Separation of Succinic Acid from Fermentation Broth Using Weak Alkaline Anion Exchange Adsorbents

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Succinic acid is one of the platform chemicals that can be bioproduced from renewable resources. Separation of succinic acid by adsorption from model solutions and fermentation broth by weak alkaline anion exchange adsorbents was studied. Adsorption capacities and regenerability of several sorts of adsorbents were tested. In a static test, the adsorbent NERCB 09 has the adsorption capacity of 0.11 g succinic acid g^{-1} at succinic acid concentrations of 5 g L^{-1}. In packed column test, its capacity was as high as 0.56 g succinic acid g^{-1} when the feeding concentration was 50 g L^{-1}. NERCB 09 showed the high selectivity toward succinate over both glucose and amino acid at acidic or neutral conditions. Langmuir/Freundlich isotherm models and pseudo-first/second-order equations were presented to simulate the adsorption behavior. Data showed that the temperature had little effect on the adsorption isotherm. Kinetic parameters suggested that about 1.5 h were sufficient for the adsorption equilibrium. The adsorbent was easily regenerated. The adsorption capacity was steady after 30 cycles and showed 96% average recovery.

1. Introduction

Succinic acid is a useful platform chemical that can be converted into many other valuable chemicals.[1] It has many applications in agriculture and food industries. Some new succinic acid derivatives have been found anti-inflammatory pharmaceutical activities.[2] Succinic acid is traditionally produced from petrochemicals, which is costly and causes some pollution problems. As the rise of crude oil price, producing succinate via fermentation has drawn great concerns.[3]

During the process of fermentation, $Ca(OH)_2$ or $CaCO_3$ is usually added to neutralize the fermentation broth and precipitate the succinate.[4] In the traditional process, a large amount of $CaSO_4$ was accumulated, which is commercially useless. Many other methods were studied to recover succinic acid from the fermentation broth.[5] The reactive extraction with amine-based extractant has been widely reported.[6−10] This method is easy to carry our via controlling pH value of the aqueous phase. However, the process is often interrupted by contaminated acids, impurities, carbon sources, protein, or salts. Electrodialysis is easily used to separate succinate from nonionized compounds with proper ion exchange membrane, although membranes are usually expensive and easily polluted.[11] An integrated system combining the electrodialysis process and the continuous fermentation has realized in situ recovery of succinic acid.[12] In addition, these processes typically yield large amounts of solid and slurry wastes that need to be further treated and properly disposed. Much improvement in recovery methods is still needed to make the whole purification process economically feasible.

Adsorption is a promising separation method for recovery of the succinic acid because adsorbents have the advantages of low price, quick recovery, and low regeneration consumption.[13,14] Ion exchange adsorption has been widely used in many organic acids separations early, for example, the adsorption capacity toward lactic acid was about 0.1−0.7 g g^{-1} dry adsorbent.[15] However, the relatively low adsorption capacity and the selectivity of adsorbents limit the industrial application in succinic acid separations. It is necessary to screen sorts of adsorbents with high capacity. Davison et al. reported that the reusable adsorption capacity of base polymer XUS 40285 and XFS 40422 was 0.06−0.07 g g^{-1} and the adsorption capacity of PVP resin Reillex 425 was 0.05−0.08 g g^{-1} at succinic acid concentrations of 5 g L^{-1}.[16] Jun et al. used modified inorganic mesoporous silica SBA-15 to separate succinic acid directly from fermentation broth. The adsorption capacity of SBA-15 could reach 0.007−0.058 g g^{-1} and it adsorbed more pyruvic acid than succinic acid.[17]

In this paper, separation of succinic acid from the fermentation broth with weak alkaline anion exchange adsorbents was studied. One kind of weak alkaline anion exchange adsorbents with good capacity was screened out after a series of batch tests. This adsorbent has the abilities of preferring the target acid over byproducts and impurities. The saturated adsorptive capacity, adsorption rate, and isothermal curve were investigated. The adsorption behavior was also conducted in a column test.

2. Materials and Method

2.1. Strain Cultivation and Fermentation Conditions. *Actinobacillus succinogenes* 130Z (ATCC 55618) was obtained from the American type Culture Collection. Seed cultures were

Table 1. Screening of Adsorbents for Succinic Acid Separation

adsorbent	type	water content (%)	capacity (g g^{-1})	regenerability (%)
NERCB 01	acrylic	66.3 ± 0.3	0.043 ± 0.002	0.88 ± 0.01
NERCB 02	acrylic	46.8 ± 0.4	0.060 ± 0.001	0.98 ± 0.02
NERCB 03	styrene	52.9 ± 0.2	0.033 ± 0.008	0.68 ± 0.01
NERCB 04	epoxy	64.1 ± 0.5	0.042 ± 0.001	0.94 ± 0.01
NERCB 05	acrylic	61.4 ± 0.2	0.034 ± 0.001	0.88 ± 0.04
NERCB 06	acrylic	69.4 ± 0.3	0.025 ± 0.001	0.19 ± 0.07
NERCB 07	polystyrene	55.6 ± 0.1	0.041 ± 0.001	0.63 ± 0.03
NERCB 08	polystyrene	65.4 ± 0.5	0.019 ± 0.004	0.52 ± 0.04
NERCB 09	polystyrene	56.0 ± 0.2	0.061 ± 0.001	0.96 ± 0.01
NERCB 10	polystyrene	70.5 ± 0.1	0.116 ± 0.006	0.91 ± 0.04
NERCB 11	polystyrene	68.6 ± 0.2	0.039 ± 0.002	0.99 ± 0.09

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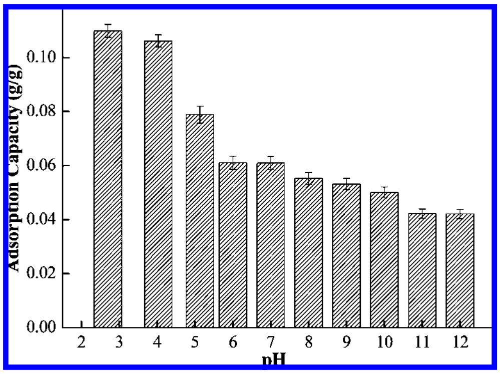

Figure 1. Effect of pH value on the adsorption capacity of NERCB 09.

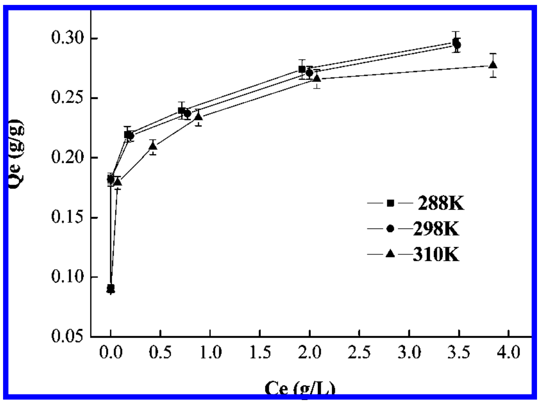

Figure 2. Effect of temperature on the adsorption isotherm of NERCB 09. C_e is the final concentrations of adsorption capacity (g L^{-1}), Q_e is the equilibrium adsorption capacity (g g^{-1}).

prepared at 37 °C and 160 rpm in an aerobic flask containing 3% (wt vol^{-1}) Tryptic soy broth (TSB) medium (pancreatic digest of casein 17 g L^{-1}, soy peptone 3 g L^{-1}, glucose 3 g L^{-1}, NaCl 5 g L^{-1}, K_2HPO_4 2.5 g L^{-1}). The flask medium contained per liter: 30 g glucose, 15 g yeast extract, 2 g urea, 2 g $MgCl_2 \cdot 6H_2O$, 1.5 g $CaCl_2$, 0.07 g $MnCl_2$, 4.4 g Na_2HPO_4, 3.3 g NaH_2PO_4, 20 g $MgCO_3$. All chemicals used in this study were of analytical level and were purchased from either OXOID (England) or Sinopharm Chemical Reagent Beijing Co., Ltd. (China) unless otherwise described.

2.2. Adsorbents Tests. All the adsorbents were provided by the National Engineering Research Center for Biotechnology (NERCB, Beijing, China). Static adsorption processes were carried out with 20 mL solutions of 5 g L^{-1} succinic acid at 25 °C. The spent adsorbent was regenerated with a 1.0 mol L^{-1} NaOH at 37 °C and treated to hydroxide. The selectivity of succinic acid against glucose was studied in 100 mL flask with fresh adsorbent with simulated succinct solutions. The amino acid tests and cycle adsorption tests were with actual fermentation broth. Calculated capacities are based on the dry weights calculated from dried samples.

2.3. Adsorption Characteristics of NERCB 09. Different pH values of succinic acid solution were chosen to determine the effect of pH on succinic acid static adsorption. The initial pH value of the solution was adjusted by solid NaOH.

2.4. Adsorption Isotherm. The equilibrium adsorption studies were conducted in a 150 mL flask. The adsorption temperatures were chosen as 288, 298, and 310 K. The pH values were 2.6, 5.0, and 7.0.

2.5. Kinetic Studies. Adsorption kinetics was studied with 200 mL solutions of succinic acid. The effects of temperature and pH value were studied. The temperatures were chosen as 288, 298, and 310 K, and the pH values were 3.0, 5.0, and 7.0.

2.6. Column Adsorption and Desorption. Adsorbent NERCB 09 was packed in a column (10 cm × 26 mm i.d.) (General Electric, GE, USA). At room temperature, to test the adsorption properties of NERCB 09, the concentrations of succinic acid solutions were 10−50 g L^{-1}. The flow rate was chosen as 1, 2.5, and 4 mL min^{-1}. The test of feeding glucose chose glucose solutions at 50, 70, and 90 g L^{-1} concentrations. The packed column had a 26 mm i.d. and was 28 cm long, and the flow rate was 4 mL min^{-1}.

2.7. Analysis Methods. Biomass concentrations were estimated from the optical density (OD) of the fermentation broth with a spectrophotometer (723N, Shanghai Precision & Scientific Instrument Co. Ltd., China) at a wavelength of 660 nm. The concentrations of glucose were measured by SBA 40D biosense analyzer (Biology Institute, Shandong Academy of Sciences, China). The concentration of amino nitrogen was analyzed by formaldehyde titration.[18] The concentrations of organic acids were measured by high-performance liquid chromatography (Agilent 1200, USA) equipped with an Agilent model DAD UV−vis detector and the ZORBAX SB-Aq column (25 cm × 4.6 mm, 5 μm; Agilent). The column was operated at 25 °C. The mobile phase was 20 mmol L^{-1} KH_2PO_4/H_3PO_4 buffer at a flow rate of 1.0 mL min^{-1}. The pH value of mobile phase was 2.7. The injection volume was 10 μL.

3. Results and Discussion

3.1. Adsorbents Screening. As shown in Table 1, a series of alkaline anion exchange adsorbents were screened. Adsorption capacities of 11 sorts of adsorbents were studied in pH 7.0, 5.0 g L^{-1} succinic acid solution at 25 °C. The ratio of the solution volume to the adsorbent was 20 mL 5 g^{-1}. The adsorbents were regenerated with 0.5 mol L^{-1} NaOH solution. Some styrene types and the epoxy types of weak alkaline anion exchange adsorbents had relatively low adsorption capacity and regenerablity, such as NERCB 03, 04. The acrylic type adsorbent NERCB 02 and the polystyrene type adsorbents NERCB 09, 10 had higher adsorption capacity and regenerablity than other adsorbents. Their capacities were 0.060, 0.061, and 0.116 g g^{-1}, respectively. Their regenerability were 98%, 96%, and 91%, respectively.

Besides the adsorption capacity toward succinic acid and regenerability, selectivity is another critical factor in the process of succinic acid separation. There are large amounts of organic acids, proteins, amino acids, and glucoses in the fermentation broth. As shown in Supporting Information Table 1, the selectivity of succinic acid against glucose was studied. In the model solutions, the concentration of succinic acid was 5.0 g L^{-1}, and the concentration of glucose was 5.0 g L^{-1}. The selectivities of NERCB 02 and NERCB 10 were 2.3 and 2.0, respectively. The selectivity of NERCB 09 is 8.93, which was much higher than any other adsorbents except NERCB 07. NERCB 07 did not adsorb glucose, but its desorption ability was poor, only 56%. The desorption ability toward succinic acid and glucose of NERCB 09 was also higher than those of NERCB 03 and NERCB 10. Davision et al. reported that the resin should preferred succinic acid against glucose, with the ratio 4:1 for Reillex 425, 4:1 for XUS 40091, and 8:1 for XUS 40285.[16] The further testing in the column showed that NERCB 09 did not adsorb glucose at all.

The selectivity of succinic acid against protein and amino acids was studied with succinic acid fermentation broth containing microbial cells. In the screening process, the literature

Table 2. Langmuir Constants of Adsorption at Different Temperatures[a]

T(K)	Langmuir eq	Q_o (g g^{-1})	K_L (L g^{-1})	R^2
288	$C_e/Q_e = 0.288 + 3.350C_e$	0.299 ± 0.002	11.641 ± 0.003	0.998 ± 0.002
298	$C_e/Q_e = 0.318 + 3.378C_e$	0.296 ± 0.001	10.632 ± 0.05	0.998 ± 0.004
310	$C_e/Q_e = 0.310 + 3.565C_e$	0.281 ± 0.003	11.500 ± 0.001	0.999 ± 0.001

[a] Langmuir equation: $C_e/Q_e = C_e/Q_0 + (1/K_L)Q_0$;[19] C_e is the final concentrations of sample adsorption capacity (g L^{-1}), Q_e is the adsorption capacity (g g^{-1}), and Q_0 and K_L are the Langmuir constants.[19,20]

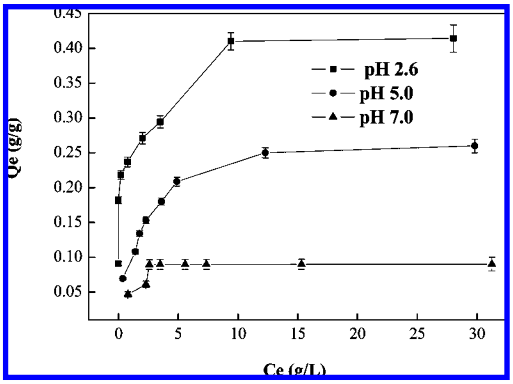

Figure 3. Effect of pH on the adsorption isotherm of NERCB 09. C_e is the final concentrations of adsorption capacity (g L^{-1}), Q_e is the equilibrium adsorption capacity (g g^{-1}).

seldom reported the selectivity screening between target acid and amino acids. However, the selectivity of succinic acid against amino acids is important especially in the in situ products removal (ISPR) process. Concentrations of amino nitrogen in the fermentation broth usually reflect the concentrations of the protein and amino acids.[18] Thus, the selectivity of succinic acid against amino nitrogen was tested. In the fermentation broth, the concentrations of succinic acid, acetic acid, formic acid, and lactic acid were 15.0, 5.2, 4.5, and 1.5 g L^{-1}, respectively. The adsorption capacity ratio of succinic acid, acetic acid, formic acid, and lactic acid was 40:1:20:0. The concentration of the amino nitrogen was 1.09 g L^{-1}. As shown in Supporting Information Table 2, the selectivity of NERCB 03 was 23.9, which was the highest than that of any other adsorbents. However, the desorption ability of succinic acid from NERCB 03 was much lower. In the present study, different adsorbents were tested for the critical properties of capacity for succinic acid, regenerability of the sorbent, and coadsorption of substrates. NERCB 09 was chosen as a suitable adsorbent for further research.

3.2. Adsorption Characteristics of NERCB 09. The pH value of fermentation broth has significant effect on the adsorption properties of anion exchange adsorbent.[13,14] As Figure 1 shown, low pH is suitable for the ion-exchange process. In acidic solution, two types of adsorption processes may exist. One is an ion-exchange process, and the other is hydrophobic adsorption. When the pH value is less than the pK_a of the organic acids, the acids usually are in the form of free acid. And the hydrophobic group of free acid may get easily close to the polystyrene hydrophobic group.

3.3. Adsorption Isotherm. The equilibrium adsorption processes were conducted in 150 mL flask at the temperature of 288, 298, and 310 K. Figure 2 showed plots of the adsorption of succinic acid on NERCB 09. As shown in Figure 2, adsorption temperature had little effect on the adsorption isotherm of NERCB 09. The adsorption capacity decreased little with the rise of temperature. According to the results, low temperature is favorable for the adsorption. The experimental equilibrium data were fitted to the Langmuir and Freundlich models. Different Freundlich (data not shown) and Langmuir constants derived from these plots are presented in Table 2. Langmuir isotherms provided very good fittings over the temperatures range studied.

As shown in Figure 3, the equilibrium adsorption capacity decreased with the rise of pH value. This result was in accordance with the result of Figure 1. The adsorption process is easily promoted by the ion-exchange process and the hydrophobic adsorption at low pH value. The experimental equilibrium data as shown in Table 3 were fitted to the Langmuir and Freundlich models.[19,20] The adsorption behavior of NERCB 09 agreed with the Langmuir isotherm model well.

3.4. Kinetic Studies. As shown in Supporting Information Figures 1 and 2, temperature had little effect on the adsorption capacity, but adsorption capacity increased dramatically when the pH value decreased. Both the pH and temperature had little effect on the adsorption rate. Kinetics parameters suggested that about 1.5 h were sufficient for the adsorption equilibrium. So, the adsorbent can be used for many practical purposes and applications that require short time adsorption. Normally adsorption kinetics can be described by two adsorption kinetic models, pseudo-first-order and pseudo-second-order equations proposed by Lagergren and Ho and McKay (data not shown).[21,22] The values of correlation coefficients were used to compare the validity of each model as shown in Table 4. The pseudo-second-order kinetic model provided very good fittings over the temperature and pH range studied, whereas the pseudo-first-order kinetic model was less accurate.

3.5. Column Adsorption. To study the dynamics of adsorption, NERCB 09 was tested for the removal of succinic acid in a packed column. The effect of flow rate on the breakthrough curve of NERCB 09 was shown in Supporting Information Figure 3. It can be seen that flow rate had little effect on the breakthrough curve after zero-dimension. Sixteen bed volumes were enough for the breakthrough after feeding. It seems that the velocity of adsorption is much faster than that of the influent stream. The effect of succinic acid concentrations on the breakthrough curve of NERCB 09 is shown in Figure 4. It can be seen that succinic acid concentrations has significant effect on the breakthrough of NERCB 09. By calculations of column parameters of Figure 4, the capacity can reach 0.56 g succinic acid g^{-1} when the feeding concentration was 50 g L^{-1}. But when the feeding concentration was 10 g L^{-1}, the capacity of adsorbents was 0.38 g succinic acid g^{-1}. The breakthrough curves of succinic acid showed the adsorption velocity of succinic acid on the adsorbent. The adsorption velocity was found to increase with the rise of concentration. The recovery of succinic acid was considerable and convenient. This successive column loading and regeneration process was repeated 30 times in a stable manner, and the average regenerability was 96% with a standard deviation (SD) value of 0.011 ± 0.004.

As shown in Figure 5, NERCB 09 was tested for the adsorption of glucose in a packed column. Glucose at different concentrations (53, 72, and 92 g L^{-1}) was fed to test the effect on the breakthrough curve of NERCB 09 at 4 mL min^{-1} at room temperature. The glucose can realize breakthrough after feeding 6 bed volume solutions of glucose. Between 6 and 10 bed

Table 3. Langmuir Constants of Adsorption at Different pH Values[a]

pH	Langmuir eq	Q_o (g g^{-1})	K_L (L g^{-1})	R^2
2.6	$C_e/Q_e = 1.381 + 2.385C_e$	0.724 ± 0.001	0.579 ± 0.002	0.999 ± 0.001
5	$C_e/Q_e = 6.195 + 3.607C_e$	0.161 ± 0.003	1.718 ± 0.002	0.999 ± 0.001
7	$C_e/Q_e = 4.507 + 11.780C_e$	0.222 ± 0.008	0.383 ± 0.003	0.984 ± 0.005

[a] Langmuir equation:[19] $C_e/Q_e = C_e/Q_0 + (1/K_L)Q_0$; C_e is the final concentrations of sample adsorption capacity (g L^{-1}), Q_e is the adsorption capacity (g g^{-1}), and Q_0 and K_L are the Langmuir constants.[19,20]

Table 4. Modeling Parameters of the Pseudo-Second-Order Equation[a]

temperature (K)	Q_e (g g^{-1})	K_2 (g g^{-1} h^{-1})	R^2	pH	Q_e (g g^{-1})	K_2 (g g^{-1} h^{-1})	R^2
288	0.319 ±0.011	48.838 ±0.013	0.999 ±0.001	3	0.263 ±0.006	33.189 ±0.013	0.999 ±0.002
298	0.263 ±0.006	33.189 ±0.013	0.999 ±0.002	5	0.198 ±0.008	33.014 ±0.010	0.999 ±0.002
310	0.311 ±0.008	81.927 ±0.006	1 ±0.003	7	0.083 ±0.006	38.134 ±0.009	0.999 ±0.003

[a] Q_e, K_t, and K_2 are the pseudo-second-order equation constants.[21,22]

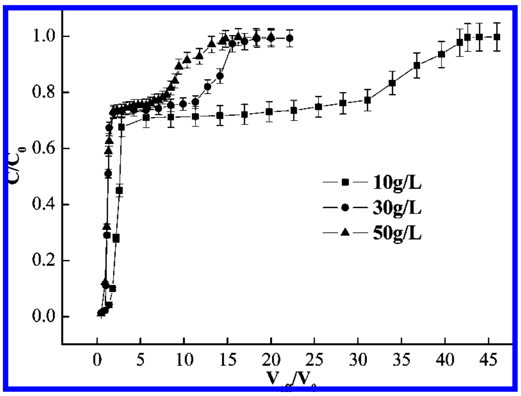

Figure 4. Effect of concentrations of succinic acid on the breakthrough curve of NERCB 09. C is the concentration of the influent stream (g L^{-1}). C_0 is the concentration of the feeding solution (g L^{-1}). V_0 is the bed volume. V_{eff} is the effective volume of the influent stream.

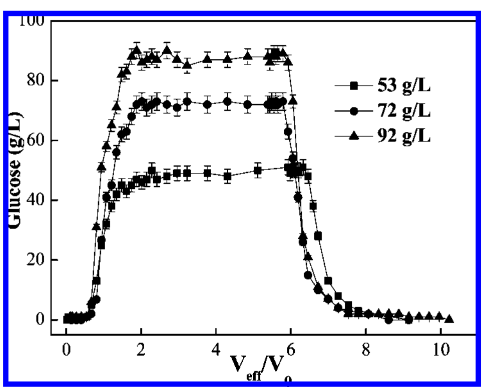

Figure 5. Effect of concentrations of glucose on the breakthrough curve of NERCB 09. V_0 is the bed volume. V_{eff} is the effective volume of the influent stream.

volumes, water was used to wash the bed. Surprisingly, the glucose can be washed out of the column totally. So succinic acid adsorption was chemical adsorption, while the glucose adsorption was physical adsorption as the adsorbent can not adsorb the glucose but only "hold" the glucose for a while.

4. Conclusion

Adsorption with weak alkaline anion exchange adsorbents was a good method to separate succinic acid from the fermentation broth. NERCB 09 was effective to separate succinic acid from the model solution and fermentation broth because it had high capacity, selectivity, and adsorption rate. The Langmuir isotherm model and a pseudo-second-order equation model were adopted for representing the static and kinetic adsorption behavior. Adsorbent NERCB 09 showed high selectivity toward succinate over glucose and amino acid at acidic and neutral conditions. The adsorption temperature had little effect on the adsorption isotherm so that adsorption operation can be carried out at a normal temperature. The adsorption rate was quick. A period of 1.5 h was sufficient for the adsorption equilibrium. The adsorbent was easily regenerated with base or acid. And the adsorption process can be integrated with a bioreactor system to realize in situ product removal.

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Supporting Information Available: Tables 1 and 2 showing additional information in the adsorbent selectivity of succinic acid toward nutrition in the broth; Figures 1 and 2 showing additional information on the effect of temperature and pH on adsorption kinetics; Figure 3 showing additional information on the effect of the flow rate on the breakthrough curve. This material is available free of charge via the Internet at http://pubs.acs.org.

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