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Purification Process for Succinic Acid Produced by Fermentation

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ABSTRACT

Succinic acid is a versatile four-carbon dicarboxylic acid. It can be used commerically as an intermediate chemical for the manufacture of 1,4-butanediol, maleic anhydride, and many other chemicals. Succinic acid can be produced by the fermentation of carbohydrates. A complete process for the production and purification of succinic acid from carbohydrates has been developed. The process includes fermentation, desalting electrodialysis, water-splitting electrodialysis, and crystallization to produce a pure crystalline succinic acid. This article will present experimental work performed in the development of this process.

Index Entries: Succinic acid; acetic acid; fermentation; electrodialysis; crystallization.

INTRODUCTION

Succinic acid and its derivatives are widely used as specialty chemicals with applications in polymers, foods, pharmaceuticals, and cosmetics. Succinic acid is currently manufactured by hydrogenation of maleic anhydride to succinic anhydride, followed by hydration to succinic acid. Succinic acid is a known plant-growth regulator (*1*). Succinic acid can be easily esterified to dimethylsuccinate, which is marketed as an environmentally friendly solvent. Succinic acid compounds also have been patented as salt-substitute flavor-enhancing agents (2).

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The availability of a low-cost succinic acid would make the manufacture of many additional chemicals attractive. Succinic acid could be a valuable four-carbon intermediate, useful for the production of 1,4-butanediol, tetrahydrofuran, and γ-butyrolactone. These processes require raw materials of high purity, since the end products are produced by chemical catalysts that can be poisoned by impurities. Using carbonylation technology, adipic acid could be produced from succinic acid feedstock. Maleic anhydride, the current source for succinic acid, could itself be produced from succinic acid. Fermentation of low-cost carbohydrates is a potential source of low-cost succinic acid.

For a fermentation-based carboxylic acid process to be economically attractive for the production of specialty and commodity chemicals, the development of low-cost fermentation must be combined with low-cost and efficient product recovery and purification methods. Anaerobic fermentations offer promise for the production of large quantities of low-cost chemicals because of their high yield on substrates and their simple scale-up requirements for very large-scale fermentations.

Fermentations showing succinic acid as a primary product have been described previously (*3–7*). The most promising anaerobic succinic acid fermentations fix carbon dioxide to increase the yield of succinic acid from glucose. A fermentation with high succinic acid yield and concentration was reported for the anaerobic microorganism *Anaerobiospirillum succiniciproducens* (ATCC 29305 and ATCC 53488) in a patent issued to Datta (*8*). Several patents describing different processes for the fermentation production of succinic acid were issued to the Michigan Biotechnology Institute recently (*9–11*).

The anaerobic fermentations that are most promising for the production of organic acids require operation at pHs where salts of the organic acids rather than the free acids are formed. However, the free acids and their derivatives are the articles of commercial interest. In addition, contaminating proteins and cell byproducts need to be removed from the free carboxylic acids because of their interference in chemical catalysis. Therefore, an effective fermentation and recovery process must remove both cells and proteins, and subsequently convert the acid salts to free acids of high purity.

Several possible alternatives exist for the preliminary recovery of succinate salts from the fermentation broth. Desalting electrodialysis was chosen because of its widespread application in industry, and because it can purify and concentrate the desired salts (*12,13*). Water-splitting electrodialysis efficiently converts salts into the corresponding alkali and acid through the use of a high-efficiency bipolar membrane (*14*).

This article presents the results of experimental work to develop a process for the production of a crystalline succinic acid. The process described in this work will use desalting electrodialysis to recover the succinate salt and water-splitting electrodialysis to split the salt into an alkali

and the succinic acid product. A novel method for recovery of a high-purity succinic acid is shown that makes the process described particularly useful for the production of a crystalline succinic acid. This method also allows for the separation and recovery of acetic acid, which is a coproduct of the fermentation.

MATERIALS AND METHODS

Preparation of Succinate Salt

Succinate salt solutions are prepared by anaerobic fermentations using a strain of *A. succiniciproducens* (deposited in the American Type Culture Collection as ATCC 29305 and redeposited under the provision of the Budapest Treaty as ATCC 53488) at 39°C in a fermentor with an initial volume of 55 L for 29 h. The fermentor used is an 80-L New Brunswick Scientific Pilot Plant Fermenter. The media contains approx 35 g/L dextrose, 10 g/L corn steep liquor, 25 ppm tryptophan, and 25 ppm cysteine-HCl reducing agent. Serial logarithmic growth-phase transfers with 5% inoculum were used to prepare active inoculum. Five percent inoculum is used to inoculate the 80-L fermentor. A continuous sparge of CO_2 maintained anaerobic conditions. The pH is maintained between 6.1 and 6.3 by addition of 3*N* sodium carbonate on a demand basis. Agitation speed was 100 rpm.

The cells in the fermentation broth may be removed by processing the broth through an AMICON DC-30 ultrafiltration unit with a hollow-fiber cartridge of 0.2-μ pore size.

Desalting Electrodialysis to Recover Succinate Salt

The sodium succinate can be concentrated and purified using desalting electrodialysis. Depending on the fermentation broth and its succinate salt concentration, desalting electrodialysis can be used to raise the succinic acid concentration above 10%. The concentrate from desalting electrodialysis was evaporated to raise the concentration of the succinate salt prior to water-splitting electrodialysis. The electrodialysis stack consists of an alternating series of anion- and cation-selective membranes separated by flow distribution gaskets as shown in Fig. 1. The membranes are bound on one end by an anolyte compartment and an anode, and on the other end by a catholyte compartment and a cathode. The stack pack used in this work is available from HPD Inc. (Naperville, IL) and is manufactured by Asahi Glass Co. (Japan). The stack pack contained the following:

10 cell pairs;
Anion membrane—AMV;
Cation membrane—CMR;

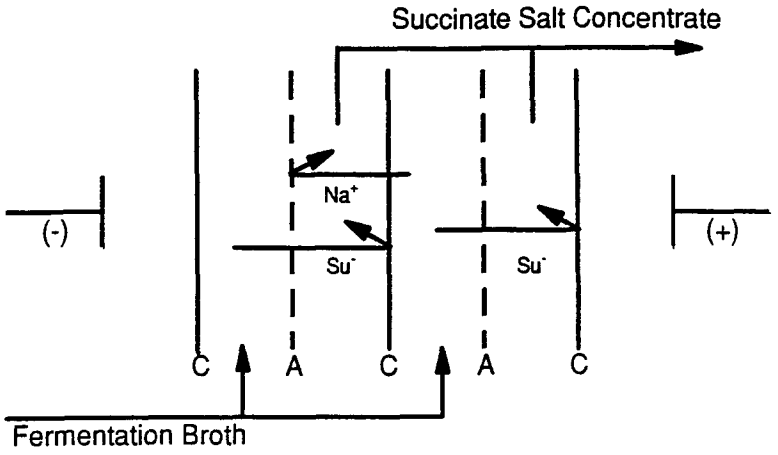

Fig. 1. Desalting electrodialysis schematic.

Effective area—178 cm^2; and
One anode and one cathode.

The electrodialysis unit consists of three independent flow systems feeding the electrodialyzer stack pack. These include the diluting stream (fermentation broth), concentrating stream (product succinate salt), and the electrolyte (1*M* sodium succinate solution).

From each flow system reservoir, solution is pumped through a valve, rotameter, pressure gage, the stack pack, and then back to the reservoir. Feed fermentation broth is supplied to alternating compartments in the membrane stack. The DC current moves the cations through the cation-exchange membranes and the anions through the anion-exchange membranes as shown in Fig. 1. The net result is that salt in the feed compartment is depleted, and the adjacent concentrating compartments are enriched with the salt.

The electrical current is supplied by a Hewlett Packard (HP) regulated DC power supply model 6268B. It is connected to the anode and cathode of the membrane stack and can produce 0–20 A and deliver 0–50 V. A Fluke A75 multimeter is used to measure the voltage drop across selected cell pairs. Two platinum wires are inserted between eight cell pairs and then connected to the voltmeter.

Conversion of Succinate Salt to Succinic Acid

A suitably concentrated, but undersaturated (<25% weight) succinate solution obtained by desalting electrodialysis can be converted into a supersaturated succinic acid solution by passing it through a water-splitting electrodialysis unit. The two-compartment stack used contains Aquatech (Warren, NJ) bipolar membranes. The stack, schematically illu-

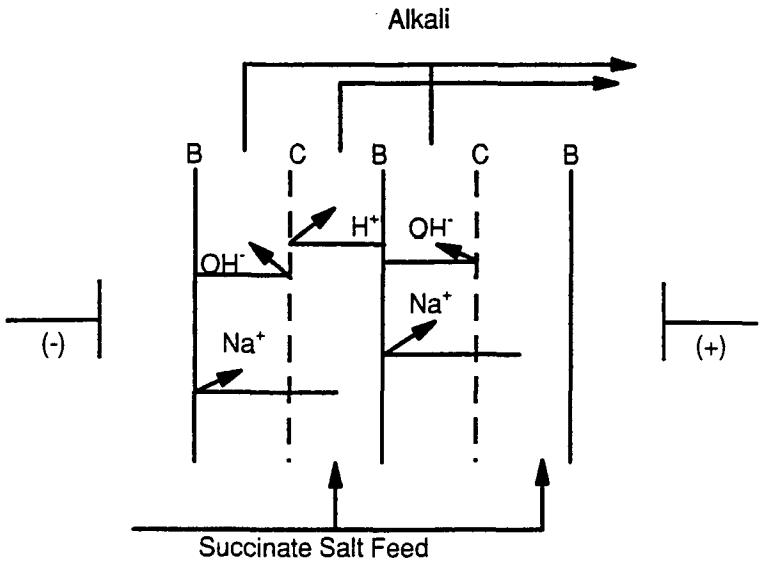

Fig. 2. Water-splitting electrodialysis schematic.

strated in Fig. 2, consists of alternating cation-permeable and bipolar membranes. The anode and cathode compartments are bound by a Nafion membrane at each end of the membrane stack.

The test membrane stack contains the following:

Eight cell pairs;
—Cation membrane; and
—Bipolar membrane;
One anode and one cathode;
Effective area 102.4 cm^2.

The unit consists of three independent flow systems feeding the electrodialyzer stack. These include the acid/salt stream (initially the sodium succinate salt stream), alkali stream (becomes more concentrated as run proceeds), and the electrode rinse stream (2.5*N* NaOH).

The acid/salt and the alkali streams are fed through alternating pairs of the membrane stack. The electrode rinse circulates through the anode and cathode compartments only. The DC current applied across the bipolar membrane causes water to split into H^+ and OH^- to carry the current across the membrane as shown in Fig. 2. Neither the cations nor the succinate ions can cross the bipolar membrane, which consists of a cation- and anion-exchange section. Correspondingly, cations are transported across the cation-exchange membrane to combine with the OH^- ions in the alkali stream. The acid/salt stream becomes progressively more acidic as the process continues.

Table 1
Process Stream Compositions (Weight % Composition, Dry Basis)

	Fermentor product	After ED	Water-splitting ED	After crystallization
Succinate	51.5	63.0	77.6	99.9
Acetate	13.2	8.8	18.6	—
Protein	9.7	0.8	0.6	0.07
Sodium	25.6	27.3	2.8	0.02
Sulfate	0.1	0.6	0.4	—
Total (g/L)	50.2	146.7	215.9	NA

Analytical Measurements

Conductivity is measured using a portable conductivity meter (Cole Parmer model 1484-10). Succinate and acetate concentrations are the anion concentration, and were measured after appropriate dilution and acidification by an HPLC method using a 1-ft long Aminex HPX87H column from Bio-Rad (Richmond, CA). Total protein content was determined on a Kjeltec Auto 1030 analyzer Kjeldahl apparatus (Tecator Inc., Herndon, VA) reported as nitrogen × 6.25%. Sulfate concentration was determined by gravimetric determination of barium sulfate precipitation. Sodium concentration was determined using an Orion SA 720 ion-selective meter and a sodium electrode.

Crystallization of Succinic Acid from Supersaturated Solution

The crystallization of succinic acid of high purity from the supersaturated solution is conducted at 30°C using 125 mL of broth obtained after water-splitting electrodialysis. The supersaturated solution is seeded with crystals of pure succinic acid in a crystallizer. The crystals of succinic acid that form are filtered and washed with cold water. The resulting crystals, when analyzed for succinate, acetate, protein, sodium, and sulfate, are found to be of high purity (about 99.9%).

RESULTS AND DISCUSSION

Effect of Impurities on Crystallization

The fermentation product was analyzed for the succinic and acetic acid, Kjeldahl protein, sodium, sulfate, and glucose. The results are shown in Table 1. Trace impurities were not quantitated. The total concentration of these components is shown in grams per liter. The fermentation broth was filtered and subjected to desalting electrodialysis. The composition of the succinate salt concentrate from desalting electrodialy-

Table 2
The Effect of Acetic Acid and Sodium Acetate on Succinic Acid Crystallization at 30°C for a Model System Designed to Mimic Broth Conditions

	Case 1	Case 2
Water, g	180	180
Sodium succinate, g	16.2	16.2
Succinic acid, g	35.4	35.4
Sodium acetate, g	6.8	—
Acetic acid, g	—	3.2
Crystal yield, g	1.22	5.17

sis is found in column two. The concentrated salts stream after desalting electrodialysis was concentrated by evaporation prior to water-splitting electrodialysis. This step is necessary to increase the salt concentration sufficiently to create a supersaturated solution at the water-splitting electrodialysis operating temperature. The main items to note are the relative compositions of the solution after water splitting and the composition of the crystalline material obtained from it. The high purity of the crystalline material indicates that crystallization is a viable means for product purification. Crystallization eliminates the need for any additional purification after water-splitting electrodialysis. The material balance around the crystallization step produced more succinic acid crystals than was expected. The following experiments were done to investigate this result.

The major impurities in the sodium succinate salt are sodium acetate and, during water-splitting electrodialysis, acetic acid. A separate set of crystallization experiments was performed to determine the effect of acetic acid/sodium acetate on succinic acid crystallization. Compositions were chosen to mimic those found after water splitting: 1.5*M* succinic acid, 0.5*M* sodium acetate, and either 0.2*M* sodium acetate or acetic acid.

The results of the acetic acid/sodium acetate impurity studies in Table 2 show four times more succinic acid crystals formed in the presence of acetic acid than sodium acetate. These results indicate acetic acid has a crystallization promotion effect. This explains the higher than expected fraction of succinic acid collected as crystalline material above. The role of the hydrogen ion in crystallization of succinic acid has been recently studied (*15*). The primary influence of the acetic acid on the enhancement of crystallization of succinic would appear to be in the shifting of the equilibrium toward the protonated form of succinic acid. The present results further substantiate the promotion of the crystallization by increased hydrogen ion concentrations.

The next three cases are used to study this phenomenon further. Sodium acetate and acetic acid are added to broth solutions, which are supersaturated. As demonstrated by Table 3, addition of acetic acid greatly

Table 3
The Effect of Added Acetic Acid and Sodium Acetate on Succinic Acid Crystallization at 30°C for the Fermentation Product

Experiment number	Case 3	Case 4	Case 5
Broth, ml	200	200	200
Sodium acetate, g	6.8	—	—
Acetic acid, g	—	3.2	—
Crystal yield, g/L	—	18.0	11.5

Table 4
Water-Splitting Electrodialysis Recovery of Succinic Acid from Fermentation Product

	Run 1	Run 2
Sodium Removal, %	78.9	81.2
Salt stream		
Initial succinate conc., g/L	78	126
Final succinate conc., g/L	91	52
Initial acetate conc., g/L	3	29
Final acetate conc., g/L	15	36
Temperature, °C	45	45
Current efficiency, %	78.9	76.2
Crystallization[a]	No	Yes
Membrane fouling	No	No

[a]Supersaturated with respect to succinic acid.

enhances yield, whereas sodium acetate causes a complete cessation of crystallization.

The ability to remove high-quality crystals from solution produced by water-splitting has several implications. Clearly, creation of supersatura tion by water-splitting is demonstrated. This phenomenon should occur in any system wherein the salt is more soluble than the acid. As can be seen in Table 4, this can be accomplished without the formation of crystals on the membrane, and current efficiency is preserved during the process. Finally, the crystallization step is not only feasible for removing impurities, but facilitated by their presence.

The recovery per pass using water-splitting electrodialysis was low with only 21.8 g/L of crystals produced. For this reason, the process should be thought of as a "stripping" crystallization, wherein the succinic acid in excess of solubility is "stripped" from solution by crystallization.

The process for the production of succinic acid identified by this work is shown in Fig. 3. This figure shows the fermentation to produce succinic acid is followed by a particulate removal step prior to desalting electro-

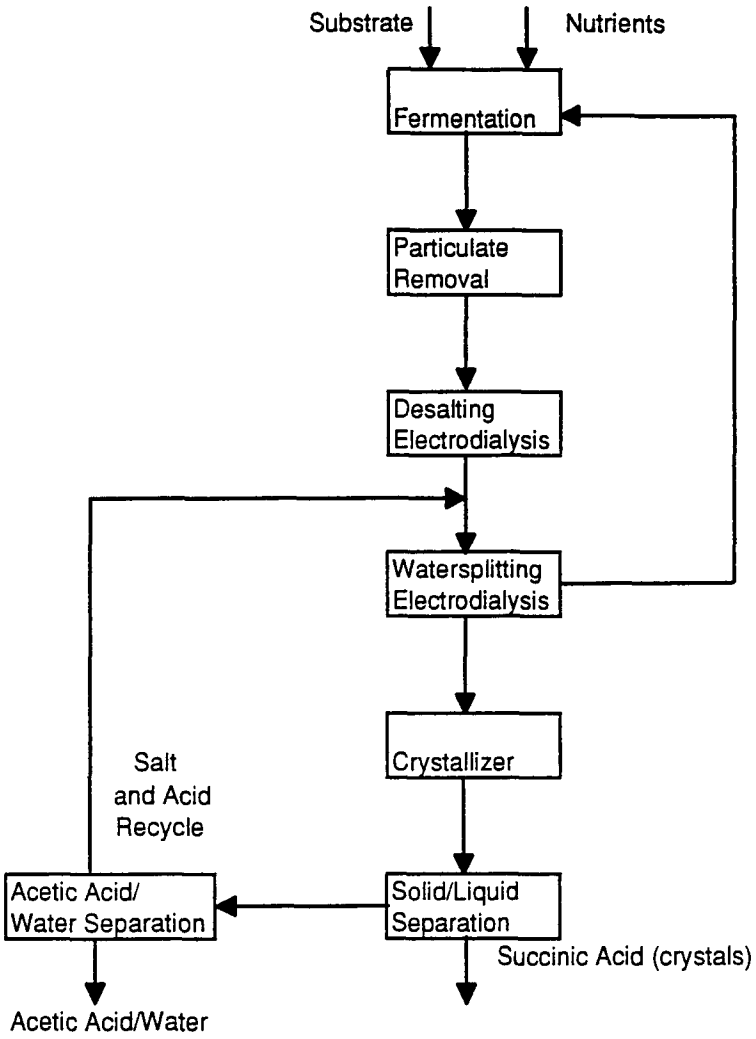

Fig. 3. Succinic acid process schematic.

dialysis. A recycle stream containing salts and acids is added to the desalting electrodialysis concentrate prior to water-splitting electrodialysis. The partially acidified stream produced by water-splitting electrodialysis is sent to a crystallizer. The alkali stream from the water-splitting electrodialysis operation is recycled to the fermentation vessels for neutralization of acids produced. In the crystallizer, a fraction of the succinic acid from the partially acidified stream crystallizes. The succinic acid crystals are removed in a solid/liquid separation operation and the remaining solution is sent to an acetic acid/water removal step. The remaining succinate and acetate salt solution is recycled back to the water-splitting electrodialysis operation.

The relationship of impurities to crystallization is quite complex. Impurities, such as amino acids and salts, are effectively excluded from the succinic acid crystals. We have discovered that the crystallization of succinic acid is inhibited by the presence of sodium acetate, whereas it is enhanced by the presence of acetic acid. This result shows that the use of water-splitting electrodialysis not only creates solutions supersaturated with respect to succinic acid, but also converts a crystallization inhibitor, sodium acetate, to a crystallization promoter, acetic acid. The process described may be applicable for the preparation of free carboxylic acids, including maleic, fumaric, or citric acid, or amino acids, such as glutamic acid.

CONCLUSIONS

The work presented shows a process capable of producing a highly purified succinic acid product based on fermentation of carbohydrates. The process uses an efficient anaerobic fermentation, desalting electrodialysis, water-splitting electrodialysis, and crystallization as the key operations for production of a high-purity succinic acid. The process can also be developed to recover the acetic acid produced.

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