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Development of vegetable oil-based emulsion liquid membrane for downstream processing of bio-succinic acid

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ABSTRACT

Succinic acid has been recognized as a useful platform chemical that can be applied in various industries. The application of bio-based succinic acid is still limited due to high downstream processing cost. In this study, vegetable oil-based emulsion liquid membrane (ELM) process is proposed to recover succinic acid from fermentation broth. The ELM system consists of three main liquid phases; external feed, membrane, and internal stripping. The liquid membrane phase was prepared by dissolving Amberlite LA2 and Span 80 in palm oil, while, the internal phase comprises of sodium carbonate solution, Na_2CO_3. The influence of feed, stripping agent and carrier concentration, treat ratio, as well as liquid membrane recycling on ELM performance were studied. The results showed 10 g/L of initial concentration, 1.0 M of Na_2CO_3, 0.7 M of Amberlite LA2, and treat ratio of 1:5 is the best condition with almost 100% recovery and enrichment of 21 times. The recycled liquid membrane concentrates the succinic acid up to 12 times. Therefore, the proposed ELM is a potential technology to extract bio-succinic acid.

1. Introduction

Succinic acid or butanedioic acid is a four-carbon dicarboxylic acid that existed as an intermediate of the tricarboxylic acid cycle (major energy-producing metabolic pathways in cells). It holds virtuous industrial applications in the pharmaceuticals, foods, cosmetics, polymer, paints, and resins as a flavoring agent, pH modifier, acidulant, antimicrobial agent, ion chelator, surfactant, and foaming agent (Tsai et al., 2012; Lam et al., 2014). Most of the commercial succinic acid is traditionally produced through the petrochemical process, which is costly and causes environmental pollution. As the rise of crude oil price and concerns of sustainable development, producing succinic acid via fermentation has drawn great interests owing to its simplicity and environmentally friendly process (Andersson et al., 2009; Isar et al., 2006). Some of anaerobic and facultative anaerobic microbes that considered as effective succinic acid producers are *Actinobacillus succinogenes*, *Mannheimia succiniciproducens*, and *Escherichia coli* (Lee et al., 2010; Chen et al., 2011; Liu et al., 2013). It is well known that there exist contaminants such as acetic, formic, and lactic acids by-product, unconsumed carbon source, as well as protein, which reduce the yield of succinic acid and cause complicated purification process (McKinlay et al., 2007). Therefore, an effective and economical downstream process is necessary to extract and recover succinic acid from fermentation broths.

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The downstream process of biologically produced succinic acid consists of product recovery, concentration, acidification, and purification. Various methods were developed to purify succinic acid from fermentation broth, such as precipitation, crystallization, solvent extraction, electrodialysis, and membrane filtration. Precipitation involves a simple operation where calcium hydroxide, $Ca(OH)_2$, is added to precipitate succinate. The yield of succinic acid could be up to 93.3% after it is purified (Yedur et al., 2001). Unfortunately, a large dosage of $Ca(OH)_2$ is needed and contribute to high operating cost. In direct crystallization, desired product can be obtained without a complicated unit operation, but constrained by low product yield. It was reported that the highest succinic acid yield were 75% and 28% from simulated and real fermentation broth, respectively (Cheng et al., 2012). Solvent extraction offers a high product yield (more than 95%) and low energy consumption (Kurzrock and Weuster, 2011), yet is still lacking due to the large quantity of extractant is required. Electrodialysis is an easy process that used to separate succinate from non-ionized compounds with proper ion exchange membrane. A separation and concentration of succinic acid from post-fermentation broth by using electrodialysis gives the efficiency of 75% (Szczygiełda et al., 2017). Anyhow, this system is usually expensive and easily polluted. On the other hand, membrane filtration produces high product yield up to 92% (Zaman et al., 2017), but membrane pollution and high costs of the device are the disadvantages.

One of the promising methods to extract succinic acid from fermentation broth is an emulsion liquid membrane (ELM) technique. It is a very unique separation method that provides various advantages, including large mass transfer area, ease of operation, low energy consumption, efficient for low solute concentration, and low energy requirement (Venkatesan and Meera, 2008; Othman et al., 2017; Jusoh et al., 2019). In principal, ELM involves the dispersion of primary emulsion containing internal and membrane phase into the external feed phase containing desired solute. The solute from the external phase diffuses through the membrane phase and chemically react with the stripping agent and remains confined in the internal phase. In most cases, a carrier is added to the membrane phase to facilitate solute transport. The success of ELM applications was widely reported in the extraction of metal ions, dyes contaminant, organic and inorganic compounds, and pharmaceutical compounds (Noah et al., 2016; Khalid et al., 2017; Othman et al., 2018).

Several attempts have been made to develop ELM purification of succinic acid (Lee, 2011, Lee and Hyun, 2010). Unfortunately, most of the ELM studies only reported on the extraction of succinic acid from aqueous simulated solution and there has been limited study on the application of ELM in fermentation broth and recovery aspect of succinic acid. Besides, the studies commonly used petroleum-based diluent in the liquid membrane formulation which is not considered environmentally friendly. Therefore, vegetable based palm cooking oil can be chosen as an alternative renewable organic diluent, as it is readily available and may contain natural surface-active agents, which could improve the stability of emulsion (Chow and Ho, 1996). In this study, the effect of several parameters on the extraction and recovery of succinic acid from fermentation broth was presented. The formulation of ELM was described by Jusoh and Othman (2016) that using Amberlite LA2 as a carrier, palm oil as a diluent, Span 80 and Tween 80 as surfactant, and Na_2CO_3 as a stripping agent.

2. Materials and methods

2.1. Materials

For fermentation purpose, *Escherichia coli* (*E. coli*, ATCC PTA-5132) was procured from the American Type Culture Collection. The strain was stored in 30% (v/v) glycerol at −80 °C. Materials such as Luria broth (LB) agar, Glycerol (99% assay), and magnesium sulphate heptahydrate ($MgSO_4{\cdot}7H_2O$) (≥99.5% assay) were used in culture media and purchased from Merck. Glucose, di-potassium hydrogen phosphate (K_2HPO_4) (99% assay), potassium dihydrogen phosphate (KH_2PO_4) (98% assay), and ammonium sulphate ($(NH_4)_2SO_4$) (99.5% assay) were procured from R&M Chemicals. Sodium hydroxide (NaOH) (98% assay) was obtained from J. T. Baker. In ELM experiment, palm oil as a diluent is commercial cooking oil (BURUH) which is obtained from Lam Soon Edible Oils. Amberlite LA-2, Trioctylamine (TOA) (≥95% assay) as a carrier and solid sodium carbonate (Na_2CO_3) (99% assay) used as an internal solution was procured from Merck. Sorbitan monooleate (Span 80) (≥60% oleic acid) and polyoxyethylenesorbitan monooleate (Tween 80) (≥58.0% oleic acid) as the surfactants, and Octanol (99.5% assay) as a modifier were obtained from Sigma Aldrich. Succinic acid (SA) (99.0% assay) was acquired from Sigma Aldrich. The reagents and solutions were used directly as received without further purification.

2.2. Succinic acid fermentation

The media for inoculum development and experiment was Luria Broth (LB) containing 1.4 g/L of K_2HPO_4, 0.6 g/L of KH_2PO_4, 3.3 g/L of $(NH_4)_2SO_4$, 0.4 g/L of $MgSO_4{\cdot}7H_2O$, and 15 g/L of yeast extract in distilled water. Every medium prepared was sterilized in autoclave (HIRAYAMA, Model HVE 50) at 121 °C for 20 min. 0.2 mL of glycerol stock culture was added into a 40 mL of LB medium in a 100 mL flask, in which the cells were aerobically incubated at 37 °C and 200 rpm for 24 h using an incubator shaker (SASTEC, Model ST 100C). Then, succinic acid production was done in batch fermentations. 40 mL of fermentation medium was prepared in 100 mL shake flask. 10 mL of glucose (30 g/L) as carbon source was prepared in universal bottles (clear glass bottles with aluminum screw cap). The prepared medium and carbon sources were sterilized at 121 °C for 20 min. After that, the carbon source with 5 mL of inoculum was added into the fermentation medium. The final solution was then incubated for 24 h at 200 rpm. During the whole process, the temperature was maintained at 37 °C and pH was controlled between 6.6 and 6.7 by addition of NaOH solution. The concentration of succinic, acetic, lactic and citric acids obtained in the fermentation broth is 1.4, 0.06, 0.04 and 0.01 g/L, respectively. For the subsequent extraction and recovery experiments from fermentation broth, the solution was concentrated to reach the desired concentration range. In addition, the final pH of the broth was slightly acidic at 5.6.

2.3. ELM extraction and recovery procedure

Emulsion was prepared by emulsifying 7.5 mL of organic liquid membrane phase (Amberlite LA2, octanol, and 5% w/v of Span 80 in palm oil) and 2.5 mL of stripping phase (Na_2CO_3 solution). Emulsification process was performed using a motor driven homogenizer (Heidolph Silent Crusher M) at 7000 rpm for 5 min (Jusoh, 2017). The prepared emulsion was dispersed

in actual succinic acid fermentation broth produced using *E. coli* AFP184 strain. After the dispersion, the solution was allowed to settle in separation funnel. The external phase at the bottom of the funnel was taken for concentration measurement. For recovery purpose, emulsion at the top of the separation funnel were demulsified at different condition (0.01–2 M of Na_2CO_3; 0.1–0.5 M of Amberlite LA2; treat ratio of 1:7–1:1) using heat induced demulsification method. The emulsion was placed in a water bath assisted with ultrasonic vibration (LIR Biotech 020S) for 10 min to initiate the demulsification process. After that, it was heated at 70 °C for 24 h for phase separation (Peng et al., 2012). The aqueous internal phase separated was collected for succinic acid concentration analysis. The experiments were repeated at least three times for every parameter investigation and the maximum standard deviation was 5%.

2.4. *Synergistic liquid membrane formulation for higher feed concentration prospect*

Extraction of succinic acid at higher concentration (100 g/L) was first investigated via synergistic system using liquid–liquid extraction (LLE) procedure. An organic phase containing mixture of Amberlite LA2 and TOA in palm oil were prepared at the concentration of 0.7 M. The obtained solution was mixed with aqueous solution of succinic acid at equal volume (20 mL) and agitated at 300 rpm for 1 h (Rahman et al., 2019). The solution was then carefully poured into a separation funnel for phase separation for about 30 min. After the settling process, aqueous phase was collected for succinic acid concentration analysis. Meanwhile, synergistic ELM system was performed using similar procedure to Section 2.3 with additional TOA as carrier in liquid membrane phase.

2.5. *Analytical method*

Concentrations of acids in the external and internal aqueous phases were measured by high-performance liquid chromatography (Agilent, USA), equipped with UV–VIS detector and the BIO-RAD AMINEX Ion Exclusion HPX-87H column (300 mm × 7.8 mm, Agilent). The column was operated at 25 °C. The mobile phase was 5 mM H_2SO_4, at a flow rate of 0.5 mL/min, with 20 μL sample injection volume. Meanwhile, pH of organic acids solutions was measured using pH meter (Mettler Toledo). The performance of extraction, recovery, and enrichment ratio were calculated using Eqs. (1)–(3):

$$\text{Extraction}(\%) = \frac{[SA]_i - [SA]_f}{[SA]_i} \times 100 \quad (1)$$

$$\text{Recovery}(\%) = \frac{[SA]_{int}}{4TR[SA]_i} \times 100 \quad (2)$$

$$\text{Enrichment ratio (ER)} = \frac{[SA]_{int}}{[SA]_i} \quad (3)$$

where $[SA]_i$ is the initial succinic acid concentration in the external phase, $[SA]_f$ is the final acid concentration in the external phase, $[SA]_{int}$ is the acid concentration in the internal phase, and TR is the treat ratio (volume ratio of external to emulsion phases).

The viscosity of the liquid membrane and primary emulsion was measured using a rotational viscometer (Cole-Parmer 98965-40, United States) equipped with a small sample adapter using TL5 spindle at room temperature, 25 ± 1 °C. 6.7 mL of liquid membrane and emulsion sample was used for each viscosity measurement. The viscosity for every sample was recorded when the torque reading was between 15% and 95%.

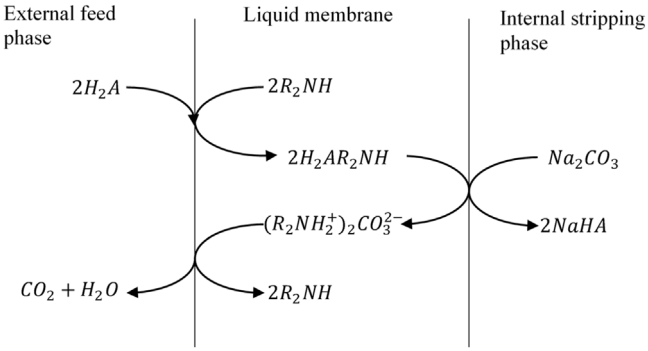

Fig. 1 – Facilitated transport mechanism of succinic acid through liquid membrane (H_2A: succinic acid; R_2NH: Amberlite LA2 as an amine carrier; H_2AR_2NH: succinic-carrier complex; Na_2CO_3: sodium carbonate).

3. Results and discussion

3.1. *Succinic acid extraction mechanism*

Carrier facilitated transport mechanism of succinic acid through liquid membrane is illustrated in Fig. 1. Succinic acid (H_2A) reacts with Amberlite LA2 (R_2NH) via acid–base reaction at the external-membrane interface to form complex of ammonium carboxylate salt (H_2AR_2NH). The complex then diffuses through the membrane into the membrane-internal interface and react with sodium carbonate (Na_2CO_3), so that succinic acid can be stripped in the internal phase.

Fourier transform infrared spectroscopy (FTIR) analysis was carried out to investigate the extraction process as shown in Fig. 2. The IR spectra of the organic phase after extracting succinic acid differ slightly from that before extraction in the fingerprint region between 550 and 400 cm^{-1} ranges, due to the formation of complexes after extraction. New band at 474 and 460 cm^{-1} exist after the extraction, which indicates bonding formation between C–O^-···$^+$N–C bond of succinic and Amberlite LA2, respectively. Besides the changes of peaks around 520–540 cm^{-1} before and after extraction is due to conjugation of solute and carrier molecule (Wade, 2013). The band at 526 and 536 cm^{-1} represents C–N and C–C bond of Amberlite LA2, which shifted to new wavenumber after extraction.

3.2. *Succinic acid extraction performance*

Fig. 3 displays the percentage of succinic acid extraction by varying the initial feed concentration from 5 to 50 g/L using previously formulated ELM (Jusoh and Othman, 2016). It is apparent that when the feed concentration was increased, there has been a significant decrease in succinic acid extraction. The peak of extraction is at 5 g/L with almost 100% of extraction was achieved, followed by 10 g/L with more than 81% of extraction. This result can be described by the fact that the concentration of solute is low compared to the larger amount of carrier provided to the system. Therefore, most of the succinic acid molecules in the external phase will react with the excess carrier to form complexes. Another possible explanation is the concentration of the stripping agent in the

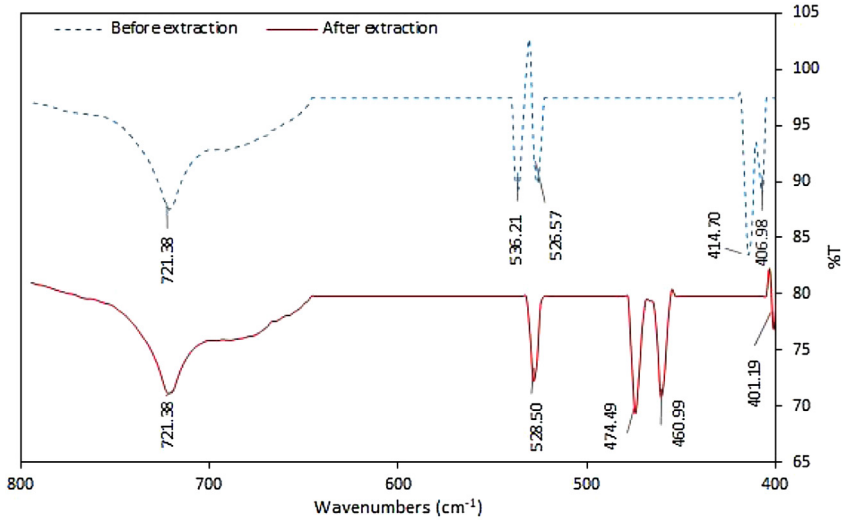

Fig. 2 – The FTIR spectra of the organic phase before and after extraction.

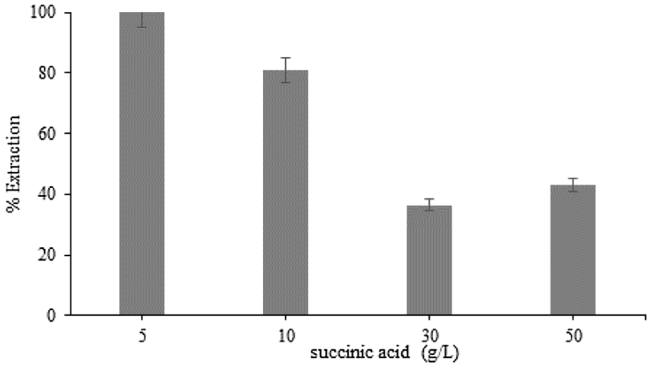

Fig. 3 – Effect of external feed phase concentration on the succinic acid extraction. (Experimental conditions: diluent: palm oil; [carrier]: 0.5 M of Amberlite LA2; surfactant: 5% w/v of Span 80 and 1% w/v of Tween 80; modifier: 10% v/v of octanol; [stripping agent]: 0.5 M of Na_2CO_3; treat ratio: 1:3).

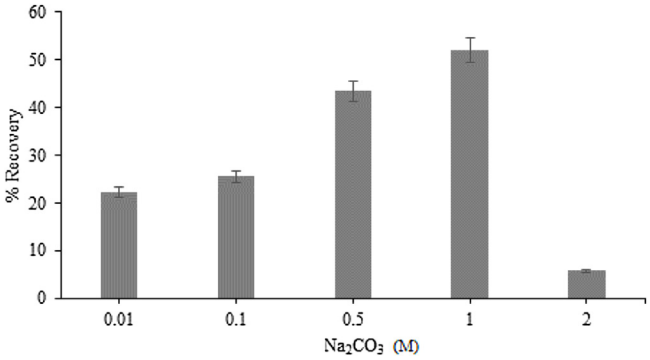

Fig. 4 – Effect of stripping agent concentration on the succinic acid recovery. (Experimental conditions: diluent: palm oil; [carrier]: 0.5 M of Amberlite LA2; surfactant: 5% w/v of Span 80 and 1% w/v of Tween 80; modifier: 10% v/v of octanol; [external feed]: 10 g/L of succinic acid; treat ratio: 1:3).

internal phase was sufficient to strip succinic acid from the complex. This leads to the release of free carrier that diffuse back and react with another solute at the external interface. Moreover, the sufficient stripping agent could also delay the accumulation of succinic acid complexes in the membrane phase that may obstruct the ELM process. These are consistent with those reported by other studies (Lee, 2011; Noah et al., 2016).

Further increase the initial concentration of succinic acid to 30 and 50 g/L, a significant decrease in extraction performance was observed to around 40% of extraction. The result is likely to be coherent with the required amount of carrier and stripping agent to transport higher amount of succinic acid. This result also could be related to the complex formation that control the external mass transfer of low solute concentration (Chakrabarty et al., 2010). Hence, the extraction rate at high concentration is lower than that at low initial succinic acid concentration. Another possible explanation for this is the internal droplets in the peripheral emulsion region are readily saturated with solute. Therefore, succinic acid complex must diffuse into a deeper region inside the emulsion globule to release solute in the internal phase. Once all the internal droplets are saturated, the succinic acid-carrier complex accumulate in the membrane phase and prevent carrier to react with another solute. These results are in agreement with those obtained Ammar et al. (2012). As the target is to perform ELM on high concentration as possible, 10 g/L of succinic acid initial concentration is chosen as the best condition to further the study.

3.3. *Recovery of succinic acid*

3.3.1. *Effect of stripping agent concentration*

A variation of Na_2CO_3 concentration in the range of 0.01–2 M was carried out to evaluate the performance of succinic acid recovery and the results obtained was depicted in Fig. 4. It can be seen that the recovery percentage is low at low stripping agent concentration from 0.01 to 0.1 M, where an average of 25% of succinic acid was recovered in the internal phase. It seems possible that this result is due to the insufficient stripping agent to strip succinic from the complexes, causing the saturation of complexes in the membrane phase. This study is in line with the findings of the previous work (Ng et al., 2010). There was a significant increase in recovery to 43% and 52% when the concentration is increased to 0.5 and 1.0 M respectively. This interesting result could be attributed to the larger reaction capacity with the stripping agent at the internal interface. As a consequence, freer carriers are generated and delay the accumulation of the complexes in the

Table 1 – Effect of Na_2CO_3 concentration on the internal phase leakage. (Experimental conditions: diluent: palm oil; carrier: 0.5 M of Amberlite LA2; surfactant: 5% w/v of Span 80 and 1% w/v of Tween 80; modifier: 10% v/v of octanol; external feed: 10 g/L of succinic acid; treat ratio: 1:3.).

Stripping agent concentration (M)	Breakage (%)
0.01	0
0.1	0
0.5	0
1.0	5
2.0	40

membrane phase. Besides, higher stripping agent concentration promoted continuous transport of succinic acid against concentration gradient between external and internal phases (Kislik, 2010). A similar result was obtained by Othman et al. (2016) and Yang et al. (2017) who found that a higher stripping agent concentration can strip more solute at the inner interface.

A further increase stripping agent concentration up to 2 M worsen the recovery performance obviously, where only 5% of succinic acid was recovered. An important issue that emerged from the data was higher difference of ionic strength between the internal and external phase was created. As a result, water transport from the external to internal phase by diffusion, and causing emulsion swelling. This phenomenon consequently leads to the thinning of membrane layer and leads to emulsion breakage and leakage of the recovered solute. Data in Table 1 proved that the increasing of stripping agent concentration causes significant increment to the internal phase leakage. This finding broadly supports the work of other studies in this area linking stripping agent concentration with recovery performance (Noah and Othman, 2017; Ooi et al., 2016). In addition, a high stripping agent concentration could also reduce the extraction performance significantly due to the hydrolysis reaction between Span 80 and stripping agent (Mortaheb et al., 2008). Thus, the effective number of surfactant molecules will be reduced. Several studies also reported the hydrolysis reaction between surfactant and stripping agent which cause partial loss of surfactant properties and leads to reduction in extraction efficiency (Chiha et al., 2010; Goyal et al., 2011). Hence, 1.0 M Na_2CO_3 was selected as the best stripping agent concentration in this process.

3.3.2. *Effect of carrier concentration*

In order to determine the effect of carrier concentration in the liquid membrane solution on succinic acid recovery, several experimental runs were performed by changing the carrier concentration in the range of 0.3–1.0 M. The results are presented in Fig. 5. It can be seen that the percentage of succinic acid recovery is slightly increase from around 49% to 52% when Amberlite LA2 concentration is increased from 0.3 to 0.5 M respectively. When 0.7 M of Amberlite LA2 was used, 100% of succinic acid recovery was achieved. This result might be explained by the fact that higher carrier concentration accelerates the transport of succinic acid into the internal phase, thereby promoting tremendous recovery process. These results corroborate the idea of Donat et al. (2017) who suggested the extraction and stripping of solute at different concentration obey consecutive first order reaction kinetics, which means the transport of solute proceeds at a rate that depends linearly on the carrier concentration. This finding

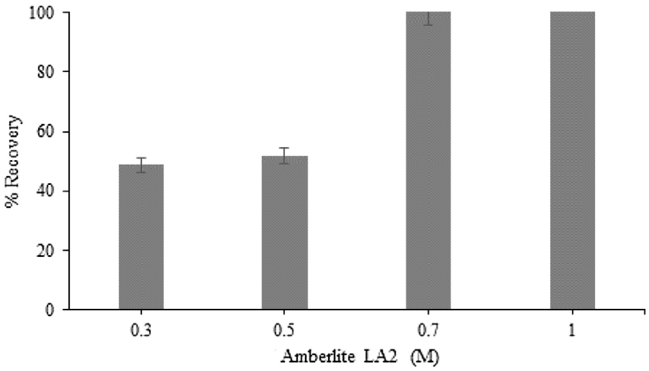

Fig. 5 – Effect of carrier concentration on the succinic acid recovery. (Experimental conditions: diluent: palm oil; surfactant: 5% w/v of Span 80 and 1% w/v of Tween 80; modifier: 10% v/v of octanol; [external feed]: 10 g/L of succinic acid; [stripping agent]: 1 M of Na_2CO_3; treat ratio: 1:3.)

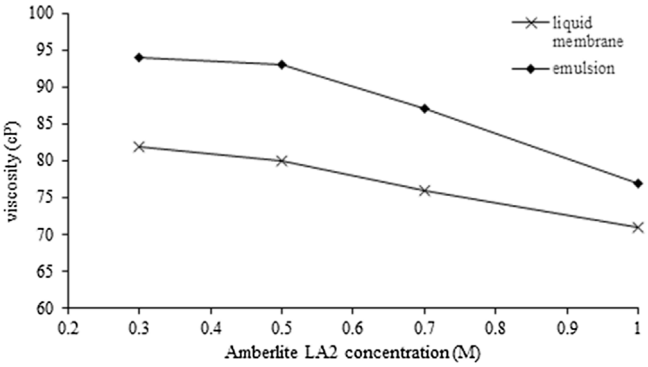

Fig. 6 – Liquid membrane and emulsion viscosity prepared at various carrier concentration.

broadly supports the work of other study in the removal of dye using ELM (Othman et al., 2011).

As more carrier is introduced in the process to 1.0 M, the recovery of succinic acid reaches plateau, indicating that all succinic acid in the external phase were transported into the internal phase. However, this trend has not previously been described. Kohli et al. (2018) reported that the membrane phase viscosity is raised with carrier concentration which lowers the diffusion of solute through the membrane and also causes bigger emulsion globules created which resulted in smaller mass transfer area between the emulsion globule and external feed phase. The rather contradictory result may be due to increasing carrier concentration in this study does not contribute to the increase in viscosity of the liquid membrane and emulsion, as the viscosity of Amberlite LA (18 cP) is much lower than palm oil (83 cP) (Jusoh, 2017). The viscosity is actually reduced with the addition of carrier as shown in Fig. 6. Lower viscosity at higher Amberlite LA2 concentration favor smaller globule formation during the dispersion process. Taken together, these results suggest that 0.7 M of Amberlite LA2 was considered to be sufficient for succinic acid recovery as it is more economically effective and was accepted as the best carrier concentration in this study.

3.3.3. *Effect of treat ratio*

Treat ratio is defined as the volume ratio of the emulsion to the external feed phase. The increment of treat ratio tends to improve the ELM performance due to increase in the capacity of the membrane and internal phase for enhanced extraction

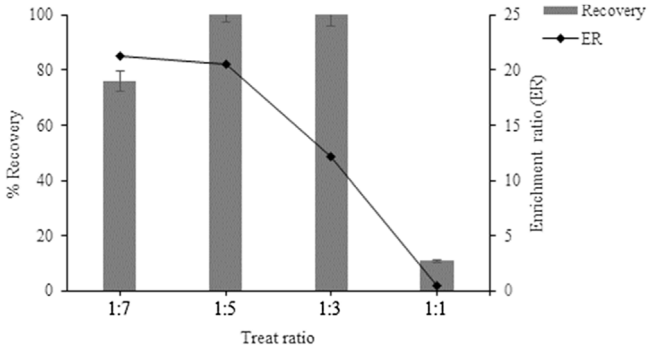

Fig. 7 – Effect of treat ratio on the succinic acid recovery. (Experimental conditions: diluent: palm oil; [carrier]: 0.5 M of Amberlite LA2; surfactant: 5% w/v of Span 80 and 1% w/v of Tween 80; modifier: 10% v/v of octanol; [external feed]: 10 g/L of succinic acid; [stripping agent]: 1 M of Na_2CO_3.)

and stripping of solute. The results obtained from varying the treat ratio from 1:7 to 1:1 can be seen in Fig. 7. During the experiment, the volume of emulsion was fixed while changing the external feed phase according to the desired ratio. Approximately 76% of succinic acid was recovered at low treat ratio (1:7). There was a clear trend of increasing recovery when the treat ratio is raised to 1:5 is used. After, the recovery is maintained at 100% using 1:3 of treat ratio. This result can be explained by the fact that increase the treat ratio will increase the capacity of the membrane and internal phase for extraction and recovery of succinic acid. The available active sites of the membrane phase per unit volume of external feed phase is basically increases with treat ratio. Consequently, it promotes the transfer of succinic acid from the external into the internal phase. In addition, an increase in treat ratio also resulted in better dispersibility of the emulsion globules (Chakraborty et al., 2010). These results agree with the finding of other studies, in which the increase of treat ratio tend to enhance the ELM performance (Goyal et al., 2011; Mehta and Mahajani, 2011).

A significant loss in succinic acid recovery was obtained with a further increase in the treat ratio. It can be seen that by far treat ratio of 1:1 resulted in the lowest value of succinic acid recovery, which is only 10%. This is owing to the difficulties to disperse the emulsion as high volume of emulsion per unit of external phase has caused the total viscosity of W/O/W emulsion to increase, hence resulting in formation of large globules. As a result, small interfacial area was created that hinder mass transfer on succinic acid from the external into the internal phase. This finding is consistent with the data obtained in previous study (Malik et al., 2011). Besides, low recovery performance also due to the leakage of the internal solution into the external phase. Larger globules leads to coalesce that induced by the effect of attractive forces between the globules larger than the repulsive force (Othman et al., 2012). For instance, large volume of emulsion it is not economically feasible as a larger volume of emulsion used can increases the overall cost of the technology (Ooi et al., 2015).

From a practical perspective, treatment higher amount of external phase is preferable for the maximum enrichment with respect to the external feed phase, provided that there are adequate stripping agent to confine all of the succinic acid in the internal phase. Besides, the least volume of the emulsion is always preferred to make it less expensive. As shown

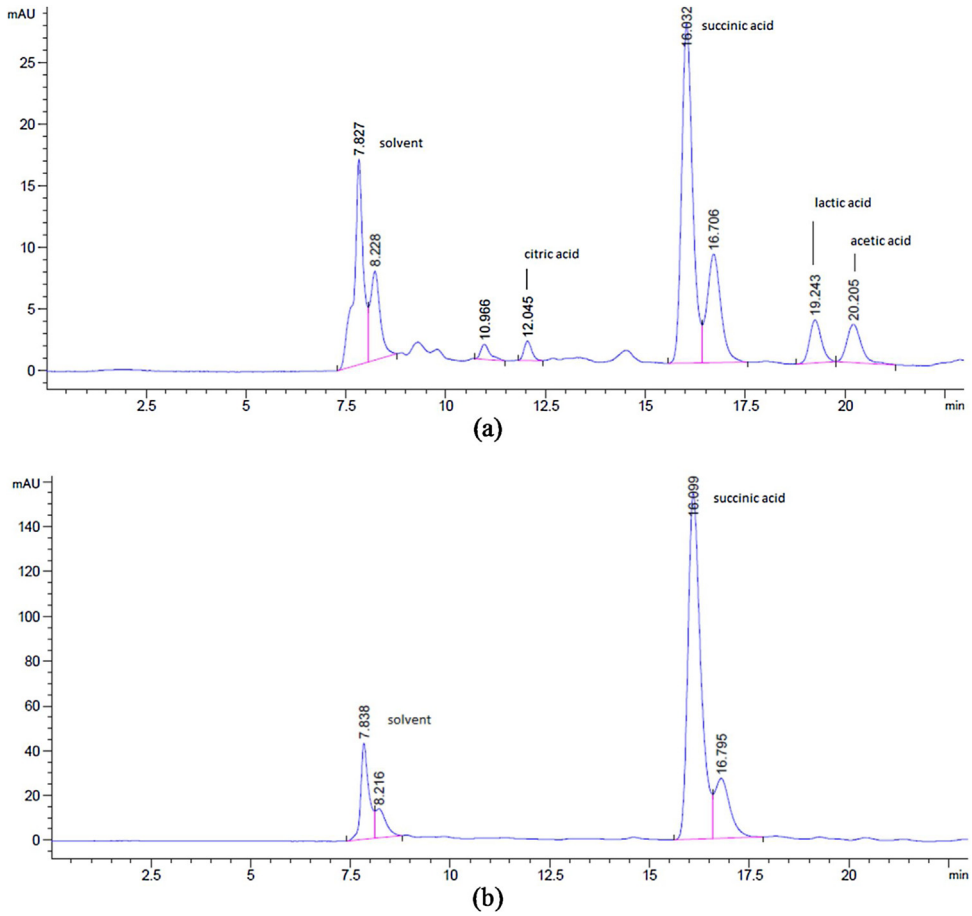

Fig. 8 – HPLC chromatogram of aqueous phase. (a) External phase before extraction, (b) internal phase after extraction.

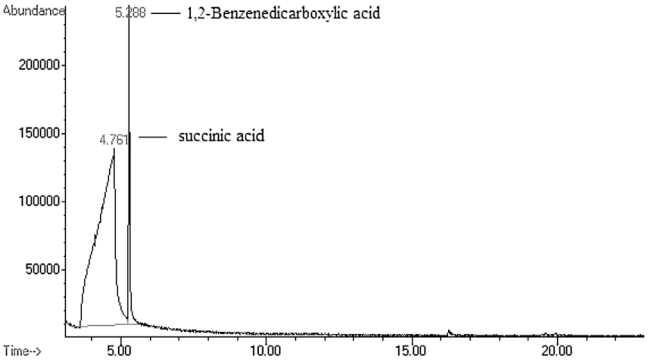

Fig. 9 – GCMS chromatogram of internal aqueous phase.

in Fig. 7, a maximum enrichment value of 21.3 was achieved when treat ratio of 1:7 is employed. Unfortunately, only 76% of succinic acid recovery was achieved at this condition. It is plausible that there is no sufficient free internal reagent to strip succinic acid from the complex. The remaining solute in the complex is somehow undesirable for liquid membrane recycle purpose. Thus, these results suggest that treat ratio of 1:5 is chosen as the best treat ratio condition as it produce comparatively high enrichment (20.5) with 100% recovery.

3.3.4. *Effect of other components in the fermentation broth*

Fig. 8 indicates the HPLC chromatogram of aqueous external phase (fermentation broth) before the extraction and internal phase (recovery phase) after the extraction process. It can be seen in that beside succinic acid, several by-products were existed in the external phase such as citric, lactic, and acetic acid. An analysis of the internal phase after extraction reveals that only succinic acid was detected in the solution. This finding suggests that the ELM formulation used selectively extract and recover succinic acid over other components. According to Hong et al. (2000), the degree of extraction of carboxylic acid is proportional to its chain length. In that case, the extractability of carboxylic acid in this study should be in the order of citric acid (6C) > succinic acid (4C) > lactic acid (3C) > acetic acid (3C). The stronger complexation of citric and succinic compared to lactic and acetic acid at the external interface and becomes an initial rate controlling step for extraction process. Contrary to the order of complexation, the decomplexation of succinic acid is easier than that of citric acid (Jusoh, 2017). Therefore, citric acid is difficult to be released into the internal phase, while succinic acid continues to be transported. As a result, almost 100% of succinic acid was recovered in the internal aqueous phase. In addition, an analysis of the internal phase through gas chromatography–mass spectrometry (GCMS) reconfirmed that succinic acid was recovered at high composition as indicate by large peak area in Fig. 9.

Furthermore, compared to extraction of succinic acid from simulated aqueous solution (Jusoh and Othman, 2016), there was no significant impact on the ELM performance in the presence of salts like K_2HPO_4, KH_2PO_4, $(NH_4)_2SO_4$, and $MgSO_4{\cdot}7H_2O$ in the fermentation broth. This result could be attributed the low concentration of ion in the fermentation broth which is less than 0.1 M. Increasing the ion concentrations might resulted in the decrease of extraction performance due to the competition of salt anions with the carboxylic groups of succinic acid (Jun et al., 2007). This is in line with previous study indicating that ionic concentration of more than 0.5 M dramatically reduce the extraction yield of succinic acid (Kurzrock and Weuster, 2011).

Table 2 – Effect of liquid membrane recycle on the recovery and enrichment of succinic acid. (Experimental conditions: diluent: palm oil; carrier: 0.7 M of Amberlite LA2; surfactant: 5% w/v of Span 80 and 1% w/v of Tween 80; modifier: 10% v/v of octanol; external feed: 10 g/L of succinic acid; stripping agent: 1 M of Na_2CO_3; treat ratio: 1:5.).

Run	1	2	3
% Recovery	100	62.4	43.2
Enrichment ratio	20	12.5	8.6

3.4. *Liquid membrane recycling*

Heat-induced demulsification was employed on the loaded emulsion so as to recycle the liquid membrane. The advantages of this method are reduction in the viscosity and density of the oil and cause an increased solubility of the surfactants, which leads to weakening of the interfacial film (Pabby et al., 2009). Table 2 presents the effect of liquid membrane recycle on the succinic acid recovery performance. The result shows that the recovery of succinic acid using fresh liquid membrane was 100% and declined to around 62% and 43% on the second and the third cycle respectively. A possible explanation for this is the emulsion made from the recycled liquid membrane is less stable compared to fresh liquid membrane. The instability is due to some of octanol as the phase modifier is vaporized during the demulsification process. Octanol (8.9 cP) was added to the membrane phase as the additive so as to reduce the viscosity of liquid membrane and improve the partitioning of succinic acid in the membrane phase (Jusoh, 2017). The application of heat during demulsification might concurrently resulted in octanol loss from the recycled liquid membrane. Because of this, the stability of the emulsion and extraction process were affected, resulting in lower succinic acid recovery.

On the third cycle, only 43% of succinic acid was recovered. It seems possible that liquid membrane used being contaminated with the loaded unstripped succinic acid complex. This is accords with Mokhtari and Pourabdollah (2013) which reported that the remaining complex in the membrane may affects the recovery of metal in ELM process. It can be hypothesized that the recycling of the liquid membrane is likely to be possible after its regeneration (make-up), such as the supplemental addition of ingredients to reproduce its previous physical and chemical composition. That is, the emulsion from the recycled liquid membrane should maintain the appropriate carrier, modifier, and surfactant concentrations, together with its viscosity. Meanwhile, it can be seen from the data that the enrichment of succinic acid is quite high (12.5), which is very desirable compared to LLE method that could not concentrate the solute. The result of the enrichment is as good as the one obtained by Othman et al. (2017) in the recovery of phenol using fresh liquid membrane.

3.5. *Prospect of succinic acid extraction*

Throughout this study, the formulated ELM is suitable to treat 10 g/L of succinic acid in the feed phase. However, the production of succinic acid was reported to reach as high as 100 g/L in the fermentation industry (Yang et al., 2007). With the interest to improve succinic acid separation from higher feed concentration, modification of liquid membrane using synergistic carrier was attempted. The synergistic extraction

Table 3 – Effect of synergistic extraction system toward succinic acid extraction performance. (Experimental conditions: diluent: palm oil; external feed: 100 g/L of succinic acid; carrier concentration: 0.7 M. LLE: treat ratio: 1:1; extraction time: 1 h. ELM: surfactant: 5% w/v of Span 80 and 1% w/v of Tween 80; modifier: 10% v/v of octanol; extraction time: 5 min; stripping agent: 1 M of Na_2CO_3; treat ratio: 1:5).

Process	Carrier	Extraction (%)
LLE	Amberlite LA2	53.1
LLE	Amberlite LA2 + TOA	86.3
ELM	Amberlite LA2 + TOA	91.7

system can occur through the interaction or cooperation of two or more carrier to produce higher effect of extraction. Rahman et al. (2019) have proved that a combined carrier provides greater effect than single carrier. Based from the carrier screening results from Jusoh (2017), TOA shows best extraction performance after Amberlite LA2. Therefore, synergistic system combining these carriers was performed and the results are presented in Table 3. In order to determine the compatibility of mixed-carrier system, LLE procedure was first carried out. It can be seen that combination of Amberlite LA2 and TOA provides better extraction performance (86.3%) compared to single Amberlite LA2 (53.1%). This is due to the use of single carrier cause small loading capacity of succinic acid in the organic phase. The ion pair association between acid radical and alkylammonium cation of both carriers are responsible for an increase of the extraction performance. The result is in agreement with other studies for the extraction of organic acid by amine extractants (Hong et al., 2001; Kumar and Thakur, 2018). It is well known that formulated liquid membrane was applied in ELM process (Othman et al., 2014). The result of this study proved that more than 91% of succinic acid was successfully extracted from aqueous phase in the ELM process.

4. Conclusion

This study has examined the feasibility of ELM process for the extraction and recovery of succinic acid from fermentation broth. The best conditions to recover succinic acid at 10 g/L of initial concentration were 1.0 M of Na_2CO_3, 0.7 M of Amberlite LA2, and treat ratio of 1:5, where almost 100% recovery and enrichment of 21 times were achieved. Besides, the ELM system with recycled liquid membrane capable to concentrate the succinic acid up to 12 times. Therefore, ELM provides a promising technology for the downstream process of bio-based succinic acid production.

Conflict of interest

The authors declare no conflict of interest.

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