succinic Acid Adsorption onto Anion-Exchange Resins

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Abstract Succinic acid is a useful chemical and its purification from fermentation broth by ion-exchange resins has widely drawn attention. In this study, pH neutralization in the process of adsorption of succinic acid from model solutions and fermentation broth by anion-exchange resin NERCB 04 has been tested. Adsorption capacity of NERCB 04 was about 0.41 g succinic acid/g resin at concentrations of succinic acid in the range of 10–50 g/L in packed column. In the process of succinic acid removal, pH of the system could also be neutralized. The neutralizing ability of the resin as a neutralizing agent has also been studied in the model cycle system and in the real fermentation cycle process. It was found that NERCB 04 showed stable adsorption capacity and pH neutralization ability after each regeneration. A certain amount of anion-exchange resin could neutralize the low pH values (pH 2–5) and maintain the system around pH 7.0. This means the anion-exchange resins have the function of neutralizing reagent in the process of adsorbing succinic acid.

Keywords Succinic acid · Adsorption · pH neutralizing agent · Resin · Fermentation

Introduction

Succinic acid and its derivatives have [1] many applications in agriculture, foods, pharmaceuticals, and cosmetics. Traditionally, succinate can be produced from petrochemicals through costly processes which may cause some pollution problems.

As the price of oil rises, production by microbial fermentation and recovery of succinic acid from the fermentation broth have been widely studied [2].

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In the process of succinic acid production, purification makes up a major part of production costs. Some recovery methods, such as the reactive extraction [3, 4], electrodialysis [5, 6], and succinate precipitation [7], have been studied. These methods consist of many laborious energy-consuming or toxic/carcinogenic intermediate steps that limit the application especially for food manufacturing or pharmaceutical industry.

Ion-exchange resin has been widely used in the purification of organic acid products [8]. Although adsorption by resins is an attractive method for the separation of succinic acid, systematic investigation on ion-exchange resins for adsorption of succinic acid is seldom reported. Davison et al. reported that adsorption capacity of base polymer XUS 40285 was only about 0.06 g succinic acid/g resin at moderate concentrations (1–5 g/L) of succinic acid [9], and the regenerability with water was not good. Moreover, many operating details of ion exchange such as pH fluctuation in the process of adsorption should be further studied both in the model systems and in the actual fermentation systems in order to improve the separation process.

In the process of fermentation, production of succinic acid from microbial strains should not be run at low pH. So the fermentation system should be neutralized by adding neutralizing agents such as NaOH or Na_2CO_3 [10]. But literature has seldom reported that the alkaline anion-exchange resins can act as a solid neutralizing reagent. As the hydroxyl group linked to the framework of alkaline anion-exchange resins, these resins have the function of pH neutralization while adsorbing targeted acids.

In this paper, adsorption of succinic acid from the model system and fermentation broth with NERCB 04 was developed in columns. The adsorption capacity in column was also presented. When succinic acid was adsorbed onto the alkaline anion-exchange resins, the ability of pH neutralization at different initial pH value of succinic acid solutions was investigated for the first time.

Materials and Methods

Materials

All chemicals used in this study were of analytical grade and were purchased from either OXOID (England) or Sinopharm Chemical Reagent Beijing Co., Ltd. (China) unless otherwise described. NERCB 04 was a multiple hydroxyl anion-exchange resin provided by the National Engineering Research Center for Biotechnology (Beijing, China), with 64.1% water content. The resin was pretreated with NaOH.

Adsorption and Desorption

Adsorbent NERCB 04 was packed in a column (GE Co., Ltd., USA) (Ø 34 mm, 10 cm height). At room temperature, the bed was first equilibrated with 10 BV (bed volume) water, then continuously loaded with a solution of succinic acid in the range of 10–50 g/L in simulated medium (pH 5.2) until the bed was fully exhausted with the succinic acid. Samples were taken with a fraction collector and analyzed by the HPLC to create a breakthrough curve. Following water washing, the spent resins were eluted and regenerated from the column by 0.5 M NaOH. The flow rate of 2.5 mL/min was controlled by the peristaltic pump in the column process.

The data of adsorption capacity from column were calculated using the Thomas equation. The equation is represented by [11, 12]

$$C/C_0 = 1/\left(1 + \exp\left(K_{\mathrm{TH}}\left(q_{\mathrm{eq}}N - C_0V_{\mathrm{eff}}\right)/Q\right)\right)$$

Where K_{TH} is the Thomas rate constant (mL/(min mg)) and Q is the volumetric flow rate (L/min). The linearized form of the Thomas equation is as follows:

$$\ln(C/C_0) = K_{\mathrm{TH}}N/Q - K_{\mathrm{TH}}V_{\mathrm{eff}}/Q$$

The kinetic coefficient K_{TH} and sorption capacity of the bed q_{eq} can be determined from a plot of $\ln((C_0/C)-1)$ against time at a given flow rate.

pH Neutralization by NERCB 04 in the Model System

The column adsorption was then coupled with the bioreactor to test the pH neutralizing ability and adsorption capacity of the resin. A peristaltic pump helped to realize the recycle of succinic acid solutions between the column (above) and a 2.5-L KBT bioreactor (Ko Bio Tech Co., Lid, Korea). In model recycle process of adsorption, about 2 g/L succinic acid (pH 2.8 and pH 5.5, respectively) solutions were added into the bioreactor periodically. The adsorption capacity and the pH value were investigated.

pH Neutralization by NaOH or NERCB 04 in Fermentation System

Bacterial strains *Actinobacillus succinogenes* 130Z (ATCC 55618) [13] was obtained from the American Type Culture Collection.

Seed cultures were prepared by growing cells at 37°C, 155 rpm in an aerobic flask containing 3% (wt/vol) tryptic soy broth (TSB) medium (pancreatic digest of casein 17 g/L, soy peptone 3 g/L, dextrose 3 g/L, NaCl 5 g/L, K_2HPO_4 2.5 g/L). The fermentation medium (per liter): 30 g glucose, 15 g yeast extract, 2 g $MgCl_2{\cdot}6H_2O$, 1.5 g $CaCl_2{\cdot}2H_2O$, and 0.07 g $MnCl_2$. Glucose was separately sterilized at 115°C for 30 min and added to the medium. The initial pH of the sterilized medium was adjusted to 7.0 with 10 M NaOH. The TSB medium was inoculated with 1 mL of glycerol stock culture and incubated at 37°C, 155 rpm in an aerobic chamber until the optical density at 660 nm (OD_{660}) was about 1.4–1.6. A 5% (vol/vol) inoculum was used.

Batch fermentation experiments were conducted under anaerobic conditions. The initial medium volume was 1 L in a 2.5-L KBT bioreactor (Ko Bio Tech Co.). The pH was measured using a glass electrode and controlled at 6.8 using 10 M NaOH or resin NERCB 04 packed in column (above). The temperature was maintained at 37°C and the agitation speed was constant at 100 rpm. The recycle of broth was realized by a peristaltic pump.

Analytical Methods

Optical density (OD) of the fermentation broth was tested with a spectrophotometer (Ubest-30, Jasco, Tokyo, Japan) at a wavelength of 660 nm to estimate the biomass concentration. The concentrations of glucose were measured by SBA 40C bio-sense analyzer (Biology Institute, Shandong Academy of Sciences, China). The concentrations of succinic acids were measured by high-performance liquid chromatography (Agilent 1200) equipped with an Agilent model VVD UV–Vis detector and the Zorbax SB-Aq column (250 mm×

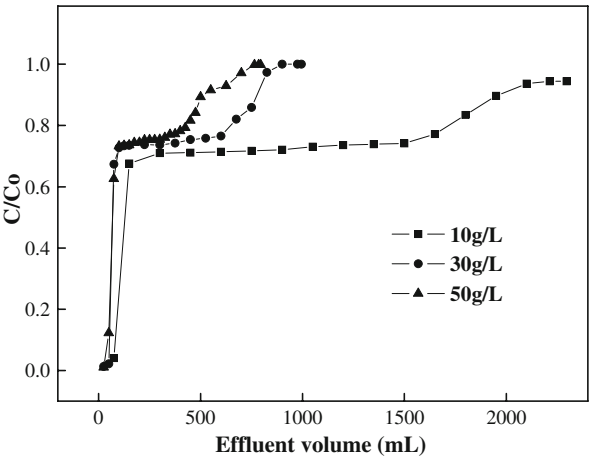


Fig. 1 Effect of succinic acid concentrations on breakthrough capacity

4.6 mm, Agilent) with pH of 2.7, 20 mmol/L KH_2PO_4 as the mobile phase. The flow rate was 1 mL/min, with a column temperature of 25°C and an injection volume of 20 μL.

Results and Discussion

The Breakthrough Curve

The effect of succinic acid concentration on the breakthrough curves was studied with the flow rate of succinic acid solution at 2.5 mL/min. The sampling concentrations of succinic acid solutions were chosen as 10 g/L, 30 g/L, and 50 g/L. As shown in Fig. 1, it took 2,212.5 mL, 900 mL, and 765 mL for the value of C/C_0 to reach 0.95, 1.0, and 1.0 respectively. It was illustrated that the breakthrough time decreased with increasing effluent concentration. The column adsorption ability was presented with the Thomas equation and was given in Table 1. As the concentrations of succinic acid increased, the values of K_{TH} increased and the values of q_{eq} increased. The data in Table 1 also showed that the max total adsorption capacity was 410.5 mg/g.

Figure 2 showed the pH values of effluent through the adsorption column. Since in the process of adsorption the hydroxyl of the resin was exchanged by the succinate, the pH values of the effluent went down. At last, the pH values of the effluent decreased to the pH value of the feeding solution. The concentrations of succinic acid have significant effects on the breakthrough time and pH values of effluents.

After washing with water till pH 7.0, the spent resins were eluted by 0.7 M NaOH and gave 97% average regenerability with the SD value 0.015±3. This successive column

Table 1 Column parameters analyzed with the Thomas equation.

Q (mL/min)	C_0 (g/L)	K_{TH} (mL/min/mg)	$q_{eq.cal}$ (mg/g)	$q_{eq.exp}$ (mg/g)
2.5	9.924	0.000344	321.2	221.705
2.5	31.002	0.000415	378.8	495.014
2.5	50.012	0.000539	410.5	511.949

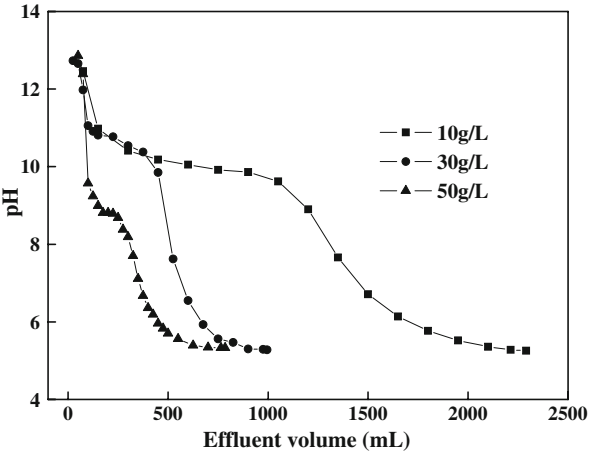
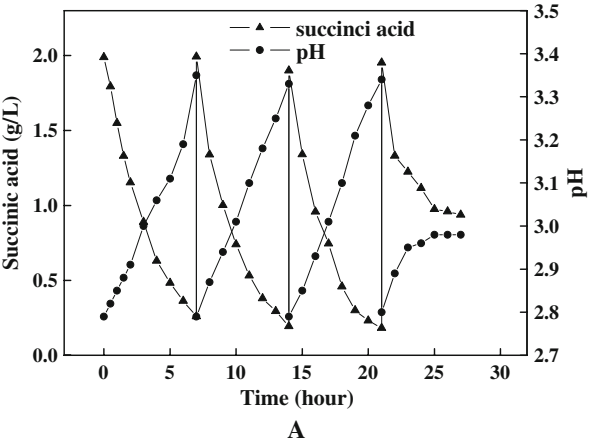


Fig. 2 Effect of succinic acid concentrations on pH curves of effluent



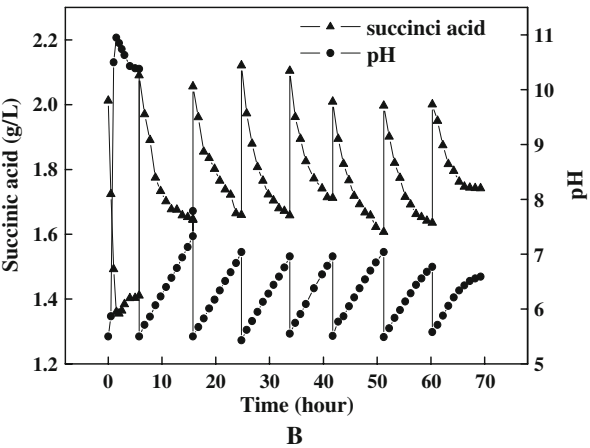


Fig. 3 Cycle adsorption of succinic acid between column and bioreactor added succinic acid solution (~2 g/L) at the initial pH 2.8 (**a**) or pH 5.5 (**b**)

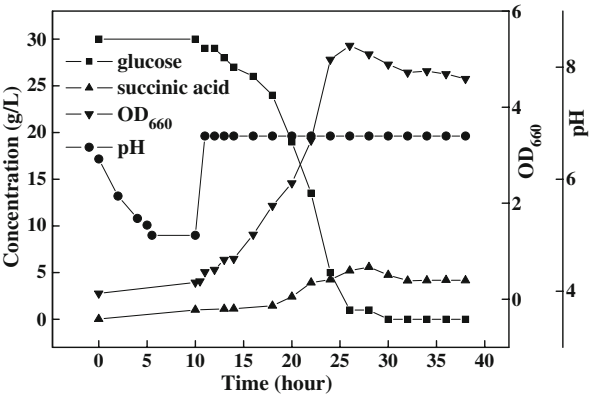


Fig. 4 Batch fermentation of succinic acid by *A. succinogenes* 130Z with NaOH as the pH neutralizing agent

loading and regeneration process was repeated for at least 30 times with stable adsorption capacity and regenerability.

pH Neutralization in Model System

To study the periodic adsorption capacity of the alkaline anion-exchange resin, the adsorption was conducted in a cycling system that coupled the column to the succinic acid solution. The periodic curves were shown in Fig. 3. The concentration of succinic acid was 2 g/L at pH 2.8 (Fig. 3a) and pH 5.5 (Fig. 3b), respectively. In each cycle, the succinic acid in the solutions was adsorbed and the pH value in the cycling system increased each time. After several cycles, the resins in the column got saturated. According to Fig. 3, the recycle times depended on the initial pH of the model solution, which can be explained by the finite activation of functional hydroxyl sites on the resin.

pH Neutralization in Real Fermentation System

In the process of fermentation without pH control, the pH value did not continue to decrease in the first 5 h until it reached pH 5.1 (Figs. 4 and 5 shown). This may be caused by the accumulation of acidic metabolites of succinic acid producers [14, 15]. In the period of low pH, the microbial cell grew slowly; the OD_{660} was less than 0.4 and the depletion of

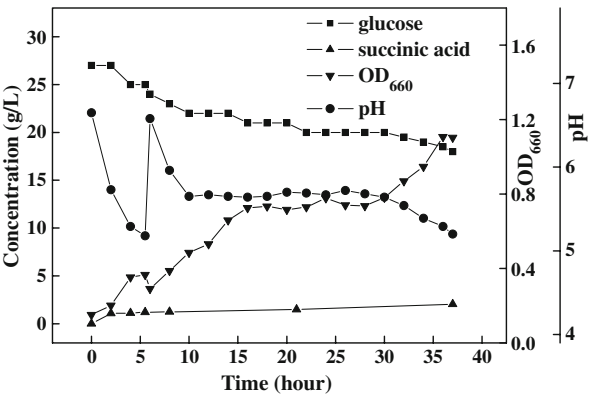


Fig. 5 Batch fermentation of succinic acid by *A. succinogenes* 130Z with alkaline anion-exchange resins as the solid pH neutralizing agent

glucose was very little. As a result, the fermentation of succinic acid could not be run at low pH, thus it should be neutralized by adding neutralizing reagents.

As shown in Fig. 4, NaOH was added to prevent the pH from dropping below 5.0. The pH was maintained at 6.77 after 10 h. The maximum concentrations of succinic acid in a batch fermentation were 5.60 g/L and productivity was 0.19 g/g glucose. To study the pH neutralization ability of the alkaline anion-exchange resin, the bioreactor was coupled with the anion-exchange column. According to Fig. 5, both the adsorption of succinic acid from the broth and the pH neutralization of the fermentation broth were realized by the resins at the same time. The total concentrations of succinic acid were 3.96 g/L and the succinic acid productivity was 0.44 g/g glucose. Although the ability of anion-exchange resin acting as a pH neutralizing reagent was less than that of NaOH, it could still play an important role in succinic acid production and pH neutralization. Otherwise, the results confirmed the need for combining pH control with succinate removal.

As shown in Fig. 5, the fermentation was inhibited because the adsorption capacity of the resin in the column got saturated. After that, the resin could not realize the function of neutralization. So the neutralizing ability depends on the number of resin packed. In the neutralizing system, the volume of resin packed in the column was only 90.7 mL, and Fig. 5 only showed one cycle of the pH neutralization by resin. After they were eluted and regenerated with NaOH, the resins were refreshed again. So the inhibition was dismissed and the cell growth was continuous in another cycle. Also, the column can be changed to a bigger one so that more counts of resins can be packed to increase the succinic acid adsorbed.

Conclusion

The feasibility of adsorption of succinic acid with alkaline anion-exchange resins was studied in model and fermentation systems. Concentrations of succinic acid have a significant effect on the breakthrough curve and ability of pH neutralization. The resin NERCB 04 was effective in removing succinic acid from solution in column. It could neutralize the pH as a solid pH buffer in the real fermentation process too. According to the roles of pH neutralization with alkaline anion-exchange resins, it can be applied as a new tool to couple in situ product removal process and in situ pH neutralization process in the organic acid fermentation industry.

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