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A STUDY OF PARAMETERS AFFECTING THE SOLVENT EXTRACTION OF LACTIC ACID FROM FERMENTATION BROTH

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Abstract - Lactic acid has recently been drawing much interest as a raw material for biodegradable polymer. One of the promising technologies for recovery of lactic acid from fermentation broth is reactive liquid–liquid extraction. Equilibrium studies on the reactive extraction of lactic acid with trioctylamine (TOA) in various organic phases and its re-extraction into aqueous solutions were carried out. In this study distribution coefficient, extractability, stripping efficiency of various active and inert diluents with TOA as extractant were investigated, which were higher for active diluents. The effects of operating temperature, speed of agitation, agitation time and diluent composition on extraction efficiency were also studied. Temperature and extraction efficiency were inversely proportional to each other, whereas extraction efficiency was little affected by speed of agitation and agitation time.
Keywords: Lactic acid; Reactive extraction; Trioctylamine; Diluents.

INTRODUCTION

Lactic acid ($CH_3CHOHCOOH$, 2-hydroxypropanoic acid) is a carboxylic acid predominantly produced by fermentation in industry. The Food and Drug Administration (FDA) have approved lactic acid and its salts to be GRAS (Generally Recognized as Safe) (Lee *et al*., 2004). It has a variety of applications in the food, agricultural, textile, chemical, pharmaceutical and cosmetic industries. It can be converted to ethanol, propylene glycol and acrylic polymers, its derivatives like lactate salts, esters, lactamides and lactonitriles have widespread applications (Wasewar *et al.,* 2002). As well as being environmentally friendly, there is a growing demand due to strict environmental laws being legislated for biodegradable polymers as a substitute for conventional plastic materials. Biodegradable copolymers are also used for the production of new materials with biomedical applications such as drug delivery systems (Han *et al.,* 2000, Harington and Hossain, 2008).

One of the main obstacles in the large-scale production of this substance is the cost of the raw material. Application of agro-industrial wastes in bioprocesses provides an alternative way to replace the refined and costly raw materials. In addition, the bulk use of such materials helps to solve many environmental hazards. However, the application of microorganisms for the production of lactic acid using cost-effective raw materials is rare. Hence, research efforts are focused on looking for new and effective nutritional sources and new progressive fermentation techniques, enabling the achievement of both high substrate conversion and high production (Jawad *et al.*, 2013).

Lactic acid is typically produced via either chemical synthesis or fermentation. Approximately

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90% of the total lactic acid produced worldwide is by bacterial fermentation. The chemical synthesis of lactic acid always leads to a racemic mixture, which is a major disadvantage (Dumbrepatil *et al*., 2008).

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 disadvantage since the vaporization of water consumes much energy. Also distillation is not useful due to the 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 for up to 50 % of the production costs (Choudhuri *et al.,* 1992; Eyal *et al.,* 1993). 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 (Shreve *et al.*, 1977). It is, therefore, reasonable to look for other methods of lactic acid recovery (Wasewar, 2005).

Several possible alternatives like an ion exchange process, adsorption, diffusion dialysis, or reactive extraction exist for the recovery of lactic acid from fermentation broth. Among these, reactive extraction is considered to be an effective separation step for the recovery of lactic acid from fermentation broth. Reactive extraction is the separation process using reaction between extractant and material extracted. The extractant in the organic phase reacts with material of the aqueous phase and the reaction complex formed is solubilised into the organic phase (Tamada *et al*., 1990; Hang and Hong, 1996; Han *et al*., 2000).

Reactive extraction using long-chain aliphatic amines has been studied as an effective and economical separation process of carboxylic acids. There are two stages in extractive separation. The first is the extraction of the acid to produce an acid-amine complex and a relatively acid-free aqueous raffinate. The second step is necessary for stripping lactic acid from the organic complex to obtain amine-free aqueous lactic acid as a product and also for simultaneously regenerating the extractant, which is recycled back to the extraction unit.

Ratchford *et al*. (1951) studied the effects of amine structure and the solvent properties. The solvation of the whole amine-acid complex is based on dipole-dipole interaction and has been found to play an important role in the neutralization reaction between acid and amine. In general; there are primary, secondary and tertiary amines in amine-type extractant. Among these amines, the tertiary amines have been widely used in reactive extraction because the primary and secondary amines tend to react irreversibly with carboxylic acids and the stripping of solvent becomes difficult (Han *et al.*, 2000).

Diluent is usually added along with the extractant to enhance its physical properties by providing general solvation and affect the extraction power of the extractant by providing specific interaction. The diluents also affect the basicity of the amine, the stability of the ion-pair (acid-amine complex) formed and its solvation (Choudhury *et al.,* 1998). The diluent may consist of one or more components, inert or active. Various active polar and proton- or electron-donating diluents (halogenated aliphatic/aromatic hydrocarbons, ketones, nitrobenzenes, higher alcohols), enhance the extraction. On the other hand, inert diluents (long chain paraffins, benzene etc.), limit the extractant capacity (Kumar *et al.,* 2008; Wasewar and Shende, 2011). In order to improve the amine's solvation power, diluents such as oleyl alcohol, chloroform, methyl isobutyl ketone and 1-octanol have been tested. The diluents affect the basicity of the amine, the stability of the acid:amine complex formed and its solvation power (Kertes and King, 1983; Tamada *et al*., 1990; Bizek *et al*., 1993; Kahya *et al*., 2001). The important parameters for the selection of suitable diluents for the amine are distribution coefficient, selectivity, toxicity, low solubility in water, viscosity, density, and stability (Wennersten, 1983; Han and Hong, 1996).

Extraction efficiency using trioctylamine with different diluents has been studied. The lactic acid extracted has to be stripped for the final production of lactic acid. The reaction of amine and acid is an exothermic reaction, so the extraction efficiency of lactic acid decreased with increasing temperature for active diluents, whereas it showed little effect for inert diluents. The extraction efficiency of lactic acid increased with increasing concentration of active diluents. Variation of inactive diluents had little influence on the extraction efficiency except for polar diluents (King, 1992; Han and Hong, 1996). The speed of agitation and time had little effect on extraction efficiency of lactic acid (Wasewar *et al.*, 2002).

The objective of our study was to study distribution coefficient, extractability, stripping efficiency of various active and inert diluents with trioctylamine (TOA) as extractant. Another aim was to study the parameters affecting the solvent extraction of lactic acid from fermentation broth like effects of operating temperature, speed of agitation, agitation time and diluent composition on extraction efficiency.

MATERIALS AND METHODS

Materials

Trioctylamine (TOA), a C 8 straight-chain tertiary amine, was obtained from Spectrum Chemical Company and was used as an extractant. 2-ethyl hexanol and 1-octanol were used as active diluents and were obtained from Hi-Media India Ltd. Hexane, xylene and toluene were used as inert diluents and were obtained from Ranbaxy India Ltd. Aqueous solutions of lactic acid was obtained from S. D. Fine Chem. Ltd., India. All these chemicals were technical grade and were used without any further purification.

Fermentation Broth

Fermentation of glucose was carried out using a mutant strain, *Lactobacillus delbrueckii* NCIM 2025. A glass-lined stirred reactor having a capacity of 20 L was used for fermentation. The fermentation was carried out at 43 °C with speed of agitation of 100 rpm. Autoclaved $CaCO_3$ was added frequently to maintain the pH of fermentation. Initially, the culture was grown in 1 L of growth medium for 4 days. The culture was then made up to a final volume of 5 L. This culture was transferred to the fermentation vessel. The fermentation was carried out for 110 hrs. The final lactic acid concentration in the fermentation broth was 36 $g.L^{-1}$. The pH of this fermentation broth was adjusted to 3.00 by adding concentrated H_2SO_4. This solution was centrifuged at 5000 rpm for 1 hour to separate calcium sulfate. The filtrate and washings were concentrated in the evaporator under vacuum to get crude lactic acid. The crude lactic acid so obtained was a viscous, dark reddish brown liquid and had impurities of fermentation. It was treated with activated charcoal and filtered to get transparent and clear dilute crude lactic acid, which was used for further work.

Experimental Procedures

Liquid-Liquid Extraction

The "Organic phase" in this paper refers to the mixture of the extractant and the diluents. In a typical experiment, 30 mL trioctylamine of the aqueous feed solution containing lactic acid, 30 mL of the organic solvent system containing 15mL of trioctylamine as extractant and 15mL of diluents were transferred to a glass vessel having a jacket for the continuous flow of water from a water bath to maintain the temperature at 0 °C. The phase mixing was carried out in this glass vessel by agitating at 4000 rpm for 2 minutes. The mixture was then centrifuged at 10000 rpm for 15 minutes and transferred to a 100 mL separatory funnel at room temperature to separate the aqueous and organic phases, which took 15-20 minutes depending upon the nature of the diluent. Volumes of both phases were measured exactly.

The lactic acid concentration in the aqueous phase and organic phase was determined by titrating with 0.5 N NaOH using phenolphthalein as an indicator. The efficiency of extraction (E) was expressed as

$$E = (1 - Ce / C_0) \times 100 \quad (1)$$

where Ce is the concentration of lactic acid in the aqueous phase after extraction and C_0 is the initial concentration of lactic acid in the aqueous phase (feed).

An E value of 100% means that all of the lactic acid in the aqueous phase has been removed and is present in the organic phase of the mixture. If no lactic acid is extracted, Ce= C_0, and E = 0 %. This definition of extraction efficiency neglects any changes in the volumes of the organic and aqueous phases upon mixing; however, no changes were observed with trioctylamine (TOA) as the extractant.

Back Extraction to Recover Lactic Acid

In back extraction, the 30 mL of organic phase of the above extraction and 50 mL of 0.5 N NaOH were transferred to a glass vessel with an agitator. Phase mixing was carried out by agitating at 4000 rpm for 5 minutes. The two phases were separated in a separatory funnel into organic phase and aqueous phase in 5-10 minutes. Volumes of both phases were measured exactly.

The lactic acid concentration freed from the organic phase was measured by titrating 20 mL of above phase with 0.5 N oxalic acid using phenolphthalein as indicator. The titration of 20 mL NaOH with 0.5 N oxalic acid was used as initial blank. By taking the difference and considering the separated volume of organic phase after extraction of lactic acid, the back extracted amount of lactic acid was measured in terms of $g.L^{-1}$.

Stripping Efficiency

The stripping efficiency was calculated in terms of percentage by considering the amount of lactic acid in extraction and back extraction.

Distribution Coefficient

Lactic acid concentration in the aqueous phase was determined by potentiometric titration using 0.1 N NaOH. The amount of lactic acid in the organic phase was obtained by mass balance. The distribution coefficient, $K_{D,}$ was calculated by taking the ratio of concentration of lactic acid in the organic phase to the concentration of lactic acid in the aqueous phase.

Effect of Extractant: Diluent Ratio on the Extraction Efficiency

The extraction of lactic acid was carried out as described above changing the volumetric concentration of trioctylamine as extractant as well as volumetric concentration of diluent to optimize their level of concentration for extraction efficiency and to study the effect of extractant: diluent ratio on the extraction efficiency.

Effect of Temperature on the Extraction Efficiency of Active Diluents and Inert Diluents

The extraction of lactic acid was carried out similarly as described above by increasing the temperature of water circulation from 0 °C to 60 °C. The effect of temperature on active diluent or inert diluent was also studied by keeping constant trioctylamine as extractant and varying the active diluent (1-octanol) or inert diluent (n-hexane).

Effect of Concentration of Active Diluents and Inert Diluents on the Extraction Efficiency

The effect of concentration of active diluents and inert diluents was measured by increasing the volumetric concentration of active diluent (1-octanol) and inert diluent (n-hexane).

Effect of Speed of Agitation on the Extraction Efficiency

Agitation was carried out using an overhead stirrer with 4-bladed propeller stirrer having a shaft length of 40 cm and stirrer diameter of 5 cm. The extraction of lactic acid was carried out as described above changing the speed of agitation from 4000 rpm to 6000 rpm keeping the time of agitation constant.

Effect of Agitation Time on the Extraction Efficiency

The extraction of lactic acid was carried out as described above changing the agitation time and keeping the speed of agitation constant.

RESULTS AND DISCUSSION

Liquid-Liquid Extraction

Table 1 shows that the extraction efficiency is high for active diluents like 1-octanol and 2-ethyl-hexanol, whereas it is low for the inert diluents like xylene, n-hexane and toluene.

The amount of lactic acid extracted and back extracted for the various active and inert diluents with trioctylamine is shown in Table 2. From the table we can see that the amounts of lactic extracted and amounts of lactic acid back extracted are closer.

Table 1: Extraction efficiency.

Extractant	Diluent	Extraction efficiency (%)
Trioctylamine	1-Octanol	96.86
Trioctylamine	2-Ethyl-hexanol	96.50
Trioctylamine	Xylene	83.69
Trioctylamine	n-Hexane	70.88
Trioctylamine	Toluene	89.39

Table 2: Stripping efficiency.

Extractant	Diluent	Extracted amount of lactic acid ($g.L^{-1}$)	Back extracted amount of lactic acid ($g.L^{-1}$)	Stripping efficiency (%)
Trioctylamine	1-Octanol	36.18	34.45	95.21
Trioctylamine	2-Ethyl-hexanol	35.67	35.37	99.15
Trioctylamine	Xylene	31.26	30.37	97.16
Trioctylamine	n-Hexane	25.97	25.71	99.02
Trioctylamine	Toluene	33.39	31.95	95.69

The stripping efficiency depends on the closeness of the extracted and back extracted amount of lactic acid. The stripping efficiency should be as high as possible, which means that the maximum amount of lactic extracted by extractant-diluent is back extracted.

Figure 1 indicates that the distribution coefficient is high for active diluents like 1-octanol and 2-ethyl-hexanol, whereas it is low for inert diluents like xylene, n-hexane and toluene. If the distribution coefficient is high for the extractant-diluent combination, this indicates that more lactic acid is extracted by the extractant-diluent complex.

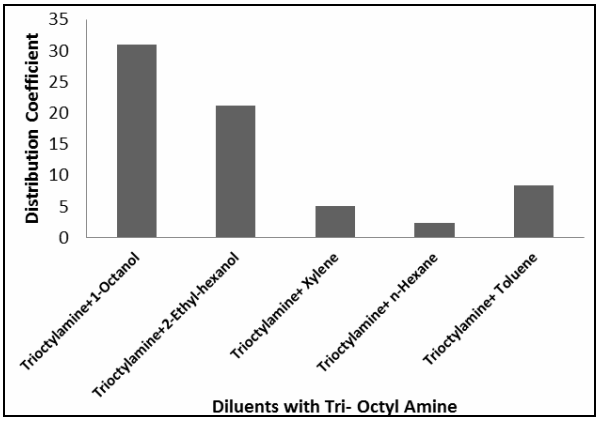

Figure 1: Variations of distribution coefficient with diluents.

Figure 2 indicates that the extraction efficiency decreases with increasing temperature for active diluents, whereas for inert diluents like n-hexane with trioctylamine it did not make any major difference in the extraction efficiency. This happens because of the exothermic reaction between amine and acid.

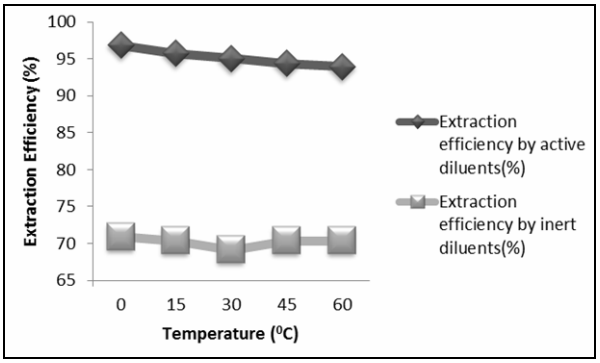

Figure 2: Effect of temperature on the extraction efficiency of active diluents (1-octanol) and inert diluents (n-hexane).

The effect of concentration of active diluents or inert diluent on the extraction efficiency is shown in Figure 3. 1-Octanol and n-hexane were used in this study as an active diluent and inert diluents, respectively. Their volumetric concentrations were increased. Figure 3 shows that the extraction efficiency is sharply increased with an increase in concentration of active diluent. Variation of inert diluent had little influence on the extraction efficiencies.

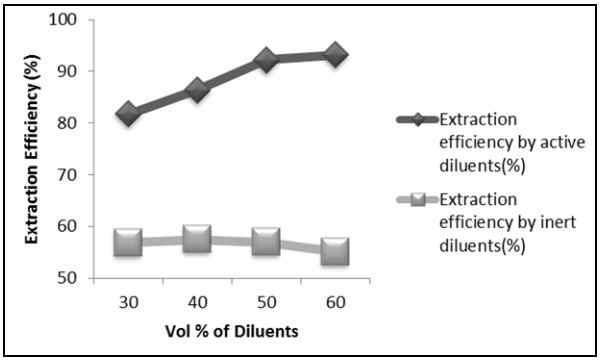

Figure 3: Effect of concentration of active diluent (1-octanol) and inert diluent (n-hexane) on the extraction efficiency.

The effect of extractant:diluent ratio on the extraction efficiency is shown in Figure 4. Trioctylamine was selected as extractant, whereas n-hexane was used as diluent to see the effect of volumetric concentration of extractant and diluent on the extraction efficiency. From the figure we can see that the maximum extraction was achieved when the volumetric concentration of extractant and diluent were equal. So for the present work the volumetric concentrations of diluent and extractant were kept constant, i.e., as 50 vol. % of TOA+50 vol. % of diluents.

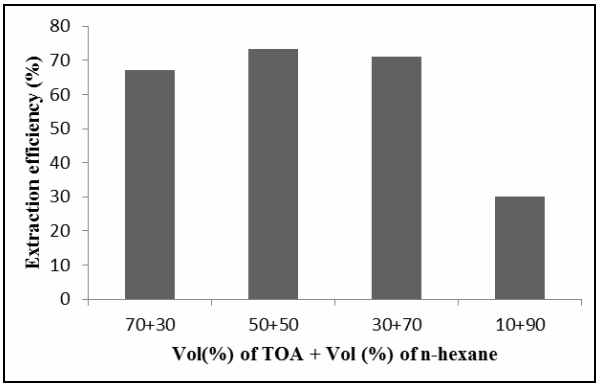

Figure 4: Effect of Vol. (%) TOA + Vol. (%) n-hexane on the extraction efficiency.

From Figures 5 and 6 we can see that the extraction efficiency is little affected by speed of agitation and agitation time.

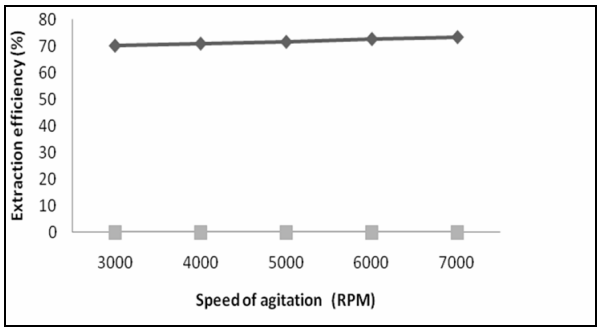

Figure 5: Effect of speed of agitation on extraction efficiency.

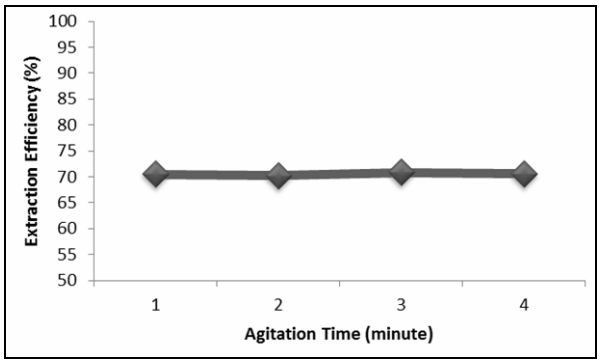

Figure 6: Effect of agitation time on extraction efficiency.

CONCLUSION

The study of reactive extraction of lactic acid with TOA dissolved in diluents indicated that the reactive extraction occurred by means of the interfacial formation of solvates between lactic acid and TOA. Different parameters like distribution coefficient, stripping efficiency, effect of extractant:diluent ratio, temperature, agitation time and agitation speed on the extraction efficiency were studied. The distribution coefficient was higher for active diluents, whereas it was low for inert diluents. So extraction efficiency was greater for the active diluents than for inert diluents. The extraction efficiency decreased with increasing temperature because of the exothermic reaction between amine and acid, whereas it did not make any major difference on the extraction efficiency in the case of inert diluents. The extraction efficiency was sharply increased with an increase in concentration of active diluent. Therefore, the solubility of the reaction complex in the active diluents, as well as the extractability of amine, is an important factor in the extraction of lactic acid. Variation of inert diluent had little influence on the extraction efficiencies. The extraction efficiency was little affected by speed of agitation and agitation time.

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