

Removal of contaminated organic acids from simulated succinic acid fermentation broth by reactive extraction process: Single- and mixed-solute solution

Chakkrit Umpuch, Songsak Sakeaw, Sunthorn Kanchanatawee & Kaemwich Jantama

To cite this article: Chakkrit Umpuch, Songsak Sakeaw, Sunthorn Kanchanatawee & Kaemwich Jantama (2016) Removal of contaminated organic acids from simulated succinic acid fermentation broth by reactive extraction process: Single- and mixed-solute solution, Separation Science and Technology, 51:4, 629-640, DOI: [10.1080/01496395.2015.1105823](https://doi.org/10.1080/01496395.2015.1105823)

To link to this article: <http://dx.doi.org/10.1080/01496395.2015.1105823>



Accepted author version posted online: 03 Nov 2015.
Published online: 03 Nov 2015.



Submit your article to this journal [↗](#)



Article views: 105



View related articles [↗](#)



View Crossmark data [↗](#)

จักรกฤษณ์ อัมพุก

Removal of contaminated organic acids from simulated succinic acid fermentation broth by reactive extraction process: Single- and mixed-solute solution

Chakkrit Umpuch^a, Songsak Sakeaw^a, Sunthorn Kanchanatawee^b, and Kaemwich Jantama^b

^aDepartment of Chemical Engineering, Ubon Ratchathani University, Warinchamrap, Ubon Ratchathani, Thailand; ^bMetabolic Engineering Research Unit, School of Biotechnology, Suranaree University of Technology, Mueng, Nakorn Ratchasima, Thailand

ABSTRACT

This work aims to study how to remove the organic acid by-products from simulated fermentation broth containing succinic acid by reactive extraction. Model solutions including single-, binary-, ternary-, and quaternary-solute solutions were used. The broths were reactively extracted using 0.25 mol TOA/kg 1-octanol under pH of 2.45–6.0. The extracted broths were then distilled under vacuum -0.017 MPa with operating temperature between 45 and 65°C. Finally, the distilled broths were crystallized at 4°C and pH of 2.0. The results showed that the purity and yield of succinic acid of 99.10% and 30.25%, respectively, were obtained.

ARTICLE HISTORY

Received 27 April 2015
Accepted 6 October 2015

KEYWORDS

Succinic acid; contaminated organic acids; reactive extraction; single-solute solution; mixed-solute solution

Introduction

Succinic acid, an organic acid, is widely used in various industries producing detergents, cosmetics and food. It gained attention as a building block for the production of poly(ethyl succinate), a biodegradable plastic (1), and is traditionally manufactured by chemical synthesis from butane, a fractional distilled product of petrol (2). Concerns regarding the energy renewal about the production of succinic acid via microbial fermentation have recently been raised. In Thailand, abundant agricultural products such as starch and sugarcane are hydrolyzed to fermentable sugar and used as substrate for microbe-producing succinic acid (3). However, the fermentation broth also produces contaminated organic acids, for example, pyruvic acid, acetic acid, formic acid, malic acid, and lactic acid resulting in a low purity of succinic acid in the broth (4). High purity of succinic acid is an important factor to obtain good mechanical properties for further applications. Thus, the fermentation broth needs to be purified to obtain sufficiently pure succinic acid (5).

Normally downstream purification costs account for 60–70% of the production costs in the fermentation-based process (5). For the recovery process of succinic acid via fermentation route to be economical, several separation methods such as electrodialysis, precipitation, reactive distillation, and esterification have been investigated for succinic acid purification from the

broth (6). In addition, reactive extraction is also an effective primary separation step for the recovery of carboxylic acids including succinic acid from a dilute fermentation broth due to its simple operation, low capital cost, and low energy consumption. The method has been developed to intensify separation by solvent extraction and represents a connection between chemicals (reaction between carboxylic acid and extractant) and physical phenomena (diffusion and solubilization of the system components). The extractant in the organic phase reacts with organic acids by means of ion-pair association in the aqueous phase and then the reaction complex is solubilized into the organic phase. Tertiary amine is widely used as an extractant for recovery of organic acids from fermentation broth (7) and 1-octanol is an active diluent for a solvating medium for organic acid-amine complex (8).

Hong and Hong (6) reported combined effective processes including reactive extraction, vacuum distillation and crystallization for succinate purification. The reactive extraction selectively removed contaminated organic acids from the fermentation broth. A vacuum distillation was used to effectively remove volatile contaminated organic acids, and the extracted fermentation broth was then concentrated. Finally, the crystallization process was applied to obtain a higher purity and colorless crystal of succinic acid. Nevertheless, the work studied only the extraction

efficiency of succinic acid in the diluted fermentation broth. The result would not be applied with a real fermentation broth in which the succinate concentration is usually high.

The extraction efficiency of carboxylic acids by reactive solvent extraction depends on pH, concentrations of carboxylic acids and amine, polarity of the diluent, and the operating temperature (6-8). However, the effect of interactions of succinic acid and other contaminated organic acids in fermentation broth on reactive extraction efficiency have been rarely studied. Furthermore, only one study of reactive extraction was previously reported (6). Also, the effect of temperature under vacuum distillation on the separation efficiency of the extracted broth has not been investigated. Therefore, this work aims to study separation and purification of high concentration of simulated fermentation broth containing succinic acid and other contaminated organic acids by three consecutive technologies, reactive extraction, vacuum distillation, and crystallization. The results from this study revealed that distribution coefficient of succinic acid in single-, binary-, ternary-, and quaternary-solutions were affected by interactions of succinic acid with other organic acids. The temperatures under vacuum distillation also affected the separation efficiency of the

extracted broth. After the purification process developed in this study, the purity of succinic acid in the crystals was found at the highest level compared to those previously published works.

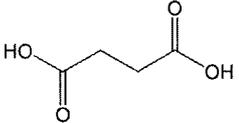
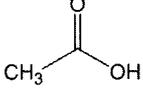
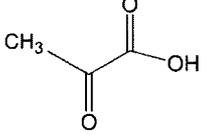
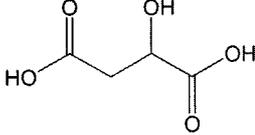
Materials and methods

Chemicals

Organic acids including succinic acid (Ajax Finechem, Australia, 99.5%), pyruvic acid (Merck, Germany, 95%), malic acid (Ajax, Australia, 99%), and acetic acid (RCL Labscan, Thailand, 99.7%) were used as stock solutions. The initial concentration of succinic acid was 0.7 M and those of acetic acid, pyruvic acid, and malic acid were 0.2 M. These concentrations were simulated according to the final composition of fermentation broth produced by *Escherichia coli* KJ122 (4). The solution pH was adjusted by adding either NaOH or HCl solution. The model solutions including single-, binary-, ternary-, and quaternary-solute solutions were studied.

Tri-n-octylamine (TOA, Merck, Germany: 93%) was used as an extractant, a straight-chain tertiary amine. In general, TOA is usually used in the form of a solution in organic diluent due to its high viscous and corrosive properties. 1-Octanol (Panreac, E.U., analytical grade), water insoluble alcohol and floats on water because it contains non-polar part of long chain alkyl group

Table 1. Properties of organic acids studied.

Name	Molecular weight (g.mol ⁻¹)	pKa ₁	pKa ₂	Boiling point (°C)	Molecular structure
Succinic acid (C ₄ H ₆ O ₄)	118.09	4.21	5.64	235	
Acetic acid (CH ₃ COOH)	60.05	4.76	-	118-119	
Pyruvic acid (C ₃ H ₄ O ₃)	88.06	2.50	-	165	
Malic acid (C ₄ H ₆ O ₅)	134.09	3.40	5.20	140	
1-Octanol (C ₈ H ₁₈ O)	130.23	-	-	195	C ₈ H ₁₇ -OH

ศกกรกฤษณ์ อัมพุก

which is dominant and another polar part of a hydroxyl group, was used as a diluent (9). The physical properties and structure of all organic acids and 1-octanol are shown in Table 1. The concentration of the extractant in a diluent of 0.25 mol TOA/kg 1-octanol providing the highest distribution coefficient for monocarboxylic acids and the lowest one for dicarboxylic acid was reported (6). In this work, the concentration of TOA in 1-octanol was fixed as 0.25 mol TOA/kg 1-octanol.

Pretreatment step of reactive extraction

The pH levels of the initial solutions varied between 2.0 and 6.0 using 0.1M NaOH (Ajax Finechem, Australia, 98%) and 0.1M HCl (J.T. Baker, China, 36.5-38.0%). 10 mL of model solutions and 10 mL of 0.25 mol TOA/kg 1-octanol were added into a 30 mL tube. The mixture was mixed by vortex at 4,000 rpm and 25°C for 15 min and then left for 24 hours to allow two complete phases of separation. The distribution coefficient (DE) was a major parameter to evaluate the extractability.

Polishing step of vacuum distillation and crystallization

The sample extracted from the aqueous phase was evaporated by a vacuum distillation for the removal of volatile carboxylic acids under the pressure of -0.017 MPa with different operating temperatures (45-65°C). Afterwards, the bottom product was brought into crystallization process for further purification at 4°C and pH 2.0. It was then dried after the recovery of precipitated crystallization, and the final purified succinic acid crystal were obtained.

Analytical method

The concentrations of organic acids were determined by High Performance Liquid Chromatography (HPLC) with a UV-VIS Detector (Shimadzu, SPD-10A VP, Japan), and VertiSepTMOA column (VERTICAL, Thailand). The mobile phase was 0.003N H₂SO₄ with a flow rate of 0.4 mL/min.

Calculations

The DE value can be calculated from Eq. (1). The parameter indicates the mass transfer between two phases. C_i^{org} is the concentration of *i* in the organic phase (M) and C_i^{aq} is the concentration of *i* in the aqueous phase (M).

$$DE = \frac{C_i^{org}}{C_i^{aq}} \quad (1)$$

To estimate the succinate/contaminated organic acid separation efficiency, one can also use the separation factor parameter, SF, expressed as:

$$SF = \frac{C_{AR}/C_{BR}}{C_{AE}/C_{BE}} = \frac{DE_i}{DE_{suc}} \quad (2)$$

where C_{AR} and C_{BR} are the succinic acid concentration and the contaminated organic acid concentration in raffinate respectively. C_{AE} and C_{BE} are the succinic acid concentration and the contaminated organic acid concentration in the extract. DE_i and DE_{suc} are the distribution coefficients of contaminated organic acids and succinic acid, respectively. SF values more than 1 means separation is possible. Values lower than 1, such as those obtained in this work, mean that the raffinate is a solution enriched in succinate, the target product, compared to the extract. On the contrary, values over 1 indicate an extract enriched with succinic acid.

The effect of the interaction between the succinate and contaminated organic acid in the mixture on the mass transfer behavior between the two immiscible phases was studied by considering deviation parameter, $\bar{\alpha}$, expressed as:

$$\bar{\alpha} = 1 - \frac{DE'}{DE} \quad (3)$$

where DE and DE' are the distribution coefficient of organic acid in the single-solute solution and DE of organic acid in the mixtures. $\bar{\alpha}$ values differ from 0 means the deviation of DE took place. Values of $\bar{\alpha}$ more than 0 such as those obtained in this work mean that more solute remained in raffinate. On the contrary, the values lower than 0 indicate more enriched solute in the extract phase.

The separation efficiency of the reactive extraction process composed of reactive extraction, vacuum distillation, and crystallization was studied in terms of percent yield and percent purity as follows:

$$Yield(\%) = \frac{W}{W_0} \times 100 \quad (4)$$

$$Purity(\%) = \frac{W}{W_t} \times 100 \quad (5)$$

where W and W_0 are the dry weight of succinic acid after each purification step (g) and the initial dry weight of succinic acid in the simulated fermentation broth (g), respectively. Also, W_t is the total dry weight of all organic acids at each purification step (g).

ศกกรณันต์ อ่วมบุญ

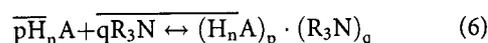
Results and discussion

Ions separation in single organic acid solutions

The extractability of TOA dissolved in 1-octanol on the reactive extraction of organic acids in single-solute solution was studied to understand mass transfer mechanisms between the two immiscible liquid phases. It was observed that the DE values of all organic acids decreased with increasing pH in the solution (Fig. 1a). For all carboxylic acids, the DE values decreased with increasing pH in solution pH. It has been well documented that TOA only reacts with undissociated organic acid (10-12). An increase of the values of pH means that the concentration of undissociated organic acid decreased which leads to reduction of the extraction. The decrease of DE in alkali medium is common and this is in accordance with much literature (13-15). It was concluded that the extractability can be influenced by the concentration of undissociated acid.

In addition, it was observed that the DE value of malic acid was highest, followed by pyruvic acid, acetic acid, and succinic acid (Fig. 1). This can be described by two hypothetical explanations. First, individual organic acid has a different ability to form an acid-

amine complex by mean of an ion-pair association. Malic acid has two carboxyl groups while pyruvic acid and acetic acid have only one carboxyl group so malic acid may have a higher ability to form an ion-pair association (16). The extraction equilibrium for carboxylic acids to form acid-amine complex can be written as a reaction (equation 6) of molecule of organic acids, H_nA ($n = 1$ for monocarboxylic acid and $n = 2$ for dicarboxylic acid) and molecule of TOA, R_3N , to form several types (p, q) complex (the notation (p, q) denotes p , the number of acid molecules, and q , the number of amine molecules, in the complex). The overall reaction is represented as follows:



where the organic phase species are marked with an overbar.

For dicarboxylic acids, the first carboxyl group forms ion-pair association with TOA so that the (1, 1) complex is the product. In addition, the second carboxyl group can be hydrogen bonded to other organic acid(s) providing (3, 1) and (2, 1) acid-amine complexes. The second carboxyl group has also possibility to hydrogen

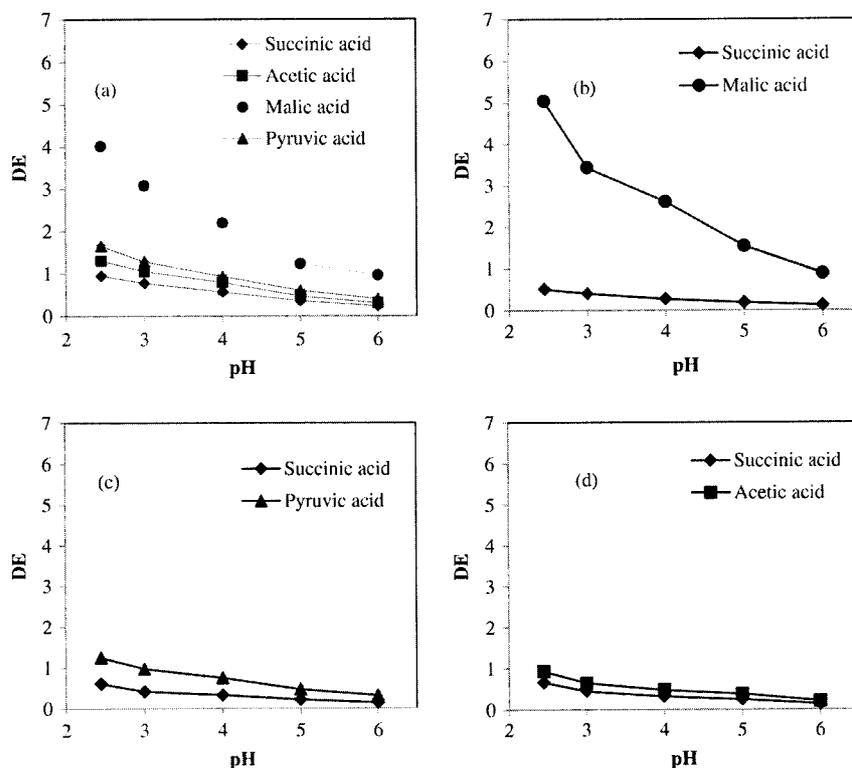


Figure 1. Effect of pH on DE of (a) organic acids in single-solute solutions and binary-solute solutions containing (b) succinic acid and malic acid, (c) succinic acid and pyruvic acid, (d) succinic acid and acetic acid: at 25°C: 0.25 mol TOA/kg 1-octanol, 0.7M of succinic acid and 0.2M of other organic acids.

Downloaded by [Imperial College London Library] at 23:14 18 May 2016

bond formation with TOA giving (1, 2) complex. For monocarboxylic acid, the carboxyl group can form ion-pair association with TOA to form (1, 1) complex. Furthermore, the carboxyl group of monocarboxylic acid is also delocalized to two partial double bond, one linking to another molecule of organic acid and another one linking to TOA by means of hydrogen bonds which provides (2, 1) complex (12). The formation of (3, 1) complex of malic acid while (2, 1) complex formation of those monocarboxylic acids could be also an explanation. The dicarboxylic acid has a tendency to aggregate when they are extracted by amines than monocarboxylic acids (17). The possible complexes between tertiary amine and malic acid are illustrated in Fig. 2 and those for pyruvic acid and acetic acid are shown in Figs. 3 and 4, respectively. Malmay et al. (2001) also found that the degree of ion-pair association of malic acid, determined by their

extraction model involving chemical association (solute and extractant reaction) and physical phenomena (diffusion and solubilization of the system components), was higher than that of a monocarboxylic acid (18). Second, an individual acid-amine complex has different solvation in organic diluent by means of specific hydrogen bonding between proton of 1-octanol and acid-amine complex. Malic acid-amine complex could be solvates which has the highest ability to form hydrogen bond with 1-octanol. Pyruvic acid-amine complex has two carbonyl groups while acetic acid-amine complex has only one, so that the intermolecular hydrogen bond with TOA of pyruvic acid-amine complex could have a higher ability than that of acetic acid. Song et al. also found that the DE of pyruvic acid was much higher than acetic acid at all pH ranges (19).

In the case of succinic acid, the formation of intermolecular hydrogen bonding of succinic acid and

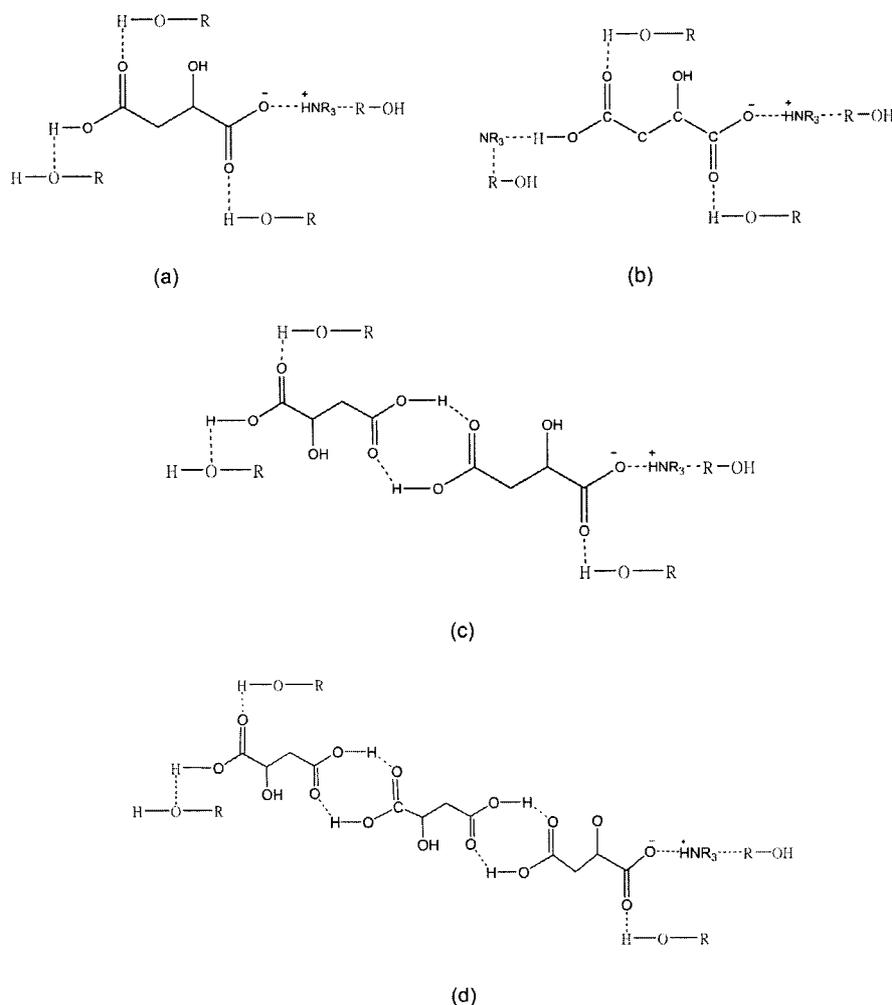


Figure 2. Malic acid-amine complexes in amine-based extraction: (a) (1, 1) complex (b) (1, 2) complex, (c) (2, 1) complex and (d) (3, 1) complex

ศักรกฤษณ์ ชัยบุญ

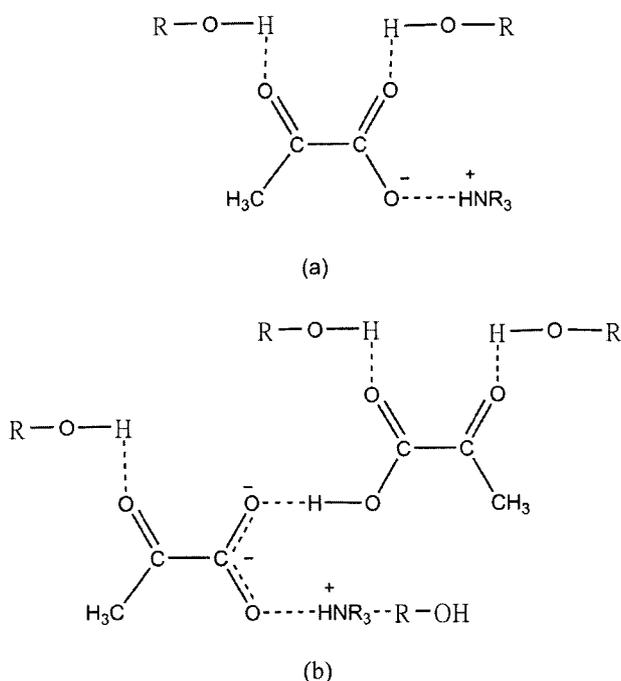


Figure 3. Pyruvic acid-amine complexes in amine-based extraction: (a) (1, 1) complex and (b) (2, 1) complex.

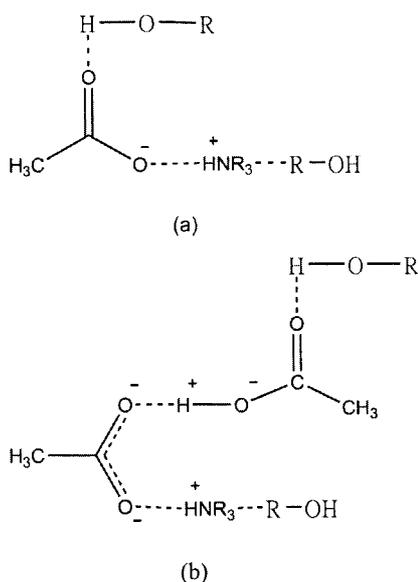


Figure 4. Acetic acid-amine complexes in amine-based extraction: (a) (1, 1) complex and (b) (2, 1) complex.

amine is not as easy as in the case of the malic acid, pyruvic acid, and acetic acid. This results in the lowest DE of succinic acid. The rotation of central C-C bond of succinic acid causing the intramolecular hydrogen bonding formation between two carboxyl groups is possible (Fig. 5). Hong and Hong described the relating

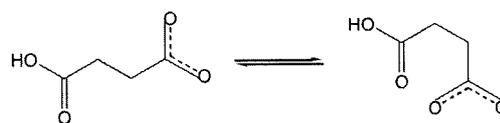


Figure 5. Two types of bisuccinate anions. (Two carboxyl groups which can or cannot form only intermolecular interactions.)

phenomenon that the structure of succinic acid was bulkier than that of acetic acid and the stable bisuccinate anion could be formed due to the intramolecular hydrogen bonding between two carboxyl groups in succinic acid (6, 20). The intramolecular hydrogen bonding formation causes less availability of carboxyl groups of succinic acid to form acid-amine complex. Furthermore, the higher DE of pyruvic acid and acetic acid compared to succinic acid comes from the fact that the dimer formation of monocarboxylic acid by means of intermolecular hydrogen bonding is easy (6). Therefore, the higher amount of acid-amine complex formation of pyruvic acid and acetic acid compared to succinic acid is a result of higher extractability of pyruvic acid and acetic acid. Therefore, the selective removal of specific acids from the simulated fermentation broth is made possible by using different degrees of dissociation of each acid with pH.

C. Umpuch et al.

In conclusion, the lowest DE of succinic acid was observed revealing that the succinic acid remained in the raffinate phase. On the contrary, other contaminant organic acid by-products were enriched in the extract phase. According to the different manner between succinic acid and other contaminants, the separation could be expected if those organic acids existed together in the mixed-solute solutions.

Ions separation in binary organic acid solutions

The reactive extraction in binary-solute solutions was studied to understand the interactions between succinic acid and another contaminated organic acid. An addition of contaminated organic acid by-product into the succinic acid solution causes a change of the DE values of all organic acids in the system compared to those in single-solute solution (Figs. 1b-1d). It was observed that the DE values of succinic acid, pyruvic acid, and acetic acid in the mixtures were lower than those in the single-solute solutions while the DE of malic acid was higher.

The existence of malic acid and succinic acid together in the binary-solute solution cause co-extraction between the two dicarboxylic acids and TOA. The acid-amine complex formation of the two dicarboxylic acids should decrease due to sharing of TOA and the two dicarboxylic acids. Consequently, lower DE of succinic acid in the mixture compared to that in single-solute solution was observed. On the other hand, the higher of DE of malic acid in binary-solute solution was unexpectedly found. This is likely attributed to the interference of succinic acid to malic acid-amine complex formation. In the mixture, a carboxyl group of succinic acid may form intermolecular hydrogen bonding with one of two carboxyl groups of malic acid which is a cause of only one carboxyl group of malic acid remaining for ion-pair association with TOA. This results in obstruction of (1, 2) malic acid-amine complex formation and a higher amount of TOA in the mixture. Thus, the DE of malic acid in the binary-solute solution was higher than that of single-solute solution resulting from higher portions of (1, 1) and (2, 1) complexes formation in the mixture. In addition, the existence of malic acid contributes intramolecular hydrogen bonding formation of succinic acid due to steric hindrance effect providing more bisuccinate anion which cannot form an ion-pair association with TOA.

The DE values of pyruvic acid and acetic acid in a binary-solute solution are little lower than those in a single-solute solution. The co-extraction between succinic acid and monocarboxylic acid causes decrease in

DE values of both acids. Thus, the carboxyl group of the monocarboxylic acids can form acid-amine complexes in lower ability compared to those in a single-solute solution. In addition, it is likely that a carboxyl group of succinic acid forms an intermolecular hydrogen bond with a proton of monocarboxylic acids; consequently, the less availability of carboxyl group of monocarboxylic acid forms hydrogen bonds with TOA (19). Jun et al. also found that the co-extraction between succinic acid and pyruvic acid to form an ion-pair association with TOA caused decrease in their DE values (5). As reported in equilibrium studies (15, 19, 20), monocarboxylic acid has higher DE and forms acid-amine complexes more easily than dicarboxylic acid because of the steric hindrance. Therefore, the presence of monocarboxylic acids, such as pyruvic and acetic acids, has an adverse effect on the extraction of succinic acid.

In summary, the existence together of two bicarboxylic acids in the mixture such as malic acid and succinic acid gives an advantage to the separation. The high amount of succinic acid remained in the raffinate while the high amount of malic acid enriched the extract. The separation between succinic acid and dicarboxylic acid is highly likely. On the other hand, the DE values of pyruvic acid, acetic acid, and succinic acid in binary-solute solutions are not much different from those observed in the single-solute solution. This indicates that the broth containing monocarboxylic acids and bicarboxylic acids does not improve the separation.

Ions separation in ternary organic acid solutions

The addition of the other two contaminated organic acid by-products into the succinic acid solution deriving ternary-solute solution could affect the DEs of all organic acids in the mixture as shown in Figs. 6a-6c for DE in ternary-solute solution. The DE values of succinic acid and acetic acid in the ternary-solute solution were lower than those in the binary-solute solution and single-solute solution while the DEs of malic acid were higher. In addition, the DE of pyruvic acid was higher than that in the binary-solute solution but it was lower than that in the single-solute solution.

In the mixture containing succinic acid, malic acid, and acetic acid, the lower DE values of succinic acid and acetic acid could be explained by the presence of three solutes in the solution causing higher ability of intermolecular hydrogen bonding between three acids and less available carboxyl groups of succinic acid and acetic acid due to steric hindrance. The higher DE of malic acid in the ternary-solute solution may be due to less degree of formation between malic acid and other

Downloaded by [Imperial College London Library] at 23:14 18 May 2016

Downloaded by [Imperial College London Library] at 23:14 18 May 2016

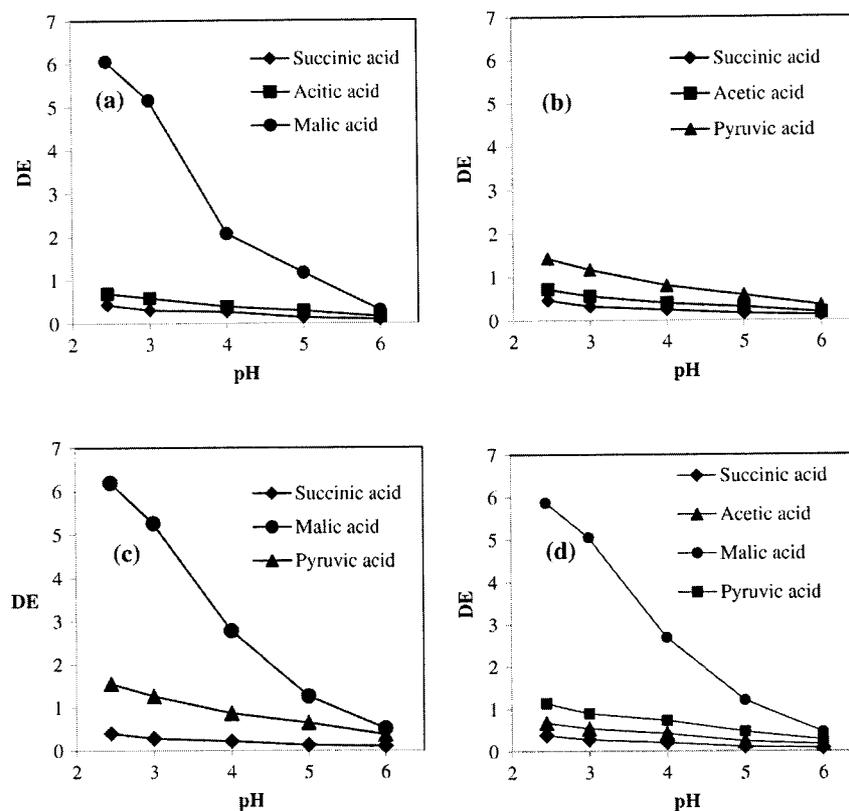


Figure 6. Effect of pH on DE of organic acids in ternary-solute solutions containing (a) succinic acid, acetic acid and malic acid, (b) succinic acid, acetic acid and pyruvic acid, (c) succinic acid, malic acid and pyruvic acid and (d) quaternary-solute solution: at 25°C: 0.25 mol TOA/kg 1-octanol, 0.7M of succinic acid and 0.2M of other organic acids

organic acids. Therefore, the increased aggregation of malic acid forming dimer or trimer acid-amine complexes in the mixture is a result (21-23).

In the mixture containing succinic acid, malic acid and pyruvic acid, the behaviors of the DE values of succinic acid and malic acid could be explained in the same manner in the system containing succinic acid, malic acid, and acetic acid. However, the DE of pyruvic acid was less affected when the malic acid was present in the system compared to that in the binary-solute solution. It was attributed to a reduced degree of formation of intermolecular hydrogen bonding between pyruvic acid and succinic acid. Increased availability of carboxyl groups of pyruvic acid causes an increase of pyruvic acid-amine complex and higher mass transfer portion in the diluent (24-26).

In the mixture containing succinic acid, acetic acid, and pyruvic acid, the behaviors of the DE values of succinic acid and malic acid can be explained in the same manner in the system containing succinic acid, malic acid, and acetic acid. The higher DE of pyruvic acid in this system can be explained by the increased degree of formation between succinic acid and acetic

acid causing a decrease in the degree of formation between succinic acid and pyruvic acid. Therefore, more available carboxyl groups of pyruvic acid occurred, thus allowing pyruvic acid to transfer to the organic phase in a higher portion than that in the binary-solute solution (27).

From the above results, the DEs of succinic acid and acetic acid in the ternary-solute solutions were lower than those in the binary-solute solution while the DE of malic acid and pyruvic acid was higher. In addition, the DE of pyruvic acid was less affected when there were other two organic acids present in the solution. The results showed that the separation efficiency between malic acid and pyruvic acid from succinic acid increased in the ternary-solute solution whereas the separation between acetic acid and succinic acid decreased.

Ion separation in quaternary organic acid solutions

The DE values of succinic acid and the other three contaminated organic acid by-products in the quaternary-solute solution were not different from those observed in the ternary-solute solution as shown in

Downloaded by [Imperial College London Library] at 23:14 18 May 2016

Fig. 6d for DE in quaternary solution. The DE values of all organic acids in the ternary- and quaternary-solute solutions were not different which may be due to the saturation of acid-amine complex in the extract phase. In addition, the presence of three organic acids in the system was enough to meet the saturation of acid-amine complexes in the diluent (28-33).

From the above results, the DEs of succinic acid, malic acid, pyruvic acid, and acetic acid in the quaternary-solute solutions were not greatly different from those observed in the ternary-solute solutions. The results showed that the separation efficiency between succinic acid and the other contaminated organic acid by-products by reactive reaction could be predicted from the results in the ternary-solute solution. In addition, the removal of contaminated organic acid by-products from the succinic acid fermentation broth by means of reactive extraction would be possible.

Selectivity and solute/solute interactions

The SF observed in the single-solute solution was likely independent of the solution pH (Fig. 7a for SF in Single-solute solution) while SF values observed in the

binary-, ternary-, and quaternary-solute solutions were significantly affected by the solutions' pH (Figs. 7b-7d for SF in ternary- and quaternary-solute solution, respectively). For all mixtures, the highest SF between malic acid and succinic acid was observed at pH 3.0 and the highest SF between pyruvic acid and succinic acid was observed at pH 5.0. From the results, the optimum solution pH could be at 5.0 because under this condition the malic acid could be greatly removed from the solution and the pyruvic acid could be removed from the solution in the highest portion. In addition, at pH 3.0 the percentage yield of succinic acid was very low (78.26%) compared to that observed at pH 5.0 (90.00%) as depicted in Table 2.

Table 3 shows the deviation parameter of organic acids in the mixed-solute solution at the solution pH of 5.0. The deviation of succinic acid in the quaternary-solute solution was highest, followed by the ternary-solute solution and binary-solute solution. This indicated that the increased amount of other contaminated organic acid by-products or system complexity caused the enrichment of succinic acid in the raffinate phase. The deviations of pyruvic acid and acetic acid that were higher than zero mean the pyruvic

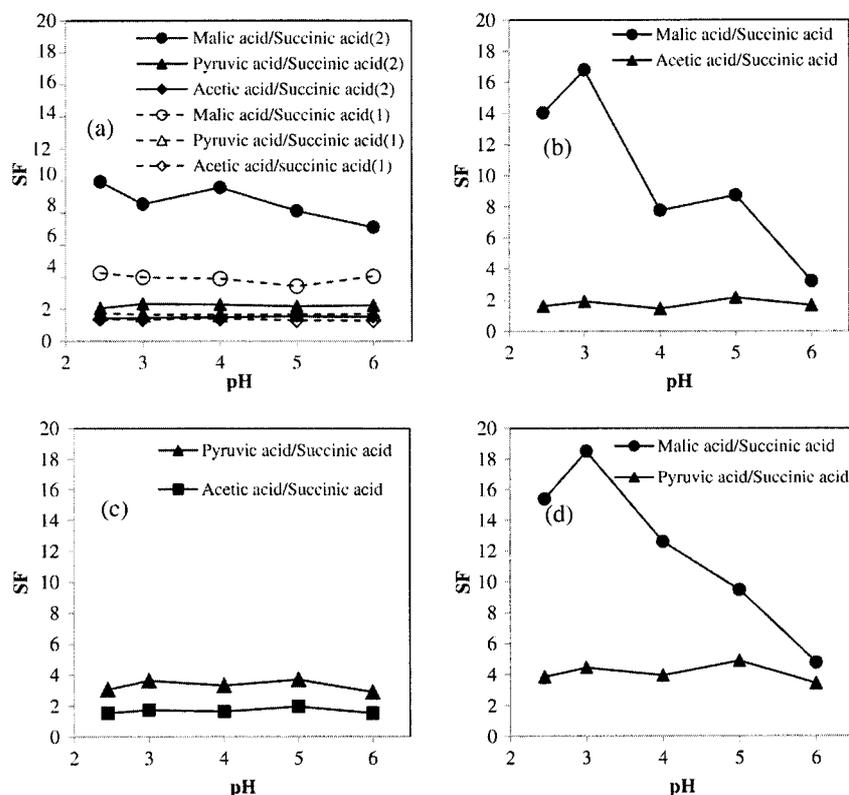


Figure 7. Separation factor of (a) three types of binary-solute solutions (2) and single-solute solutions (1), and three types of ternary-solute solutions containing (b) succinic acid, acetic acid and malic acid, (c) succinic acid, acetic acid and pyruvic acid, (d) succinic acid, malic acid and pyruvic acid: at 25°C: 0.25 mol TOA/kg 1-octanol, 0.7M of succinic acid and 0.2M of other organic acids.

Downloaded by [Imperial College London Library] at 23:14 18 May 2016

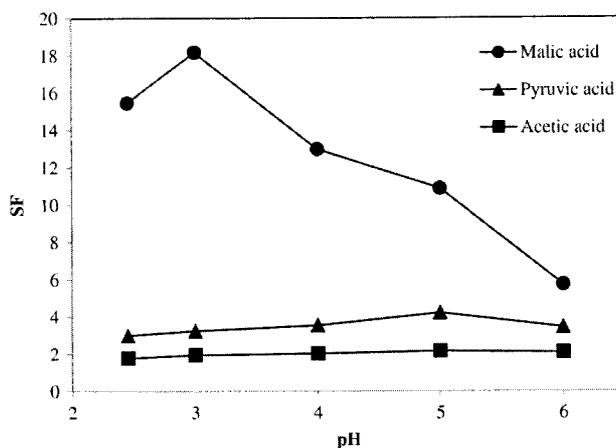


Figure 8. Separation factor of quaternary-solute solutions: at 25°C: 0.25 mol TOA/kg 1-octanol, 0.7M of succinic acid and 0.2M of other organic acids.

Table 2. The percent purity and percent yield of succinic acid with the complex separation process from the quaternary-solute solution.

pH	Distillated temp. (°C)	Distillate volume (mL)	After reactive extraction		After vacuum distillation		After crystallization	
			% Purity	% Yield	% Purity	% Yield	% Purity	% Yield
2.45	-	-	78.43	72.48	-	-	-	-
3.0	-	-	75.23	78.26	-	-	-	-
4.0	-	-	71.75	82.83	-	-	-	-
6.0	-	-	63.36	92.60	-	-	-	-
5.0	45	155	67.76	90.00	71.40	93.62	94.16	12.68
	50	145	67.76	90.00	71.58	89.34	95.96	16.93
	55	122	67.76	90.00	73.82	84.91	97.03	17.35
	60	100	67.76	90.00	74.81	79.01	98.30	25.40
	65	77	67.76	90.00	75.29	56.97	99.10	30.25

Table 3. The \bar{a} (deviation parameter) of organic acids in mixed-solute solutions at pH 5.0.

Solutions	Compositions	\bar{a}			
		Suc	Mal	Pyr	Ace
Binary-solute solution	Suc + Mal	0.48	-0.15	-	-
	Suc + Pyr	0.41	-	0.23	-
	Suc + Ace	0.38	-	-	0.32
Ternary-solute solution	Suc + Mal + Pyr	0.60	-0.22	0.02	-
	Suc + Mal + Ace	0.58	-0.08	-	0.46
	Suc + Pyr + Ace	0.54	-	0.11	0.43
Quaternary-solute solution	Suc + Mal + Pyr + Ace	0.64	-0.16	0.27	0.47

acid and acetic acid were enriched in raffinate phase. On the other hand, the deviation of malic acid was a negative value, which means malic acid remained in the extract phase. The effect of the presence of malic acid in the system could affect the DE of succinic acid in a greater portion than that in the presence of pyruvic acid and acetic acid. On the other hand, the presence of succinic acid showed a smaller effect on the DE of other contaminated organic

acids. From the above results, the increase of solution complexity showed the high removal possibility of malic acid from the mixture whereas the pyruvic acid and acetic acid had low removal potential by reactive extraction.

Polishing step of vacuum distillation and crystallization

Based on the former results, the study investigated the development of the complex separation process, reactive extraction, vacuum distillation, and crystallization, for the effective production of succinic acid from the simulated fermentation broth. Table 4 shows the recovery, yield, and purity of each component using the complex separation process from the broth. The pretreatment step of the reactive extraction selectively removed contaminated organic acids such as malic acid and pyruvic acid from the simulated fermentation broth.

After reactive extraction, the aqueous phase at pH 5.0 was then vacuum distilled at the temperatures ranging from 45 to 65°C for 2.0 h. Volatile impurities such as malic acid, pyruvic acid, and acetic acid and a diluent as 1-octanol were effectively removed. The bottom product after vacuum distillation was concentrated two to three-fold and then was brought to the crystallization process. Table 2 shows that the increase of distillation temperature under vacuum condition causes higher evaporation of all solutes and diluents. However, the succinic acid remains in the bottom product were higher than others. This results in higher purity of succinic acid in the bottom product. The highest percentage purity of succinic acid (75.29%) and the lowest percentage yield (56.97%) of distillate were observed in the distillate obtained under 65°C (Table 4).

For the production of further purified succinic acid, crystallization, as the final purification process, was

ศันสนีย์ คุ้มชู

Table 4. The recovery, yield and purity of all components using the complex separation process from the quaternary-solute solution.

Fermentation products	Initial solution		After reactive extraction (pH 5.0)		After vacuum distillation (65°C)		After crystallization	
	g ^a	wt.% ^b	g ^a	wt.% ^b	g ^a	wt.% ^b	g ^a	wt.% ^b
Succinic acid	11.67	65.64	11.62	68.04	6.62	75.29	3.53	99.10
Malic acid	2.46	13.83	1.09	6.38	0.53	5.97	-	-
Pyruvic acid	1.51	8.47	1.44	8.40	0.63	7.18	-	-
Acetic acid	2.14	12.06	2.93	17.17	1.02	11.56	0.03	0.84
Total	17.78	100	17.08	100	8.79	100	3.56	100

a Dry weight of each organic acid in basis of 100 ml fermentation broth (g).

b Weight percentage for dry weight of each organic acid to total dry weight of sample (%).

Table 5. Fermentation broth compositions, separation method, purity percentage and yield percentage.

Fermentation products	Separation methods	%	%	References
		Purity	Yield	
Suc 22.3 g/L, Pyr 7.0 g/L and Ace 1.8 g/L	Reactive extraction (0.25 mol TOA/ kg 1-octanol), vacuum distillation and crystallization (pH 2.0 and 4.0°C)	99.8	73.1	(9)
Suc 50 g/L and Ace 18.8 g/L	Reactive extraction (0.25 mol TOA/ kg 1-octanol)	83.3	100	(6)
Suc 13.64 g/L, Pyr 4.82 g/L and Ace 0.372 g/L	Reactive extraction (0.25 mol TOA/ kg 1-octanol), vacuum distillation and crystallization (pH 2.0 and 4.0°C)	99.5	67.05	(10)
Suc 11.67 g/L, Mal 2.46 g/L, Pyr 1.51 g/L and Ace 2.14 g/L	Reactive extraction (0.25 mol TOA/ kg 1-octanol), vacuum distillation and crystallization (pH 2.0 and 4.0°C)	99.1	56.19	This work
Suc 51.46 g/L, Ace 23.82 g/L, Lac 11.17 g/L, and residual glucose 14.57 g/L	Salting out (acetone/ammonium sulfate) and crystallization (pH 2.0 and 4.0°C)	95	65	(23)
Suc 97.8 g/L, For 23.5 g/L, Lac 5.1 g/L, and 17.4 g/L	Crystallization (pH 2.0 and 4.0 °C)	92	55	(18)

*Remark: Suc is succinic acid, Ace is acetic acid, Pyr is pyruvic acid, Mal is malic acid, Lac is lactic acid and Fac is formic acid.

carried out at pH 2.0 and 4°C. The solubility of succinic acid in the bottom product from the vacuum distillation step decreased due to acidification and a drop in temperature to obtain succinic acid crystal. The highest percentage purity of 99.10% and the highest percent yield of 30.25% were observed by using the initial solution as the bottom product obtained at 65°C. Crystallization completely removed malic acid and pyruvic acid from the system and a very small amount of acetic acid was left in the crystal (Table 4) (12). In addition, the purity and yield of succinic acid in the crystal increased purity of the bottom product (Table 2). This study produced high purity of succinic acid crystal compared with other literatures as listed in Table 5. Although a very low percentage yield was observed in this study, the feed compositions of other literatures in Table 5 did not contain a contaminated dicarboxylic acid such as malic acid. The interference of malic acid may cause a low yield of succinic acid compared to these literatures.

Conclusions

The complex separation process including reactive extraction, vacuum distillation, and crystallization successfully purified succinic acid from the contaminated organic acids in the simulated fermentation broth. A final purity of 99.10% and a final yield of 30.25% of succinic acid crystal were observed. The contaminated organic acids were effectively removed by reactive extraction, especially

malic acid. The separation process used in this study was a simpler and more effective purification method than previously published works in which the production costs were higher and purification steps were also required.

The intermolecular bonding between succinic acid and other contaminated organic acids and the selective removal of acetic acid needs to be further studied. The variation of pressure in vacuum distillation should also be further studied to obtain higher yield of the distilled broth. In addition, the crystallization process needs to be refined to increase the yield of the crystal.

Acknowledgements

The authors express their sincere gratitude to Bob Tremayne, from the Office of International Relations at Ubon Ratchathani University, for assistance with English.

Funding

The research was financially supported by the National Research Council of Thailand in 2011 with contact number of PK2553-165.

References

- [1] Kondratowicz, F. Ł.; Ukielski, R. (2009) Synthesis and hydrolytic degradation of poly(ethylene succinate) and poly(ethylene terephthalate) copolymers. *Polym. Degrad. Stabil.*, 94: 375–382.

ศันสนีย์ วัฒนกุล

- [2] Song, H.; Lee, S. Y. (2006) Production of succinic acid by bacterial fermentation. *Enzyme Microb. Tech.*, 39: 352–361.
- [3] Orjuela, A.; Yanez, A. J.; Peereboom, L.; Lira, C. T.; Miller, D. J. (2011) A novel process for recovery of fermentation-derived succinic acid. *Sep. Purif. Technol.*, 83: 31–37.
- [4] Jantama, K.; Zhang, X.; Moore, J. C.; Shamugam, K. T.; Svoronos, S. A.; Ingram, L. O. (2008) Eliminating side products and increasing succinate yields in engineered strains of *Escherichia Coli*. *Biotechnol. Bioeng.*, 101(5): 881–893.
- [5] Andres Castillo Martinez, F.; Marcos Balciunas, E.; Manuel Salgado, J.; Manuel Domínguez González, J.; Converti, A.; Pinheiro de Souza, O. (2013) Lactic acid properties, applications and production: A review. *Trends in Food Sci. Technol.*, 30: 70–83.
- [6] Hong, Y. K.; Hong, W. H. (2005) Removal of acetic acid from aqueous solutions containing succinic acid and acetic acid by tri-n-octylamine. *Sep. Purif. Technol.*, 42: 151–157.
- [7] Hong, Y. K.; Hong W. H. (2004) Influence of chain length of tertiary amines on extractability and chemical interactions in reactive extraction of succinic acid. *Korean J. Chem. Eng.*, 21(2): 488–493.
- [8] Tamada J. A.; Kertes, A. S.; King, C. J. (1990) Extraction of carboxylic acids with amine extractants. I. Equilibria and law of mass action modeling. *Ind. Eng. Chem. Res.*, 29: 1319–26.
- [9] Huh, Y. S.; Jun, Y. S.; Hong, Y. K.; Song, H.; Lee, S. Y.; Hong, W. H. (2006) Effective purification of succinic acid from fermentation broth produced by *Mannheimia succiniciproducers*. *Process Biochem.* 41: 1461–1465.
- [10] Song, H.; Huh, Y. S.; Lee, S. Y.; Hong, W. H.; Hong, Y. K. (2007) Recovery of succinic acid produced by fermentation of a metabolically engineered *Mannheimia succiniciproducers* strain. *J. Biotechnol.* 132(4): 445–452.
- [11] Hong, Y. K.; Hong, W. H.; Han, D. H. (2001) Application of Reactive Extraction to recovery of carboxylic acids. *Biotechnol. Bioprocess Eng.* 6: 386–394.
- [12] Wang, M.; Qin, W.; Dai, Y. (2005) Extraction mechanism and behavior of malic acid with tri-octylamine (TOA). *Separ. Sci. Technol.*, 39(1): 185–198.
- [13] Jun Y. S.; Lee, E. Z.; Huh, Y. S.; Hong, Y. K.; Hong, W. H.; Lee, S. Y. (2007) Kinetic study for the extraction of succinic acid with TOA in fermentation broth; effects of pH, salt and contaminated acid. *Biochem. Eng. J.*, 36: 8–13
- [14] Hong, Y. K.; Hong, W. H. (2000) Extraction of succinic acid with 1-octanol/n-heptane of mixed tertiary amine. *Bioprocess Eng.* 23(5): 535–538.
- [15] Hong Y. K.; Hong, W. H.; Chang, H. N. (2000) Selective extraction of succinic acid from binary mixture of succinic acid and acetic acid. *Biotechnol. Letters.* 22: 871–874.
- [16] Kirsh, T.; Maurer, G. (1997). Distribution of binary mixtures of citric, acetic, and oxalic acids between water and organic solutions of tri-n-octylamine. Part I. Organic solvent toluene. *Fluid Phase Equilib.*, 131: 213–231.
- [17] Tuyun, A. F.; Uslu, H. (2011) Investigation of picolinic acid extraction by trioctylamine. *Int. J.Chem. React. Eng.* 9: A29.
- [18] Malmay, G.; Albet, J.; Putranto, A. M. H.; Molinier, J. (2001) Recovery of carboxylic acids from aqueous solutions by liquid-liquid extraction with a triisooctylamine diluent system. *Braz. J. Chem. Eng.* 18(4): 441–447.
- [19] Kim, B. S.; Hong, Y. K.; Hong, W. H. (2004) Effect of salts on the extraction characteristics of succinic acid by predispersed solvent extraction. *Biotechnol. Bioprocess Eng.*, 9: 207–211.
- [20] Huh Y.S.; Hong Y. K.; Hong, W.H.; Chang, H.N. (2004) Selective extraction of acetic acid from the fermentation broth produced by *Mannheimia succiniciproucens*. *Biotechnol. Letters*, 26: 1581–1584.
- [21] Juang, R. S.; Hung, R. H. (1996) Comparison of extraction equilibria of succinic and tartaric acids from aqueous solutions with tri-n-octylamine. *Int. J. Chem. React. Eng.* 35: 1944–1950.
- [22] Keshav, A.; Wasewar, K. L.; Chand, S. (2010) Reactive extraction of propionic acid using tri-n-octylamine. *Chem. Eng. Commun.*, 197: 606–626.
- [23] Sun, Y.; Yan, L.; Fu, H.; Xiu, Z. (2014) Salting-out extraction and crystallization of succinic acid from fermentation broths. *Process biochem.* 49(3): 506–511.
- [24] Orjuela, A.; Yanez, A. J.; Peereboom, L.; Lira, C. T.; Mille, D. J. (2011) A novel process for recovery of fermentation-derived succinic acid. *Sep. Purif. Technol.*, 83: 31–37.
- [25] Rasrendra, C. B.; Girisuta, B.; van de Bovenkamp, H. H.; Winkelman, J. G. M.; Leijenhurst, E. J.; Venderbosch, R. H.; Windt, M.; Meier, D.; Heeres, H. J. (2011) Recovery of acetic acid from an aqueous pyrolysis oil phase by reactive extraction using tri-n-octylamine. *Chem. Eng. J.* 176-177: 244–252.
- [26] Kloetzer, L.; Galaction, A.-I.; Webb, C.; Vlysidis, A.; Cascaval, D. (2012) Selective extraction of carboxylic acids obtained by succinic acid fermentation. *N. Biotechnol.*, 29, supplement, 23-26 September 2012, S223–S224.
- [27] Matsumoto, M.; Otono, T.; Kondo, K. (2001) Synergistic extraction of organic acids with tri-n-octylamine and tri-n-butylphosphate. *Sep. Purif. Technol.*, 24: 337–342.
- [28] Caşcaval, D.; Kloetzer, L.; Galaction, A.-I.; Vlysidis, A.; Webb, C. (2013) Fractionation of Carboxylic acids mixture obtained by succinic acid fermentation using reactive extraction. *Separ. Sci. Technol.*, 48(4): 634–643.
- [29] Hasan, U. (2009) Reactive extraction of formic acid by using Trioctylamine (TOA). *Separ. Sci. Technol.*, 44(8): 1784–1798.
- [30] Han D. H.; Hong W. H. (1996) Reactive extraction of lactic acid with trioctylamine/methylene chloride/n-hexane. *Separ. Sci. Technol.*, 31(8): 1123–1135.
- [31] Datta, D.; Kumar, S. (2013) Reactive extraction of pyridine carboxylic acids with N,N-dioctyl-octan-1-amine: experimental and theoretical studies. *Separ. Sci. Tech.*, 48(6): 898–908.
- [32] Liu, Y.-S.; Dai, Y.-Y. (2003) Distribution behavior of α -amino acids and aminobenzoic acid by extraction with trioctylamine. *Separ. Sci. Technol.* 38(5): 1217–1228.
- [33] Li, Q.; Wang, D.; Wu, Y.; Li, W.; Zhang, Y.; Xing, J.; Su, Z. (2010) One step recovery of succinic acid from fermentation broths by crystallization. *Sep. Purif. Technol.* 72: 294–300.

Umpuch C. 2016