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EXTRACTION OF SUCCINIC ACID FROM REAL FERMENTATION BROTH BY USING EMULSION LIQUID MEMBRANE PROCESS

(Pengekstrakan Asid Suksinik daripada Larutan Penapaian Sebenar dengan Menggunakan Proses Membran Cecair Emulsi)

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Abstract

Succinic acid is listed as one of the twelve building block chemicals based on the ease of production through a biotechnological approach and potential to derive various chemicals. The application of bio-based succinic acid is still limited due to high downstream processing costs. One of the potential methods to recover succinic acid is emulsion liquid membrane (ELM). The ELM system consists of three main liquid phases; external feed, membrane, and internal. In this study, the membrane phase was prepared by dissolving Amberlite LA2 as a carrier, sorbitan monooleate (Span 80) and polyoxyethylenesorbitan monooleate (Tween 80) as surfactants in commercial grade palm oil, while the internal phase comprised of sodium carbonate solution, Na_2CO_3. The influence of emulsifying time, agitation speed, and agitation time on the water-in-oil-in-water (W/O/W) emulsion stability were studied. The most stable condition was implemented on various external phase concentrations to study the extraction and recovery performances. The results showed that the most stable emulsion was obtained at 5 minutes of emulsifying time, 300 rpm of agitation speed and 3 minutes of agitation time. The emulsion produced was able to provide a balanced result between stability and ELM performance as it was able to extract almost 100% of succinic acid with 98% recovery. The finding of this study showed ELM process as the potential technology to extract succinic acid produced from fermentation.

Keywords: succinic acid, fermentation broth, emulsion liquid membrane, stability, extraction

Abstrak

Asid suksinik telah disenaraikan sebagai salah satu daripada dua belas bahan kimia asas berdasarkan kepada kemudahan pengeluaran melalui pendekatan bioteknologi dan berpotensi untuk menerbitkan pelbagai bahan kimia. Penggunaan asid bio-suksinik masih terhad disebabkan oleh kos pemprosesan hiliran yang tinggi. Salah satu daripada kaedah yang berpotensi untuk memperoleh asid suksinik ialah proses membran cecair emulsi (ELM). Sistem ELM terdiri daripada tiga fasa cecair utama; suapan luaran, membran, dan dalaman. Dalam kajian ini, fasa membran telah disediakan dengan melarutkan pembawa Amberlite LA2, surfaktan Span 80 dan Tween 80 di dalam minyak kelapa sawit komersial, manakala fasa dalaman terdiri daripada larutan natrium karbonat, Na_2CO_3. Kesan masa pengemulsian, kelajuan pengadukan, dan masa pengadukan ke atas kestabilan air-dalam-minyak-dalam air (W/O/W) telah dikaji. Keadaan emulsi yang paling stabil telah digunakan pada pelbagai kepekatan fasa luaran untuk mengkaji prestasi pengekstrakan dan perolehan. Keputusan menunjukkan keadaan emulsi yang paling stabil didapati pada 5 minit masa pengemulsian, pada 300 rpm kelajuan pengadukan, dan 3 minit masa pengadukan. Emulsi yang dihasilkan dapat memberikan hasil yang seimbang di antara kestabilan dan prestasi ELM kerana ia dapat mengekstrak hampir 100% asid suksinik dengan perolehan sebanyak 98%. Hasil daripada kajian ini menunjukkan bahawa proses ELM adalah teknologi yang berpotensi untuk mengekstrak asid suksinik yang dihasilkan daripada proses penapaian.

Kata kunci: asid suksinik, larutan penapaian, membran cecair emulsi, kestabilan, pengekstrakan

Introduction

Succinic acid is a C4 di-carboxylic acid with the IUPAC name of butanedioic acid. It is recognised as one of top twelve building block chemicals that can be produced through biological processes [1]. Succinic acid is a key compound in producing numerous important chemicals, such as the raw materials of pharmaceutical and food products, as the intermediate of chemical synthesis of biodegradable plastics, green solvents, detergents, and surfactants, and also as an ingredient to stimulate animal and plant growth [2, 3]. Most of the commercial succinic acid is currently produced through petrochemical process, which causes environmental pollution and the concerns of sustainable developments [4]. As to preserve the environment, studies are directed towards producing succinic acid by fermentation. Many anaerobic and facultative anaerobic microbes, for instance *Actinobacillus succinogenes*, *Mannheimia succiniciproducens*, and *Escherichia coli*, produce succinic acid as the fermentation end product [5, 6]. It is well known that there are contaminants such as acetic and formic acids by-product, unconsumed carbon source, and protein, which reduce the yield of succinic acid and cause difficult purification process. Therefore, an effective and economical separation process is required to recover succinic acid from fermentation broth.

Various separation methods were developed for the separation and purification of succinic acid, including crystallization, precipitation, solvent extraction, and membrane filtration [5, 7, 8]. In direct crystallization, the desired product can be obtained without a complicated unit operation, but it is constrained by low product yield. Precipitation comprises a simple operation where calcium hydroxide, $Ca(OH)_{2,}$ is added to precipitate succinate. However, a large dosage of $Ca(OH)_2$ is required, thus contributes to high operating cost. Solvent extraction offers high product yield and low energy consumption, yet is still lacking due to large quantity of extractant is required. While membrane filtration produces high product purity, the membrane pollution and high costs of the device are the disadvantages.

One of the promising methods to extract succinic acid from fermentation broth is emulsion liquid membrane (ELM). 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 [9, 10]. In principal, ELM involves the dispersion of the primary emulsion that contains the internal and membrane phases, into the external phase that holds the desired solute. The solute from the external phase diffuses through the membrane phase and chemically reacts with the stripping agent, then remains confined in the internal phase. In most cases, a carrier is added into the membrane phase to facilitate solute transport. The success of ELM applications is widely reported in the extraction of metal ions, dyes contaminant, organic and inorganic compounds, and pharmaceutical compounds [11-14].

However, the industrial application of ELM is limited due to emulsion stability problem which affects the extraction efficiency. Unstable emulsion occurs in the form of breakage or swelling. Emulsion breakage causes the release of internal stripping solution and extracted solute, which reduce the extraction performance [15]. During swelling, water molecules from the external phase are transferred into the internal phase and the extracted solute concentration in the internal phase is diluted. Emulsion swells are the results of emulsion volume increment and consequently trigger the emulsion globules and reduce the extraction efficiency [16]. The emulsion is considered stable when neither breakage nor swelling phenomenon occurs.

In this study, the effect of several parameters on the emulsion stability of succinic acid extraction system is presented. The formulation of ELM was described by Jusoh et al. [17] where, Amberlite LA2 was used as the carrier, palm oil as the diluent, Span 80 and Tween 80 as surfactants, and Na_2CO_3 as the stripping agent. In addition, research on ELM extraction of succinic acid mostly covers the extraction from simulated solution, but very limited from real fermentation broth [18]. Therefore, the performance of ELM in the present study was evaluated for succinic acid extraction and recovery from real fermentation broth.

Materials and Methods

Materials

Palm oil as the diluent is a commercial cooking oil (BURUH) from Lam Soon Edible Oils. Amberlite LA2 as the carrier was procured from MERCK. Solid sodium carbonate (Na_2CO_3) (99% assay) used as the internal solution was purchased from Merck. Sorbitan monooleate (Span 80) and polyoxyethylenesorbitan monooleate (Tween 80) were used as surfactants and obtained from Sigma Aldrich. Succinic acid (SA) (99.0% assay) was also acquired from Sigma Aldrich. The reagents and solutions were used directly as- received without further purification.

Emulsion stability study

A two-step emulsification procedure, consisting of primary emulsion preparation and dispersion process, was implemented to investigate the stability of emulsion. The internal and membrane phases (0.05 M Amberlite LA2 and 3% (w/v) Span 80 in palm oil) were emulsified by using a motor driven homogeniser (Heidolph Silent Crusher M, Germany) at 7000 rpm for 3 min to 9 min. The emulsion was then dispersed into succinic acid external solution containing 1% (w/v) Tween 80 and agitated at 200 rpm to 500 rpm for 1 minute to 7 minutes by using a digital mixer system (Cole-Parmer EW-50006-00, Germany). Upon completion, the solution was transferred into a separation funnel and allowed to settle. A small drop of emulsion was taken and placed under a microscope (Olympus CX31, Japan) to access the state of aggregation of the emulsion globules. The external solution volume was measured to determine the emulsion stability *via* Equation 1.

$$\text{Breakage/Swelling (\%)} = \frac{V_{e,f} - V_{e,i}}{V_{int}} x100 \quad (1)$$

where $V_{e,f}$ is the volume of external phase after extraction, $V_{e,i}$ is the volume of external phase before extraction, and V_{int} is the volume of internal phase.

Extraction and recovery performance

Emulsion, prepared at the most stable condition, was dispersed in real succinic acid fermentation broth produced by using *E. coli* AFP184 strain containing 1.4 g/L of succinic acid [19]. Prior to fermentation, the strain was stored in 30% (v/v) glycerol at -80 ^{o}C. The inoculum and production medium used were 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 $MgSO_4.7H_2O$, and 15 g/L of yeast extract in distilled water. The prepared medium was sterilised in an autoclave (HIRAYAMA, Model HVE 50) at 121 °C for 20 minutes. An amount 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 hours by using an incubator shaker (SASTEC, Model ST 100C). Then, the succinic acid production was done in batch fermentations. An amount 40 ml of fermentation medium was prepared in a 100 mL shake flask, while 10 mL of glucose as carbon source was prepared in a universal bottle. The prepared medium and carbon source were sterilised at 121 °C for 20 minutes. After that, the carbon source with 5 mL of inoculum was added into the fermentation medium. The final solution was then incubated at 200 rpm for 24 hours. During the whole process, the temperature was maintained at 37 °C and pH was controlled between 6.6 and 6.7 with the addition of NaOH.

In order to investigate the performance of ELM, the succinic acid concentration was adjusted (5-50 g/L) with the addition of pure succinic acid. After dispersion, the solution was allowed to settle in a separation funnel. The external phase at the bottom of the funnel was taken for concentration measurement. For recovery purpose, heat induced demulsification method was applied to break the emulsion at the top of the separation funnel. The emulsion was placed in a water bath and assisted with ultrasonic vibration (LIR Biotech 020S) for 10 minutes to initiate the demulsification process. After that, it was heated at 70 °C for 24 hours for phase separation [20]. The separated aqueous phase was collected for succinic acid concentration analysis. The performance of extraction, recovery, and enrichment ratio were calculated by using Equations 2 to 4:

$$\text{Extraction (\%)} = \frac{[SA]_i - [SA]_f}{[SA]_i} x100 \quad (2)$$

$$\text{Recovery (\%)} = \frac{[SA]_{int}}{4TR[SA]_i} x100 \quad (3)$$

$$\text{Enrichment ratio} = \frac{[SA]_{int}}{[SA]_i} \quad (4)$$

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.

Analytical procedures

A Mettler Toledo pH meter was used for pH measurement of the succinic acid fermentation broth. Meanwhile, succinic acid concentration was determined by using the high performance liquid chromatography (HPLC) (Agilent 1260 Infinity, Germany) with an ion exchange column (Aminex HPX-87H, 300 mm x 7.8 mm, brand X). A five-point calibration curve for succinic acid was prepared by using a known concentration of succinic acid standard. This step also helped to determine the retention time (time required to go off the column) of succinic acid, which was around 16 minutes in this study. The concentration of succinic acid in the sample was determined by comparing the peak areas with those of the standard curves. The measurement was performed *via* UV detection at 210 nm. Prior to analysis, each sample was filtred through 0.45 µm Whatman puradisc syringe filter into 2 mL of HPLC vials. 0.005 M of sulphuric acid (H_2SO_4) at a flow rate of 0.5 mL/ min was used as the mobile phase.

Emulsion droplets and globule size were measured by using an optical microscope (Olympus CX31) equipped with a camera that was connected to a computer. A small drop of emulsion was placed on a glass slide which was later being placed on the microscope stage. The focus of the microscope was adjusted accordingly so as to obtain a clear emulsion image. Each image was captured at the magnification of 400 times within 10 minutes after the sample preparation process. The size of the emulsion was determined by taking the average size of 40 droplets by using the VImage 2014 software and expressed as the Sauter mean diameter (D_{32}) as defined in Equation 5 [21].

$$D_{32} = \frac{\sum(n_i . d_i^3)}{\sum(n_i . d_i^2)} \quad (5)$$

where n_i represents the number of drops of the class i of the droplet diameter, d_i.

Results and Discussion

Analysis of succinic acid fermentation broth

Succinic acid was characterized by using HPLC. The concentration of succinic acid measured was 1.4 g/L from the fermentation of glucose by *E. coli*. The yield of succinic acid obtained is considered very low as a higher amount can be produced through fermentation condition optimization. It was discovered that, under optimum condition, the fermentation process was capable of producing succinic acid up to 50 g/L [22]. Meanwhile, the interest of this study is to obtain the fermentation broth, regardless of the succinic acid yield. In order to investigate the performance of ELM extraction, pure succinic acid was added into the solution up to the desired concentration. Other compounds, such as unconsumed glucose, and small amount of by-products also existed in the fermentation solution as shown in Figure 1. In addition, the pH of the broth was slightly acidic at 5.6. Basically, the accumulation of succinic acid in the fermentation broth inevitably led to the lowering of the pH in the fermentation medium. Maintaining the pH of the fermentation in a suitable range for the microorganism is one of the significant factors that affect the yield of succinic acid.

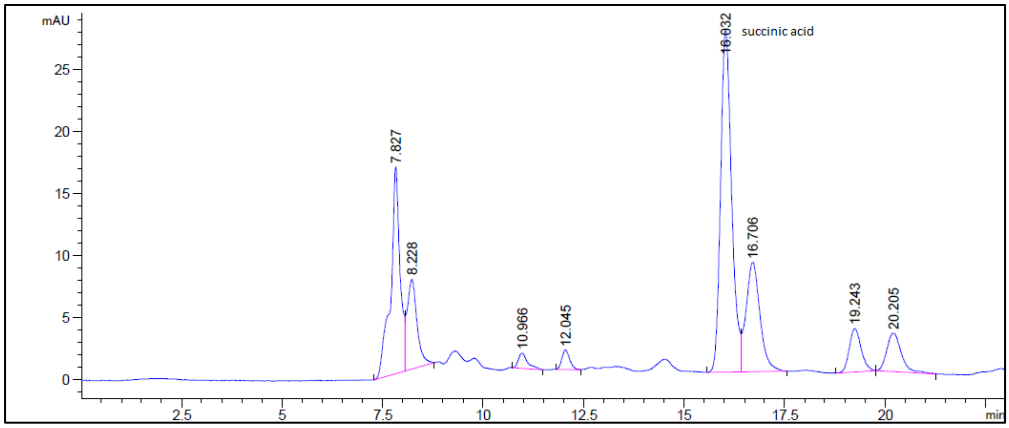


Figure 1. HPLC chromatogram of succinic acid fermentation broth

Emulsion stability study

Table 1 presents the effect of emulsification time, as well as agitation speed and time on the emulsion breakage. The experiments were performed in duplicates and the investigation was carried out one factor at a time with the best condition of each factor was used in the subsequent experiment.

Table 1. Effect of parameters on emulsion breakage. [diluent: palm oil; [Amberlite LA2]: 0.05M; Octanol: 10%; [Na_2CO_3]: 0.5M; O/I: 3/1; homogenizer speed: 7000 rpm; Tween 80: 1%; Span 80: 3%]

Parameters	**Condition**	**Emulsion Breakage (%)**
Emulsification time (minute)	3	40
	5	0
	7	40
	9	40
Agitation speed (rpm)	200	40
	300	0
	400	40
	500	60
Text C	1	40
	3	0
	5	40
	7	60

The influence of emulsification time was studied in the range of 3 to 9 minutes. It was found that the most stable emulsion was at 5 minutes of emulsification time where no breakage was observed. Meanwhile, emulsification time of both shorter and longer than 5 minutes resulted in higher breakage which was 40%. A higher breakage at short emulsification time happened because the time provided was insufficient to disperse the internal in the membrane phase. The configuration of surfactant at the interfacial area was unorganized and the interfacial tension was slightly reduced. This led to poor droplet formation with unbalanced size distribution obtained, as shown in Figure 2(a). Consequently, the emulsion broke easily when dispersed in the external phase. This is in agreement with the study by Gaikwad and Pandit [23] which indicated that a short emulsification time is insufficient to produce desirable emulsion droplet and leads to high breakage percentage. The intensity of the solution was enhanced at a longer

emulsification time with more internal phase entrapped in the membrane phase. Besides that, greater surface tension was reduced at 5 minutes of emulsification time, which contributed to uniform droplet distribution with smaller size at 1.33 µm as presented in Figure 2(b) and Table 2. A study by Sabry et al. [24] stated that when the droplets are smaller, they will take a longer time to coalesce. Therefore, the emulsion is more stable and the breakage phenomenon is hindered during emulsion dispersion. However, a further increase in the emulsification time (7 and 9 minutes) will damage the emulsion stability with 40% breakage. Intense emulsification leads to the formation of higher number of small emulsion droplets, as displayed in Figure 2(c) and Figure 2(d). The droplets tend to coalesce with one another and form larger sizes, such as 1.41 µm and 1.66 µm as listed in Table 2. This is in accordance with another study by Djenouhat et al. [25] that observed coalescence of internal droplets at a prolonged emulsification time. In this study, 5 minutes was chosen as the best emulsification time to form a stable emulsion.

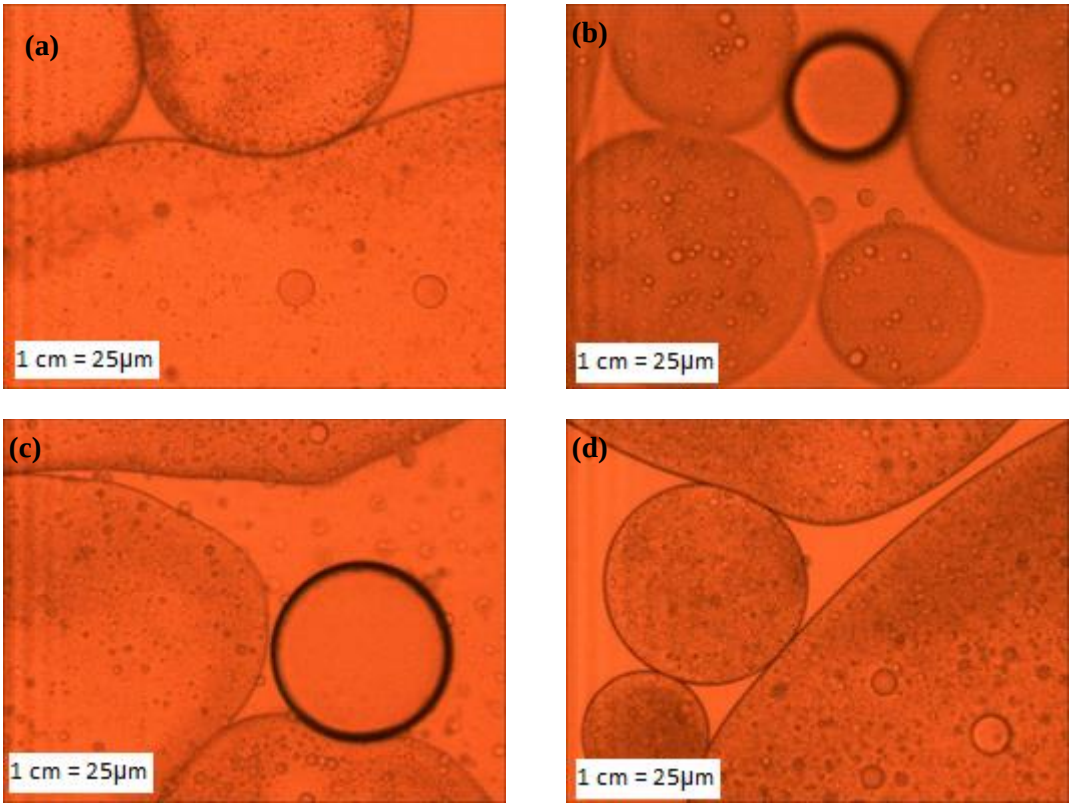


Figure 2. Microscopic images of emulsion at different emulsification time (a) 3 minutes, (b) 5 minutes, (c) 7 minutes and (d) 9 minutes (magnification 400x)

Table 2. Effect of emulsification time on emulsion droplets size

Agitation Time (minutes)	Diameter of Droplets (µm)
1	1.35
3	1.33
5	1.41
7	1.66

Besides that, Table 1 displays the results of the effect of agitation speed from 200 rpm to 500 rpm. It is apparent from this table that the most stable emulsion happens at 300 rpm. The emulsion was found unstable at lower agitation speed, where 40% of emulsion breakage was recorded. The result can be explained by the fact that the shear energy provided was insufficient to disperse the emulsion in the external phase. Consequently, larger globules were formed and they tended to coalesce with one another [23]. As can be seen in Figure 3(a) and Table 3, the size of emulsion globules formed at 200 rpm is larger (52.67 µm) compared to other agitation speeds. This result reflects those of Othman et al. [26] who also found that large globules were formed when low agitation speed was applied. Apart from that, larger emulsion globules could also reduce extraction performance since they increase the membrane thickness together with the small mass transfer area. Looking at Figure 3(b), it can be seen that smaller size of emulsion globules was formed at 36.48 µm. This can hinder the coalescence phenomenon between the emulsion globules that contributes to a more stable system. A further increase in agitation speed (400 rpm and 500 rpm) produced smaller emulsion globules at 24.42 µm and 17.66 µm, respectively. Unfortunately, the emulsion was found unstable where 40% and 60% of breakage were observed. This result could be attributed to the thinning of interfacial film and favored rapid coalescence of emulsion globules. Furthermore, the application of higher speed beyond a certain limit exposed the emulsion to high shear and led to globules rupture. These results seem to be consistent with another research which found that high agitation speed could damage the emulsion [12, 16]. Taken together, 300 rpm of agitation speed was chosen as the best condition in this study.

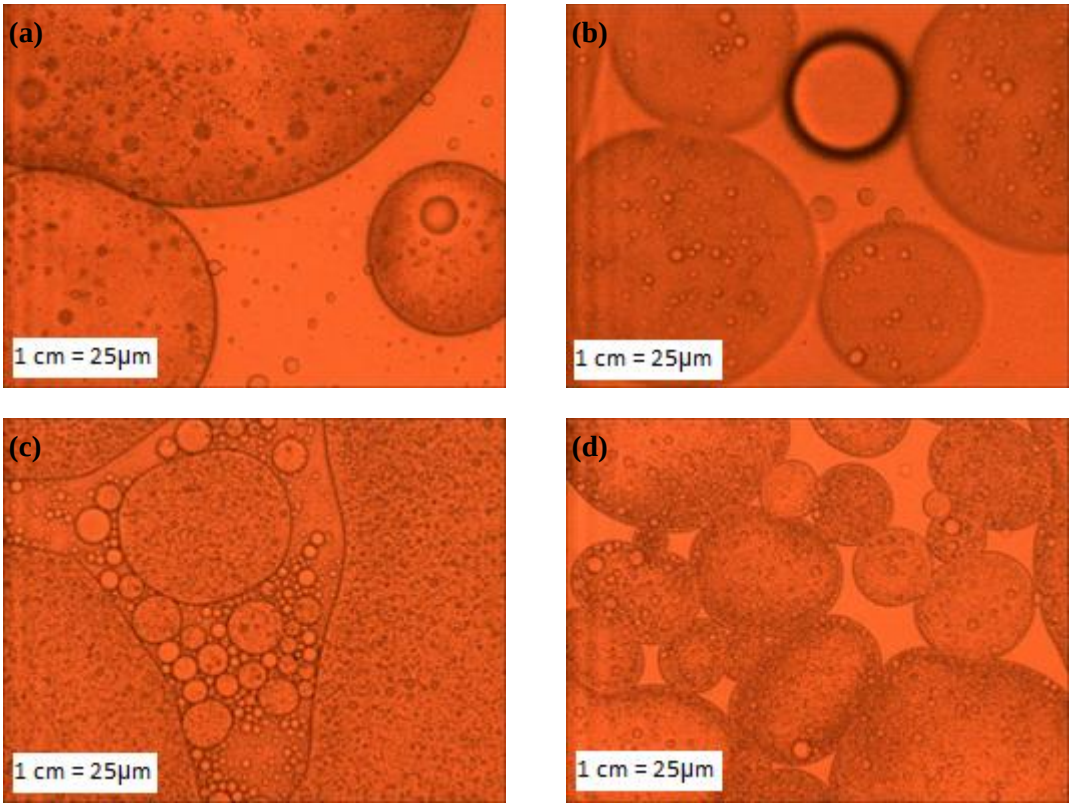
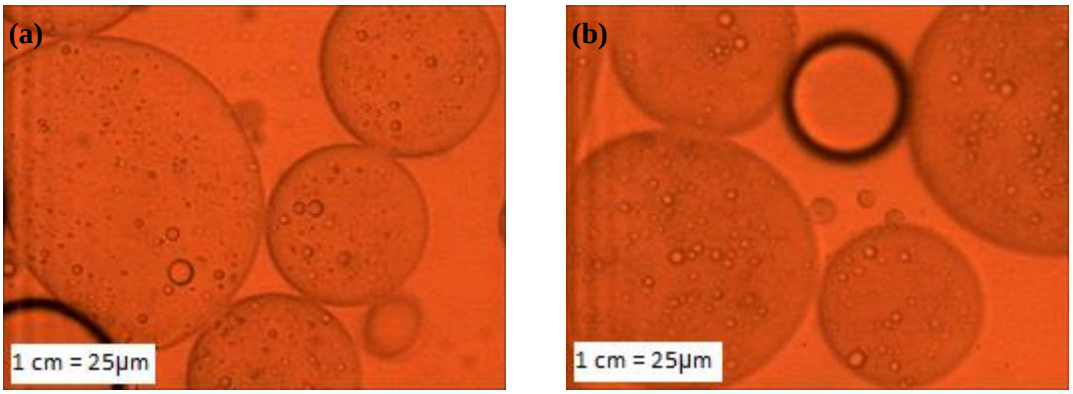


Figure 3. Microscopic images of emulsion at different agitation speed (a) 200, (b) 300, (c) 400 and (d) 500 (magnification 400x)

Table 3. Effect of agitation speed on size of emulsion globules

Agitation Speed (rpm)	Diameter of Globules (µm)
200	52.67
300	36.48
400	24.42
500	17.66

In addition, the effect of agitation time from 1 to 7 minutes was investigated. As can be seen from Table 1, emulsion stability improves when the agitation time is increased from 1 to 3 minutes, where 40% and no breakage can be observed, respectively. This happens because the shorter agitation time is insufficient to disperse primary emulsion in the external phase. As a consequence, a larger size of emulsion globules (42.77 µm) is formed as depicted in Figure 4(a). Consequently, coalescence phenomenon could occur within a short time [24]. As mentioned previously, large size of emulsion globules is also undesirable since it could decrease the extraction performance. A further increase in the agitation time (3 minutes) revealed that a stable emulsion was obtained with smaller globule size of 36.48 µm. The interface between the primary emulsion and external phase was basically deformed to the extent that large globules were formed. These large globules were subsequently broken up into smaller ones with time. As a result, the emulsion produced was more stable owing to smaller globules that will take more time to coalesce. A further increment in the agitation time (5 and 7 minutes) had worsen the emulsion stability, where 40% and 60% of emulsion breakage were recorded, respectively. As indicated earlier, longer agitation time favored the dispersion of smaller emulsion globules. Unfortunately, this condition led to rapid coalescence of small globules which later caused breakage. While Table 4 shows that smaller sizes of emulsion globules are obtained at 34.19 µm and 33.18 µm, Figure 4(c) and Figure 4 (d) demonstrate that larger globule size distribution is the result of coalescence. The findings are consistent with that of Kulkarni and Mahajani [27] who reported emulsion breakage at a prolonged time in the ELM process of molybdenum extraction. Thus, 3 min of agitation time was selected as the best condition.



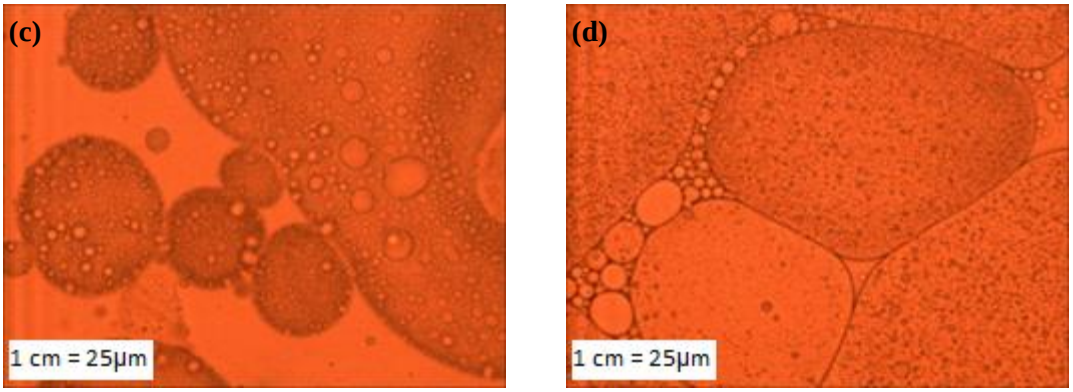


Figure 4. Microscopic images of emulsion at different emulsification time (a) 1 minute, (b) 3 minute, (c) 5 minute and (d) 7 minute (magnification 400x)

Table 4. Effect of agitation time on size of emulsion globules

Agitation Time (minutes)	Diameter of Globules (µm)
1	42.77
3	36.48
5	34.19
7	33.18

Succinic acid extraction and recovery performance

In order to determine ELM performance on succinic acid, the primary emulsion was dispersed in the fermentation broth at the best stability condition. The transport of succinic acid is governed by non-equilibrium mass transfer, which is due to the chemical concentration gradient [28]. The transfer of succinic acid through the liquid membrane consisted of two steps, which involved the reaction at the external and internal interfaces [29]. At the external interface, the succinic acid reacted with Amberlite LA2 and formed a complex. Following the reaction, the complex diffused through the membrane phase into the internal phase. The succinic acid was then released into the internal phase by stripping reaction with Na_2CO_3. Once completed, Amberlite LA2 was freed and diffused back to the external interface.

The effect of succinic acid concentration on the extraction (by varying it from 5 to 50 g/L) and the recovery performance were investigated and the results are shown in Figure 5. The graph shows that when the external concentration is increased, a marked decline can be seen in both extraction and recovery. It can also be seen that, by far the best condition is at 5 g/L of initial external feed concentration, where almost 100% of extraction and 94% of recovery are achieved. At this condition, the concentration of succinic acid recovered in the internal phase is 11.3 enrichment compared to the initial concentration in the external phase, as shown in Table 5. This intriguing result can be described by the fact that the concentration of solute is low compared to the larger amount of carrier. Another possible explanation is the concentration of the stripping agent in the internal phase is sufficient to strip succinic acid from the complex. This leads to the release of free carrier that reacts with another solute at the external interface. Moreover, the sufficient stripping agent could also delay the accumulation of succinic acid complex in the membrane phase that may obstruct the ELM process. These are consistent with those reported by Lee [30] and Noah et al. [31].

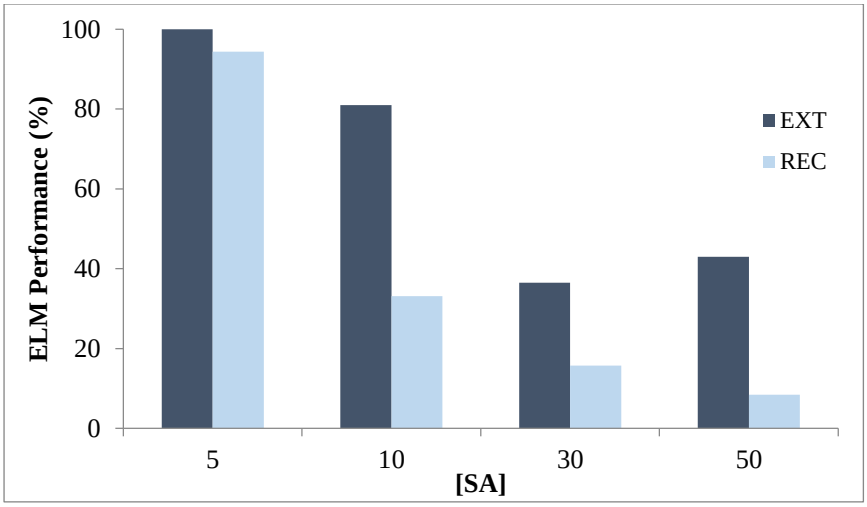


Figure 5. Succinic acid extraction and recovery performance at different initial concentration. [diluent: palm oil; [Amberlite LA2]: 0.05M; Octanol: 10%; [Na_2CO_3]: 0.5M; O/I: 3/1; homogenizer speed: 7000 rpm; emulsification time: 5 minutes; Tween 80: 1%; Span 80: 3%; agitation speed: 300 rpm; agitation time: 3 minutes]

Table 5. Enrichment of the recovered succinic acid

Initial Concentration of Succinic Acid in External Phase (g/L)	Final Concentration of Succinic Acid in Internal Phase (g/L)	Enrichment Ratio
5	58.98	11.3
10	39.7	4.0
30	62.87	1.9
50	56.21	1.0

When the initial concentration was increased to 10 g/L, the degree of extraction decreased slightly to 81%. After that, there was a significant decrease in the performance of ELM when the initial concentration was raised up to 50 g/L, where only around 40% of succinic acid extraction was attained. The result was likely to be coherent with the required amount of carrier to transport the higher amount of succinic acid. This result also could be related to the complex formation that controlled the external mass transfer of low solute concentration [32]. Hence, the extraction rate at high concentration was lower than that at low initial succinic acid concentration. On the other hand, a clear reduction of the recovery performance to only 33% with four times enrichment was identified at 10 g/L of initial feed concentration in this analysis. Less than 16% of recovery with enrichment ratio lower than 2 of succinic acid was recovered when the initial concentration was raised to 50 g/L. An important concept that emerged at high initial concentration was the transport of solute could decrease largely owing to the reduced capacity of internal phase to strip the transported solute. Moreover, the internal droplets in the peripheral emulsion region were 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 were saturated, the succinic acid-carrier complex accumulated in the membrane phase and prevented the carrier to react with another solute. These results are in agreement with those obtained by Chakraborty et al. [33] and Ammar et al. [34]. In this study, it can be concluded that 5 g/L is the best condition for recovery of succinic acid from fermentation broth.

Conclusion

This study presented the feasibility of succinic acid recovery by using ELM from real fermentation broth. The best operating conditions for emulsion stability were 5 minutes of emulsification time, 300 rpm of agitation speed, and 3 minutes of agitation time. The ELM performance from 5 g/L of succinic acid in fermentation broth was almost 100% extraction, 94% recovery, and 11 times enrichment. Thus, the ELM process is very promising to be applied in downstream processing of bio-based succinic acid production.

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