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# Succinic Acid Adsorption from Fermentation Broth and Regeneration

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## Abstract

More than 25 sorbents were tested for uptake of succinic acid from aqueous solutions. The best resins were then tested for successive loading and regeneration using hot water. The key desired properties for an ideal sorbent are high capacity, complete stable regenerability, and specificity for the product. The best resins have a stable capacity of about 0.06 g of succinic acid/g of resin at moderate concentrations (1–5 g/L) of succinic acid. Several sorbents were tested more exhaustively for uptake of succinic acid and for successive loading and regeneration using hot water. One resin, XUS 40285, has a good stable isotherm capacity, prefers succinate over glucose, and has good capacities at both acidic and neutral pH. Succinic acid was removed from simulated media containing salts, succinic acid, acetic acid, and sugar using a packed column of sorbent resin, XUS 40285. The fermentation byproduct, acetate, was completely separated from succinate. A simple hot water regeneration successfully concentrated succinate from 10 g/L (inlet) to 40–110 g/L in the effluent. If successful, this would lower separation costs by reducing the need for chemicals for the initial purification step. Despite promising initial results of good capacity (0.06 g of succinic/g of sorbent), 70% recovery using hot water, and a recovered concentration of >100 g/L, this regeneration was not stable over 10 cycles in the column. Alternative regeneration schemes using acid and base were examined. Two (XUS 40285 and XFS-40422) showed both good stable capacities for succinic acid over 10 cycles and >95% recovery in a batch operation using a modified extraction procedure combining acid and hot water washes. These resins showed comparable results with actual broth.

**Index Entries:** Succinic acid; sorbent; adsorption; hot water regeneration; glucose; fermentation broth.

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## Introduction

Many industrial organic acids can be produced by fermentation, such as acetic, citric, and lactic acids. Succinic acid is a dicarboxylic acid of potential industrial interest as a platform chemical (1–3). Separation and purification of succinic acid by adsorption was tested to replace current precipitation methods and their associated waste disposal problems. Succinic acid is a valuable intermediate value chemical with a moderate market. For succinic acid to have an economic and energy impact, it will need to become a commodity chemical intermediate with a much lower price. This target price has been estimated to be between $0.22 and $0.30/lb ($0.48–$0.66/kg) and is potentially achievable with advanced technology *(1)*. At this price, succinic acid can be catalytically upgraded into other higher valued chemicals such as tetrahydrofuran, 1,4-butanediol, γ-butyrolactone, 2-pyrrolidinone, and *N*-methylpyrrolidinone.

There is no current commercial biologic process for the production of succinic acid. In past laboratory systems, when succinic acid has been produced by fermentation, lime is added to the fermentation medium to neutralize the acid, yielding calcium succinate *(2)*. The calcium succinate salt then precipitates out of the solution. Subsequently, sulfuric acid is added to the salt to produce the free soluble succinic acid and solid calcium sulfate (gypsum). The acid is then purified with several washings over a sorbent to remove impurities. The disposal of the solid waste is both a directly economic and an environmental concern, as is the cost of the raw materials. Some key process-related problems have been identified as follows: (1) the separation of dilute product streams and the related costs of recovery, (2) the elimination of the salt waste from the current purification process, and (3) the reduction of inhibition to the product succinic acid on the fermentation itself. Acetic acid is also a byproduct of the fermentation of glucose by *Anaerobiospirillium succiniciproducens*; almost 1 mol of acetate will be produced for every 2 mol of succinate *(3)*. Under certain cultivation conditions by a mutant *Escherichia coli,* lesser amounts of acetate can be produced *(4,5)*. This byproduct will also need to be separated.

Adsorption has shown good potential and some data have been gathered for the distribution properties of other carboxylic acids, including acetic, lactic, and citric acids. These sorbents were tested with succinic acid. The key desired properties for an ideal sorbent are high capacity, complete low-cost stable regenerability, and specificity for the product. The economics of the adsorption process have not been evaluated in detail; however, as a stand-alone primary separation, an ultimate goal of a final concentration of >100 g of succinic acid/L was chosen as likely to be economically feasible if achieved.

Solid sorbents have been used and studied for subsequent product recovery from fermentation broth *(6)*. Sorbents were used in a subsequent fluidized-bed contactor for product recovery from fermentation broths *(7)*. Kaufman et al. *(8)* tested a variety of sorbents for lactic acid. Garcia *(6)*

and Garcia and King *(9)* have reviewed the use of some polymeric adsorbents for acetic acid. Many of these sorbents are weak base resins such as poly(4-vinylpyridine) (PVP). PVP showed good capacities for a variety of carboxylic acids *(10)*; succinic acid was not tested. King and colleagues *(9,11–15)* have shown that the sorption is very pH dependent owing to different binding of dissociated and undissociated carboxylic acids. The resins work best at pH less than the $pK_a$ of the acid where the organic acid is primarily in the undissociated or protonated form. The $pK_a$ values of succinic acid are 4.2 and 5.6. Sorption data for succinic acid have been reported *(11,13–15)* with strong pH effects and maximum capacities of 0.2–0.45 g of succinic acid / g of sorbent at acidic pH. Unfortunately, the pH that is typically required for the fermentation (i.e., pH 5.0–7.0) is above the acidic pH that is optimal for most adsorbents. Following King, we use the generic terms *sorption* and *sorbent* to indicate our observational approach and not to presume whether the mechanism is strictly absorption or adsorption.

Competition from other solutes may also be important. In particular, glucose in the fermentation broth has been shown to be sorbed *(16,17)*. This may decrease the total capacity available and the product purity. Solute anions, such as phosphate and sulfate, can also compete for binding sites on basic resins *(11)*.

It may also be economical to remove the inhibitory product directly from the ongoing fermentation by extraction, membranes, or sorption. The use of sorption with simultaneous fermentation and separation for succinic acid has not been investigated. Separation has been used to enhance other organic acid fermentations through *in situ* separation or separation from a recycled side stream. Solid sorbents have been added directly to batch fermentations *(18,19)*. Seevarantnam et al. *(20)* tested a sorbent in the solvent phase to enhance recovery of lactic acid from free cell batch culture. A sorption column was also used to remove lactate from a recycled side stream in a free-cell continuously stirred tank reactor *(21)*. Continuous sorption for *in situ* separation in a biparticle fermentor was successful in enhancing the production of lactic acid *(16,22)*. Recovery in this system was tested with hot water *(16)*.

The extraction of the desired product from the sorbent and the stable regeneration of the resin for further use are essential. The regeneration of some of these sorbents by methanol has also been tested *(23)*. Hot water regeneration has been proposed as well *(24)*. Back extraction with trimethylamine has been tested for succinic acid with consideration of formation of a succinate ester *(13,25)*.

In the present study, various sorbents were tested for the critical properties of capacity for succinic acid, regenerability of the sorbent, and coadsorption of substrates. These criteria were evaluated in order to choose a suitable sorbent for use with either a primary purification step or an *in situ* separation and fermentation. Other factors that were considered were the pH, temperature, and potential ability to concentrate the product.

Table 1
Initial Screening of Sorbents for Uptake of Succinic Acid with First Loading Cycle Capacity Values at 25ºC [a]

| Sorbent | Source [b] | Type | Equilibrium (g succinic acid/L) | Capacity (g/g) |
|---|---|---|---|---|
| XUS 40285 | Dow | Weak base polymer | 2.53 | 0.06 |
| XUS 40091 | Dow | Weak base | 3.98 | 0.04 |
| XUS 40323 | Dow | Strong base | 3.78 | 0.04 |
| XUS 40283 | Dow | Strong base | 1.98 | 0.06 |
| XUS 43432 | Dow | Weak base | 2.81 | 0.03 |
| XUS 40196 | Dow | Strong base | 4.88 | 0.02 |
| XUS 40189 | Dow | Strong base | 2.03 | 0.05 |
| XFS-40422 | Dow | Polymer | 1.50 | 0.07 |
| IRA-93 | RH | Weak base macroreticular | 1.03 | 0.07 |
| IRA-35 | RH | Weak base macroreticular | 0.79 | 0.11 |
| XAD-4 | RH | Weak base polymer | 3.53 | 0.04 |
| XAD-7 [c] | RH | Weak base polymer | 4.80 | 0.04 |
| Dow-11 MXBD | Dow | Strong base | 5.01 | 0.01 |
| Dowex 1x2 | Dow | Weak base | 2.77 | 0.06 |
| Marathon WBA | Dow | Strong base macroreticular | 2.04 | 0.04 |
| MSA-1 | Dow | Strong acid PVP | 3.98 | 0.04 |
| MSA-2 | Dow | Strong acid PVP | 3.99 | 0.02 |
| Reillex 425 | Reilly | PVP | 1.74 | 0.05 |
| Reillex 425 | Reilly | PVP | 4.8 | 0.08 |
| Reillex HPQ | Reilly | Hydrophobic molecular sieve | 1.03 | 0.06 |
| Reillex 402 [c] | Reilly | Polymer | 3.90 | 0.02 |
| Silicalite powder [c] | Linde | Hydrophobic molecular sieve | 3.68 | 0.07 |
| Silicalite pellet [c] | Linde | Hydrophobic molecular sieve | 4.01 | 0.05 |
| | | with binder | 4.01 | 0.05 |
| AG-3 [c] | Bio-Rad | Styrene | 4.10 | 0.02 |
| AG-1 [c] | Bio-Rad | Styrene quaternary amine | 3.99 | 0.03 |
| MWA-1 | Dow | Tertiary amine macroreticular | 4.81 | 0.02 |

[a] Fifty-milliliters of a solution of acidic succinic acid (~5 g/L) was equilibrated overnight with a known amount of sorbent (~5 grams wet wt). Initial and final concentrations were measured. Calculated capacities are based on the dry weights calculated from dried samples. Most sorbents were preconditioned by washing in 0.01 *M* HCl and triple washing in 50 mL of water at 90ºC. The wash pH was 6.5–7.8.

[b] RH, Rohm & Haas; Linde, Union Carbide.

[c] These sorbents were not preconditioned.

## Materials and Methods

### *Resins*

Sorbents were selected from a variety of sources: sorbents previously studied for lactic acids at Oak Ridge National Laboratory; the literature; and sorbents provided by interested industries such as Rohm & Haas, Dow, Union Carbide, and Reilly. The sources are listed in Table 1.

### *Batch Screening*

The initial screening was performed in batch tests. In each test, a known amount (usually 5 g wet wt) of sorbent was contacted with a known volume (usually 50 mL) of an aqueous solution containing the organic acid made from succinic acid crystals. The initial concentration varied between 1 and 50 g/L, with a typical value of approx 5 g/L. The mixture was agitated and allowed to equilibrate overnight and analyzed with the resin's capacity being determined by the difference between the measured initial and equilibrium concentrations. A separate sample of the sorbent was also dried at 90°C; this measured wet/dry ratio of each sorbent was used to calculate the true dry weight, which was used in all capacity calculations. Dried samples were unsuitable for tests themselves owing to greatly reduced wettability. The first tests were performed at ambient 25°C at acidic pH (approx 2.0); later tests were performed at neutral pH (6.0) in a simulated fermentation medium. The simulated medium contained: 1–50 g/L succinic acid as stated; 5 g/L of yeast extract (Difco; Detroit, MI); 0.3 g/L of $MgSO_4$; 0.5 g/L of $(NH_4)_2SO_4$; 0.2 $K_2HPO_4$; 0.2 g/L of $KH_2PO_4$; and 0, 5, or 40 g/L of glucose.

All chemicals were laboratory grade and dissolved in distilled water. The medium was sterilized by autoclaving, and sorption experiments were performed in sealed 150-mL serum bottles. Subsequent tests for *in situ* use may need to be performed at mesophilic fermentation temperatures. In a similar manner, the sorbents were also tested for their capacity for the substrate of the possible fermentation, glucose, at a concentration of about 5 g/L. Frequently the glucose was included in the fermentation medium for competitive sorption.

### *Regeneration with Hot Water*

The best resins were then tested for successive loading and regeneration. Acid and bases are conventionally used to extract and regenerate sorbents. However, the standard regeneration technique was hot water. Hot water regeneration was considered to avoid environmental and raw material costs associated with solvent stripping or acid regeneration. The resins were loaded as already described, except that actual medium containing salts and nutrients was used. After loading with succinic acid and determining the capacity, the bulk medium was removed and replaced with a known amount (20–40 mL) of pure water. The resin was then shaken for more than 4 h at 90°C, and the amount extracted was determined by analyzing the bulk solution. The final pH was near 5.0. This cycle was repeated several times with the results calculated at intervals of either 3, 5, or 10 cycles. In later tests, acid regeneration of certain sorbents was studied using 1 *M* HCl. In a few limited tests, a sequential combination of dilute base (0.01 *M* NaOH) and hot water was used; *see* Results for details.

### *Column Tests*

Some resins were tested for in-process removal of succinic acid. Industrial-scale sorption is performed in columns. A packed column (1-cm id,

30-cm length) of the sorbent was contacted with a stream of succinic acid in simulated medium. The total column volume including connections was measured as 75 mL. Samples were taken with a fraction collector and analyzed to create a breakthrough curve. The feed was continued until the column was fully loaded with the succinic acid. The column was then heated to 90°C and stripped with hot water while collecting samples with the fraction collector. This allowed estimation of the concentration factor possible and the fraction recovered. A process scheme would use sorbent columns in series. As breakthrough occurs in the first column, the effluent would be introduced into the second column. Once the first column is fully loaded, the feed stream would be diverted to the second column directly and the first column would be regenerated while the second column continues to be loaded. Depending on the regeneration time, an additional column might be needed in the series.

### *Analytical Methods*

Succinic acid concentrations were analyzed using a Dionex 4500i ion chromatograph. Some effort was spent on developing an accurate, reproducible method using an Ion-Pax AS-10 column, a suppressor module, and a PED electroconductivity detector. A carbonate trap is required to prevent interference from the carbonate peak. This technique can also measure other organic acids and anions such as phosphate. Care must be taken to maintain standards and to store samples. An alternative analytic method was developed using gas chromatography for the fermentation experiments and to confirm the analysis. This method also allows the detection of alcohols such as ethanol and butanol and other organic acids such as lactic and acetic. The method uses a packed column of Chromosorb 101 at 200°C with a helium carrier and an FID detector after sample preparation with metaphosphoric acid. The glucose and l-lactate were measured with a Yellow Springs Instruments glucose analyzer.

## Results

### *Batch Screening*

Twenty-five sorbents were screened for uptake of succinic acid. Table 1 lists the initial capacity for each of the sorbents as well as the type and manufacturer of the sorbents.

The capacities ranged from 0.02 to 0.11 g succinic acid/g of dry sorbent at the measured equilibrium succinic acid concentration. In order to have complete removal, a high binding affinity and capacity at low concentration is desired. This screen was performed at moderate concentrations in order to quickly identify sorbents with good capacities at lower concentrations. These are not maximum capacities reported elsewhere. Isotherms were measured for several sorbents that agreed with the literature and followed Langmuir isotherms (data not shown). For example, Reillex 425 has a good capacity (0.08 g/g at 5 g/L) and very good binding affinity

Table 2
Repetitive Sorption of Succinic Acid in Media Capacities After Successive Loading and Regeneration with Hot Water[a]

| Sorbent | Loading cycle | Equilibrium concentration (g/L) | Capacity (g/g) |
|---|---|---|---|
| Reillex 425 | 1 | 3.56 | 0.050 |
| | 5 | 2.67 | 0.040 |
| XUS 40091 | 1 | 0.51 | 0.080 |
| | 3 | 2.74 | 0.050 |
| XUS 40285 | 1 | 1.20 | 0.079 |
| | 3 | 2.41 | 0.062 |
| XAD-4 | 1 | 4.20 | 0.040 |
| | 3 | 5.34 | 0.020 |
| IRA-35 | 1 | 0.13 | 0.305 |
| | 3 | 3.56 | 0.010 |
| Silicalite powder | 1 | 3.68 | 0.075 |
| | 3 | 4.11 | 0.015 |
| IRA-93 | 1 | 0.19 | 0.320 |
| | 3 | 4.45 | 0.010 |
| Dowex 1x2,16-100 | 1 | 2.77 | 0.060 |
| | 5 | 3.98 | 0.030 |
| Reillex HPQ | 1 | 1.03 | 0.060 |
| | 5 | 4.03 | 0.020 |
| XUS 40283 | 1 | 1.98 | 0.060 |
| | 5 | 3.55 | 0.040 |
| XFS-40422 | 1 | 1.50 | 0.070 |
| | 5 | 2.70 | 0.050 |

[a]Fifty milliliters of a solution of succinic acid in medium was equilibrated overnight with a known amount of sorbent (~5 g) in a manner similar to that in Table 1. Resins were regenerated in hot water at 90°C and capacities calculated using dry weights.

(*K* approx 0.8 g/L). The requirement for a high binding affinity and capacity at low concentration can be partially addressed by use in a packed column as discussed later. Total capacity and regenerability then are critical factors. Since the possibility of *in situ* separation was being considered, the effect of the substrate glucose was also used to screen sorbents.

Approximately half of the better resins were then tested for successive loading of succinic acid in simulated medium and regeneration using hot water. The final pH was near pH 5.0 This cycle was repeated for multiple cycles. Some of the hot water regeneration results are excerpted in Table 2. The best resins (Reillex 425, XUS 40285, and XUS 40091) have a stable capacity of about 0.06 g of succinic acid/g of resin. MWA-1 was not included in these tests because of its lower capacity; however, it was reexamined in later

tests for stability. These capacities were measured at moderate to low equilibrium concentration of succinic acid; and , thus these capacities are not the maximum loadings for these resins, which have been reported by other investigators. The use of a lower succinic acid concentration for the screening gave a measurement of the specificity as well as the capacity without performing a complete isotherm for each sorbent. Because nearly complete recovery is required for most commercial separation, the capacity at the lower concentrations will be important. Other resins had initially higher capacities (e.g., IRA-35), but the capacities were unstable and their capacity rapidly fell to 0.01 g/g. In these cases, it was unclear whether the resin itself was unstable at 90°C or whether the binding sites never released the sorbate (incomplete regeneration). About 50–80% of the succinic acid was removed in each of these cycles. Some of the resins with the highest initial capacity were unstable under the regeneration temperatures; other resins (e.g., Silicalite) did not release the acid under this procedure.

In a similar manner, the sorbents were tested for their capacity for the substrate of the planned fermentation, glucose. Glucose was found to adsorb with all tested resins (data not shown). Maximum glucose capacities ranged from 0.01 to 0.04 g/g. However, the resin preferred succinic acid at a ratio near 4:1 for Reillex 425, 4:1 for XUS 40091, and 8:1 for XUS 40285. Sorbents that preferentially sorbed glucose over succinic acid were eliminated from further testing.

The possible impact of solution pH was also examined by testing two resins more exhaustively for uptake of succinic acid and successive loading and regeneration using hot water. One resin, XUS 40285, has a good stable isotherm capacity, prefers succinate over glucose, and has good capacities at both acidic and neutral pH. Sorption isotherms were constructed for the Dow resin XUS 40285 for uptake of succinic acid after three cycles of loading and regeneration with hot water. These tests were performed at both acidic and neutral pH. The resin has a stable capacity of approx 0.06 g of succinic acid/g of resin at moderate succinic acid concentrations.

Tests were performed in a simulated medium, containing salts and nutrients, as described earlier at an ambient temperature of 25°C, and at both acidic pH (approx 2.0) and neutral pH (approx 6.0). The final pH value was adjusted to the desired value with HCl and allowed to reequilibrate. After loading with succinic acid and determining the capacity, the bulk medium was removed and replaced with a known amount of hot water. The resin was then shaken for more than 4 h at 90Ÿ°C, and the amount extracted was determined by analyzing the bulk solution. The final pH of the aqueous wash was near 5.0. This load/wash cycle was repeated three times. About 70–80% of the succinic acid was removed in each of these cycles. In a similar manner, the sorbents were tested for their regenerated capacity for the fermentation substrate, glucose (*see* Table 3). The resin prefers succinic acid despite an excess of glucose under these conditions. The ion chromatograph analyses indicated that phosphate and sulfate from the medium were adsorbed. This agrees with the findings of Tung and King *(11)*.

Table 3
Repetitive Competitive Sorption of Succinic Acid in Medium with Glucose at pH 2.0 and 6.0[a]

| pH | Succinate (g/L) | Capacity (g succinate/g) | Capacity (g glucose/g) |
|---|---|---|---|
| Resin XUS 40285 | | | |
| pH 2.0 | 1.44 | 0.041 | 0.013 |
| | 2.34 | 0.053 | 0.015 |
| | 3.88 | 0.061 | 0.020 |
| pH 6.0 | 1.44 | 0.048 | 0.011 |
| | 2.66 | 0.059 | 0.013 |
| | 3.48 | 0.066 | 0.022 |
| Resin MWA-1 | | | |
| pH 2.0 | 1.28 | 0.043 | 0.023 |
| | 3.55 | 0.047 | 0.022 |
| | 4.81 | 0.060 | 0.021 |
| pH 6.0 | 1.21 | 0.046 | 0.024 |
| | 2.70 | 0.053 | 0.024 |
| | 4.14 | 0.060 | 0.025 |

[a]Fifty milliliters of a solution of acidic succinic acid (~5 g/L) was equilibrated overnight with a known amount of sorbent (~5 grams wet wt). Initial and final concentrations were measured at each cycle. Between cycles the sorbent was regenerated with hot water. Calculated capacities are based on the dry weights estimated from separate dried samples. The reported capacities are from the third successive loading and are based on the direct measured sorption in that cycle, not on the cumulative capacity.

Sorption isotherms were also constructed for another resin, MWA-1, for uptake of succinic acid and regeneration with hot water. The isotherms of the final capacities of MWA-1 were similar to those of the XUS 40285, with a maximum capacity of about 0.05 at both 2.0 and 6.0. About 70% of the succinic acid was removed in each of these cycles. In a similar manner, the sorbent was tested for its regenerated capacity for the fermentation substrate, glucose, (Table 3). Glucose was also found to competitively adsorb; however, MWA-1 prefers succinic acid despite an excess of glucose under these conditions. In comparison with the XUS 40285 resin, MWA-1 has a very comparable succinic acid sorption isotherm in terms of capacity and pH; it has a somewhat less regenerability in hot water. MWA-1 also has about twice the affinity for glucose, which would disqualify it for optimal use in simultaneous fermentation and separation. In a batch fermentation, the glucose could desorb as its concentration falls and the conversion could proceed to completion; however, in an extractive fermentation, the continuous removal of the resin would also remove a significant amount of the substrate.

### Packed Column Tests

The sorption results from the batch tests just reported indicated good potential for the separation and purification of succinic acid. The key desired properties for an ideal sorbent are high capacity, complete stable regenerability, and specificity for the product. Two of the best sorbents identified by our screening and provided by industry were tested more exhaustively for uptake of succinic acid and for successive loading and regeneration using hot water. These resins, MWA-1 and XUS 40285, had capacities of approx 0.06 g of succinic acid/g of resin over three cycles. They have good capacities at both acidic and neutral pH. This should allow efficient removal of succinic acid from the fermentation broth. Both prefer to adsorb succinate over glucose. This will have no impact on sorption when used as a primary purification step but is necessary if *in situ* separation is to be used.

Two resins were tested for the removal of succinic acid from simulated medium on a packed column of sorbent to simulate an actual process on a small scale. It is important to test the sorption with medium, because salts and other nutrients can interfere with the sorption. Table 4 presents the results for XUS 40285; MWA-1 was comparable. This indicates that either sorbent can remove succinic acid efficiently from the fermentation broth without direct loss of product. Both columns were then stripped or regenerated with hot water. Stripping with hot water recovered 70–80% of the succinic acid from the XUS 40285 resin whereas less (50–60%) was recovered from the MWA-1. The XUS 40285 column was stripped with 2 column volumes of hot water with eluent concentrations up to 49 g/L. Succinic acid was concentrated on average to 40 g/L in the XUS resin by this operation and to 30 g/L by the MWA-1. The 10-fold concentration factor bodes well for the use of sorbents to purify the fermentation broth.

Subsequent tests used streams containing 10, 5, and 2 g/L of succinic acid and acetic acid. Acetic acid is a byproduct of the fermentation and should be in the broth at less than a 1/4 weight ratio. Here, we used an equal concentration of acetic acid and succinic acid as a more stringent screen. Figure 1A shows the contacting that resulted for 10 g/L on XUS 40285. Breakthrough of succinic acid occurred after more than 20 column volumes were passed through. The column was fully loaded with succinic acid, then heated to 90°C and stripped with hot water (Fig. 1B). The regeneration was almost finished in the first 2 column volumes with a maximum concentration of 133 g/L and 70% of total recovery. The consistent recovery at more than 10-fold concentration bodes well for the use of sorbents to purify the fermentation broth. Concentrations of >100 g/L are needed from the primary separation step before subsequent concentration. It can be noted that acetic acid was minimally adsorbed and thus almost completely separated from succinic acid. The acetic acid concentration in the regenerant was always <1 g/L. Succinic acid was also partially purified from the salts by this procedure. This purification will decrease the load on subsequent secondary purification methods such as ion exchange. Methods to concentrate and use the dilute acetic acid or recycle the tailings have not been considered to date.

Table 4
Succinic Acid Sorption on Columns of XUS 40285

| | Loading at breakthrough | | Loading at saturation | | Regeneration with hot water | | | |
|---|---|---|---|---|---|---|---|---|
| Feed (g/L) | Column vol | Capacity (g/g) average | Column vol | Capacity (g/g) | Column vol | Recovery on first cycle (%) | Maximum concentration (g/L) | Average concentration (g/L) |
| 2 | 31.0 | 0.11 | 37 | 0.12 | 3.1 | 60% | 41 | 18 |
| 5 | 16.0 | 0.13 | 34 | 0.20 | 2.8 | 71% | 59 | 28 |
| 10 | 9.3 | 0.16 | 34 | 0.36 | 3.3 | 71% | 133 | 43 |

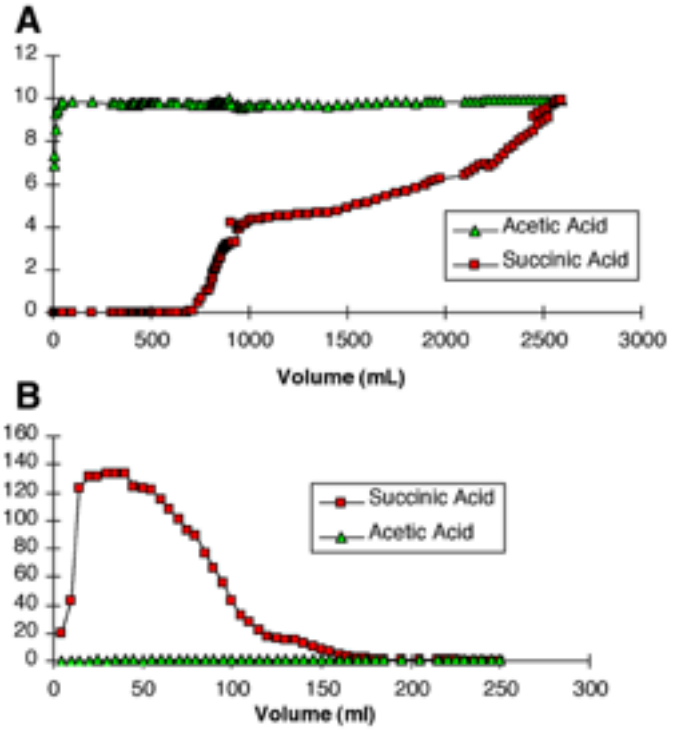


Fig. 1. **(A)** Loading and breakthrough profile for packed column of sorbent (XUS 40285) exposed to 10 g/L of succinic acid and 10 g/L of acetic acid in simulated media. The column volume was 75 mL and the pH of the feed was 6.0. The pH fell to 5.2 during the breakthrough and slowly rose as the bed loaded. **(B)** Elution profile of same column with water at 90°C.

These tests will need to be repeated at higher concentration and for multiple loading and regeneration cycles. At the *n*th cycle, the recovery per cycle needs to be near 100%, even if the capacity is lower. The stability of this resin to this procedure must be confirmed as well. Regeneration also was tested by the use of acid. We wished to minimize this because acid regeneration will require subsequent neutralization. The next experiments extended long-term tests of the use in a packed absorber column of the best resin from the previous screening, a Dow polymeric sorbent, XUS 40285. This resin was tested in a packed column with simulated medium containing salts, 40 g/L of succinic acid, acetic acid, and sugar. The column was loaded with succinic acid and then extracted with hot water at 90°C. The results using a hot water regeneration step were very promising, comparable to those in Fig. 1; however, succinic acid was no longer extracted after 10 repeated cycles of loading and hot water regeneration.

This resin has the characteristics of a capacity of 0.06 g of succinic acid/g of sorbent. With extraction using only hot water, 70–80% of the succinic

acid was recovered per run in the first cycles and concentrated more than fivefold. The long-term regenerability of this hot water method is in question. There are several possible explanations for this. It may be that the hot water regeneration step is not sufficient to remove all of the succinic acid. This residual succinic acid slowly builds up and blocks the binding sites. It may also be that the heat allows the succinic acid to bind more tightly to the resin.

More traditional acid and base regeneration steps were tested for this high-capacity resin. The column of sorbent XUS 40285 was loaded with succinic acid at 35 g/L and extracted with 1 *M* HCl. Eighty-one percent of the succinic acid was recovered; however, there was no increased concentration of the succinic acid in the final product stream. The recovered product stream must be at least as concentrated as the fermentation broth and should be significantly concentrated.

### *Batch Acid Regeneration*

Despite promising initial results of good capacity (0.06 g of succinic acid/ g of sorbent), 70% recovery using hot water, and a recovered concentration of >100 g/L, the hot water regeneration was not stable over 10 cycles in a packed column. Therefore, alternative regeneration schemes using acid and base were examined for the best resins and more sorbents were reexamined at this time.

Tests were performed with both simulated broth containing succinic acid at various concentrations and actual broth provided by MBI. Seven resins were tested for regenerability and stability with acid; XUS 40285, Dowex 1x2, XUS 40283, XUS 440323, XFS-40422, IRA-35, and IRA-93. Previous results had shown a decrease in capacity with repeated hot water regeneration. It is essential for economical operation that the organic acid recovery be >90% and that the sorbents be stable for at least 20 cycles (based on industrial comments). Several resins were tested for stability with a single-step dilute-acid regeneration. The resins were either low capacity after five cycles or had incomplete recovery of the succinic acid (data not shown). Therefore, we modified the procedure to extract the succinic acid first with dilute base, then hot water.

### *Combined Base/Hot Water Regenration*

In the modified procedure, dilute base was used followed by three hot water washes. The resins were preconditioned in 0.01 *M* HCl overnight and then rinsed three times in water. Dry and wet weights of representative samples were measured to allow the capacities to be calculated with estimated dry weights. The resins were contacted overnight with 5 g/L of succinic acid in simulated medium also containing 5 g/L of glucose. Glucose was added to check for interference as well as for possible use in an *in situ* separation from the ongoing fermentation. The extraction procedure was with 0.01 *M* NaOH for 3 h at 40°C followed by three washes

of 90°C water. To determine the total percentage recovered, the succinate from these four steps were combined. This was followed by another cycle of loading. The samples from the first, fifth, and tenth cycles were analyzed. The capacities of four sorbents remained above 0.04 g of succinic acid/g of dry sorbent for the 10 cycles (*see* Table 5). Two of the resins (XUS 40285 and XFS 40422) had both good stability and recovery of succinic acid of >95%. However, the first extraction step would release the organic acid as an aqueous salt, sodium succinate. Based on this and earlier data, the XUS 40285 and the XFS 40422 resins appear to be the most promising for further column process testing. Proper operation of sorbent columns for extraction can concentrate the product and, therefore, simplify further purification steps.

## Discussion

More than 25 sorbents were tested for uptake of succinic acid. The best resins were then tested for successive loading and regeneration using hot water. The key desired properties for an ideal sorbent are high capacity, complete stable regenerability, and specificity for the product. The best resins have a stable capacity of approx 0.06 g of succinic acid/g of resin at moderate concentrations (1–5 g/L) of succinic acid. Tung and King *(11)* reported first-cycle maximum capacities at pH 6.0 between 0.0 and 0.08 g/g for weak base resins and 0.22 and 0.25 g/g for strong base resins. The strong base resins needed reactive regeneration. From discussions with individuals in the industry, we estimate that the final resin will need a capacity of >0.05 g/g; however, the regenerability and stability as well as the concentration factor are more important.

Several sorbents were tested more exhaustively for uptake of succinic acid and for successive loading and regeneration using hot water. One resin, XUS 40285, has a good stable isotherm capacity, prefers succinate over glucose, and has good capacities at both acidic and neutral pH.

Succinic acid was removed from medium on a packed column of sorbent. The resin XUS 40285 was tested in a packed column with simulated medium containing salts, succinic acid, acetic acid, and sugar. The packed column completely separated the fermentation byproduct, acetate, from succinate. A simple hot water regeneration successfully concentrated succinate from 10 g/L (inlet) to 40–110 g/L in the effluent with a pH of about 5.0. The end pH indicates that succinate salt was sorbed from the "medium" and released in a partially acidic form by the hot water regeneration. If successful, this would lower separation costs by reducing the need for chemicals for the initial purification step.

Despite promising initial results of good capacity (0.06 g of succinic acid/ g of sorbent), 70% recovery using hot water, and a recovered concentration of >100 g/L, this regeneration was not stable over 10 cycles. Alternative regeneration schemes using acid and base were examined and more sorbents screened. Tests were performed with simulated broth containing succinic acid at various concentrations.

Table 5
Successive Cycles of Batch Loading of Succinic Acid
and Regeneration with Both Dilute Base and Hot Water[a]

| Resin | Capacity (cycle 5) (g succinic acid/g resin) | Capacity (cycle 10) (g succinic acid/g resin) | Glucose capacity (g/g resin) | Recovery (% succinic acid after 10 successive cycles of loading and regeneration) | |
|---|---|---|---|---|---|
| | | | | Recovered in first step of base | Combined recovery from base and hot water steps |
| XUS 40285 | 0.05 | 0.04 | 0.025 | 81 | 97 |
| Dowex 1x2 | 0.05 | 0.05 | 0.038 | 38 | 74 |
| XUS 40283 | 0.05 | 0.03 | 0.03 | 59 | 90 |
| IRA-35 | 0.08 | 0.03 | 0.042 | 44 | 90 |
| IRA-93 | 0.06 | 0.05 | 0.032 | 60 | 78 |
| XUS 40323 | 0.03 | 0.02 | 0.031 | 8 | 81 |
| XFS-40422 | 0.06 | 0.05 | 0.034 | 85 | 97 |

[a] NaOH 0.01 *M* followed by three steps of hot water.

Seven of the most promising resins were tested for regenerability and stability using a modified extraction procedure combining acid and hot water washes. Two (XUS 40285 and XFS-40422) showed both good stable capacities for succinic acid over 10 cycles and >95% recovery in a batch operation.

These results show that sorption is a technical possibility for succinic acid separation. In particular, sorption remains an excellent candidate for *in situ* separation. However, targets for capacity, regeneration, and concentration were not met in these preliminary studies. Additional process tests focusing on regeneration and succinic acid concentration will be required. Therefore, these and other economic considerations not reported here have favored membrane or crystallization methods such as described in the referenced patents *(3,26–28)*; these approaches were continued in the larger succinic acid fermentation project *(29,30)*. The sorption data are presented as a baseline for other researchers in the examination of organic acid separation schemes.

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