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Recovery of Organic Acids from Fermentation Broths

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Abstract

Rising concerns over the use of fossil resources have generated renewed interest in the production of commodity chemicals via fermentation. Organic acids are a particularly attractive target because their functionality enables downstream catalytic upgrading to a variety of compounds. In this article, we survey how common technical issues are addressed in the recovery schemes for several organic acids. We present results for the recovery of acetate using a new method based on amine complexation. Our reactive separation scheme produces a high-purity product, is energy efficient, and avoids the coproduction of a waste salt coproduct, all prerequisites for a large-scale production process.

Index Entries: Organic acid recovery; acetic acid recovery; indirect ethanol process; gypsum; citric acid.

Introduction

Most commodity chemicals today are derived from fossil resources using synthetic routes. Organic acids are an attractive target for process development efforts in the emergent renewable-based biorefinery industry. Their functionality enables downstream catalytic upgrading to a variety of useful compounds including alcohols, aldehydes, ketones, esters, and olefins. Process concepts in which organic acids are used as intermediates in chemical production via combined fermentation and chemical synthesis schemes may eventually displace existing all-synthetic routes as the environmental and national security costs for the use of fossil resources rise.

Figure 1A shows some simple interconversions of generic organic acids and related derivatives. These transformations are applicable to a wide range of organic acids. To make the discussion more concrete, Fig. 1B illustrates the network for R = CH_3 (i.e., acetic acid). The number of products that can be reached by chemical transformations of this simple organic acid is surprising. Although not all the transformations shown in Fig. 1B are economically feasible, they are all technically feasible, and many of the transformations either are or were at one time the basis for commercial operations.

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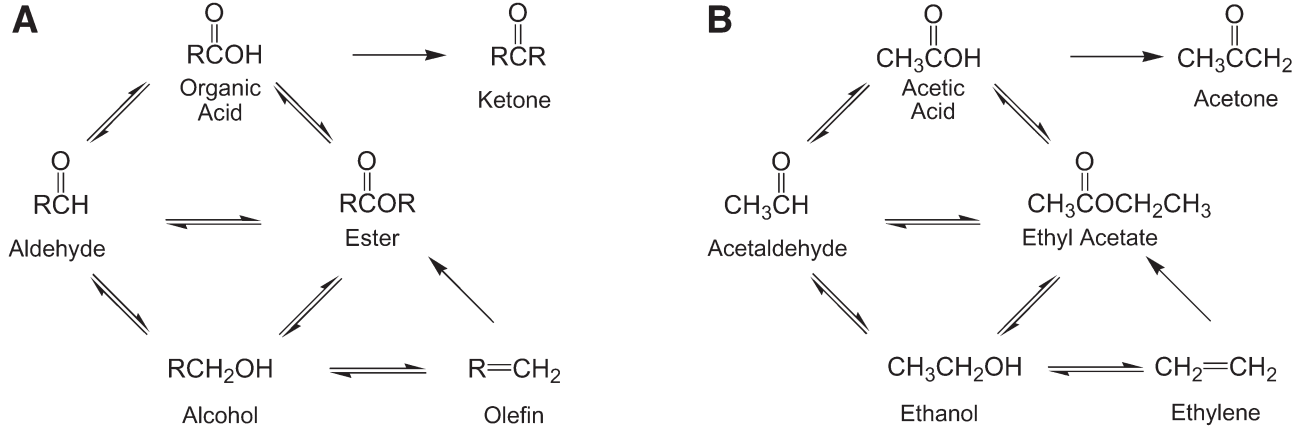

Fig. 1. Interconversions of an organic acid and derivatives: **(A)** generic case; **(B)** acetic acid and related C_2 derivatives.

Acetic acid, acetaldehyde, and ethanol are related through an oxidation/reduction sequence. Common oxidants or reductants can be used to interconvert these three species. Acetone was produced from acetic acid by dry distillation of the calcium salt prior to World War I *(1)*, after which this route was displaced by the acetone-butanol fermentation process (which, in turn, was displaced by synthetic routes). Today, intentional commercial production of ethyl acetate occurs by several routes depending on the local feedstock situation. These include esterification of acetic acid with ethanol, Tischenko condensation of 2 mol of acetaldehyde, addition of acetic acid to ethylene, or subjecting of ethanol to dehydrogenation conditions to produce ethyl acetate directly without the need for acetic acid *(2,3)*. Most ethanol today is produced by direct fermentation of carbohydrates, but synthetic production by hydration of ethylene is also commercially practiced. An intriguing possibility, ethanol production by hydrogenolysis of acetate esters *(4)*, is discussed later in this article. Conversely, ethylene can be readily derived from ethanol via dehydration *(5)*, the preferred route for ethylene production prior to the rise of the petrochemical industry in the 1950s.

A wide range of organic acids can potentially be produced by fermentation using known microorganisms, yet today nearly all organic acids are produced by synthetic means. Some reasons why fermentation-based routes have not been competitive with synthetic routes are related to the technical difficulties associated with recovery of organic acids from fermentation broths. Three common technical issues—high-purity specifications for the final product, energy-efficient means of handling the dilute broths, and avoidance of the coproduction of stoichiometric amounts of salts and other wastes—must be addressed to enable commodity-scale production of organic acids via fermentation.

Consider the case of lactic acid production. Sugars are converted into lactate using *Lactobacillus acidophilus, Rhizopus oryzae*, or other lactate-producing microorganisms. These organisms are often inhibited by low pH. To achieve high yield, the pH of the fermentation has to be kept near neutral by the addition of a base. Because the fermentation is conducted at a pH above the pK_a of the acid, the fermentation produces a dilute solution of the organic acid salt, rather than the organic acid in its protonated form. The salt is highly water soluble, has a negligible vapor pressure, and the carbonyl group is unreactive.

Recovery schemes based on the formation and subsequent hydrolysis of lactate esters have dominated commercial practice since the 1960s. The clarified broth from fermentation is first acidified by the direct addition of a strong acid such as H_2SO_4, thus protonating the acid and making the carbonyl group reactive. When either lime or calcium carbonate is used as the base for pH control in fermentation, the addition of the strong acid during recovery produces a gypsum precipitate. The gypsum is removed by filtration and the broth is then further concentrated by evaporation. An alcohol, such as methanol or ethanol, is added and the mixture is heated

to form the ester. The ester is sufficiently volatile that it can be carried overhead while most of the broth impurities remain in the bottom product. Lactic acid is recovered by hydrolysis of the purified ester followed by some polishing steps to guarantee that the final product meets heat-stable–water-white specifications for the market-dominant US pharmacopoeia grade. The alcohol generated during hydrolysis is recycled back to esterification *(6,7)*.

At least one positive purification step (i.e., a separation step in which the desired species undergoes a preferential phase change leaving impurities behind, as opposed to a negative purification step in which impurities undergo a preferential phase change leaving the desired species behind) is required to meet purity requirements demanded by today's marketplace. For lactic acid, this positive purification step is the formation and subsequent distillation of the lactate ester. Unfortunately this recovery scheme does not score well on energy efficiency because evaporation, even when performed in a multieffect system, is a brute force way to deal with the dilute nature of the broth. This scheme also fails to avoid the production of stoichiometric amounts of a salt coproduct. One-half mole of gypsum ($CaSO_4 \cdot 2H_2O$) is produced per mole of lactic acid, equivalent to 0.96 kg of gypsum/kg of lactic acid. This coproduct is of low value, and its fate is an environmental constraint that prevents lactic acid from achieving true commodity status. The coproduct gypsum is often stockpiled, land-filled, or otherwise disposed into undemanding applications such as concrete filler.

Much effort has been expended to avoid the salt issue including: the careful selection of cations and anions to give a more desirable salt coproduct such as ammonium sulfate *(8)*, the use of recovery schemes that include the use of weakly acidic ion-exchange resins to reduce the amount of salt coproduct *(9,10)*, the use of recovery schemes based on the thermal decomposition of the ammonium organic acid salt *(11,12)*, the use of bipolar electrodialysis *(13)*, and the use of CO_2 as an acidulant along with the formation of an amine complex in a reactive extraction process *(14)*.

Citric acid is another organic acid produced by fermentation in large quantities today. It is traditionally made by a low-pH fungal fermentation of carbohydrates using *Aspergillus niger*. Older citric acid recovery technology, known as the lime-H_2SO_4 process, is still used in some commercial facilities. After clarification to remove cell mass, calcium hydroxide is added to the broth to precipitate calcium citrate, in the form of either tricalcium citrate or dicalcium citrate, depending on the details of the process implementation. The calcium citrate is filtered and then resolubilized by the addition of H_2SO_4 to form citric acid plus a gypsum precipitate. The gypsum is removed by filtration, and the filtrate is subjected to further polishing, evaporative crystallization, drying, and packaging steps to produce the final citric acid product *(15–17)*. The dilute nature of the broth is handled efficiently by precipitating calcium citrate early in the recovery scheme. This precipitation step, along with the final evaporative crystallization step, also serves as pos-

itive purification. The lime- H_2SO_4 process, like the lactic acid process, suffers from the use of stoichiometric amounts of mineral acid and base and the production of stoichiometric amounts of a salt coproduct. The dicalcium citrate version of the process has the advantage of one-third less salt coproduction and improved filtration properties for the crystals.

Newer citric acid recovery technology takes advantage of the fact that the fermentation takes place at a pH below the lowest pK_a of the acid. At this low pH, the acid is present in its protonated form. It is reactive and will form a complex with weakly basic tertiary amines. Thus, a reactive extraction in which the clarified broth is contacted with an organic solvent containing trilaurylamine or similar water-immiscible tertiary amine will transfer the citric acid from the aqueous phase to the organic phase in the form of an amine:citric acid complex. The organic phase is then heated and back extracted with high-temperature water to dissociate the amine: citric acid complex, leaving the free amine in the organic phase for recycle to the forward extraction step while transferring the free citric acid to the hot aqueous phase *(18)*. Additional polishing, evaporative crystallization, drying, and packaging steps are used to produce the final product.

The amine extraction process has the advantage of negligible consumption of mineral acids and bases and negligible production of a salt byproduct. The technology also handles the dilute nature of the broth efficiently, because reactive extraction is used early in the recovery scheme to remove preferentially the citric acid from the broth as a positive purification step, creating a pure product and eliminating the need to evaporate large quantities of water.

In general, bacterial fermentations are much more sensitive to pH than fungal fermentations. Many organic acids are readily produced using bacterial strains. Finding general recovery schemes that solve the three technical issues cited earlier remain long-term goals for organic acid recovery research. In the rest of this article, we focus on the recovery of acetate from a near-neutral pH aqueous solution as a model system.

Busche and colleagues at Du Pont *(19,20)* and Partin and Heise *(21)* have summarized the recovery of fermentation-derived acetate. Here, we focus on the work of Urbas *(22)* at CPC International. He used CO_2 for acidification and amine complexes for the recovery of acetic acid. Tributylamine (TBA) is normally immiscible with water, but the TBA:acetic acid (TBA:HAc) complex is water soluble. When a dilute aqueous solution of calcium acetate at near-neutral pH is mixed with TBA, and then CO_2 is bubbled through the mixture, the following reaction occurs at near-ambient temperatures:

$$Ca(Ac)_2 + H_2O + CO_2 + 2\ TBA \Rightarrow 2\ TBA{:}HAc + CaCO_3$$

The use of a stoichiometric amount of TBA produces a single liquid phase containing the TBA:HAc complex. The reaction is driven to the right, because $CaCO_3$ precipitates on formation. In one embodiment, the aqueous amine complex is extracted into an organic solvent; the solvent is

stripped off; and the complex is thermally split apart, giving the acetic acid product and regenerating both the solvent and amine for recycle. Urbas preferred the use of low boiling, nonreactive solvents that do not form an azeotrope with acetic acid. Strong preference was given to chloroform, since it has a favorable distribution coefficient for the extraction step.

The thermal regeneration reaction is difficult in practice, leading to a viscous intractable residue and low yield of acetic acid. Furthermore, the use of chlorinated solvents, such as chloroform, would be problematic at industrial scale. Here, we present results on another scheme for recovery of the amine complex from the aqueous solution. The amine complex is extracted with an alcoholic solvent followed by esterification and distillation of the organic extract. During esterification, the following reaction produces the ester directly from the amine complex:

$$\text{TBA:HAc} + \text{ROH} \Rightarrow \text{TBA} + \text{RAc} + \text{H}_2\text{O}$$

The reaction is pulled to the right by continuous removal of water. After completion of the esterification step, the ester is isolated by distillation and the excess alcohol and amine are recycled. The acetate ester can either be sold as a final product or undergo further chemical transformations similar to those shown in Fig. 1B.

Materials and Methods

Chemicals

Laboratory deionized water was used for creating all aqueous feedstocks. Instrument-grade CO_2 was used for acidification. All other materials were American Chemical Society reagent grade.

Analytical Methods

Concentrations of TBA:HAc in both aqueous and organic solutions were determined by potentiometric titration of 10–20-mL samples diluted with 30 mL of methanol and titrated with standardized KOH in methanol following the method of Ricker et al. *(23)*. The water content of the organic phases was determined by the addition of excess toluene to a weighed sample of the organic phase. The resultant water phase was recovered and weighed, and the water content of the original sample was calculated by mass balance.

Acidification Experiments

Five hundred milliliters of an aqueous calcium acetate solution (0.6 molar as acetate) was added to a 1-L graduated cylinder, and the pH was adjusted to 6.9–7.0 using acetic acid. A 5% molar excess of TBA was added, and then the solution was sparged with CO_2 for 30 min at ambient pressure. The solution was filtered, and the $CaCO_3$ cake was washed once with water, washed again with acetone, dried, and then weighed.

Extraction Experiments

All extraction experiments were conducted at room temperature (25°C). For solvent screening, 100 g of an aqueous mixture containing 4.08 g of acetic acid (HAc) and 12.56 g of TBA was mixed in a separatory funnel with 100 g of organic solvent. The mixture was shaken by hand and then allowed to separate. Each phase was recovered and weighed. Samples were taken and analyzed for TBA:HAc and water. A similar procedure was used to generate data for the *n*-pentanol phase diagram except the starting concentration of TBA:HAc was varied to generate three tie-lines.

Esterification Experiments

The esterification reactions were conducted at atmospheric pressure (~630 mmHg at our laboratory in Colorado) in a glass still consisting of an electric heating mantle, a 1-L round-bottomed flask, a vacuum-jacketed 30-cm distillation column packed with 4 × 4 mm glass rings, and an overhead condenser and product splitter allowing the removal of a variable amount of distillate and return of reflux to the column.

Four hundred fifty grams of a room temperature solution containing a 3:1 molar ratio of alcohol to TBA:HAc complex was added to the still. The catalyzed run with *n*-hexanol included H_2SO_4 in the starting solution at a 0.1:1 molar ratio with respect to the TBA:HAc complex. The heating mantle was turned on, and approx 30 min later the solution began to boil. Water formed a second phase in the overheads as the reaction progressed. The water was collected and the volume recorded over time. Conversion was estimated as the percentage of the maximum theoretical water if all of the acetic acid were converted to ester and confirmed by titration of residual TBA:HAc in the still pot samples.

Results

The acidification experiments were conducted four times with $CaCO_3$ yields ranging from 91.0 to 96.1% of theoretical. The resulting $CaCO_3$ precipitates were easy to filter and wash. A fine white powder was generated in all cases.

Primary separation was achieved quickly (<30 s) for all solvents included in the solvent extraction screening experiments. Secondary separation was also very quick for all solvents, taking only a few minutes to obtain clear solutions in both phases. Table 1 reports the distribution coefficients and selectivities. The distribution coefficient, defined as the ratio of the mass fraction of TBA:HAc in the organic phase divided by the mass fraction of TBA:HAc in the aqueous phase, is a measure of the concentrating power of the solvent. Selectivity, defined as the distribution coefficient times the ratio of the mass fraction of water in the aqueous phase divided by the mass fraction of water in the organic phase, is a

Table 1
Solvent Screening Results

Solvent	Distribution coefficient	Selectivity	Esterification yield at 4 h (% theoretical)
n-Butanol	2.40	8.79	12.6
n-Pentanol	1.45	14.77	38.1
n-Hexanol	1.13	19.26	82.7/93.6 (no catalyst/H_2SO_4 catalyst)
n-Octanol	0.75	21.91	88.8

measure of the solvent's ability to separate preferentially the TBA:HAc complex from water. Higher selectivity implies that less water is dragged along into the organic extract, which, in turn, means that less energy is needed to remove free water in the downstream esterification step.

The solvent screening experiments showed that there is a trade-off between distribution coefficient and selectivity. Low molecular weight alcohols have more favorable distribution, but the mutual solubility of water with the low molecular weight alcohol lowers selectivity. Both *n*-pentanol and *n*-hexanol have favorable distribution coefficients and high selectivity.

Figure 2 is an experimentally measured phase diagram using *n*-pentanol as the extractant. The two-phase region is fairly broad and the tie-lines have a favorable slope. Distribution coefficients become more favorable at higher TBA:HAc concentrations, which leads to a concave-up equilibrium line when the data are plotted as mass fraction TBA:HAc in the organic layer (*y*-axis) vs mass fraction TBA:HAc in the aqueous layer (*x*-axis). Nonetheless, a good process design will avoid the potential pinch between the equilibrium curve and the operation line and requires only a few stages to produce a concentrated TBA:HAc extract from a dilute aqueous solution.

Figure 3 plots the water generated during esterification and reports the observed range of pot temperatures during the time the solutions were boiling. The pot temperature rose over time, further evidence of reaction. The curves in Fig. 3 are useful for comparing rates but can only be qualitatively used to compare yield because different molar amounts of TBA:HAc were present in the starting solutions, differing amounts of materials were taken for samples over the course of the experiment, and the starting solvents had different levels of initial free water. The conversion values in Table 1, estimated from the water production and confirmed by titration, are more useful for comparing yields.

Both esterification rate and yield increased with increasing molecular weight of the alcohol. Rather than being related to the chain length of the alcohol, this improvement in performance was probably caused by the

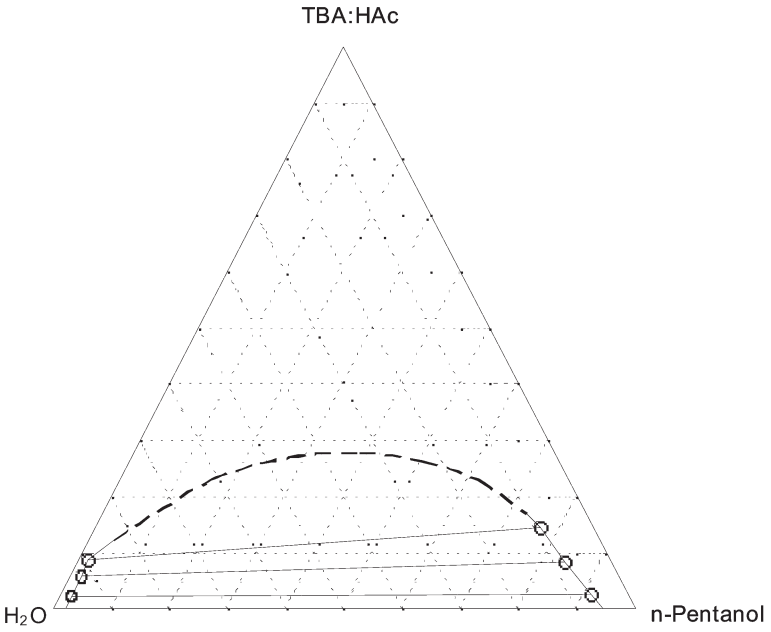

Fig. 2. Phase diagram for water TBA:HAc + *n*-pentanol at 25°C (weight basis).

higher boiling point and thus higher reaction temperature for the higher molecular weight alcohols. Adequate esterification rate and yield could probably be achieved with the lower molecular weight alcohols if the reaction was conducted at an elevated pressure. The pressures required are not extreme; for example, *n*-butanol will boil at 170°C and 482.6 kPa, well within the range of industrial importance.

Esterification rate and yield can also be improved by using a catalyst. Comparison of the noncatalyzed *n*-hexanol run with the catalyzed run shows that H_2SO_4 is potentially a good catalyst, although the ultimate fate of any homogeneous catalyst has to be worked out for any potential industrial process. Solid catalysts, both Brϕnsted and Lewis acids, may be easier to work with because catalyst recovery is not an issue.

Discussion

Figure 4 is a conceptual flow sheet of a recovery process that meets the goals outlined in the Introduction. The near-neutral pH aqueous solution of calcium acetate is mixed with a stoichiometric amount of TBA and acidified with CO_2. The resulting $CaCO_3$ precipitate is recycled back to fermentation for pH control, resulting in little net salt production for the process. The aqueous filtrate is extracted with an alcoholic solvent, providing both a positive purification and a concentrating step. The organic extract undergoes esterification with water distilled overhead. The bottom product is fractionated to give the acetate ester product and recycle streams for the alcohol and amine. The aqueous raffinate from the extraction is stripped of

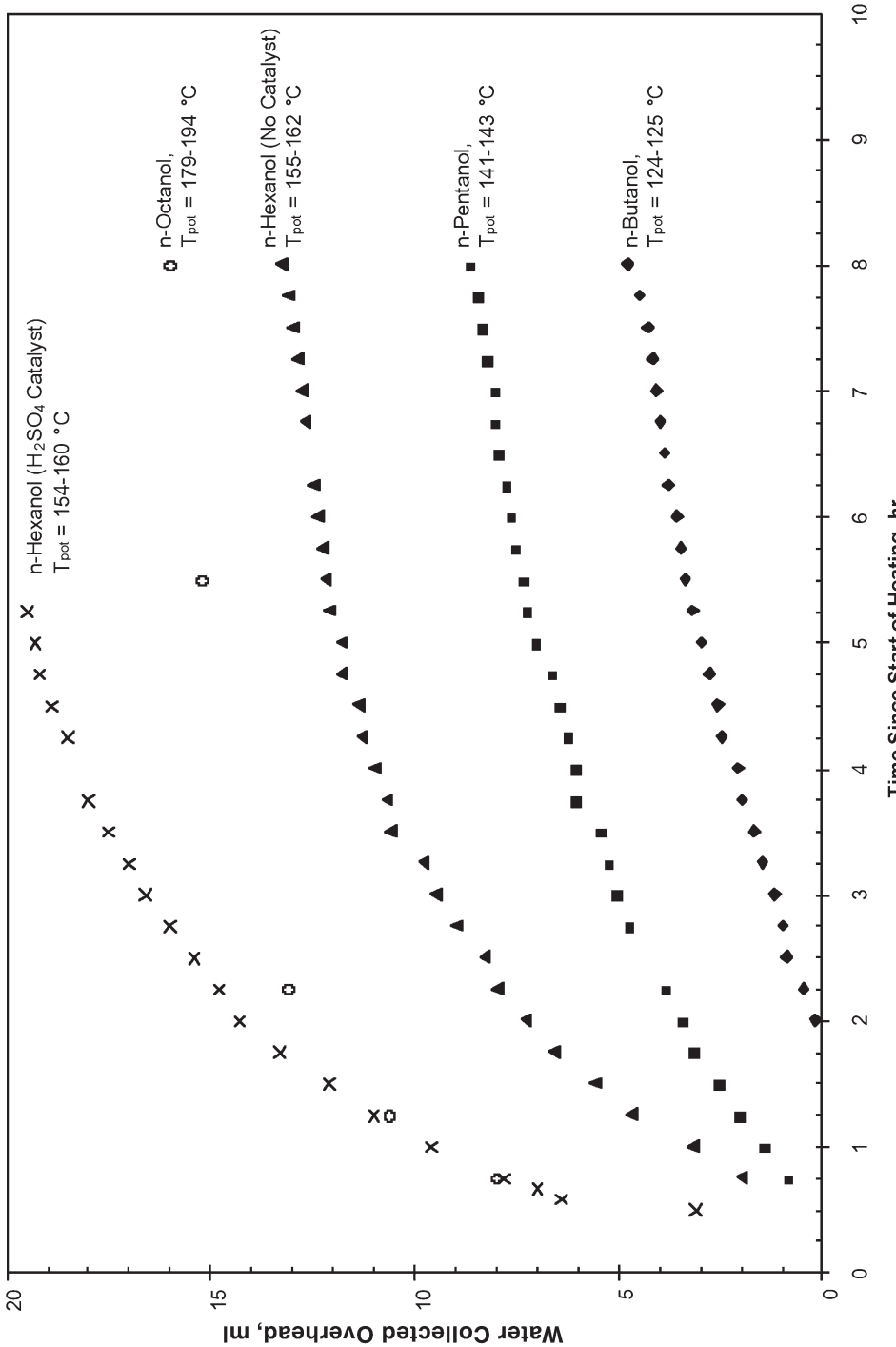

Fig. 3. Relative rates of esterification (boiling begins at ~30 min).

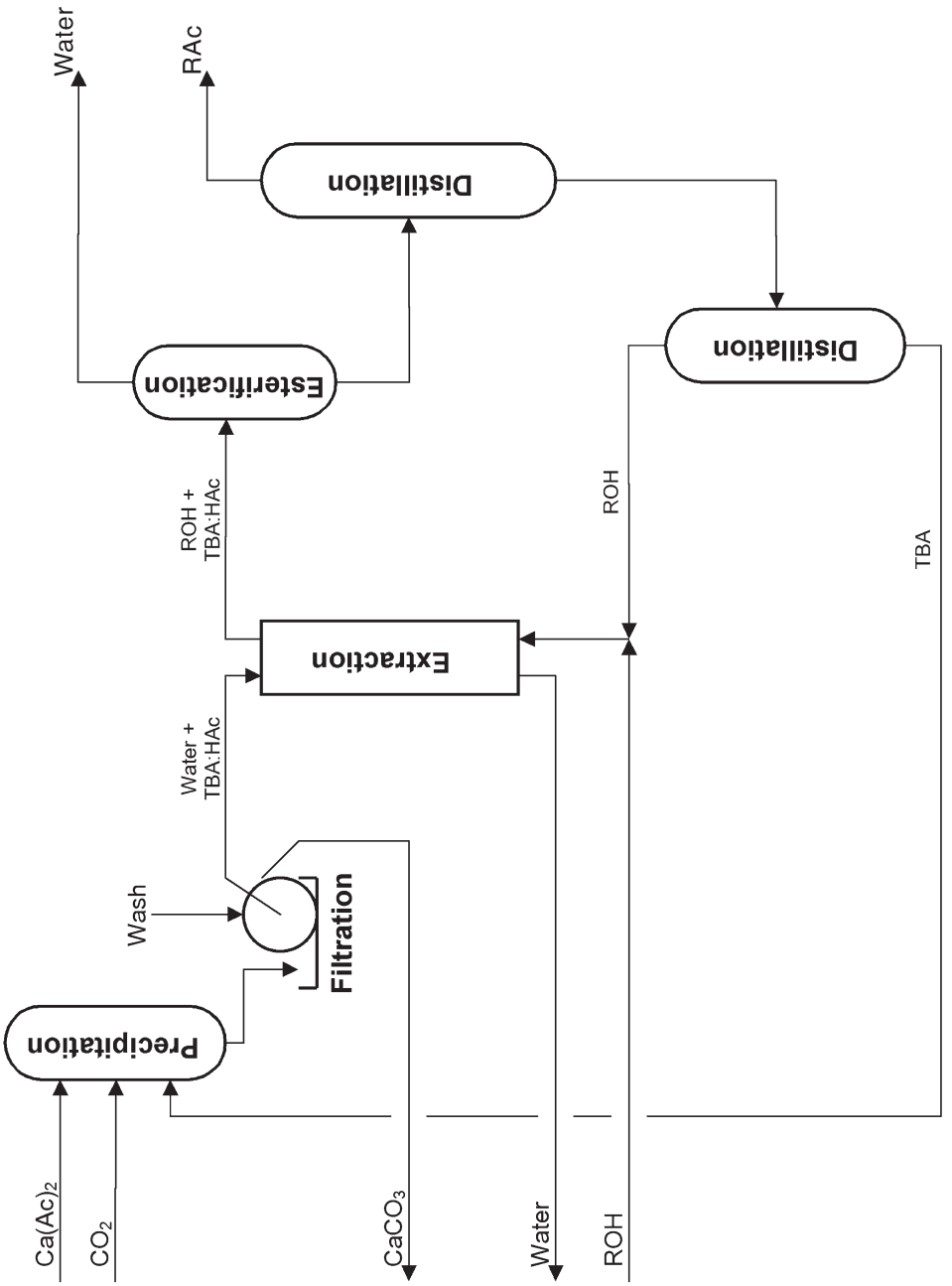

Fig. 4. Conceptual process sketch for recovery.

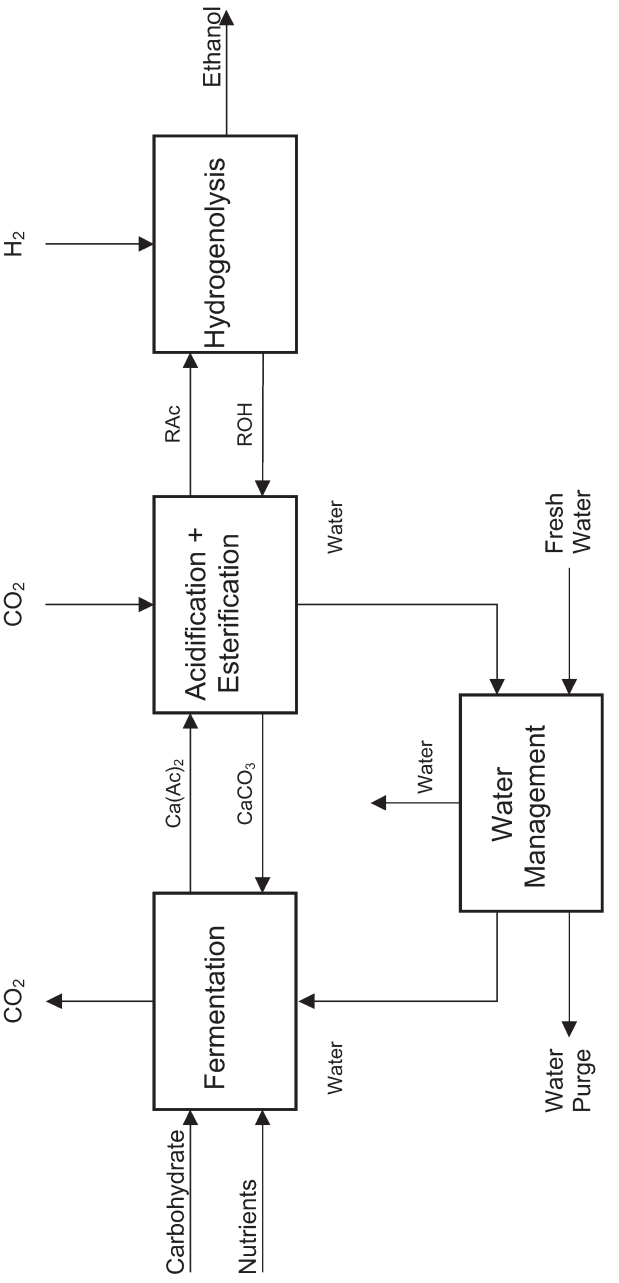

Fig. 5. ZeaChem indirect ethanol process.

residual solvent and further processed as needed to recycle water and nutrients to the fermentation or otherwise prepare the stream for discharge to the environment.

The real driver behind this work has been our interest in high-yield indirect methods for the production of ethanol from sugars *(24)*. Homoacetogens are bacteria that have the unique ability to transform both five- and six-carbon sugars into acetate at near 100% carbon yield *(25)*. Figure 5 illustrates how the recovery process described herein could be combined with an acetogenic fermentation step and a hydrogenolysis step to produce ethanol. An ethanol production process with a theoretical maximum yield of 3 mol of ethanol/mol of six-carbon sugar or 2.5 mol of ethanol/mol of five-carbon sugar results. The extra energy required for this improvement in theoretical yield is supplied by hydrogen, which, in turn, could be produced by gasification of lignin-rich fermentation residues or other biomass resources. Combining sugar and syngas platforms in this way has the potential to create high-yield renewable energy processes that integrate well with biorefinery concepts. Further refinement of the organic acid recovery scheme will be key to implementing this strategy.

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