Separation of Lactic Acid: Recent Advances

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Carboxylic acids are important commercial products. The requirements of carboxylic acids (lactic acid, citric acid, propionic acid etc.) are increasing every year. Therefore, it is important to have an efficient recovery method following the production of carboxylic acid. At present most of the manufacturers use the conventional method of recovery, which is the calcium hydroxide precipitation method. This method of recovery is expensive and unfriendly to the environment as it consumes lime and sulphuric acid and also produces a large quantity of calcium sulphate sludge as solid waste. It is, therefore, reasonable to look for other methods of recovery for carboxylic acid. Lactic acid is used in food, chemical and pharmaceutical fields, and a raw material for the production of biodegradable polylactic acid, both, substitutes for conventional plastic materials and new materials of specific uses, such as controlled drug delivery or artificial prostheses. This short review focuses on the developments of recovery of lactic acid from fermentation broth.

Keywords:
Carboxylic acid, lactic acid, separation, conventional methods, new developments

Introduction

Lactic acid is one of the carboxylic acid successfully developed by fermentation process. Lactic acid is an important chemical, which can be converted to ethanol, acrylic polymers, and polyesters. Lactic acid copolymers are used in packaging and have the advantage of being biodegradable[12,20,112] and, both, substitutes for conventional plastic materials and new materials of specific uses, such as controlled drug delivery or artificial prostheses.[54]

Exploitation of lactic acid for production of biodegradable polymers is one among the recent applications. There is a continuing interest in more efficient process for fermentation production of lactic acid, its recovery and purification. The recovery and purification are very important steps because they have significant influence on quality of lactic acid and its final price.

The world market of lactic acid is growing every year, and the level of production was estimated at around 150 millions lb* per year[1] and the worldwide growth is believed by some observers to be 12-15 % per year.[4] In December 1994, market prices in the US for both fermentation and synthetic food-grade 50 and 88 % lactic acid were $ 0.71 and $ 1.15 per lb ($ 1.56-2.53 per kg), respectively. Technical grade 88 % lactic acid was quoted at $ 1.12 per lb ($ 2.47 per kg).[2] In April 2003, market prices in the US for 88 % food-grade and technical-grade lactic acid were $ 0.77 and $ 0.7 per lb, respectively. The 50 % solution for food-grade lactic acid was $ 0.59 per lb (Chemical Economics Handbook, 2003). The prices were lowered by 50 % in last decade, illustrating the economics of the scale based on the increasing use of lactic acid.

The conventional recovery processes of lactic acid from fermentation broth are quite complicated. Separation of lactic acid from dilute wastewater or fermentation broths using evaporation has an economic problem since the vaporization of water consumes much energy. Also distillation is not useful due to non-volatility of lactic acid. In conventional processes, precipitation of calcium lactate using calcium hydroxide has the following steps: precipitation, filtration, addition of sulfuric acid, purification using activated carbon, evaporation and crystallization. Separation and final purification stages account up to 50 % of the production costs.[15,25,26] Thus, this method of recovery is expensive and unfriendly to the environment as it consumes lime and sulphuric acid and also produces a large quantity of calcium sulphate sludge as solid waste.[77] It is, therefore, reasonable to look for other methods of lactic acid recovery.

The fermented medium contains either pure lactic aid or its salt or the mixture of the two. A class of advantageous processing approaches in-

* lb, 1 pound = 0.543 kg

volves removal of lactic acid from the fermentation broth or other mixture, while leaving the soluble lactate behind in the fermentation broth. The separation can, in some instance occur within the fermenter or it can be conducted on solution material removed from the fermenter.

A number of processes for lactic acid recovery from fermentation broth without precipitation have been studied and reported in the literature: solvent extraction,[7,8,28,43,44,45,86,87,91,96,104,105,106,113,114] membrane bioreactor,[38,39,60,93] liquid surfactant membrane extraction,[82] adsorption,[41,48,115] direct distillation,[18] electrodialysis,[11,29,32,51,62] reverse osmosis,[92] anion exchange,[111] etc.

The choice of the separation process should be based on the efficient and economical usage of these extractants.[71] In the present paper, various methods of separation of lactic acid are presented and focus is given on the new developments of recovery of lactic acid from fermentation broth.

Chemical synthesis

The commercial chemical synthesis process for lactic acid production is based on lactonitrile, which is used to be a by-product of acrylonitrile synthesis. It involves the base-catalyzed addition of hydrogen cyanide to acetaldehyde to produce lactonitrile.[66] This is a liquid-phase reaction and occurs at atmospheric pressures. The crude lactonitrile is then recovered and purified by distillation and is hydrolyzed to lactic acid using either concentrated hydrochloric or sulfuric acid, producing the corresponding ammonium salt as a by-product. The crude lactic acid is esterified with methanol, producing methyl lactate. The latter is recovered and purified by distillation and hydrolyzed by water under acid catalysts to produce lactic acid, which is further concentrated, purified, and shipped under different product classifications and methanol, which is recycled.[46]

Addition of hydrogen cyanide:

$$\underset{\text{acetaldehyde}}{CH_3CHO} + \underset{\text{hydrogen cyanide}}{HCN} \xrightarrow{\text{Catalysts}} \underset{\text{lactonitrile}}{CH_3{-}CHOH{-}CN}$$

Hydrolysis by H_2SO_4:

$$\underset{\text{lactonitrile}}{CH_3CHOHCN} + 2\,\underset{\text{water}}{H_2O} + \frac{1}{2}\,\underset{\text{sulfuric acid}}{H_2SO_4} \longrightarrow$$

$$\longrightarrow \underset{\text{lactic acid}}{CH_3CHOHCOOH} + \frac{1}{2}\,\underset{\text{ammonium salt}}{(NH_4)_2SO_4}$$

Esterification:

$$\underset{\text{lactic acid}}{CH_3CHOHCOOH} + \underset{\text{methanol}}{CH_3OH} \longrightarrow$$

$$\longrightarrow \underset{\text{methyl lactate}}{CH_3CHOHCOOCH_3} + H_2O$$

Hydrolysis by H_2O:

$$\underset{\text{methyl lactate}}{CH_3CHOHCOOCH_3} + H_2O \longrightarrow$$

$$\longrightarrow \underset{\text{lactic acid}}{CH_3CHOHCOOH} + \underset{\text{methanol}}{CH_3OH}$$

Two companies Musashino, Japan and Sterling Chemicals Inc., USA are using this technology.

Other possible chemical synthesis methods for lactic acids are degradation of sugars; oxidation of propylene glycol; reaction of acetaldehyde, carbon monoxide, and water at elevated temperatures and pressures; hydrolysis of chloropropionic acid; nitric acid oxidation of propylene; etc. None of these are technically and economically viable process.[31]

Conventional methods

Commercially, lactic acid is manufactured by controlled fermentation of the hexose sugars from molasses, corn or milk.[78] Since 1930 only, the lactic acid has been produced commercially from the milk by-product whey. In 1930's about 2 billion lb (1 pound, lb. = 0.543 kg) of dry whey was produced annually from cheese or casein production, and about 1 billion lb was wasted. In 1950's about 12 billion lb of dry whey was produced annually from cheese or casein production, but less than 2.5 billion pounds is employed in food, feeds, or the production of lactose, much of the rest being wasted or fed to animals.[80]

The convention process for lactic acid production can be described by

Fermentation and neutralization:

$$\underset{\text{carbohydrate}}{C_6H_{12}O_6} + \underset{\text{calcium hydroxide}}{Ca(OH)_2} \longrightarrow$$

$$\longrightarrow \underset{\text{calcium lactate}}{(CH_3CHOHCOO^-)\ Ca^{2+}} + 2H_2O$$

Hydrolysis by H_2SO_4:

$$(CH_3CHOHCOO^-)_2Ca^{2+} + H_2SO_4 \longrightarrow$$
calcium lactate sulphuric acid

$$\longrightarrow 2\ CH_3CHOHCOOH + CaSO_4$$
lactic acid calcium sulphate

Esterification:

$$CH_3CHOHCOOH + CH_3OH \longrightarrow$$
lactic acid methanol

$$\longrightarrow CH_3CHOHCOOCH_3 + H_2O$$
methyl lactate

Hydrolysis by H_2O:

$$CH_3CHOHCOOCH_3 + H_2O \longrightarrow$$
methyl lactate

$$\longrightarrow CH_3CHOHCOOH + CH_3OH$$
lactic acid methanol

The broth containing calcium lactate is filtered to remove cells, carbon treated, evaporated and acidified with sulphuric acid to get lactic acid and calcium sulphate. The insoluble calcium sulphate is removed by filtration; lactic acid is obtained by hydrolysis, esterification, distillation, and hydrolysis.

The classical commercial procedure for the lactic acid by fermentation consists in fermenting a mash of a carbohydrate substrate together with suitable nutrients in the presence of an excess of calcium carbonate. The lactic acid as formed reacts with the calcium carbonate, producing calcium lactate carbon dioxide thus preventing the pH (general range 5.0-6.0) in the fermentation from becoming so low as to inhibit bacterial action. The thermophilic (high-temperature-thriving bacteria, the general strain used here exhibits optimum activity at 50 °C) type of *Lactobacillus delbruckii* is generally preferred. This eliminates most contamination problems and permits the use of a medium, which is pasteurized rather than sterilized. However, this bacterium will not ferment the lactose or milk sugar where a mixed culture of *L. bulgaricus* and mycoderm are necessary.[103]

Details and flowsheet of a process used by the Sheffield Products Co. are given by *Burton*[13] and summarized by *Prescott* and *Dunn*.[66] The filtered liquor (after filtration of fermentation of whey broth) from fermenter was treated with carbon under slightly alkaline and then under slightly acidic conditions. The crude calcium lactate liquor was then evaporated under vacuum. Technical grade acid was made from this liquor after evaporation, acidification, and filtration of the precipitated calcium sulfate, carbon treatment and heavy metals precipitation. To make higher grades of product the liquor was cooled, crystallized, and washed. The mother liquor and wash water were also cooled, crystallized, and washed. The crystal were redissolved and similarly recrystallized as in earlier steps to create pure grades. Acids of different purity were made from the different grades of crystals by dissolution in water, acidification, calcium sulfate precipitation, filtration, evaporation, carbon treatment, and heavy metals precipitation.[94]

Shreve[79,80] has given the following calcium precipitation process. After the fermentation is completed, where yields of 85 % lactic acid based on the mass of the fermentable sugar are normal. This solution is first alkalized with calcium hydroxide and boiled. Magnesium hydroxide precipitates – the magnesium having entered via the water – also limestone and other nutrients and ingredients. These are filtered off. The filtrate containing the calcium lactate is decomposed by sulfuric acid to regenerate the lactic acid. Calcium sulfate is filtered off and the filtrate consists of approx w = 10 % solution of crude lactic acid. Technical lactic acid is manufactured from the calcium lactate as produced in fermentation or after decolorization. The finer grades are made from calcium lactate that has been crystallized at least once; for making acids from 35 to 50 % mass fraction, the "building up" operation is used. Here crystals of calcium lactates are necessary for the 50 % grade. For stronger acids, a vacuum concentration in stainless steel or glass-lined evaporators is needed.[79,80]

Peckham[63] describes a process for the purification of lactic acid by calcium lactate precipitation. The fermenter liquor is filtered and evaporated to w = 25 % lactic acid. The calcium lactate is then crystallized and separated from the mother liquor. The mother liquor can be used for technical acid.

In the calcium precipitation process, the separation and final purification stages account for up to 50 % of the production costs[15,25] and also produces a large quantity of calcium sulphate sludge as solid waste.[77] It is, therefore, reasonable to look for other methods of lactic acid recovery.

The American Maize-Products Co. has produced lactic acid from glucose by the fermentation of relatively pure sugars with minimal amounts of nitrogenous nutrients.[94] Details of this process are given by *Inskeep* et al.[34] The similar process was used by the Clinton Company and described by *Peckham*[63] After the fermentation, fermentation broth is filtered. Activated vegetable carbon is used to bleach the calcium lactate for production of food grade lactic acid and no carbon treatment is re-

quired for technical grade lactic acid. Then the calcium lactate is evaporated to a 37 % mass fraction at 70 °C and 0.57 bar concentrated lactate, then treated with φ = 63 % sulfuric acid and the calcium sulfate precipitate is removed by a continuous filter and sent back to the first filter, which treats fermenter liquor. The filtered acid is then treated with activated carbon from the filter cakes of carbon treatments. The lactic acid is then evaporated from 8 % to 52 % or 82 %. Technical grade acid then diluted to 50 % or 80 % and treated with sodium sulfide to remove heavy metals. Edible grade acid is diluted to 50 % or 80 %, then bleached with activated carbon and treated with sodium sulfide to remove heavy metals. Then it is bleached fourth time with activated carbon before packaging. This process is not widely known.[94]

Bansal et al.[9] has given another recovery process for lactic acid. The fermented broth is generally heated to 70 °C to kill the bacteria and then acidified with sulfuric acid to pH 1.8. The precipitated salts and biomass are removed by filtration and the resulting liquor is treated with activated charcoal to remove any dyes. The clarified lactic liquor is then ion exchanged and concentrated to 80 %.[9]

Both, microfiltration[5,6] and ultrafiltration[90] have been used in downstream purification of lactic acid fermentation broths.

Bailey et al.[5,6] describe the use of a continuous centrifuge or a ceramic crossflow microfilter to separate bacterial cells from hydrolyzed cheese whey permeating medium after lactic acid fermentation.

Alternatives to conventional process

Electrodialysis

Electrodialysis is a process where ion exchange membranes are used for removing ions from an aqueous solution under the driving force of electrical field. Electrodialysis is applied to remove salts from solutions or to concentrate ionic substances. A special type of electrodialysis is water-splitting electrodialysis. Instead of anion exchange membranes in desalting, bipolar membranes are used in watersplitting electrodialysis. Water-splitting electrodialysis is applied to electroconversion of salts to the corresponding acids. There are two different methods for recovery of lactic acid. It is a twostage electrodialysis method in the first case and electrodialysis with double exchange reaction in the second case. In the first step of desalting, sodium lactate is recovered, purified and concentrated, in the water-splitting or acidification step, lactic acid is regenerated from sodium lactate, and sodium hydroxide is recovered and purified.

Yao and *Toda*,[116] *Hongo* et al.,[32] *Ishizaki* et al.,[117,118] *Nomura* et al.,[62,119-121] *Vonkataveesuk* et al.,[122] *Czytko*,[123] *Van*,[124] *Boyaval* et al.,[11] *de-Raucourt*,[125] *Miura* et al.,[126] *Yamamoto*[127] reported the electrodialysis fermentation method which had been applied to lactic acid production with favorable results by various bacteria. Electrodialysis has also been used by other authors to remove the lactic acid produced by fermentation.[128] While it may increase the fermentation rate by up to 60 %,[129] the approach faces the membrane fouling, deionization of the fermentation broth, and a higher operating cost.[130]

Hongo et al.[32] proposed the possibility of electrodialysis for *in situ* recovery of lactic acid to reduce product inhibition in batch fermentation. In electrodialysis fermentation, the amount of produced lactic acid was about 5.5 times greater than that produced in non-pH-controlled fermentation. They concluded that these good results were obtained on account of alleviating the lactic acid inhibitory effect by electrodialysis fermentation. However, the fouling of anion-exchange membranes by cells was observed in electrodialysis fermentation. *Boyaval* et al.[11] performed continuous fermentation using electrodialysis unit.

Desalting electrodialysis requires low amounts of energy to recover, purify, and concentrate lactate salts from crude fermentation broths.[27] *Glassnar* and *Datta*[27] introduced a two-stage electrodialysis method, desalting of lactate salt from fermentation broth, and acidification of the purified lactate salt by water splitting electrodialysis for the recovery of lactic acid from fermentation broth. Advance in water splitting electrodialysis membranes enable the efficient production of protons and hydroxyl ions from water and can thus produce acid and base from a salt solution. Using an osmotolerant strain of lactic acid bacteria and a configuration of desalting electrodialysis, water splitting electrodialysis and ion exchange purification steps, a concentrated lactic acid product, containing less than 0.1 % of proteinaceous impurities, could be produced from carbohydrate fermentation.

Heriban et al.[23] showed that lactate was concentrated four times by double exchange reaction electrodialysis. *Siebold* et al.[81] carried out the comparative study of production and recovery of lactic acid by extraction and electrodialysis. They concluded that the recovery of the free lactic acid by electrodialysis is very promising. The overall yield of the lactic acid production with electrodialysis amounts to about 70 %, which is higher than extraction. *Lee* et al.[51] carried out the experimental

study on a two-stage process for lactic acid recovery, which consists of desalting electrodialysis and water-splitting electrodialysis. They measured limiting current densities at various lactate concentrations in the feed solution for the determination of the condition for switching from constant-current mode to constant-voltage mode in the desalting electrodialysis. The relationship between the electrical resistance of membrane stack and the lactate concentration was identified. The amount of water transferred, due to electroosmosis which caused volume change in the feed and permeate solution, was also experimentally determined. Based on the experimental results, they developed mathematical model, in which time changes in the feed and permeate volumes and the electrical resistance were considered. Model predictions of lactate concentration, volume changes, switching time, and energy consumption were in good agreement with the experimental data.

Xuemei et al.[128] studied the lactic acid production, using immobilized *oryzae* in a three-phase fluidized-bed with simultaneous product separation by electrodialysis. The specific productivity and the yield in electrodialysis fermentation process, operated in continuous feeding mode, were almost the same as that in $CaCO_3$-buffered fermentation process. They developed a mathematical model for this process to describe the simultaneous fermentation and product separation using electrodialysis.

Kim and *Moon*[131] investigated one-stage electrodialysis (ED) for lactic acid recovery with two- and three-compartment water-splitting ED (WSED), using various ion-exchange membranes in order to overcome the inefficiency of two-stage ED, which consists of desalting ED for recovery and partial purification and subsequent WSED for acidification. The two-compartment WSED had a low current efficiency and high energy consumption in spite of a simple stack configuration. A three-compartment WSED successfully converted sodium lactate in the fermentation broth, into lactic acid, and sodium hydroxide with average yields of 96 % and 93 %, respectively. In relation to lactic acid purification, of the membranes tested, the highest glucose rejection, 98.3 %, was achieved using a PC 100D membrane. The CMS membrane rejected magnesium and calcium at levels as high as 81.7 % and 78.5 %, respectively. They concluded that the three-compartment WSED with properly chosen membranes, enabled lactic acid to be recovered directly from the fermentation broth.

Danner et al.[132] investigated the integrated continuous cell recycle cultivation using ultra-filtration membrane bioreactor coupled with on-line electrodialysis to study the performance of lactic acid production and simultaneous pre-purification. They found that the addition of supplements, like yeast extract and peptone, severely influence product formation. Integration of mono-polar ED with the MBR systems yields lactate solutions with concentrations of up to 115 g dm^{-3}. Because of the low substrate feed mass concentrations (less than 50 g dm^{-3}), stack energy consumption was positive with an average of 0.49 kW h kg^{-1} lactate.

Adsorption

Lactic acid may be recovered by the adsorption of lactic acid on solid adsorbent or by the adsorption of lactate on ion exchange resins.[94] *Sugimoto* et al.[85] patented a process for the production of lactic acid in which strongly acidic and alkaline ion exchange resins were used to separate the acid from the broth.

Kawabata et al.[42] separated carboxylic acid by using a polymer adsorbent of pyridine skeletal structure and a cross-linked structure. The polymer adsorbent showed good selectivity and high adsorption capacity for carboxylic acids even in the presence of inorganic salts. The selected elutants were aliphatic alcohol, aliphatic ketone, and carboxylic ester.

Kulprathipanja and *Oroshar*[47] recovered lactic acid from fermentation broth by using anion polymeric adsorbents, which were strong, moderate, or weak basic anion exchange resins, adsorbing lactic acid below its pK_a. For tertiary amine and pyridine-function-containing ion exchange resin, the lone electron pair of the nitrogen atom enables nitrogen atom to form hydrogen bond by sulfate ion. IRA-400, strongly basic quaternary ammonium ion exchange resin has positive charge and can form ionic bond with sulfate ion. The sulfate form of quaternary ammonium of anion exchange resin has a weakly basic property and can adsorb lactic acid through acid-base interaction. Consequently, the adsorption of lactic acid is not affected by inorganic salt in fermentation broth.

Srivastava et al.[84] separated lactic acid using IRA-400 column coupled with fermenter. This study was focused on improving fermentation yield and the separation performance of IRA-400 was not studied. The Amberlite IRA-400 resin has proper size and high adsorption property for recovery of lactic acid and it can adsorb lactic acid in wide pH range.

Zihao and *Kefeng*[111] examined an anion exchange method for lactic acid recovered from lactic acid – glucose solution in an ion-exchange membrane – based extractive fermentation system. They found that the separation method with anion exchange resins may be used in the production of lac-

tic acid by fermentation. *Dai* and *King*[19] studied the selectivity between lactic acid and glucose during recovery of lactic acid with basic extractants and polymeric sorbents. They found that extraction with Alamine 336 provides a much higher selectivity, but a lower efficacy, than the polymeric sorbents.

Evangelista and *Nikolov*[24] recovered lactic acid from fermentation broth by weak base polymer adsorbents MWA-1, IRA-35, and VI-15. The pH for the adsorption of lactic acid was below its pK_a, and fermentation broth was acidified by using cation exchange resin instead of using inorganic acid to eliminate possible competition between inorganic acid and lactate in the subsequent adsorption steps. Methanol and 5 % NH_4OH were used as elutants. Though 1.5 times of bed volume of 5 % NH_4OH could recover all the adsorbed lactic acid from MWA-1 column, product purity was not high. However, 6.8 times of bed volume of methanol could completely desorb lactic acid from VI-15 anion exchange resin with higher purity.

Monteagudo and *Aldavero*[59] investigated the lactic acid production in a continuous fermenter-ion exchange resin system and compared with conventional fermentation. The principle of this method is to remove the lactate during the course of fermentation as it is formed by adsorption to an anion exchange resin (Amberlite IRA-420) in the carbonate form and to overcome its inhibitory effects on lactic acid bacteria by maintaining low lactate concentrations in the medium. Ammonium lactate was formed by percolating ammonium carbonate solution through this resin and it was converted to lactic acid by treatment with a cation exchange resin (Amberlite IR-120) in hydrogen form. Compared with a conventional fermentation, this fermentation-ion exchange resin system enhanced the fermentation, controlled the pH, and showed the remarkable effect of increasing the yields of lactic acid from sucrose and biomass from sucrose, due to complete utilization of sucrose.[59]

Many adsorbents have been examined for lactic acid removal from fermentation.[3,21,58,110] Compared with extraction, adsorption offers the advantage of low or no negative effects to cells.[3,21] Adsorption is also potentially simpler and cheaper than electrodialysis. However, ion-exchange resins also remove essential anions other than lactate from the broth. Non-ion-exchange adsorbents deserve more attention.

Chen and *Ju*[133] reported the adsorption characteristics of lactic acid and lactate on polyvinylpyridine (PVP) and activated carbon. *Chen* and *Ju*[17] evaluated polyvinylpyridine (PVP) and activated carbon for coupled lactic acid fermentation and adsorption, to prevent the product concentration from reaching inhibitory levels. The lactic acid production doubled as a result of periodical circulation of the fermentation broth through a PVP adsorption column. Each adsorption-regeneration cycle caused about 14 % loss of the adsorption capacity, thus limiting the practical use of this rather expensive adsorbent. Activated carbon was found much more effective than PVP in lactic acid and lactate adsorption.

Raya-Tonetti et al.[134] used a strong anionic exchange resin (Amberlite IRA) to recover lactic acid directly from fermentation in an upflow fluidized bed column. They found that the resin did not alter its binding capacity after 23 cycles.

Sosa et al.[135] measured the static adsorption isotherm over a strong anionic exchange resin, Amberlite TM IRA-400, and quantified the static binding capacity parameters for lactic acid recovery. Early recovery of lactic acid was performed in a liquid solid fluidized bed, with the resin as the solid adsorbent, and the dynamic adsorption capacity was calculated. Good agreement was found between static and dynamic binding capacity values. The fluidized bed height was twice the settled bed height and the overall process was controlled by the liquid solid mass transfer. This operation was also simulated by continuously well stirred tanks arranged in series and superficial solid deactivation as in a gas solid catalytic reactor. The deactivation process takes into account liquid channeling and agglomerations of solid induced by the viscosity of the broth and also by the cells during the adsorption.

Chen and *Ju*[17] studied the coupled fermentation and adsorption to prevent the product concentration from reaching inhibitory levels for lactic acid production. They used polyvinylpyridine (PVP) and activated carbon as an adsorbent. Lactic acid production doubled as a result of periodical circulation of the fermentation broth through a PVP adsorption column. The adsorbent was then regenerated and the adsorbed lactate harvested by passing 0.1 mol dm^{-3} NaOH through the column. However, each adsorption–regeneration cycle caused about 14 % loss of the adsorption capacity, thus limiting the practical use of this rather expensive adsorbent. Activated carbon was found much more effective than PVP in lactic acid and lactate adsorption. The cells of *Lactobacillus delbrueckii* subsp. *delbrueckii* (LDD) also had strong tendency to adsorb on the carbon. Therefore, they studied using an activated carbon column for simultaneous cell immobilization and lactate adsorption, in a semi-batch process with periodical medium replacement.

Cao et al.[14] studied the adsorption of lactic acid on IRA-400, strongly basic quaternary ammonium

anionic exchange resin, at the pH above and below the pK_a of lactic acid. The adsorption isotherm, breakthrough curve, washing condition, elution condition, and column separation process for lactic acid were described. Recovery experiment coupled with fermentation was carried out successfully by using a column without autoclaving.

Ye et al.[110] developed and studied a novel integrated fermentation system in which cross-flow filtration was coupled to an anion exchange resin column to achieve biomass recycle and broth reuse for lactic acid fermentation. They reused spent broth for three consecutive biomass recycle fermentation with no significant decrease in fermentation performance.

Reverse osmosis

Reverse osmosis has also studied for recovering lactic acid from fermentation broths [83,74]. They concluded that the reverse osmosis could effectively concentrate lactic acid from 10 to 120 g dm^{-3} at a 6.9 MPa transmembrane pressure at energy use lower than multiple effect evaporators.

Reactive extraction

Although, conventional method is the most used technique on the large-scale processes, solvent extraction has been developed for the separation of lactic acid. Many investigators have investigated the reactive extraction of lactic acid into an immiscible extractant/solvent phase. In such processes lactic acid is first being extracted from fermentation broth by the extractant and then recovered from the solvent by back extraction into another solvent. Amine extractant has been found to be prospective method of separation of carboxylic acids from aqueous solutions. Lactic acid can be readily extracted into a number of organic solvents with high molecular mass aliphatic amines and phosphorous bonded oxygen donor solvents, exhibiting particularly good selectivity. Besides high capacity, high selectivity, low prices and non-toxic substance, because of the use in food industry, three important criteria have been established for solvent selection: 1. high distribution coefficient for lactic acid, 2. easy back extraction and regeneration, and 3. low tendency to emulsion formation.

Kertes and *King*[43] categorized the organic solvents for extraction into three major types: 1. conventional oxygen-bearing and hydrocarbon extractants, 2. phosphorus-bonded oxygen-bearing extractants, and 3. high molecular mass aliphatic amines. Solvent extraction with conventional solvents such as alcohols, ketones, ethers, and aliphatic hydrocarbons is not effective when applied to dilute, carboxylic acid solutions, because of the low aqueous activity of carboxylic acids resulting in low distribution coefficients.[109] However, carboxylic acid extractions with organophosphates, such as trioctylphosphine oxide (TOPO) and tri-n-butyl phosphate (TBP), and aliphatic amines have large distribution coefficients. Aliphatic amines are slightly more effective and less expensive than phosphorus-bonded oxygen bearing extractants.[96] Several aliphatic amines have been used successfully to extract carboxylic acids.[43,69,70,86,87,96,98,99,100,102,107]

Primary amines give a high mutual solubility with water. Secondary amines can give quite high values of K_D, but are subject to amide formation during regeneration by distillation. For tertiary amine extractants, K_D typically exhibits a maximum value at an intermediate solvent composition. This behavior apparently reflects the combined effects of mass action for the chemical reaction on one hand, and the activity coefficient of the reaction complex in the solvent mixture, on the other hand.[69]

Few patents are available in this context[5,6,7,8,37,150-155] and only one or two of these appear to have been practiced commercially. Numbers of literature studies are available on the reactive extraction of lactic acid.

Jenemann[37] described a continuous countercurrent solvent extraction procedure based on isopropyl ether in a patent assigned to du Pont. This process has been practiced on a commercial scale with modifications by Croda Browmans Chemicals Ltd. in the United Kingdom.[10,55]

Bailey et al.[5,6] used the tertiary amine Adogen 364 in 60-75 % isobutyl heptyl ketone as the preferred system for extraction of lactic acid from cheese whey permeate fermentation after removal of *L. casie* cells and suspended solids.

In a patent assigned to Purdue Research Foundation and Reilly Industries, *Iyer* et al.[36] described the use of solid-phase polymer having tertiary amine groups in an extractive fermentation to absorb lactic acid. Either *Lactobacillus* spp. Or *R. oryzae* can be used in this fermentation.

Wang et al.[95] proposed using a hollow fiber hydrophobic membrane between the solvent and aqueous phases of a nondispersive extraction process for lactic acid recovery. The solvent system trioctyl phosphine oxide (TOPO) in kerosene, while effective for extraction, clogged the membrane with TOPO crystal when it was exposed to air. Also, *Hano* et al.[136] measured the extraction equilibrium of lactic acid with tri-n-octyl phosphine oxide (TOPO).

Using model lactic acid solutions, a quaternary ammonium salt (Aliquat 336) gave the best extraction at pH 5 or 6 and 35 °C, the usual conditions for lactose fermentation by *L. casei*.[49] The optimum mass concentration of this extractant for liquid

membrane extraction of a 3 g dm^{-3} lactic acid feed solution was φ = 5 % in n-octane.[50]

Kyuchoukov et al.[137] proposed a novel method for the extraction of lactic acid by means of a modified extractant. They treated successfully a quaternary ammonium salt (Aliquat 336), dissolved in 1-decanol and n-dodecane with different concentrations of ammonium carbonate for replacement of the chloride anion with a carbonate one. They found that the carbonate form of Aliquat 336 is more efficient than the classical chloride one.

Wasewar et al.[98,99,100] studied the reactive extraction of lactic acid using Alamine 336 in MIBK, octanol and decanol, and suggested the back extraction process using trimethyl amine (TMA). They found that 99 % recovery is possible using TMA.

Yabannavar and *Wang*[104,105,106,107,108] developed an extractive fermentation system for removing lactic acid continuously from glucose fermentation by *L. delbrueckii*. The extractant system showing the least toxicity to the cells was 15 % Alamine 336 in oleyl alcohol. The cells were protected from the solvent by immobilization. The lactic acid productivity was 12 g dm^{-3} gel h^{-1} compared with 7 g dm^{-3} gel h^{-1} for a control fermentation without solvent. A final product mass concentration of 90 g dm^{-3} was obtained by back extraction with sodium hydroxide.

In the extractive fermentation of glucose by *L. delbrueckii* NRRL B-445 (*L. rhamnosus*), amines, such as Adogen 464, Aliquat 336, Tri-n-octylamine (TOA) and TOPO, were toxic to cells.[76] The hydrophobic resin Bonopore in paraffin oil showed no toxicity in batch cultures. However, the yield of lactic acid was lower than that of a conventional batch fermentation that may have resulted from absorption of essential nutrients by the Bonopore resin.

Yang et al.[109] studied the interaction of carboxylic acids with tertiary and quaternary amines. The quaternary amine Aliquat 336 extracted, both, dissociated and undissociated forms of acids, whereas the tertiary amine Alamine 336 extracted only the undissociated acid. The polar diluent, octanol increased the extracting power of Alamine 336 by providing more solvating capacity for the nonpolar amine. In contrast, neither the polar nor the nonpolar diluent was active when used with Aliquat 336.[109]

Choudhury et al.[16] studied lactic acid extraction with two extractants, namely, trioctylamine (TOA) and Aliquat-336, in three diluents (MIBK, octanol and paraffin liquid). Among the extractants, TOA was found to be better extractant than Aliquat 336 in all the experiments.

Hong and *Hong*[138] used the mixture of tripropylamine (TPA) and trioctylamine (TOA) dissolved in 1-octanol/n-heptane in the reactive extraction of lactic acid in aqueous solution. They obtained maximum distribution coefficient in the range from 6:4 to 8:2 mass ratio of $\zeta_{TPA/TOA}$ at w = 5 % lactic acid in aqueous phase and their extraction efficiencies were above 90 %. By introducing TPA into TOA, the third phase formation could be overcome, thereby; the settling time is shorter than in the case of TOA, only.

Jarvinen et al.[139] examined the separation of lactic acid from complex fermentation broth. They used 40 % tertiary amine Hostarex A327 (tri-n-octyl/n-decylamine) in decanol for extraction and over 50 % yield was obtained in a single step of extraction.

Malmary et al.[140] investigated the mechanism for extraction of lactic acid from water by a long-chain aliphatic tertiary amine (tertiary alkylamines) in solution with organic diluents (1-octanol + n-heptane). The experiments showed that the partition coefficient for a particular organic acid depends on the kind of solute, notably when the acid concentration in the aqueous phase is low. A mathematical model, where, both, chemical association and physical distribution are taken into consideration, is proposed. The model suggests that the various complexes obtained between amine and organic acids contribute to the distribution of the solute between the coexisting phases in equilibrium.

Matsumoto et al.[141] examined synergistic extraction system of lactic acid to develop on *in situ* extractive fermentation process. The addition of tri-n-butyl phosphate(TBP) to the extraction system of lactic acid (HA) with tri-n-octylamine (TOA) diluted by hexane causes a large synergism. Extraction reaction with the mixed extractant is interpreted quite well, based on the formation of mixed complex, HA-TOA-2TBP.

Extraction data for solvent with lactic acid and water and some data for solvent with crude lactic acid fermentation liquors are presented.[31,53,67,101] The effect of adding inorganic salts to the aqueous phase and the distribution coefficients of sucrose and lactose with several solvents was examined by *Weiser* and *Geankoplis*.[101]

The effect of temperature on the distribution coefficient has also been studied.[56,88] They found that the distribution constant and equilibrium complexation constant decreases with increasing temperature i.e. extraction decreases with increasing temperature. The complexation reactions in the organic phase involve a proton transfer reaction or hydrogen bond formation and are expected to be exothermic.[88] The complex formation increases the

order of the system and hence the entropy should decreases. Therefore, as the temperature increases, the amount of acid extracted decreases.[87] It is generally believed that a temperature increase has an adverse effect on the extraction of metallic ions due to a decrease in the stability of the species at higher temperatures.[35] The same may be applicable for the acids also.

Generally the organic phase extracts more acids than would be expected on the basis of (1:1) lactic acid – amine complex.[73,98,99] This is a common behavior, especially for mono carboxylic acids. The formation of (2:1) and (3:1) lactic acid – amine complexes depends on the lactic acid concentration in the aqueous phase, and the ratio of (1:1) to (2:1) complex formation is diluent dependent.[86] Different diluents solvate the various complexes and the amine to different extents, thereby changing the activity coefficients.

Many fermentations operate best or only work at all, under conditions where the pH exceeds the pK_a of the lactic acid being produced. However, most extractants work efficiently only at acidic pHs, and acidogenic anaerobes generally have poor growth rates at low pH. It is thus important to find an extractant that will work well at a relatively high pH. Furthermore, it is essential to understand the effects of pH on extraction as well as on the fermentation before an extractive fermentation process can be designed.[33] Effect of pH was studied by *Yang* et al.[109] and *Choudhury* et al.[16] The K_D value increased with decrease in the pH except at extremely high or low pHs, where K_D does not change significantly with pH.[109] They concluded that a lower pH favors the extraction of lactic acid. Generally, distribution coefficient is constant for low concentration of lactic acid and decreases for higher concentration.[98,99] Hence, it is beneficial to carry out reactive extraction at lower lactic acid concentration for high distribution coefficient, which require less amount of extractant and also avoid the product inhibition of microorganisms due to the acid.

San-Martin et al.[72,73] carried out several experiments to determine the distribution equilibrium of lactic acid to study the influence of salts and lactose in the extraction of lactic acid. Their results indicated that the extraction of lactic acid with Alamine 336 dissolved in toluene is not affected by lactose and less lactic acid is extracted by the organic phase in the presence of chlorine.

Matsumoto et al.[56] investigated the extraction kinetics of organic acids with tri-n-octylphosphine oxide (TOPO) to determine the extraction mechanism. They used two-film theory for kinetic study. They found that the extraction rate is limited by the mass transfer through organic phase. *Hironaka* et al.[30] studied the extraction and stripping kinetics of lactic acid in extractive fermentation using tri-*n*-octylmethylammonium chloride, a quaternary ammonium salt as an extractant, and oleyl alcohol as a diluent. They examined the dependences of extraction rate on initial lactic acid and extractant concentrations. They used two-film theory for kinetic studies. Diffusion through the organic film was found to be the rate-determining step because of the fairly high viscosity of the organic phase.

Wasewar et al.[98,99,100] studied the kinetics of reactive extraction of lactic acid using Alamine 336 in various diluents (MIBK, decanol, and octanol). They used the theory of extraction accompanied by a chemical reaction.[23] They found that the reaction was fast reaction with zero order in Alamine 336 and first order in lactic acid. Also *Wasewar* et al.[97] studied the kinetics for the back extraction of lactic acid using aqueous trimethylamine (TMA).

Tik et al.[91] investigated the extractive fermentation using immobilized *Lactobacillus delbrueckii* in the presence of sunflower oil and Alamine 336 with oleyl alcohol. They investigated the effects of oleyl alcohol (φ = 33.3 %), immobilization, and immobilization in the presence of sunflower oil (5, 10, 15 %). A maximum total lactic acid concentration (2.5 times that of without extraction) was obtained when 15 % Alamine 336 in oleyl alcohol, together with immobilized cells with 15 % sunflower oil, was used. Coimmobilization with sunflower oil probably affected the metabolism of the microorganism. Fats and oils are used as carbon sources and they are broken down to glycerol and fatty acids. Fatty acids are used as the source of ATP while Glycerol is converted to pyruvate via glycolysis. Then, lactate is formed from pyruvate under the anaerobic conditions.[52] Therefore, lactic acid production increased with the increase in sunflower oil concentration. The sunflower oil can also extract Alamine 336 that diffused into the gel and prevent the toxic effect of the solvent. These are the reasons why sunflower oil was used in the extractive fermentation experiments.[91]

Various processes were suggested for back extraction of lactic acid from loaded organic phase, such as, using NaOH,[105] using HCl,[105] using distillation and ammonia,[40] using trimethylamine (TMA),[65,97] temperature swing regeneration,[88] diluent swing regeneration[7,89] and gas antisolvent induced regeneration.[57] It was found that the regeneration of lactic acid from the loaded organic phase by gas-antisolvent-induced method is the best suitable method because this process does not require any toxic material and also the energy requirement is low because of the lack of a distillation step compared to the other processes

Liquid membrane / aqueous two phase system

A supported liquid membrane (SLM), which uses a porous membrane support soaked with complexing carriers to separate feed and strip phases, represent one of the feasible type of liquid membranes.[61,64]

Sirman et al.[82] studied the separation of citric and lactic acids by an SLM containing Alamine 336 and concluded that citric acid has an overall extraction rate higher than the lactic acid. *Reisinger* and *Marr*[68] examined the separation of organic acids from fermentation broth by a liquid surfactant membrane (LSM) containing Amberlite LA-2 (a secondary amine). They found that in addition to lactic acid other monocarboxylic acids can be separated and purified, and indicated that for di-and tricarboxylic acids the carrier contents of the membrane phase must be adapted to the slower extraction kinetics to achieve fast permeation.

An emulsion liquid membranes system, consisting of the amine Alamine 336 and the surfactant Span 80 in n-heptane paraffin, was evaluated from extracting lactic acid from *L. delbruckii* NRRL B-445 fermentation broth after cell removal.[75] Alamine 336 had a lower selectivity for lactic acid than desirable owing to its possible binding to other competing solutes.

Aqueous two-phase systems have been used for the production of lactic acid.[142–148] However, an even distribution of lactic acid between two phases, together with the cost of polymers, makes this process concept economically nonviable.

Dissing and *Mattiesson*[22] investigated an aqueous polyethyleneimine (PEI) – hydroxyethylcellulose (HEC) two phase system for the extractive fermentation production of lactic acid from glucose by *L. lactis*. Lactic acid partition into the PEI-rich bottom phase, whereas the cells accumulated in the HEC top phase or at the interface.

A phase system composed of a polyelectrolyte, poly(ethyleneimine) (PEI), and a neutral polymer, hydroxyethylcellulose (HEC), has been found suitable for extractive fermentation of lactic acid.[22,144] Since PEI is positively charged, it can form an ion pair with the lactate produced during fermentation, and lactate can be partitioned in favour of the PEI-rich phase as soon as it is formed in an extractive fermentation. Successful use of other phase systems has also been reported in the literature such as an ethylene oxide / propylene oxide-dextran T40 ATPS[146] and PEG / hydroxypropyl starch (HPS) and a random copolymer of ethylene oxide and propylene oxide (EO-PO) / HPS[145] for production of lactic acid. A new family of polymer conjugates is proposed to overcome constraints in the applicability of aqueous two-phase systems for the recovery of lactic acid[148]. Polyethylene glycol-polyethylenimine (PEI) conjugates and ethylene oxide propylene oxide-PEI (EOPO-PEI) conjugates were synthesized and mixed with fractionated dextran or crude hydrolyzed starch. Lactic acid partitioned to the top conjugate-rich phase of the new aqueous two-phase systems. They found that the lactic acid partition coefficient was 2.1 in 10 % EOPO-PEI-8 % DEX systems containing 2 % phosphate.

Juang, *Huang*[38] and *Juang* et al.[39] explored the separation mechanism of lactic acid and citric acid in aqueous stream using supported liquid membrane (hydrophobic PVDF microporous membrane). They examined the effects of temperature and composition of strip phase. Separation factor was calculated to discuss quantitatively separation characteristics and to obtain optimal operating conditions.

Hollow fiber membrane

Membrane extraction seems to be the very candidate for extractive fermentation of lactic acid.[93] Moreover, it overcomes many drawbacks of the classics which have plagued us for long, and offers other numerous advantages, such as i) no fear of back mixing, ii) no direct exposure of microbes to extraction reagents, thereby ensuring biocompatibility, iii) no need for agitation, iv) potentially high efficiency, etc.[93] For the above reasons, membrane extraction can be considered a very promising alternative to the conventional solvent extraction for separation and purification of lactic acid. Here, the dispersive free condition can be easily attained as long as an appropriate pressure difference is maintained between the two phases.[93]

Tong et al.[93] used the microporous hollow fiber membrane device for the extraction of lactic acid. They selected tri-n-octylmethylammonium chloride dissolved in oleyl alcohol as the optimum extraction reagents for the extractive fermentation of lactic acid. They accomplished the satisfactory recovery of lactic acid from both aqueous solution and actual fermentation broth, signifying the great potential of integrating the membrane extraction with fermentation process.

Huang et al.[149] successfully developed an energy-efficient hollow-fiber membrane extraction process to separate and recover lactic acid produced in fermentation. Continuous extraction of lactic acid from a simulated aqueous stream was achieved by using Alamine 336 in 2-octanol contained in a hollow-fiber membrane extractor. In this process, the extractant was simultaneously regenerated by stripping with NaOH in a second membrane extractor, and the final product is a concentrated lactate salt solution. The extraction rate increased linearly with an increase in the Alamine 336 content in the

solvent (from 5 to 40 %). Increasing the concentration of the undissociated lactic acid in the feed solution by, either, increasing the lactate mass concentration (from 5 to 40 g dm^{-3}) or decreasing the solution pH (from 5.0 to 4.0), also increased the extraction rate.

Discussion

Above-mentioned methods have some advantages and disadvantages. Conventional calcium precipitation method is simple and reliable but it is expensive and unfriendly to the environment as it consumes lime and sulphuric acid and also produces a large quantity of calcium sulphate sludge as solid waste. Electrodialysis and dialysis have good potential and have advantage of simultaneous separation and concentration. Electrodialysis and dialysis has the problem of membrane fouling which requires frequent cleaning of the dialyzer. Also, very large cost dialysis units, even greater than the cost of the fermenter vessel, would be required for a commercial scale operation. Electrodialysis gives a higher extent of lactic acid separation but with increased power and energy consumption. Also, by-product salt from the ion exchange regeneration is formed. Adsorption or ion exchange process requires regeneration of ion exchange resin and adjustment of feed pH to increase the sorption efficiency requiring large amount of chemicals In reverse osmosis, there is a tendency to form emulsion and complexity of operation. Distillation is the well-established and reliable technology but it has a drawback; there is a formation of high boiling esters and dimmers. In hollow fiber membrane extraction process, membrane has tendency to form emulsion but has advantage of large interfacial surface area for mass transfer in a compact unit. Interest in liquid surfactant membranes for biochemical separations has focused on their potential advantages. The main advantage of liquid surfactant membranes over other separation techniques is the large surface area available for mass transfer, which results in a fast rate of separation. In spite of these apparent advantages, very few industrial applications have been reported so far. Several drawbacks were shown to hinder implementation, manly complexity of operation and swelling in liquid surfactant membrane. The use of supported liquid membranes for the recovery of lactic acid offers unique advantages. Some of the advantages are lower energy consumption, higher separation factors in a single stage, and the ability to concentrate lactic acid during the separation. However, supported liquid membrane often suffers from membrane instability. For continuous separation of products membrane bioreactors can be used, which enhance the productivity and avoids toxicity due to extractant by immobilization of biocatalyst in membrane. In membrane bioreactor cleaning and sterilization are very difficult. Reactive extraction is a closed loop process and proper combination of extractant and diluent and proper choice of back extraction process yields high productivity. Also practically all data of reactive extraction is available for commercial design. In reactive extraction, most of the extractant works efficiently at low pH while most microbes give higher productivity at higher pH. Also most of the solvents are toxic towards microbes. Hence, further improvement in the extractant-solvent and microbes is needed i.e. immobilization of microbes and development of extractant-solvent system.

Conclusion

Biosynthesis processes for lactic acid are product inhibited. The productivity of these fermentation processes can be significantly increased by *in-situ* recovery of lactic acid from fermentation broths by reactive extraction. It is important to have an efficient and economic and low waste residual disposal process for the separation of lactic acid from the fermentation broth. Although, commercial process of lactic acid separation are based on classical method of separation, the work done on solvent extraction of lactic acid is promising.

Future scope

Amine extraction has been found to be a prospective method of separation of lactic acid from aqueous solution. A good starting point for developing new extractive recovery processes for the fermentation products should be the application of novel, more powerful extractant such as Alamine 336. The use of nontoxic substance is the main demand. Many interesting problems have been left for future work.

Extractant system toxicity against the microorganism plays an important role in the separation of fermentation product from fermentation broth using reactive extraction. It can be prevented by proper immobilization of microorganisms and an environment conducive to high activity. Generally these microorganisms are stable at high pH and do not survive at lower pH i.e. at higher lactic acid concentration. Therefore, it is necessary to focus towards the development of microorganisms which can be active at lower pH. Further, lower pH i.e. higher lactic acid concentration of fermentation broth yields higher lactic acid concentration in the

extractant organic phase, which reduces the cost of further purification.

Commercialization of any process is the key success for the developed new technology/process and the process is mainly commercialized on the basis of its economical evaluation. Economical evaluation data of various processes of lactic acid production and its recovery are not available. Therefore, it is necessary to focus on the economical evaluation of various processes of lactic acid production and its recovery for the economical comparison.

List of simbols

c – concentration, mol dm^{-3}
w – mass fraction, g dm^{-3}
φ – volume fraction, %
γ – mass concentration, g dm^{-3}
ζ_{m_1/m_2} – mass ratio, m_1/m_2

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