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Review

Recovery Processes of Organic Acids from Fermentation Broths in the Biomass-Based Industry

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The new movement towards green chemistry and renewable feedstocks makes microbial production of chemicals more competitive. Among the numerous chemicals, organic acids are more attractive targets for process development efforts in the renewable-based biorefinery industry. However, most of the production costs in microbial processes are higher than that in chemical processes, among which over 60% are generated by separation processes. Therefore, the research of separation and purification processes is important for a promising biorefinery industry. This review highlights the progress of recovery processes in the separation and purification of organic acids, including their advantages and disadvantages, current situation, and future prospects in terms of recovery yields and industrial application.
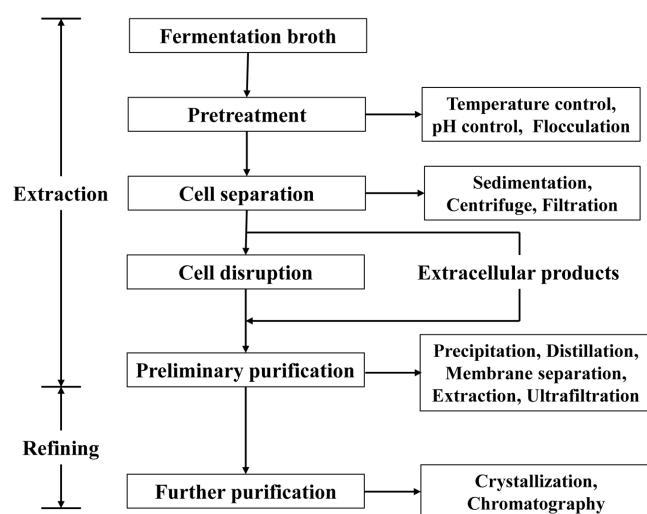
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Introduction

Traditionally, commodity organics were mainly derived from fossil feedstock using synthetic routes, which often suffer from low yield, complicated work-up, or toxic reagents. As a result of the shortage of fossil resources and development of the biorefinery industry, the biological routes for organics are becoming more and more competitive. Among various organics, organic acids are one of the most attractive targets in biological routes using biomass as feedstock. Their functionalities make them extremely useful as platform materials, and they are less toxic to the host cells than corresponding alkanes, alcohols, or aldehydes. Moreover, their highly oxidized nature makes biosynthesis of organic acids more atom economy than other organics, because the co-products adenosine triphosphate (ATP) and

triphosphopyridine nucleotide (NADPH) in biological routes can be utilized by microorganisms. Since the last century, lactic acid and citric acid have been produced from fermentation broths successfully [35]. The market size of lactic acid would be up to 367.3 thousand metric tons by 2017, and that of acid is over 1.4 million tons per year [16, 57]. A variety of organic acids can be converted from corn by biological fermentation [9, 33].

The biological routes for the industrial production of organic acids are mainly fermentation through appropriate bacteria species or genetic engineering of the species. The main shortage of the fermentation-based routes is the technical difficulties associated with costly recovery of organic acids. Just like the recovery processes in chemical routes, high purity of the final product and avoidance of the co-production are the important influencing factors.

Fig. 1. General flow of separation from fermentation broth. The organic acids are mainly extracellular products, and therefore, this review focusses on the recovery process of extracellular products. Precipitation, distillation, membrane separation, extraction, and ultrafiltration are mainly used in preliminary purification, whereas, chromatography and crystallization are mainly applied in refining of organic acids [19, 28].

Moreover, energy-efficient means of handing the dilute broths must be addressed to enable commodity-scale production of organic acids via fermentation [15, 21].

A number of processes for organic acids recovery from broth have been studied in the literature. The proposed techniques mainly include precipitation, chromatography, membrane separation, extraction, and distillation [18, 48, 60]. The general steps of the separation process can be seen in Fig. 1 [20, 29]. The first step of downstream processing is often cell separation to separate cell debris, proteins, *etc.* from fermentation broths. To obtain a satisfactory separation of organic acid, two or more unit operations may be used. All of these methods for organic acid recovery are presented below and evaluated briefly in terms of their advantages and disadvantages.

Separation Technology

Precipitation

Precipitation is a classical method for the recovery of organic acids from broth, which has been used for the isolation of lactic acid and citric acid industrially since the last century. Precipitation can recover organic acids from a bulk of fermentation broths efficiently, which makes it more competitive, especially in preliminary purification.

Taking calcium precipitation as an example, organic acids are often separated by four steps. First, the fermentation liquid is filtered to obtain the mother liquor and to remove impurities, and then $Ca(OH)_2$ or $CaCO_3$ is added to the mother liquor with agitating. Second, the calcium salt of organic acid is filtered off from the fermentation. Third, the calcium salt is treated with a high concentration of sulfuric acid to free the desired acid. Afterwards, the purified acid is obtained by further purification processes [32, 56].

Heding and Gupta [19] and Pazouki and Panda [44] found the optimum condition for precipitation of citric acid was at 50°C for 20 min, and citric acid can be recovered with a yield of almost 100%. The research of Min *et al.* [40] indicated the molar ratio of calcium lactate to sulfuric acid, which played an important role in the isolation of lactic acid with precipitation. At the optimal conditions, the yield of lactic acid was up to 92% [40]. Some patents also studied the separation of organic acids from broth by precipitation [4, 10, 11].

Precipitation with ammonia is another method for the recovery of organic acids. Diammonium succinate can be generated by changing the cation in the succinate salt with ammonia or adding an ammonia-based titration agent. Afterwards, the diammonium salt of succinic acid reacts with sulfuric acid to produce succinic acid. The yield of succinic acid could be up to 93.3% after it is purified [4].

After years of development, precipitation is now a well-established technique. The main advantages of precipitation are that it is highly selective, has no phase transition, and has high product purity. Meanwhile, finding proper precipitants for the products is the key factor for this method. As for the industrial-scale calcium precipitation process, when a one molar amount of organic acid is converted, an equal amount of $Ca(OH)_2/CaCO_3$ and H_2SO_4 are consumed, and low valuable calcium sulfate is formed [32, 44]. The constitutive consumption makes the cost difficult to reduce. Besides this, reusable precipitants should be developed, which will make it more competitive.

Chromatography

This method relies mainly on ion-exchange or adsorptive properties of resin to the products. It is a well-established operation for the final purification of organic acids from aqueous solution, especially for refining of the products. The physical and chemical properties of resin are stable, and insoluble in acid, alkali, or organic solvents. The resin has a good selectivity for organic acids, and the separation process involves low energy consumption with no phase transition [43, 59]. The most used resins in recovery

processes are mainly divided into two categories: ion-exchange resins and macroporous adsorption resins [5].

Polymers that contain suitable substituted acidic or basic groups are often taken for resins. The particle size, degree of cross-linking, and exchange ions all can determine the exchange capacity [17]. The ion-exchange resin is wildly applied in bioseparation, which is thought to be low energy-consuming [7]. Different from ion-exchange resins, macroporous adsorption resins mainly rely on the van der Waals' force, hydrogen bond, and dipole ion interaction.

Zhou *et al.* [60] applied poly(4-vinylpyridine) resin for the separation of lactic acid from fermentation broths. The yield and purity reached 95% and 88%, respectively [60]. Tong *et al.* [52] indicated that a weak anion-exchange resin could be used in the purification process of lactic acid. The results also indicated that the recovery yield could be enhanced when the pH of the fermentation broths was between 5.0 and 6.0. The yield and purity were up to 82.6% and 92.2%, respectively [52].

Macroporous adsorption resins are applied for separation of long-chain dibasic acid and succinic acid from broth efficiently. A kind of nonpolar macroporous adsorption resin was improved to separate dibasic acids of different chain length but with similar property from broth, by Zang *et al.* [59]. Davison *et al.* [12] found excellent performance of a resin (XUS 40285) from 25 sorbents, which had a stable capacity of succinic acid at either acidic or neutral pH. It could be used more than 10 times and the yield of succinic acid was up to 95% [12].

High capacity, quick recovery, low regeneration consumption, and specificity for the desired product are the most wanted properties of resin sorbents [34]. Chromatography does not produce the co-product as calcium sulfate, and the yields of products are high. However, there will be a large amount of waste liquor produced and high consumption of salt in the elution process. Moreover, the exchange capacity of the resin will be weakened with increase of time [1].

Membrane Separation

Membrane technologies have been used in the recovery process of organic acids because of their adaptability and selectivity. With the development of recovery processes and material technology, membrane separation attracts more attentions, especially in the in situ product removal technology. A membrane is essentially a thin artificial or natural barrier, which permits selective mass transport of solutes or solvents across the barrier to achieve the physical separation and enrichment purposes. High purity and high yield can be obtained by membrane separation. The main membrane filtrations used for the separation of organic acids includes microfiltration, ultrafiltration, nanofiltration (NF), reverse osmosis, pervaporation, and electrodialysis [9, 37, 53].

Kang employed NF for the recovery of succinate from simulated broth, and they found the NF membrane had a lower rejection to monovalent anions than divalent ions. NF was also used to isolate lactic acid from clarified broth. The ratio of lactate ions to non-ionized lactic acid could affect the rejection and permeation of the NF membrane. Therefore, pH was an important factor to determine the effects of NF on separation [16].

Pervaporation technology is an attractive membrane technique for the separation of close-boiling substances, especially for azeotrope separation. However, the flux of the pervaporation membrane is usually less than $2{,}000 \ g/(m^2{\bullet}h)$, which is the typical barrier encounter in the development of pervaporation. In this field, there is a large gap between the pervaporation membrane and the conventional membrane technologies [25, 53].

As far back as the early 1970s, electrodialysis has been applied for the separation of citric acid from broth. The bipolar membrane is an outstanding feature of electrodialysis. In this way, cation and anion selective layers are alternatively used to form diluted and concentrated flows [7]. A two-stage electrodialysis was developed by Hábová *et al.* [18] for separation of lactic acid, with the energy consumption of 1.5 kWh per 1 kg in the whole step. However, high energy consumption still barred its application [18].

Other membrane separation methods can also be implemented to separate organic acids from broth. The emulsion liquid membrane system was applied for removal of acetic acid from succinic acid fermentation broths. The degree of extraction of acetic acid was more than 95% and an enrichment ratio of 3.0 was obtained by Lee *et al.* [32]. Although membrane separation methods may realize highly efficient separation, the process is high energy-consuming, and the membrane is expensive. Moreover, the separation efficiency of a membrane may decrease with an increase of organic acid concentration. Membrane pollution is another obstacle, leading to by-product salt formation during the ion-exchange process [7, 56].

Liquid-Liquid Extraction

Liquid-liquid extraction is a common method used to separate compounds by their relative solubility in two different immiscible liquids, which holds an important status for separation of mixtures in the biochemical industry [9, 29]. Here, three commonly used methods for separation

are reviewed.

Solvent extraction. Solvent extraction is a highly efficient separation technology. It is also low energy-consuming [29], which makes it a traditional recovery process. Moreover, it can be used in the in situ product removal technology. Ethyl acetate, diethyl ether, and diethyl ether-hexane mixtures were tested to separate acetic acid from fermentation broths. Studies showed that ethyl acetate had a better effect than diethyl ether and the mixture [24].

To meet the requirements of food and medical applications, wiped film distillation technology is often combined with calcium salt precipitation processes to separate and purify L-lactic acid from broth. However, the impurities are inclined to pollute the evaporated surface and the high vacuum increases operating costs. A novel purification process for L-lactic acid was developed by Chen *et al.* [8]. They adopted butyl alcohol for L-lactic acid extraction between the simple calcium salt precipitation and the short-path distillation. This new process with solvent extraction was successful and high-quality L-lactic acid could be obtained without extremely low pressure. Compared with previous processes, the purity and yield of L-lactic acid were improved and could be up to 91.6% and 61.73%, respectively [8].
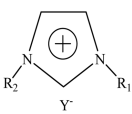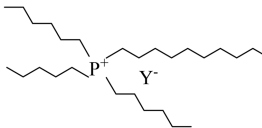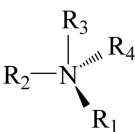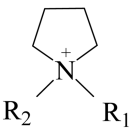
Reactive extraction. To make the desired product extracted more easily, the product is sometimes converted into another compound for extraction. This is reactive extraction, which is easily operated by controlling the pH value of the two phases [34]. The processes associated include hydrolysis, complexation, dissociation, and ion association in two phase, and finally phase equilibrium. The most used extractants are hydrocarbon solvents, phosphorous solvents, and aliphatic amines, among which aliphatic amines have been found to be a prospective solvent for the separation of organic acid from aqueous solution [3, 58].

A tri-*n*-octylamine (TOA)/oleyl alcohol and TOA/1-octanol system was adopted to reactively extract lactic acid and succinic acid from broths [21]. The final purity and yield of succinic acid were up to 99.8% and 73.1%, respectively. Kurzrock and Weuster-Botz [30] found that polarity and functional groups of the solvent and stability of the amine-acid complex were the important factors to affect the yield. Another research indicated that the phase-transfer catalyst aliquat 336 (methyltrioctylammomium chloride) could improve extraction performance in lactic acid separation [58]. Ren *et al.* [48] developed a new reactive extraction without any diluents and vacuum evaporation stripping to avoid the secondary pollution caused by various diluents addition. This method was easier to conduct and more effective in separation of short-chain fatty acids [48]. Esterification and hydrolysis can also be used for reactive extraction and purification of lactic acid from broth, as shown by Sun *et al.* [50]. This process also combined a rectifying column, which enhanced the esterification and hydrolysis efficiency [50].

Ionic liquid extraction. Ionic liquids (ILs), a group of organic salts, have drawn considerable attention and been

Table 1. Several common ionic liquids used for extraction.

Ionic liquids	Chemical structure	Separated substances	References
Imidazolium-based ionic liquids		L-Lactic and succinic acids, tryptophan, phenylalanine, tyrosine	[31, 55]
Phosphonium-based ionic liquids		L-Lactic, L-malic, and succinic acids	[38, 42]
Quaternary ammonium ionic liquids		Organics and rare earths	[27, 39]
Pyrrolidinium-based ionic liquids		Acetic acid and aromatic hydrocarbons	[13]

developed rapidly [31, 51, 55]. Most of them are imidazolium, [31, 55], quaternary phosphate [38, 42], or quaternary ammonium salts [27, 39], which are non-volatile, non-flammable, and liquid in a wide range of temperatures (Table 1) [13]. They are also chemically stable and have a lower viscosity and higher density than many organic solvents. The good extractabilities for organic compounds make ILs promising alternatives to traditional organic solvents [31, 51].

Oliveira *et al.* [42] applied phosphonium-based hydrophobic ILs for the separation of L-lactic, L-malic, and succinic acids from aqueous solutions. These ILs showed a better extraction ability to extract short-chain organic acids from aqueous solution than traditionally used organic extractions. The recovery could reach 73% under appropriate pH [42]. Martak and Schlosser [38] proved industrial processed phosphonium-based ILs have a better performance than the traditional organic solvents used in the separation of lactic acid [38]. Wang *et al*. [55] found that the pH of aqueous phases, anion nature, and alkyl chain length on the cation of the ILs can strongly affect the IL/water partition coefficients of acids. Although many progresses were made about IL extraction, the cost of ILs is still the obstacle [55].

Liquid-liquid extraction has been researched a lot regarding its application for the separation of organic acids from fermentation broths [20]. Liquid-liquid extraction has outstanding advantages, such as a short cycle, and faster mass transfer between the liquid than solid phase. To be more effective and green, the desired properties of solvents should be a high distribution coefficient, high selectivity to the desired product, little or no miscibility with the feed solution, non-toxicity to microorganisms, and high stability [22, 29]. Besides this, the pH should be taken into consideration and a drop in pH value should be avoided.

Distillation

Distillation is a method of separating a mixture based on differences in volatility of the components. As one of the most conventional processes for chemical separations, distillation not only can be used in the beginning of the separation but also in the last step combined with crystallization. Even if other techniques are available to separate organic acid from fermentation broths, distillation still remains an important alternative technology [14], especially in the refining. In general, distillation is effective at a low concentration of organic acid, whereas it becomes inefficient at a high concentration, especially when it is near the azeotropic point [20].

Kumar *et al*. [28] once used a continuous reactive distillation method for the recovery of lactic acid, which should less energy consumption and higher selectivity than conventional discontinuous processes [28]. In reactive distillation, methanol was chosen to form methyl lactate to decrease azeotrope, which was then removed from the aqueous solution. The reaction between methanol and lactic acid was catalyzed by an acidic cation-exchange resin

Table 2. The main organic acids produced by fermentation broths and their recovery processes.

Production	Recovery processes	Annual yield	Applications	References
Lactic acid	Calcium salt precipitation (mainly), reactive extraction, membrane separation	367.3 Thousand metric tons by 2017, according to new report by Global Industry Analysts, Inc.	Important platform chemicals in food and beverages, cosmetics, pharmaceutical, biodegradable plastics	[16, 52]
Citric acid	Precipitation (classical process), ion exchange, solvent extraction	Over 1.4 million tons per year and fermentation comprises over 90% of the world production	Foods, beverages and confectionery, pharmaceuticals, industrial applications, potential building block	[44, 49]
Succinic acid	Precipitation, direct crystallization, extraction, adsorption, electrodialysis	Annual market size is over 400 million US $, and will be over 1 billion US $ by 2015 globally	Foods, cosmetics, and solvents; can also be applied as new biodegradable polymer, surfactants, and detergents	[29, 34]
Gluconic acid	Anion-exchange resin	Global yield is more than 6,000 tons annually	Gluconic acid and its salts often used in food, beverage, and pharmaceutical industries	[47,49]
Itaconic acid	Evaporation and crystallization, ultrafiltration, solvent extraction	Global output has been over 80,000 tons per year	Often used in coatings, adhesives, binders, and is a substitute for acrylic and methacrylic acids	[41]

and this reversible reaction was the following:

$$\text{Methanol} + \text{lactic acid} \leftrightarrow \text{Methyl lactate} \quad (1)$$

Research revealed that lactic acid could be effectively recovered by a single-stage reactive distillation for esterification and multistage reactive distillation column for hydrolysis [28, 36].

Extractive distillation with ionic liquid was also proposed by Blahušiak *et al.* [6], to separate an organic acid. Butyric acid was extracted by phosphonium IL, which was then regenerated by two-stage short-path distillation. The yield of butyric acid was up to 89%. The significant difference of this process is that the product is in the form of free acid rather than its salt [6].

Distillation is one of the most commonly used separation methods for different volatile components. Because the carbonyl in their structure has a strong adsorb-electron effect, most organic acids have a higher boiling point than water. Thus, reactive and extractive distillations are more competitive in the organic acid recovery process. Besides this, vacuum distillation is commonly used in experiments and in industrial production to realize cost reduction.

In Situ Product Removal Technology

In situ product removal (ISPR) is the fast removal of organic acid, thereby preventing its subsequent interference with cellular or medium components. It couples fermentation with separation, including extraction, resin, and membrane, to realize a continuous process. Moreover, the remove of products can minimize the toxicity of products to microorganisms. The study of ISPR has been widely concerned in the separation process of organic acids from fermentation broths [9, 29, 45].

In situ separation of lactic acid from broth by ion-exchange resin was investigated by Ataei and Vasheghani-Farahani [2] and the productivity of the extractive fermentation was about 4.3 times higher than that of conventional processes at the optimum temperature [2]. In situ separation of lactic acid with period electrodialysis was applied to reduce lactic acid end-product inhibition and avoid cell death. The fermentation time and lactic acid production rate were improved to a significant extent [54].

ISPR allows a continuous fermentation to be conducted. By removing acids from the broth promptly, the product inhibition is reduced and pH is controlled, leading to higher utilization of feedstock, improvement of productivity, reduction of downstream processing load, and recovery of cost. An ideal ISPR has low energy consumption and needs minimal chemical addition. Obviously, continuous fermentation and in situ product removal are more efficient than traditional batch production and deserves more attention in the future [9, 46].

Industrial Development

Currently, the raw materials used for chemical synthesis are hardly able to support the chemical industry, and the demand for organic acids is increasing with the rapid development of the economy, and the fermentation process has become an available way to obtain the desired products. The most important chemicals produced directly by fermentation include lactic acid, citric acid, glutamic acid, and L-lysine (Table 2), and gluconic acid and itaconic acid are also produced on the tons scale [23, 41, 47, 49].

The main factor that has restrained the application of fermentation routes in organic acid production is the cost, in which the recovery and concentration of desired acids can represent up to 60%. The three main products (lactic acid, citric acid, and succinic acid, Table 2) produced by fermentation broths are mainly recovered by precipitation, which indicates it is still the most economical for industrial-scale production. Meanwhile, resin and extraction were the main methods for the products produced on the scale of a few tons.

Conclusions and Perspectives

Biological fermentation has been found to be a promising method for the production of organic acids from renewable biomass. However, it is still a challenge to efficiently separate the organic acids from a mix of multiple diluted components and reduce the purity of other organic acids with similar properties to a minimum. Current methodologies used in recovery processes all have their limitations, and their improvements are especially needed with regard to yield, purity, and energy consumption. Therefore, there is a need to develop a process that should ideally be simple to carry out and allow the purification of organic acids directly from the fermentation broths. Besides this, the emergence of new materials and the development of technologies would boost the recovery processes, which would make the biological process more competitive than the chemical routes and promote the development of green chemistry.

Acknowledgments

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