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Post-Fermentation Recovery of Biobased Carboxylic Acids

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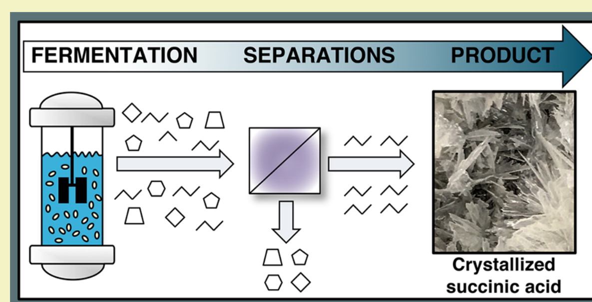
Supporting Information

ABSTRACT: Carboxylic acids are common products produced from the bioconversion of renewable feedstocks. In these processes the separation of the acid product from fermentation broth is the most energy and cost intensive unit operation. Thus, the development of robust, scalable separation approaches that can be applied to a variety of carboxylates is of critical importance to the development of processes that utilize carboxylic acids as platform chemicals. Here we report a batch separation method that includes cell and particulate removal, cation exchange, activated carbon treatment, dewatering with a polymer resin, and product recovery. This method is demonstrated on two unique fermentation broths both derived from corn stover hydrolysate to separate neat succinic and propionic acid. For succinic acid, a crystallization yield of 91% with a product purity of 99.93% was achieved. To our knowledge this is the highest reported crystallization yield and purity for the recovery of succinic acid. Additionally, the method requires approximately 50% less energy compared to standard evaporative crystallization approaches. For propionic acid, neat liquid product was obtained with a distillation yield of 80% and purity of 98%. These excellent results achieved in terms of yield and purity for succinic and propionic acid, two acids with widely different physical properties, from chemically complex hydrolysate broth demonstrates the effective and robust nature of this approach.



KEYWORDS: *Biochemicals, Separations, Carboxylic acid recovery, Fermentation product recovery*

INTRODUCTION

Carboxylic acids are common chemicals that can be produced biologically from a variety of renewable feedstocks, such as carbohydrates, glycerol, and waste gases. The relative ease of carboxylate production from microbes has generated much interest in using them as a platform to create a variety of renewable chemicals and fuels.[1−3] Recently, this approach has yielded successful demonstrations of producing renewable fuels and commodity chemicals.[1−6] Furthermore, the promising trajectory of metabolic engineering and continued advances in large-scale bioprocess development have improved the economic outlook of these processes and allowed industrial operations to be developed for the production of targets, such as succinic and lactic acid. However, among the unit operations employed, the major cost driver is in the separation of the acid from dilute fermentation broth, accounting for approximately 20−40% of the product cost.[7] The typical method for carboxylic acid recovery is dependent on the target acid produced. However, the sequence of steps involved generally includes some combination of (1) filtration, (2) color removal, (3) salt breaking, (4) evaporative dewatering, and (5) neat product recovery (chromatography, distillation, crystallization, etc.).[8] Given the large proportion of production cost associated with separations, it is important that scalable, cost-effective methods are developed for the recovery of carboxylic acids from fermentation broth, and to that end, modifications and alternative approaches to the typical sequence described above have been the subject of several publications.[9−11]

In this article, we report a modified sequence of five steps, shown in Figure 1, for the recovery of neat carboxylic acids from fermentation broth that, to our knowledge, achieve the highest reported crystallization yields for succinic acid and also produces good results for the recovery of propionic acid. These steps include (1) broth centrifugation and filtration (for cell, particulate, and protein removal), (2) cation exchange (salt removal and acidification), (3) activated carbon treatment (color removal), (4) nonthermal dewatering using a poly(4-vinylpyridine) (PVP) resin, and (5) a final product recovery step, which is chosen on the basis of the physical properties of the targeted acid. The key findings are that the order of operations is critical to increasing the overall broth-to-product yield. Specifically, cation exchange should be performed first to lower the amount of activated carbon required. This reduces waste and increases the overall yield by decreasing product loss due to adsorption on the carbon. The second key finding is that nonthermal dewatering in lieu of evaporative dewatering is

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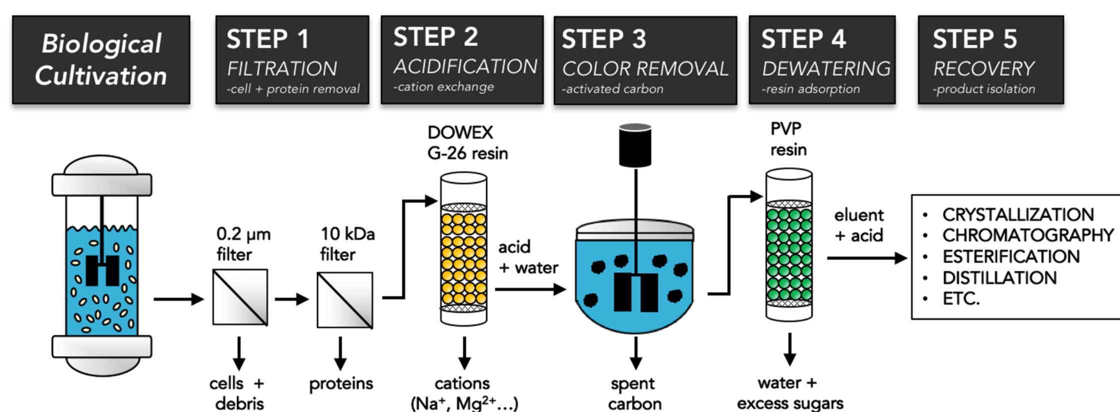

Figure 1. Illustration of the five-step method for recovering carboxylic acids from fermentation broth. In step 1, broth is subjected to centrifugation and filtration, first with a 0.2 μm filter and then a 10 kDa filter to remove debris, cells, and protein. In step 2, the broth is subjected to cation exchange to remove the cation from the salt and generate the free acid. In step 3, color-inducing compounds are further removed through treatment with activated carbon. In step 4, water and excess sugars are removed via adsorption of the free acids to a PVP resin. Finally, in step 5, the target acid is recovered in neat form by elution from the PVP resin with a polar solvent (e.g., methanol, acetone, etc.) and recovered via a method tailored to the physical properties of the acid. Exemplary methods include crystallization, chromatography, esterification, and distillation.

more energy efficient, and its selectivity toward acids results in a highly pure product.

We demonstrate this approach on two common acids produced from the fermentation of sugars, succinic acid and propionic acid. These acids form an excellent, comparative case study because they have significantly different physical properties, and the strains used to produce them are heterofermentative, meaning that minor acids are also present. Namely, succinic acid is a solid C4 diacid at standard conditions with a solubility in water of ~100 g/L, while propionic acid is a liquid C3 monoacid at standard conditions that is completely miscible in water. Due to the differences in physical properties of these target acids, the final recovery step is different for each case.

For succinic acid, we employ a cold crystallization in methanol, achieving a crystallization yield of 91% and colorless crystals with a purity of 99.93%. To our knowledge, these are the highest reported crystallization yields and purities for the recovery of succinic acid from fermentation broth.

For propionic acid, a spinning band distillation system was used to recover propionic acid from the PVP elution solvent (acetone). Recovered propionic acid was obtained at a distillation yield of 80% and a purity of 98%.

Additionally, the separation methods that we report here are the result of recovering these acids from corn stover hydrolysate fermentations, which are inherently more difficult broths to handle given the increased chemical complexity of hydrolysates compared to fermentations conducted with clean sugars. The demonstrated success of this approach on hydrolysate-based fermentation broths indicates the robust nature of this process and suggests that this method could likely achieve improved results for fermentations with refined substrates (glucose, sucrose, etc.). These results also highlight the utility of these methods as important tools for researchers operating at the bench scale and can be used to enable research into downstream catalytic conversion of these acids into value-added chemicals and fuels, as this field is growing rapidly. Lastly, we provide a discussion on how these methods can be scaled for industrial applications.

EXPERIMENTAL SECTION

Production of C5-Rich Hydrolysate for Succinic Acid Production. The production of xylose-rich hydrolysate was previously described by Salvachúa et al.[12] In brief, corn stover was knife-milled and deacetylated with 0.4% (w/w) NaOH. After deacetylation, the spent caustic liquor was drained from the vessel, and the solids were rinsed with water and subjected to a dilute acid pretreatment with H_2SO_4 (8 g H_2SO_4/kg biomass). Pretreated, deacetylated solids were pressed to obtain the C5-hydrolysate. The hydrolysate was neutralized with NaOH (10 N) and filter-sterilized for further succinic acid fermentations.

Production of C5/C6-Hydrolysate for Propionic Acid Production. The preparation of C5−C6-hydrolysate was previously described by Wang et al.[13] In brief, corn stover was hammer-milled and deacetylated using a dilute NaOH solution (0.4% w/w). Following deacetylation, remaining solids were rinsed with water and then mixed with dilute H_2SO_4 solution to achieve a 0.8% (w/w) acid concentration for dilute acid pretreatment. After pretreatment, the slurry stream was neutralized and enzymatically hydrolyzed using Novozymes Cellic CTec2. The soluble sugar stream was separated from lignin solids, neutralized with NaOH (10 N), and filter-sterilized for further propionic acid fermentations. The composition of each hydrolysate is shown in Table 1.

Table 1. Composition of C5-Rich and C5/C6 Corn Stover Hydrolysates.

	hydrolysate composition (g/L)	
component	C5-rich	C6/C5
glucose	13.60	89.10
xylose	99.00	56.70
arabinose	6.60	7.80
acetic acid	15.60	1.80
furfural	3.80	0.90
HMF	1.76	0.14

Microorganism and Fermentation Conditions To Produce Succinic Acid. *Actinobacillus succinogenes* 130Z (ATCC 55618) was revived from glycerol stocks by inoculating 1 mL in 100 mL sterile capped bottles containing 50 mL of tryptic soy broth (TSB). Cells were incubated for ~16 h at 37 °C and 200 rpm and then transferred into 0.5-L working volume BioStat-Q Plus fermentors with 300 mL of TSB and 4% xylose at an initial OD_{600} of 0.05 (seed culture). The pH was controlled at 6.8 via supplementation of 4 N NaOH, the temperature at 37 °C, and the agitation at 300 rpm. CO_2 was sparged

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at 0.03 vvm to ensure anaerobic fermentation and enhance succinate yields.[14] After ∼15 h of fermentation, cells were inoculated in 9 different fermenters (0.5-L working volume BioStat-Q Plus) with 300 mL of media at an initial OD of 0.1. The fermentation media contained 6 g/L yeast extract, 10 g/L corn steep liquor (the latter prepared as previously described by Salvachúa et al.),[12] 0.3 g/L Na_2HPO_4, 1.4 g/L NaH_2PO_4, 1.5 g/L K_2HPO_4, 1.4 g/L sodium acetate, 1 g/L NaCl, 0.2 g/L $MgCl_2·6H_2O$, 0.2 g/L $CaCl_2·2H_2O$, and diluted C5-hydrolysate to yield an initial sugar concentration of 55 g/L sugars. Fermentations were controlled at conditions the same as the seed culture. Succinate titer (g/L) variability among the nine biological replicates was ±2.6%.

Microorganism and Fermentation Conditions To Produce Propionic Acid. *P. acidipropionici* ATCC 4875 was revived from glycerol stocks by inoculating 1 mL into a 100 mL sealed serum bottle containing 25 mL seed medium. Seed media consisted of 40 g/L glucose, 10 g/L yeast extract, 5 g/L tryptic soy broth, 0.48 g/L K_2HPO_4, 0.98 g/L KH_2PO_4, and 0.05 g/L $MnSO_4$. After incubating the cells at 30 °C for 48 h and 200 rpm, 1.5 mL cell culture was transferred into a 250 mL serum bottle containing 100 mL of fresh seed medium for 40 h, at 30 °C, and 200 rpm. Cell pellets were then collected through centrifugation at 6000*g* for 10 min at 4 °C. Cell pellets were resuspended and inoculated into a 10 L bioreactor (New Brunswick BioFlo/CelliGen 310 bioreactor, Eppendorf) containing 9.5 L of fermentation medium at an initial OD_{600} of 0.6. The fermentation medium contained 13.9 g/L yeast extract, 0.48 g/L K_2HPO_4, 0.98 g/L KH_2PO_4, 0.05 g/L $MnSO_4$, and diluted C6/C5 corn stover hydrolysate to yield an initial sugar concentration of 125 g/L (75.3 g/L glucose, 44.3 g/L xylose, and 6.5 g/L arabinose). The fermentation was conducted at 30 °C with constant agitation at 400 rpm. The pH was controlled at 6.0 by the addition of 4 N NaOH. Anaerobic conditions were maintained by sparging 0.1 vvm N_2 in the medium during the fermentation process.

Cell Particulate and Protein Removal. Ten liters of each broth was centrifuged at 12 000*g* for 30 min in 1 L centrifuge bottles (ThermoFisher Scientific, Waltham MA). After 30 min, a thick cell cake was formed, and the liquid broth was decanted off of the cake and vacuum filtered through a PTFE filter with a pore size of 0.2 μm (ThermoFisher Scientific Nalgene, Waltham MA). The filter-sterilized broth was then filtered through a hollow fiber cartridge filter with a 10 kDa pore size (GE Healthcare Bio-Sciences Corp., Westborough MA) to remove proteins. After each filtration, the total mass of liquid was measured, and a 0.5 mL sample was taken for analysis (see the Analytical Methods section). The mass and measured concentration from each step was used to determine the yield of acid after each separation step.

Cation Exchange. The DOWEX G-26 resin (Sigma-Aldrich, lot #MKBX1809 V) was cleaned and pretreated by adding 600 g of dry resin to a large beaker, which was covered with 10 wt % sulfuric acid and stirred for 1 h. The resin and sulfuric acid solution was then slurried into a 1 L glass column with a glass frit at the base of the column. Six bed volumes (BV) of ultra high purity (UHP) water were added and gravity drained rapidly through the resin bed using a stopcock at the bottom of the column. The pH of the effluent water was tested using pH paper, and additional water was rinsed through the column, if necessary, until the pH of the effluent water was neutral. Then, 200 mL of the wet resin was then removed from the column and added to a large beaker, and 1 L of filtered fermentation broth was added to the beaker and stirred with the resin for 1 h. This batch exchange was necessary to release CO_2 gas from the broth before it was passed through the column.

The pre-exchanged broth and resin were then poured on top of the resin column and drained through at a rate of ∼ 50 mL/min. As the broth drained through the column, additional water was added on top of the broth to ensure that the column did not run dry. The black-colored broth was collected as it eluted from the column. Collection of the broth stopped when the black color of the eluent disappeared.

The resin was regenerated by first rinsing the resin in the column rapidly with 2 BV of water. Next, 2 BV 10 wt % sulfuric acid was passed through the column at a rate of 40 mL/min. The sulfuric acid was rinsed out of the column first with 1 BV of water at a rate of 25 mL/min, followed by rapidly rinsing the column with 6 BV of water. The effluent water was confirmed to be of neutral pH, indicating the sulfuric acid had been fully removed from the column.

Activated Carbon Treatment. For the data shown in Figure 2A−E, 1, 3, 5, or 7 w/v% activated carbon (Sigma-Aldrich, lot #MKBS9039 V) was added to 20 mL samples of broth that had been ion exchanged and broth that had not been ion exchanged. The mixtures were then stirred vigorously for 4 h. The carbon was removed via vacuum filtration through a 0.2 μm PTFE filter. The treated 20 mL samples were analyzed for color inducing impurities as a function of carbon loading via the analytical methods described below. From these results, the optimal carbon loadings were determined, and the entire 10 L of remaining broth was ion exchanged and treated with 3 wt % activated carbon for 4 h.

Dewatering with PVP Resin. The loading capacity of the target acids, succinic and propionic acid, on the PVP resin were determined by generating breakthrough curves (Figures 3A and S4A). For these experiments, 5 g (∼6 mL wetted volume) of dry resin (Sigma-Aldrich Reillex 425, lot #MKBR9896) was stirred in 200-proof ethanol for 20 min. The ethanol was vacuum filtered off. and the resin was slurried into a 25 mL buret in UHP water with a quartz wool plug at the base of the column. The column was allowed to settle and then was rinsed with 10 BV of UHP water. Then, 5−6 BV of cation-exchanged and activated carbon treated broth was added on top of the resin and drained through at a rate of 3 BV/h (0.4 mL/min), a rate suggested by Kawabata et al.[15] Effluent samples were taken at intervals between 0.3−0.6 BV and analyzed for acid concentrations as described in the analytical methods below. The breakthrough point was determined as the midpoint between the first data point that measures a detectable amount of target acid and the previous data point that did not measure any concentration of target acid. Using the breakthrough point, the loading capacity of the resin was determined, in mg of the target acid/g dry resin using eq 1 below.

$$\text{loading capacity} = \frac{V_{\text{effluent}} \times C_{\text{acid}}}{m_{\text{PVP resin}}} \quad (1)$$

In eq 1, V_{effluent} is the total volume in mL of effluent that was collected up to the breakthrough point, C_{acid} is the concentration of the target acid in the broth in mg/mL, and $m_{\text{PVP resin}}$ is the dry mass of the PVP resin present in the column.

Elution profiles (Figures 3B and S4B) were constructed to determine the minimum volume of elution solvent required to completely remove the target acid from the column. Here, 5 g of dry PVP resin was slurried in a 25 mL buret as described above. The resin was loaded with acid by passing an appropriate volume of pretreated broth through the column, determined from eq 1, in order to reach the loading capacity. The 4−5 BV of the elution solvent (methanol for succinic acid and acetone for propionic acid) was passed through the column at a rate of 3 BV/h. Effluent samples were taken at intervals between 0.3−0.6 BV and analyzed for acid concentrations as described in the analytical methods below. The minimum volume of eluent necessary to elute the target acid was determined by the first elution fraction to yield a concentration of the target acid below 0.2 wt %.

For the succinic acid broth, the loading capacity determined above in eq 1 was used to scale up the procedure and process 1 L of fermentation broth. Here, 1 L of acidified and activated carbon-treated broth was loaded onto the PVP resin in a 1 L glass column. The broth was then drained at a rate of 3 BV/h until the liquid level was just above the bed of resin. The minimum volume of methanol (for succinic acid) or acetone (for propionic acid) determined by the elution profile, described above, was measured and gently loaded onto the column. Eluent was drained at a rate of 3 BV/h, and the first 0.25 BV was set aside, as this volume contains mostly the remaining water from the influent broth. This portion was analyzed for succinic acid and propionic acid content to quantify any product loss.

For the propionic acid broth, the loading capacity determined above in eq 1 was used to scale up the procedure. Similarly, 1 L of

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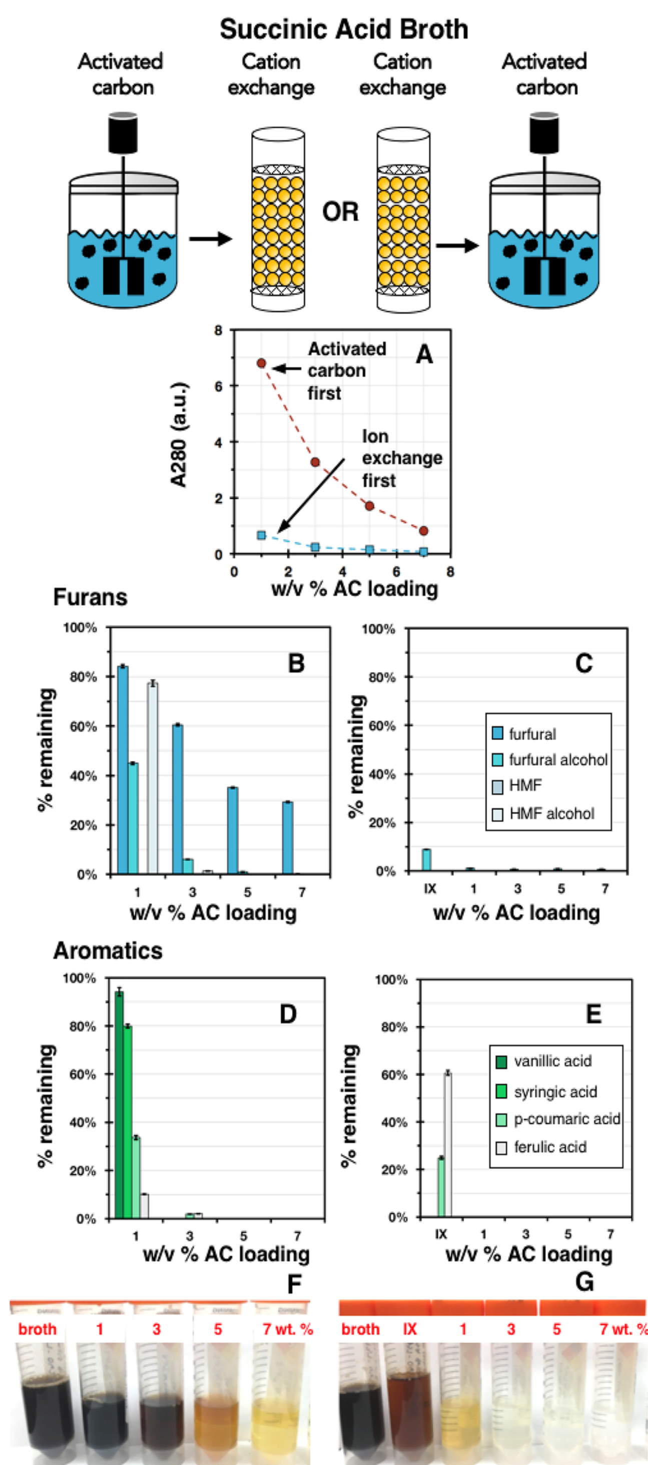

Figure 2. Color removal effect of treating succinic acid fermentation broth with activated carbon compared to first cation exchanging the broth then treating with activated carbon. (A) UV−vis measurement of fermentation broth treated with the two procedures above. (B, C) The percentage of furans remaining in the broth, relative to the results in Table 2, following activated carbon treatment (B) and following IX first before activated carbon treatment (C). (D, E) The percentage of aromatic compounds following activated carbon treatment at various loadings (D) and following IX first before activated carbon treatment (E). (F, G) An image displaying the color differences in the broths treated through both methods.

acidified and activated carbon treated broth was loaded onto the PVP resin in a 1 L glass column. The broth was then drained at a rate of 3 BV/h until the liquid level was just above the bed of resin. The minimum volume of acetone determined by the elution profile, described above, was measured and gently loaded onto the column. Eluent was drained at 3 BV/h, and the first 0.25 BV was set aside, as this volume contains the remaining water from the influent broth. This portion was analyzed for propionic acid content (product loss). Then, ~90% of the acetone was removed via rotary evaporation, and the acetone collected in the trap was analyzed for propionic acid that may have carried over during the acetone solvent removal.

Succinic Acid Recovery via Crystallization. The methanol eluent from the PVP resin was concentrated via rotary evaporation, until crystals of succinic acid began to form on the flask. Crystals were redissolved by gently heating and stirring the methanol solution. Succinic acid was crystallized by placing the warm flask containing the crystallization liquor in a −40 °C cold bath of acetonitrile and dry ice for 4 h. A K-type thermocouple was used to measure the bath and solution temperature. After crystallization appeared to be complete, the methanol was vacuum filtered until no liquid was dripping into the filter flask. The succinic acid was then dried in a vacuum oven overnight at 40 °C. The crystallization yield was determined by dividing the mass of succinic acid eluted off the column by the dry weight of succinic acid obtained. The purity of succinic acid was determined by differential scanning calorimetry (DSC) as described below.

Propionic Acid Recovery via Distillation. A spinning band 800 Micro Distillation System (B/R Instrument Corporation, Easton, MD), equipped with a vacuum pump (Edwards, Burgess Hill, England) was used to distill the acetic acid−propionic acid mixture obtained from the liquid−liquid extraction. The acetic acid was distilled off at a reflux ratio of 30 at "atmospheric pressure" (0.82 atm in Golden, CO) with the Teflon spinning band operating at 5000 rpm (~30 equiv distillation trays). The remaining propionic acid was carried over as a distillate to ensure purity. Distillate fractions were collected, and purities were determined by HPLC as described below.

Analytical Methods. Analysis of acid content in the samples was performed on an Agilent 1200 LC system equipped with a G1362A refractive index detector (RID) (Agilent Technologies, Santa Clara, CA). Each sample was injected undiluted at a volume of 20 μL into an BioRad Aminex HPX-87H 9 μm, 7.8 mm × 300 mm column (BioRad, Hercules, CA) using a column and RID detector temperature of 55 °C. Compounds were separated utilizing an isocratic flow of 0.01 N H_2SO_4 in water at 0.6 mL/min for a total run time of 27 min. Sugar concentrations were measured using an ICS-5000 + HPLC system (Dionex Corp., Sunnyvale, CA, USA). Samples were diluted to a quantifiable range, and 10 μL was injected on to a CarboPac SA-10 Dionex carbohydrates column (4 mm × 250 mm) equipped with a CarboPac SA-10 guard column (4 mm × 50 mm). Sugars were separated using an isocratic flow of 1 mM potassium hydroxide at 1.5 mL/min for 15 min at 45 °C. Aromatic and furan concentrations were analyzed using an Agilent 1100 LC system equipped with a G1315B diode array detector and an Ion Trap SL (Agilent Technologies, Palo Alto, CA) mass spectrometer (MS) with in-line electrospray ionization (ESI). Each sample was injected undiluted at a volume of 50 μL into an YMC C30 Carotenoid 3 μm, 4.6 mm × 150 mm column (YMC America, Allentown, PA) at an oven temperature of 30 °C. The HPLC solvent regime consisted of eluent (A), water modified with 0.03% formic acid, and eluent (B), 9:1 acetonitrile and water also modified with 0.03% formic acid. At a flow rate of 0.7 mL/min, the gradient chromatography was as follows: 0−3 min, 0% B; 16 min, 7% B; 21 min, 8.5% B; 34 min, 10% B; 46 min, 25% B; 51−54 min, 30% B; 61 min, 50% B; and the last 64−75 min, 100% B before equilibrium. Flow from the HPLC-DAD was directly routed to the ESI-MS ion trap. The DAD was used to monitor chromatography at 210 nm for a direct comparison to MS data. Source and ion trap conditions were as previously described.[16] For each chromatographic analytical method, a minimum of five calibration levels was used from the response of authentic standards, resulting in a coefficient of determination (R^2) of 0.995 or better for

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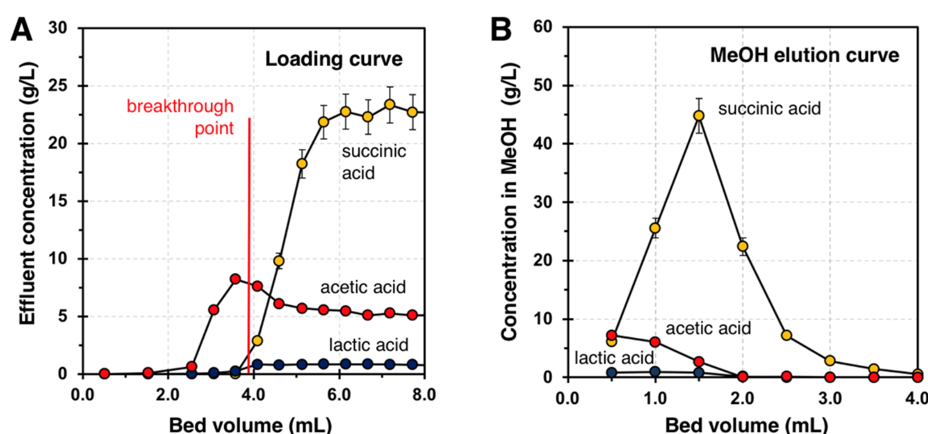

Figure 3. (A) Loading curve for succinic acid broth passed through a 6 mL wetted BV of PVP resin (5 g dry bed mass of PVP). Here, the broth drained through the column was analyzed for the concentrations of major acidic components (acetic acid, lactic acid, and succinic acid). The broth was collected in fractions, and concentrations of each sample are reported as BV on the *x*-axis. (B) Elution curve of the major acidic components desorbed from the resin using methanol.

each analyte. The UV absorbance measurement in Figure 2A was performed on a Thermo Scientific Nanodrop 8000 spectrophotometer. Each sample was diluted with DI water as necessary to bring the absorbance within a range of 0.0−6.0 au

DSC for Purity Analysis. Purity was determined via melting point depression, as measured by modulated digital scanning calorimetry (MDSC) on a TA Instruments Q200 MDSC. Samples were prepared by measuring out ~20 mg of sample into a TA Instruments Aluminum Hematic T-Zero Pan. For succinic acid, the samples were tested from 160 to 200 °C at a ramp rate of 3 °C/min with a modulation period of 40 s and a modulation amplitude of 0.12 °C.

RESULTS

Fermentation Broths. Fermentation broth containing 38.7 g/L succinic acid was obtained from *Actinobacillus succinogenes* fermentation of C5-rich corn stover hydrolysate, and a separate fermentation broth containing 35.6 g/L propionic acid was obtained from *Propionibacterium acidipropionici* fermentation of C5/C6 corn stover hydrolysate, similar to our previously published work.[12,13] The concentrations of carboxylic acids (including secondary acids produced by the heterofermentative microbes), unfermented sugars, furans, and trace aromatics are reported in Table 2 for each broth. The furans and aromatics are generated from sugar degradation reactions and lignin, respectively, that occur during acid pretreatment of corn stover.

In both broths, the presence of furans and aromatics creates a black color (Figure S1). To highlight the drastic color difference between a hydrolysate fermentation and a refined glucose fermentation, two broths are shown side by side in Figure S1. In the biobased chemical industry, the economical removal of discoloration remains challenging. These compounds are often present at trace levels but are sufficiently potent to discolor the final product (generally a yellow tint). Yellow color in the final product is aesthetically undesirable when using the recovered chemical in downstream products, particularly when producing polymers. For example, when polymerizing succinic acid with 1,4-butanediol to produce poly(butylene succinate) (PBS), the removal of the yellow coloring beforehand is key to achieve reproducible dyeability of the final product. Thus, the black color of the hydrolysate broth necessitates development of a more effective color removal process in lieu of a simple activated carbon treatment.

Table 2. Measured Composition of the Original Broths prior to the Unit Operations in Figure 1[a]

	succinic acid broth (g/L)	propionic acid broth (g/L)
Acids		
succinic acid	38.7	5.90
propionic acid	N.D.[b]	35.6
acetic acid	9.46	7.38
lactic acid	0.51	N.D.
formic acid	0.37	0.68
Sugars		
glucose	N.D.	N.D.
xylose	0.07	0.69
galactose	0.64	N.D.
arabinose	0.21	N.D.
Furans		
furfural	0.02	N.D.
furfural alcohol	0.39	0.13
HMF	N.D.	0.08
HMF alcohol	0.04	0.41
Aromatics		
p-coumaric acid	0.09	0.08
ferulic acid	0.08	0.01
4-hydroxybenzaldehyde	0.01	2.0 E^{-3}
syringic acid	2.0 E^{-4}	7.0 E^{-3}
vanillic acid	8.0 E^{-4}	0.01
caffeic acid	0.01	0.02

[a]Compounds are grouped as acids, furans, and aromatics. [b]N.D. = not detected.

Color Removal Effect of the Ion-Exchange Resin and Activated Carbon. The removal of color bodies from fermentation broths is commonly achieved through activated carbon treatment.[17] However, activated carbon will absorb a percentage of the target carboxylate from the broth and thus will lower the overall product yield. To quantify the losses associated with activated carbon treatment, increased loadings of activated carbon were added to samples of both the succinic acid broth and the propionic acid broth at 1, 3, 5, and 7 wt % loadings, and the carboxylic acid concentrations of the treated

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samples were measured post treatment. The results are shown in Figure S2 and demonstrate that, in the succinic acid broth at 1 w/v% activated carbon treatment, 6% of the succinic acid is lost to absorption to the activated carbon, while a 7 w/v% activated carbon treatment results in a 31% loss of succinic acid. Similarly, for the propionic acid broth, 4% of the propionic acid is lost at a 1 w/v% activated carbon treatment, with 21% lost at a 7 w/v% activated carbon treatment. Thus, approaches to color removal that minimize the amount of activated carbon required are needed to increase the overall product recovery yield.

To that end, the amount of activated carbon required to sufficiently remove color bodies can be reduced by first acidifying the broth with cation exchange resin before activated carbon treatment. Cation exchange is generally employed as a salt breaking step wherein the cation (in this case sodium) of the carboxylic acid is transferred to the resin in exchange for a proton. However, the cation exchange resin can serve a dual purpose to decolor the broth in addition to serving as the salt breaking step. To demonstrate the color removal effect of the cation exchange resin, broth was treated with increasing activated carbon loadings from 1 to 7 w/v% and compared to broth that had been cation exchanged first (as described in the Experimental section) and then treated with increasing activated carbon loadings. The results for the succinic acid broth are shown in Figure 2, and the results of the propionic acid broth are shown in Figure S3.

In Figure 2A, UV absorbance measurements at 280 nm are shown for broth that has been treated with increasing activated carbon loadings alone and for broth that has been cation exchanged first and then treated with increasing activated carbon loadings. The UV measurement at 280 nm tracks the relative amount of aromatic components present in the broth, which contributes to the color. From Figure 2A, it is clear that the broth that is cation exchanged first before activated carbon treatment exhibits a significantly lower absorbance at 280 nm across all activated carbon loadings. Even at 1 wt % activated carbon loading, the cation exchanged broth exhibits an absorbance measurement of 0.65 au, while the broth that has not been cation exchanged exhibits an absorbance of 6.8., approximately a 10-fold decrease in absorbance intensity at 280 nm is achieved by cation exchanging the succinic acid broth before activated carbon treatment.

Results in Figure 2B,C display the percentage of furans as a function of activated carbon loading used, relative to the concentrations in Table 2 for untreated broth. In Figure 2B, broth that has been treated with various loadings of activated carbon still retains a significant furan content. Furfural remains at 30% of its initial concentration even after a 7 w/v% treatment with activated carbon. In Figure 2C, broth that has been cation exchanged first before activated carbon treatment contains no measurable concentration of any furan after a 1 w/v% activated carbon treatment. Results in Figure 2D,E show aromatic content derived from lignin compounds and demonstrate similar trends to those reported in Figure 2B,C. Activated carbon treatment alone results in Figure 2D indicate at least a 3 w/v% activated carbon is needed to reduce the aromatic content below detection limits. Results in Figure 2E show that cation exchange before activated carbon lowers the needed activated carbon load to 1 w/v% to reduce the aromatic content below detection limits.

We note that only select furanic- and lignin-derived aromatic compounds were tracked and quantified in the results shown in Figure 2. These results suggest that a 1 w/v% activated carbon loading is sufficient to remove all of the measured furanic and aromatic components if the broth is cation exchanged first (compared to >7 wt % activated carbon needed if the broth is not ion exchanged first). However, the UV absorbance results shown in Figure 2A reflect a larger group of aromatic color inducing compounds in excess of what was identified and quantified in Figure 2B–E. In Figure 2A, the A_{280} signal decreases from 0.65 to 0.22 between a 1 and 3 w/v% activated carbon treatment and is essentially constant at loadings greater than 3 w/v%. These results appear to track the visual images of the broth in Figure 2F,G that show the broth treated with increasing activated carbon loadings (Figure 2F) compared to broth cation exchanged followed by treatment with increasing activated carbon loadings (Figure 2G). In Figure 2G, there is little to no color in the broth that has been cation exchanged and treated with activated carbon at 3 w/v% or greater loadings. As such, the remainder of both the succinic acid broth and the propionic acid broth (~10 L) was processed by first cation exchange and then treated with 3 w/v% of activated carbon. This yielded decolored broths at pH ~2.5–3.0 with the composition shown in Table S1. After this treatment, the concentration of succinic acid is 24.7 g/L (Table S1) and is the result of excess water present in the cation exchange column diluting the broth (similar results were obtained for the propionic acid broth Table S1). The gravimetric yield on recovered succinic acid from cation exchange and activated carbon treatment was found to be 89%.

Dewatering with PVP Resin. After cation exchange and activated carbon treatment (steps 2–3 in Figure 1), the broths are acidified, and color-inducing impurities are sufficiently removed, leaving dilute free acid (1–10 wt %) in an aqueous solution. Recovery of the acid through distillation at this point is not energetically practical given that carboxylic acids have boiling points higher than that of water. Thus, to remove the acids from water, the broths are passed through a solid adsorbent that selectively adsorbs acids. Two weak-base adsorbents work well for this purpose, PVP[9] and polybenzimidazole (PBI).[18] These adsorbents are known to selectively retain carboxylic acids through an ion-pair interaction, and elution can be performed with a polar solvent (e.g., alcohols, acetone, etc.).[15] We note the PBI resin is reported to have a greater capacity for carboxylic acid adsorption over PVP.[15] However, commercial PVP resins are more readily available from standard chemical suppliers, and thus we utilize PVP in this work.

Breakthrough and elution curves measured in Figure 3 are used to size the amount of PVP resin and methanol eluent needed to dewater the target acid from the broth. Figure 3A displays the breakthrough curve for the succinic acid broth passed through a 6 mL wetted BV of PVP (described in detail in the Experimental section). In Figure 3A, we define the breakthrough point as the midpoint between the first data point that measures a detectable amount of succinic acid and the previous data point that did not measure any concentration of succinic acid. In Figure 3A, the breakthrough point defined in this way is measured at 3.9 BV for the succinic acid broth and 1.4 BV for the propionic acid broth (Figure S4A). Using the breakthrough point, the known concentration of target acid in the influent, and the mass of dry PVP loaded into the column, the loading capacity of the target acid on the resin can be determined using eq 1 (Experimental section). This analysis

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Table 3. Succinic Acid Crystallization Methods Reported in the Literature Compared to the Method Reported in This Work[a]

substrate	A.C.[b] loading (w/v%)	crystallization yield (%)	purity (%)	method	ref
hydrolysate	3.0	91	99.93	filtration > cation EX > AC > PVP > crystallization	this work
glucose	12.5	89.5	99.0	filtration > AC > cation EX > crystallization	9
glucose	N/A	86.53	99.18	H_2SO_4 acidification > microfiltration > nanofiltration > crystallization	10
glucose	10.0	85.4	96.7	salting out extraction > filtration > AC > vacuum distillation > crystallization	11
glucose	N/A	77.0	99.9	filtration > electrodialysis > crystallization	20
glucose	N/A	73.1	99.8	liquid−liquid extraction of byproduct acids > HCl acidification > crystallization	21
glucose	20.0	70.0	90.0	filtration > AC > calcium succinate precipitation > ion exchange > crystallization	22
hydrolysate	12.5	45.0	28.0	filtration > AC > HCl acidification > crystallization	23

[a]Here, activated carbon loadings used are listed along with crystallization yields and purities achieved. A brief description of the process used in each reference is also given for comparison. [b]AC = activated carbon.

yields a loading capacity of 106 mg of succinic acid/g of dry PVP.

The total amount of eluent needed to desorb the acid from the column is determined for each target acid through the measurement of an elution curve. Figure 3B displays the elution curve measured for succinic acid desorbed from the resin by passing methanol through the column. In Figure 3B, we find that 4 BV of methanol are needed to completely remove the adsorbed succinic acid from the resin. After elution, succinic acid is present in the methanol eluent at a concentration of 22 g/L.

Similarly, the cation-exchanged and color-removed broth containing propionic acid was loaded onto 6 mL (wetted BV) of PVP, and loading and elution curves were measured (Figure S4). From this analysis, a loading capacity of 85 mg of propionic acid/g of dry PVP was determined. Elution from the resin was performed with acetone[15] as the eluent, finding 2.5 BV of acetone are required to fully recover propionic acid from the resin (Figure S4B). Thus, the concentration of propionic acid in the acetone eluent is 28 g/L.

Acetone was chosen as the eluent because propionic acid is recovered from the eluent via distillation (discussed below), and the boiling point of acetone is 56 °C (at standard conditions), sufficiently different from propionic acid (BP 118 °C, S.C.) such that acetone can be evaporated from the acids without significant loss of the target compound.

Crystallization of Succinic Acid. Table 3 summarizes the reported crystallization yields and purities from previous studies and our work. The separation approach that is reported in this work is distinct from the approaches summarized in Table 3 because succinic acid is eluted from the PVP resin using methanol. Thus, the crystallization of succinic acid occurs in a methanol solvent rather than water. The benefit of using methanol as a crystallization solvent is 2-fold, (1) methanol can be cooled to temperatures lower than that of water before freezing, allowing succinic acid crystals to be driven out of solution in very high yields, and (2) methanol requires less energy to evaporate than water, thus improving the energy efficiency of an evaporative crystallization operation.

Accordingly, 1.5 L of wet methanol eluent from the PVP resin containing ∼22 g/L of succinic acid in methanol and ∼10 wt % water was evaporated to its saturation point where small crystals began to form on the flask (∼180 g/L).[19] The flask was then warmed slightly (∼25−30 °C) to fully solubilize the succinic acid. Succinic acid was then recovered through crystallization by submerging the flask in a −40 °C cold bath of acetonitrile and dry ice for 4 h. The crystals where recovered via vacuum filtration and dried for 12 h in a vacuum oven (Figure S5). On a dry mass basis, this crystallization procedure achieved a 91% yield of succinic acid from the methanol solution, and the purity was determined via DSC to be 99.93%. To our knowledge, these are the highest crystallization yields and purities obtained compared to other reported crystallization methods (Table 3).

In addition to the high yields and purities, this approach is more energy efficient than a crystallization operation that occurs in water. In the above procedure, 1.32 L of methanol was evaporated to generate the crystallization solution. Using the heat capacity and latent heat of vaporization of methanol this requires 1321 kJ of energy. In comparison, if the aqueous acidified solution from Step 3 (Figure 1) was evaporatively crystallized, it would require ∼2592 kJ of energy. Thus, the nonthermal dewatering step accomplished using the PVP resin allows for high yields and purities on a single pass crystallization and requires ∼50% less energy compared to crystallization in the aqueous solution from Step 3 (Figure 1).

Lastly, it is critically important that the activated carbon loading used for color removal is held to a minimum to maintain a high overall broth-to-product yield. As discussed above, at an activated carbon treatment of 7 w/v%, approximately 31% of the succinic acid product is lost to adsorption on the carbon (Figure S2). In the succinic acid recovery procedure demonstrated here, a 3 w/v% activated carbon treatment was used, losing 11% of the initial product to adsorption on the carbon. Couple this loss with the 91% crystallization yield in methanol, demonstrated above, and the overall broth-to-product yield for succinic acid recovery from our procedure is 81%. Reports in the literature for succinic acid recovery generally do not list an overall broth-to-product yield but rather report a crystallization yield only (Table 3), making it difficult to compare overall yields. The exception is ref 11, which reports an overall broth-to-product yield of 65%. Interestingly, ref 11 also finds a succinic acid recovery of 97.53% during a 10 w/v% treatment of activated carbon. This is drastically higher than the <70% recovery predicted from the results of this work, shown in Figure S2A. The much higher recovery of succinic acid during activated carbon treatment found in ref 11 is likely due to the activated carbon treatment occurring in the organic extractant (acetone and ammonium sulfate) instead of water. In this solution, the affinity for succinic acid to the carbon appears much lower than when activated carbon treatment occurs in water. In all other studies reported in Table 3, activated carbon treatment occurs in aqueous solutions. Thus, using the reported activated carbon loadings listed in those studies (Table 3), the lowest activated

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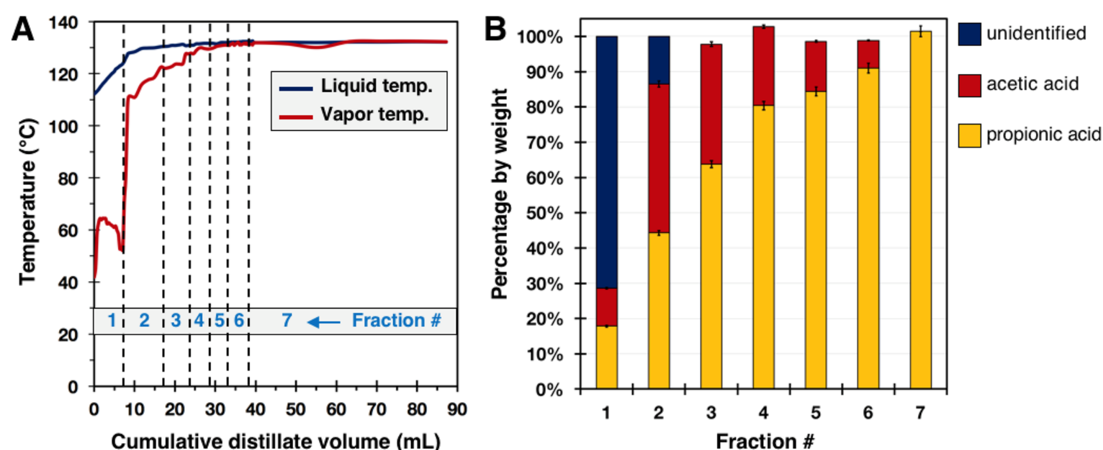

Figure 4. (A) Vapor and liquid temperature as a function of the cumulative distillate volume collected during distillation of the dried 87 mL of propionic acid eluent. The fractions collected are labeled numerically toward the bottom of the graph. The volume of each fraction is denoted between the dashed vertical lines. (B) Percent composition of each fraction was determined by liquid chromatography (LC).

carbon loading used appears to be 10 w/v%. Even at this loading, in water, well over 31% of the succinic acid product would be lost to carbon adsorption, (Figure S2A) and accordingly, one can expect overall broth-to-product yields from those previous studies in Table 3 to be much less than 70%. Thus, the results of this study and those in Table 3 highlight the importance in the order of operations for maintaining high broth-to-product yields. Namely, the use of cation exchange first before activated carbon treatment in order to lower the amount of activated carbon needed for color removal.

Recovery of Propionic Acid through Distillation. Four liters of cation-exchanged and activated carbon-treated propionic acid broth was loaded onto PVP resin and eluted off with acetone as described above. Acetone was removed from the eluent via rotoevaporation, which left an 844 g solution composed of 14% propionic acid, 4% acetic acid, 6% acetone, and 77% water. The propionic acid was recovered with a 91% yield from this PVP desorption step. The significant amount of water remaining is the result of incomplete dewatering.[24] It is likely, that water trapped in the pores of the resin was released into the acetone during elution. Complete removal of the water is necessary before distillation to avoid azeotrope formation with the propionic acid[25] that would ultimately prevent recovery of pure propionic acid. Thus, to remove the remaining water, an extraction using 1.4 L of dichloromethane (DCM) was performed. After extraction, the DCM phase was decanted and evaporated leaving a 105 g solution containing 75% propionic acid, 9% acetic acid, 9% water, and 8% unidentified (likely acetone, see below). To remove the remaining water, 10 g of drying salts (Na_2SO_4) were added to the solution and removed via vacuum filtration. These incomplete dewatering during PVP elution is an unfortunate reality when using batch column chromatography at these bench scales. The further drying steps described above likely would not be needed with the continuous processing equipment discussed below. The dried solution was then distilled using a spinning band system with 30 theoretical stages.

The vapor and liquid temperature traces during distillation are displayed in Figure 4A as a function of the cumulative distillate volume collected, and dashed lines in Figure 4A represent the fractions collected. Figure 4B displays the percent composition of the collected fractions. In fraction 1, approximately 70% of the mass of fraction #1 is unidentified. However, this is likely acetone, given that the average vapor temperature of fraction 1 is 58 °C, which is close to the boiling point of acetone (56 °C). Subsequent fractions 2−6 contain a mixture of acetic acid and propionic acid, which is expected from a batch distillation process. Fraction 7 contains pure propionic acid. After distillation, fractions 4−7 were combined, resulting in a 98% pure propionic acid solution with a propionic acid distillation yield of 80%.

The overall broth-to-product yield from this procedure is 59%. Specifically, 94% of the original propionic acid was recovered during the activated carbon treatment, 90% was recovered during the PVP adsorption and elution process, 87% was recovered after the extraction and drying steps, and 80% was recovered from the dewatered solution during the distillation operation.

In the literature, the method that is often reported for propionic/acetic acid separation from fermentation broth is the liquid−liquid extraction of the two acids into an organic phase containing a high molecular weight amine (e.g., trioctylamine) solved in a hydrophobic alcohol (e.g., oleyl alcohol).[26] The carboxylic acids are extracted into the organic phase through the formation of an ion pair with the amine.[27] After extraction, acetic and propionic acid can be recovered by thermally breaking the ion-pair in the organic phase via distillation. However, distillation of the organic phase yields a mixture of propionic acid, acetic acid, and some coextracted water.[28] The subsequent separation of these two compounds to recover neat propionic acid is a difficult separation to achieve, and in the instances reported in the literature, propionic acid is not recovered as a neat product. Thus, the yield and purity have not, to our knowledge, been reported before, and this prevents comparison to the yields and purities that we report in this article.

DISCUSSION

The procedure described above yields excellent bench-scale recovery yields and purity for the target carboxylic acids from fermentation broth, including with nontarget minor acids from heterofermentative carboxylate mixtures, and highlights the importance of the order of operations for achieving high broth-to-product yields. Here, we further provide a discussion of how

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to scale and improve these methods to continuous operations for industrial scale application. In general, the five-step procedure outlined in Figure 1 remains the same; however, specialized equipment is needed to complete each operation at scale.[8]

The first step where cell and particulates are removed is commonly achieved using tangential flow filtration with ceramic membranes[29] or by using rotating disc ceramic membranes[30] with pore sizes below 0.2 μm. Rotating systems increase broth throughput by decreasing filter fouling potential compared to the commonly used static filters used at the bench scale. Furthermore, ceramic membranes have several operational advantages. For example, ceramic membranes are more hydrophilic than polymer membranes, leading to less irreversible fouling, the excellent chemical resistance of ceramic membranes enables low pH operation, and the use of harsher cleaning methods to restore the initial flux of the membrane. These advantages lead to a longer membrane lifetime and less downtime.[31]

The second step, is the acidification of the broth to a pH below the pK_a of the target carboxylic acid. Recent work has shown this step is the most costly operation in the downstream separation of bioacids.[32] As such, some industrial bioacid processes are trending toward development of microbes that operate in low pH environments (e.g., for lactic acid[33] and succinic acid[34]). In these low pH fermentations, the salt breaking step is not needed in the downstream recovery operations. However, most fermentations and most laboratory level fermentations still occur at a pH above the pK_a of the target acid, and a salt breaking step is required.[35,36] In the instance when a salt breaking step is required at scale, cation exchange is performed in the continuous countercurrent cation exchange system.[37]

Color removal via activated carbon in step three, is a problematic step to implement at the industrial scale. As discussed above, the use of activated carbon results in significant product loss from adsorption. To that end, in this work, we demonstrated the importance of cation exchange first before activated carbon treatment to utilize the color removal capacity of the resin and allow lower loadings of activated carbon to be used. Here physical adsorption of the color body to the hydrophobic polymer backbone of the resin is likely the mechanism for color removal.[38] During regeneration of the resin with 10 wt.% sulfuric acid, the color bodies are washed off. In this work, the same 600 g of resin was regenerated 20 times to process the total 20 L of fermentation broth. We observed no loss in performance throughout these regeneration cycles.

Still, the 3 w/v% loading of activated carbon used in this study resulted in a 11% loss of succinic acid and a 6% loss of propionic acid during workup of the respective broths. This renders the use of activated carbon as a color removal option difficult to justify for industrial production. Therefore, alternative decoloring methods that do not result in product loss must be considered. Some alternative methods include the use of anion exchange resins[39] as a decoloring step before cation exchange, the pH swing precipitation followed by bleaching described by Fruchey[40] could also be higher yielding than activated carbon, and the use of ultra and nanofiltration[41] also appears promising. Studies are clearly needed in this area that focus on the yield and effectiveness of alternative decoloring methods compared to activated carbon.

Regarding the fourth step, dewatering using PVP adsorption and elution (Step 4, Figure 1) can be automated for large scale processing using a simulated moving bed (SMB).[42] However, modifications must be made to achieve complete dewatering before elution occurs. For example, a wash step of the resin bed using a hydrophobic liquid (e.g., petroleum ether) could be used before the alcohol elution to flush water out of the void volume in the resin bed. The benefits of complete dewatering using an SMB with an incorporated wash step to remove water are substantial. In the case of this work, if complete dewatering was achieved with PVP for the propionic acid broth, the subsequent DCM extraction and drying salts would not be required. This would result in the overall broth-to-product yield increasing from 59 to 70%. Furthermore, recent work has shown that the methanol eluent can be recovered from a SMB operation using <1% of the heating value of the carboxylic acid.[43]

Beyond post cultivation separation operations, in situ product recovery (ISPR) schemes may offer the next generation technology to significantly lower separation costs and time, while increasing fermentation titers, productivities, and yields.[44−52] These separation technologies are a form of process intensification wherein the extracellular product is separated in situ as it is produced in the fermentation broth. This ameliorates end product inhibition to the culture, allowing for greater productivities and titers in smaller fermentation volumes compared to a batch process. However, these methods are more complex to integrate at the bench scale, but their benefits highlight the importance of process intensification by combining separation operations directly with the bioprocess. As separation technology continues to advance, ISPR will likely see more use in lieu of multistep post cultivation operations for the production of bioproducts.

CONCLUSION

In this work, a method for separation of carboxylic acids from fermentation broth is detailed that contains a sequence of five steps. This method was applied to separate succinic acid and propionic acid from their respective broths. Overall broth-to-product yields were 81% for succinic acid and 59% for propionic acid and, the importance of cation exchange first before activated carbon treatment is detailed as a means to lower the required amount of activated carbon needed and limit yield losses to carbon adsorption. For succinic acid, a single pass crystallization yield of 91% with a product purity of 99.93% was achieved. To our knowledge, these are the highest yields and purities reported for the separation of succinic acid from fermentation broth. For propionic acid, distillation yields were 80%, resulting in a product purity of 98%. The simplicity of this method presents itself well as a method for researchers to recover carboxylic acids from fermentation broths for bench-scale laboratory testing. However, clear opportunities exist for the development of alternative decoloring methods to activated carbon that are higher yielding and for improving the dewatering efficiency of PVP columns.

ASSOCIATED CONTENT

Supporting Information

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Additional figures and tables referenced in the above text (PDF)

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Notes

The authors declare no competing financial interest.

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