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Turbiscan Lab® Expert analysis of the biological demulsification of a water-in-oil emulsion by two biodemulsifiers

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ABSTRACT

The long-term destabilization process of a water-in-oil emulsion was investigated with two different biodemulsifiers produced under different culture conditions by *Alcaligenes* sp. S-XJ-1. Biodemulsifier I was obtained by using paraffin as substrate at initial culture pH of 10 and biodemulsifier II was produced with waste frying oils at pH of 7. The former exhibited higher demulsifying ability and interfacial activity than the latter. Bottle test, microscopy and Turbiscan Lab® Expert were used to investigate the biological demulsification process. It was found that biodemulsifiers' ability to decrease the interfacial tension played a more important role in demulsification than their ability to decrease the surface tension. Owing to their amphiphilic nature, demulsification process began with the adsorption of the biodemulsifiers onto the water–oil interface. Then the biodemulsifiers reacted with the emulsifiers because of their interfacial activity. As a result, thin liquid film was removed from the surface of dispersed droplets and coalescence occurred. This led to the settling of the dispersed droplets and the clarification of the continuous phase. Turbiscan Lab® Expert can be used to evaluate the demulsification efficiency and to analyze the destabilization process of different biodemulsifiers. It is a rapid and accurate method to screen high-efficiency demulsifiers from other bioproducts.

1. Introduction

Biodemulsifiers synthesized by microorganisms can break emulsions, which has many applications in the petroleum industry [1], food processing [2] and environmental protection [3]. Most emulsions produced in petroleum exploration are water-in-oil (W/O) emulsions. Large quantities of demulsifiers are needed to separate water from these emulsions so as to lower water content in oil [4]. Because of their interfacial activity, demulsifiers can adsorb onto the oil–water interface and thus induce demulsification [5]. Compared with chemical demulsifiers, biodemulsifiers are more efficient at demulsification, create no secondary pollution, and perform well under extreme conditions [3]. So far biodemulsifiers are either found in demulsifying bacterial cells [6] or occasionally in their extracellular metabolites [7].

However, biodemulsifers are more complicated than chemical demulsifier regarding their working mechanism and production. Previous observations showed that various demulsifying strains and different culture conditions have impact on the demulsification performance of biodemulsifiers. For example, Nadarajah et al. found three strains of *Acinetobacter* sp. showed a higher demulsification ratio than *Pseudomonas* sp. and *Al. Latus* did [8]. Park et al. showed that aerial spores cultivated with different media had very different demulsifying abilities [9]. Additionally, compared to chemical demulsifiers with definite structure and composition, the performance of demulsifying bacterial cells are influenced by more factors, such as cell surface hydrophobicity [10] and surface charge of cells [3] and, etc. The mechanism of demulsification induced by bacterial strains has not been thoroughly investigated in previous studies [1]. Bottle test is widely used in screening chemical demulsifiers in the petroleum industry because of its operational simplicity [11]. The volume of the breakage emulsion in a bottle test is determined by naked eyes at set time intervals, and used to calculate a demulsification ratio [12]. Although it is simple, the data is subject to bias of analyzer. Moreover, the test can only showed the different demulsification performance of demulsifiers but provided little information to explain the difference and the demulsification process. More sophisticated methods are needed to facilitate the understanding of the mechanism of demulsification.

To date, methods for monitoring the demulsification process have not been well developed. There are some means for evaluating emulsion stability, such as optical analysis with microscopy and spectroscopy [13], turbidity analysis and particle size analysis [14]. However, these means are only applicable to diluted emulsions in order to have acceptable accuracy [13]. Thus they are not suitable for investigating the demulsification process. The Turbiscan optical analyzer is an instrument, which can be used for real-time monitoring of concentrated and opaque emulsions. Its working mechanism

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is based on multiple light scattering analysis [14,15]. The Turbiscan can be used to observe reversible (creaming and sedimentation) and irreversible (coalescence and segregation) destabilization phenomena in the sample without the need of dilution. It can detect the changes of phase much earlier and also in a simpler way than other means [16]. Transmission and backscattering variation in an emulsion are recorded over time, and this has been successfully used to investigate the stability and homogeneity of a liquid mixture. Turbiscan has been used in many studies in chemical industry [17] and pharmaceutical industry [15] but not in the demulsification process. It is likely that Turbiscan would be able to reveal more details of the biological demulsification process induced by biodemulsifiers.

In this study, bottle test, microscopy, and Turbiscan methods were used for analysis of the biological demulsification process. With the aid of the Turbiscan optical analyzer, we hope that more details will be revealed in the destabilization process of W/O emulsion and some clues can be found to answer the performance difference of two different biodemulsifier.

2. Materials and methods

2.1. Bacterial strain and growth conditions

Alcaligenes sp. S-XJ-1 (CGMCC No. 2142) was isolated from petroleum-contaminated soil and was kept at −4 °C in an agar slant culture [18]. It was inoculated into 100 mL of nutrient broth medium (5.0 g L^{-1} beef extract, 10.0 g L^{-1} peptone, 5.0 g L^{-1} NaCl, pH 7.0) for enrichment for 72 h. The fermented broth (10 mL) was transferred into 100 mL of modified mineral salts medium (MMSM) for another 7 d of cultivation. The MMSM composition is detailed elsewhere [18]. The influence of carbon source and pH were studied by adjusting the initial pH values of the culture media to 5, 6, 7, 8, 9, 10, 11 or 12 with 6 mol L^{-1} HCl and 2 mol L^{-1} NaOH, and adding paraffin (4%, percentage by volume) or waste frying oil (4%, percentage by volume) as the carbon source.

2.2. Biomass

After fermentation, any carbon source residue was removed by mixing the fermented broth (50 mL) with 20 mL of carbon tetrachloride in a separating funnel. The solution was then left to separate for 20 min. The bacteria were harvested by centrifugation (19,800 ×g, 10 min, 4 °C) from the water-pellet phase and thoroughly cleaned with distilled water. After lyophilization at −50 °C for 24 h, the freeze-dried cell powder was weighed to identify the biomass.

2.3. Evaluation of demulsification performance

The W/O model emulsion was prepared according to an established protocol [12]. Aviation kerosene was mixed with the emulsifiers, Span 80 (0.1%, percentage by mass) and Tween 80 (1.9%, percentage by mass), which were purchased from Shanghai Shenyu Pharmaceutical and Chemical Limited Company, China. The aviation kerosene was then mixed with distilled water at a volume ratio of 3:2 at 10,000 rpm for 3.5 min using a high speed emulsifying machine (WL-500CY, Shanghai Wei Yu Mechanical and Electrical Manufacture Limited Company, China). The emulsion type was identified by the Oil Red *O* test as described by Lee and Lee [19]. The fresh emulsion had an emulsion breaking ratio of <10% at 35 °C after 24 h.

A cell suspension of each biodemulsifiers was prepared by suspending the dry powder biodemulsifier in distilled water to achieve a solution at a concentration of 5000 mg L^{-1}. Before dosing, the samples were ultrasonically vibrated for 3 min to ensure full dispersion.

In the demulsification test, 2 mL of the prepared cell suspension was added to a 20 mL graduated test tube containing 18 mL of the fresh model emulsion. The test tubes were vigorously inverted 200 times to achieve complete mixing and then left undisturbed in a water bath at 35 °C. The volumes of separated oil (on the top phase), separated water (on the bottom phase), and residual emulsion were recorded at set time intervals. The demulsification performance was evaluated using the oil separation ratio, water separation ratio and demulsification ratio (or emulsion breaking ratio) according to the following equations:

$$\text{Oil separation ratio} = \frac{\text{Kerosene volume (on the top)}}{\text{Kerosene volume in original emulsion}} \times 100\% \quad (1)$$

$$\text{Water separation ratio} = \frac{\text{Water volume (on the bottom)}}{\text{Water volume in original emulsion} + \text{Added sample volume}} \times 100\% \quad (2)$$

$$\text{Demulsification ratio} = \left(1 - \frac{\text{Remaining emulsion volume}}{\text{Original emulsion volume} + \text{Added sample volume}}\right) \times 100\% \quad (3)$$

A blank test was conducted with 2 mL distilled water, and this had an emulsion breaking ratio of <10% after 24 h.

2.4. Measurement of surface tension

The surface tension of the cell suspension was measured using a Du Nouy ring tensionmeter (DT-102, Zibo Huakun Electrical Equipment Limited Company, China) according to the procedure described by Bodour and Miller-Maier [20].

2.5. Measurement of interfacial tension

The oil–water interfacial tension was measured by a platinum ring tensiometer (JK99, Shanghai Zhongchen Digital Equipment Ltd. Co., China) at ambient temperature according to the procedure described by Wen et al. [18]. The oil phase was produced by dissolving the emulsifiers (2%, percentage by mass) into the kerosene. The Span80 and Tween 80 mass ratio was 19:1, which corresponds to that used in preparation of W/O model emulsion (Section 2.3). The water phase was a suspension of the dry powder biodemulsifier at concentrations of 0, 50, 100, 500, 1000, 2500, 5000, 7500, and 10,000 mg L^{-1}.

2.6. Demulsification analysis using microphotography

Static microscopic images of the residual emulsion remaining at different time intervals were acquired according to the procedure described by Wen et al. [18]. The two demulsifiers were dosed at 500 mg L^{-1} into the emulsion as described in Section 2.3. At set time intervals, the residual emulsion remaining in the middle of each test tube was collected using a 250 μL pipette and then observed under the optical microscope (Leica DMLB, Leica Microsystems, Germany) equipped with a color camera (JVC TK-C1380 CCD camera).

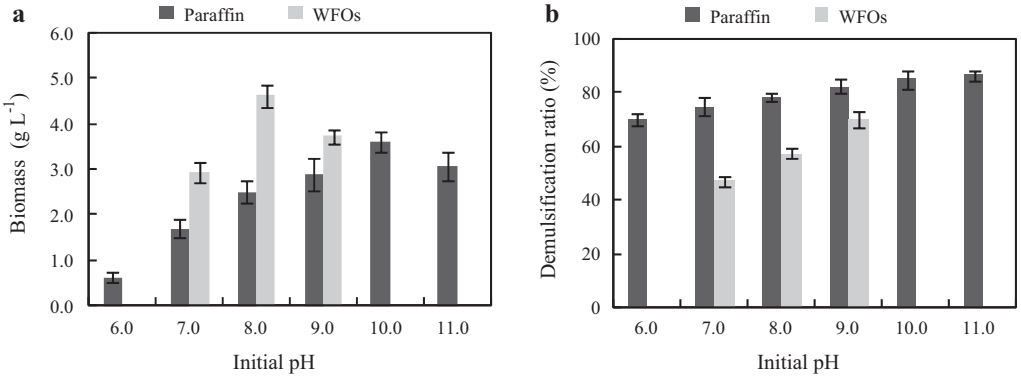

Fig. 1. Influence of carbon source (paraffin or waste frying oil, WFOs) and initial culture pH on production of biodemulsifiers: (a) yield of biodemulsifier, and (b) demulsification ratio.

2.7. Demulsification analysis using Turbiscan Lab® Expert

The emulsion destabilization induced by two biodemulsifiers was investigated using a Turbiscan Lab® Expert (Formulaction, France). For comparison with the bottle test results, the samples were prepared according to the procedure described as Section 2.3. Two biodemulsifiers were separately mixed with emulsion at the same concentration of 500 mg L^{-1}, and then transferred to a flat-bottomed glass cylindrical sample cell for measurement. Emulsion destabilization was analyzed using transmission (*T*) and backscattering (*BS*) profiles, with emulsion sample scans at 880 nm every 30 min over 24 h.

The Turbiscan can be used for optical characterization of a liquid dispersion, and includes a pulsed near infrared light source (λ = 880 nm) and two synchronous detectors: a transmission detector and a back scattering detector. The transmission detector receives the light going across the sample (at 0° from the incident beam) and the back scattering detector receives the light scattered backward by the sample (at 135° from the incident beam) [15]. The principle of this measurement is based on variation of the droplet volume fraction (migration) or mean size (coalescence), which results in variation of the *BS* and *T* signals [16]. The variation of the delta transmission signal (ΔT) and delta backscattering signal (ΔBS) were calculated as the difference between *T* or *BS* at 0 h and at a given time. A plot is produced of these results with ΔT or ΔBS on the *y*-axis and the sample height (*h*, mm) on the *x*-axis. A sample height of *h* = 0 mm corresponds to the bottom of the measurement cell. For comprehensive analysis, the obtained flux profile was interpreted and the size of a dispersed droplet was calculated using the Turbiscan Lab® Expert software according to the operational manual.

3. Results and discussion

3.1. Production of biodemulsifiers under different conditions

We previously reported that a demulsifying strain of *Dietzia* sp. S-JS-1 exhibited demulsifying ability when cultivated using waste frying oils as economical carbon source [21]. In this study, another demulsifying strain of *Alcaligenes* sp. S-XJ-1could also use waste frying oil as carbon source, which was compared to that produced using paraffin as carbon source. The initial culture pH greatly influenced the synthesis of biodemulsifier with waste frying oil or paraffin as the carbon source (Fig. 1). This finding agrees with the result of Rufino et al., who found that a moderate pH was required for *Candida lipolytica* growth in industrial residues [22]. Thus, regulation of the culture pH is crucial when producing biodemulsifiers or biosurfactants. Under the same initial culture pH, the produced biodemulsifiers had largely different biomass and demulsification ratios when different substances were used as the carbon source. The biomass produced with waste frying oil was higher than that produced with paraffin, but the trend for the demulsifying performance was opposite to this. Abouseoud et al. found that when olive oil was used as the carbon source, the bacteria produced more biosurfactants than that produced using hexadecane was the carbon source [23]. This could occur because it is more difficult for the S-XJ-1 strain to utilize the hydrophobic paraffin than waste oil. To enhance its contact and use of paraffin, the bacterial strain had to regulate its cell surface properties, which increased its HET hydrophobicity of the cell surface and thus enhanced its demulsifying ability.

Two biodemulsifiers with great demulsification difference were selected in order to analyze their demulsification process. Biodemulsifier I was obtained by using paraffin as substrate at initial culture pH of 10 and biodemulsifier II was produced with waste frying oils at pH of 7.

3.2. Comparison of demulsification efficiency using the bottle test

The demulsification efficiency of the two demulsifiers was firstly evaluated in the bottle test. The emulsion separation ratio of biodemulsifier I and biodemulsifier II after 24 h was 85% and 47%, respectively (Fig. 2). Moreover, biodemulsifier I initiated emulsion separation earlier than biodemulsifier II did. Emulsion destabilization occurred at a high rate at hour 3–4 with biodemulsifier I, while at hour 9–11 with biodemulsifier II. The bottle test showed that the demulsifying abilities of the two biodemulsifiers were very different.

3.3. Comparison on surface and interfacial activity of the two biodemulsifiers

Some studies have shown that the physiochemical properties of the demulsifying strains, such as the cell surface hydrophobicity [10] and charge of cell surface [3], greatly influence their demulsification ability. However, few studies focus on the surface and interfacial activity of biodemulsifiers as the research of chemical demulsifiers did. It has been proven that the demulsification abilities of chemical demulsifiers are related to their surface and interfacial activity [24]. So in this study, the surface tension and interfacial tension of the suspension of the demulsifying strains were measured and shown in Fig. 3. Dispersions of the two biodemulsifiers (at the concentration of 10,000 mg L^{-1}) decreased the surface tension of distilled water to 44 mN m^{-1}, and biodemulsifier II had higher surface activity than biodemulsifier I. This indicates that both of the biodemulsifiers were amphiphilic,

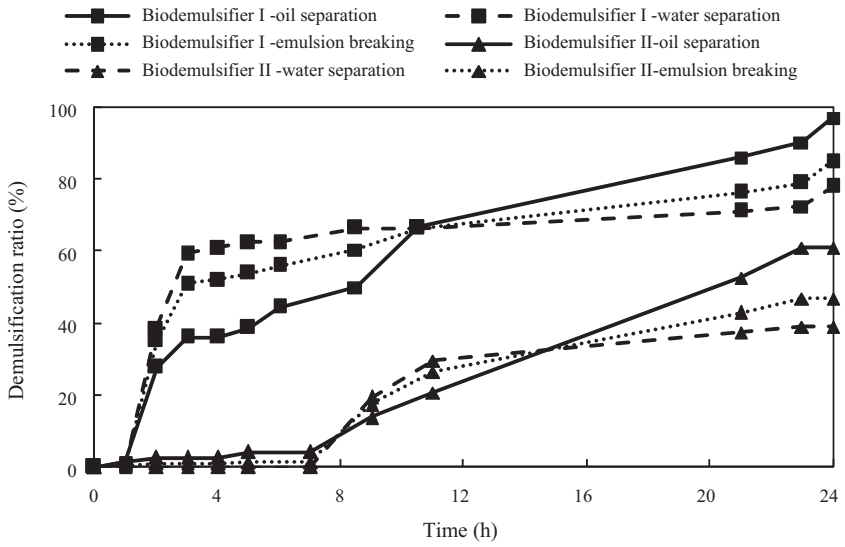

Fig. 2. Bottle test evaluation of the demulsification efficiency of the two biodemulsifiers (I and II).

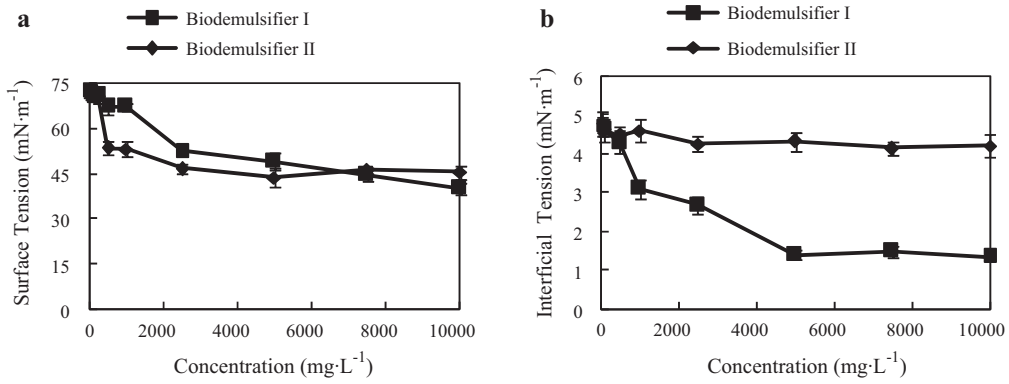

Fig. 3. Comparison of (a) surface tension, and (b) interfacial tension with the two biodemulsifiers (I and II).

which could assist in their adsorption onto the oil–water interface. Compared with biodemulsifier II, biodemulsifier I exhibited higher interfacial activity and was able to reduce the interfacial tension as low as 1.34 mN m^{-1} at the concentration of 5000 mg L^{-1}. Chemical demulsifiers with higher interfacial tension have been proven to enhance breakup of the interfacial film and to increase the coalescence of droplets [24]. Deng et al. found that the chemical demulsifier DODY68 had a high demulsification ratio and it also decreased the water–oil interfacial tension to 1.35 mN m^{-1} [25]. The observation of biodemulsifier I in this study indicated that the demulsifying ability of biodemulsifiers is also more affected by their interfacial activity than by their surface activity.

3.4. Comparison of droplet changes of the emulsion using microscopy

To study the changes of emulsion droplets, microscopy was used to observe the emulsion during demulsification process at different time intervals. The emulsion was initially a homogenous system with many droplets dispersed in oil (at hour 0) before biodemulsifier I was added into the emulsion (Fig. 4). As demulsification went on, the dispersed droplets gradually grew in size. At hour 24, the diameter of the largest droplet was about 5 times larger of the initial diameter of the droplet. The microscopy picture of emulsion with the dosage of biodemulsifier II is shown in Fig. 5. Compared with biodemulsifier I, the change in the size of dispersed droplets was slower and less significant. For example, there was little difference in droplet size at 7 h and 20 h. These results agreed with observations in the bottle test (Fig. 2), where demulsification was faster with biodemulsifier I than with biodemulsifier II. This difference in demulsification speed can be explained by the lower ability of biodemulsifier II to decrease the water–oil interfacial tension. Consequently, it could not destroy the interfacial film as efficiently as biodemulsifier I, and it was more difficult for the dispersed droplets to coalesce into larger droplets.

3.5. Comparison of the demulsification process using Turbiscan

3.5.1. Comparison on the real-time stability of entire emulsion

Predicting the real-time destabilization of an emulsion is of interest. Both the bottle test and microscopy cannot be used for that purpose because they cannot detect real-time coalescence of the droplets. As a non-destructive method, Turbiscan analysis does not need sample dilution. Moreover, it provides real-time information on the destabilization process [15]. To better visualize the Turbiscan signal, transmission and backscattering profiles at hour 0 were used as a baseline and were subtracted from the profiles at other times. The delta transmission and delta backscattering profiles of biodemulsifier I and II are shown in Figs. 6 and 7, respectively. The change in each delta transmission signal increased during the scan (24 h) over the entire height of the sample. The change of transmission signals was greater in the separated oil (upper phase of

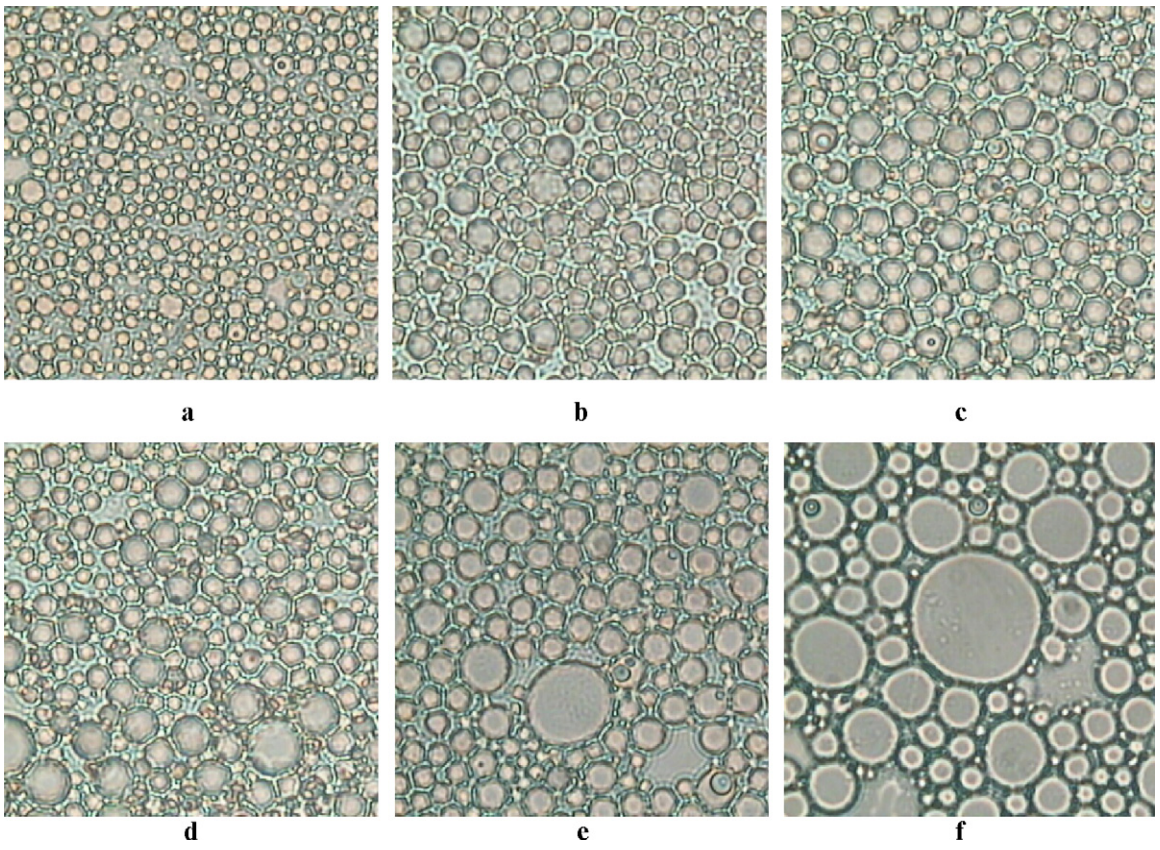

Fig. 4. Microscopy (400× magnification) of the residual emulsion with biodemulsifier I at (a) 0 h, (b) 2 h, (c) 4 h, (d) 7 h, (e) 20 h and (f) 24 h after initiation of the demulsification process.

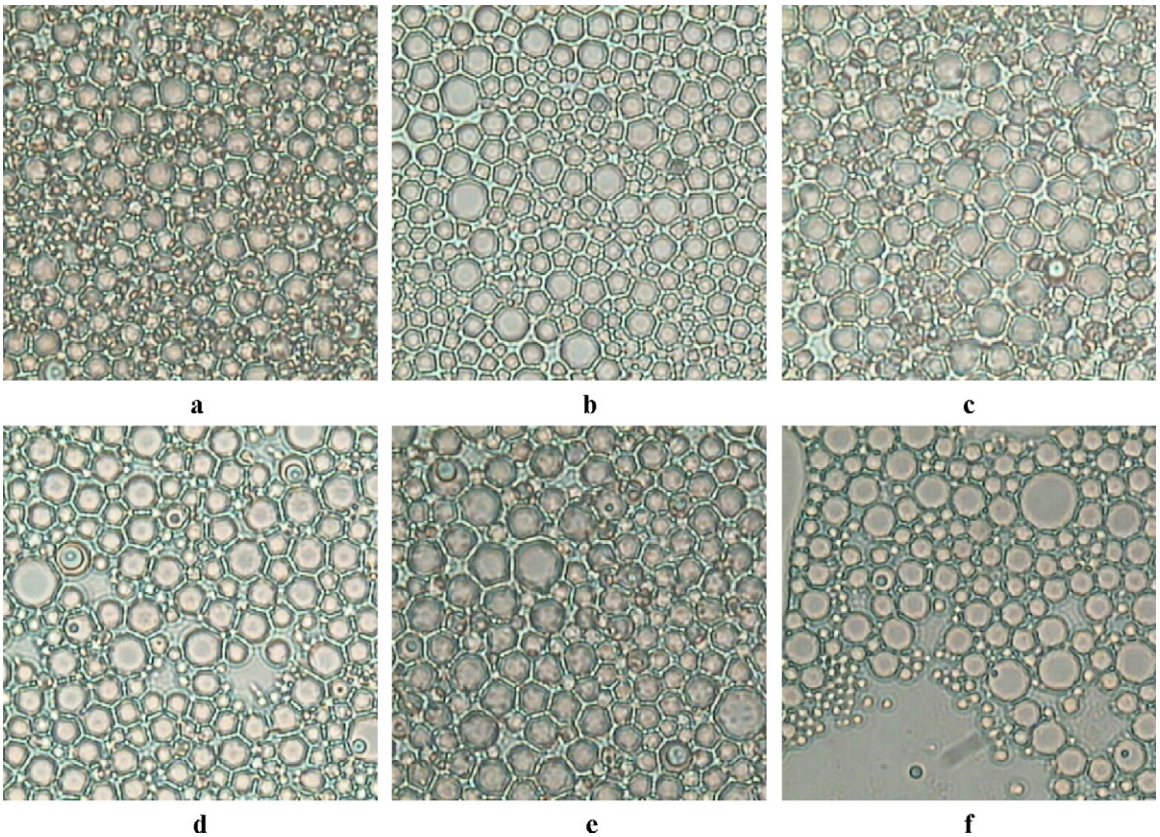

Fig. 5. Microscopy (400× magnification) of the residual emulsion with biodemulsifier II at (a) 0 h, (b) 2 h, (c) 4 h, (d) 7 h, (e) 20 h and (f) 24 h after initiation of the demulsification process.

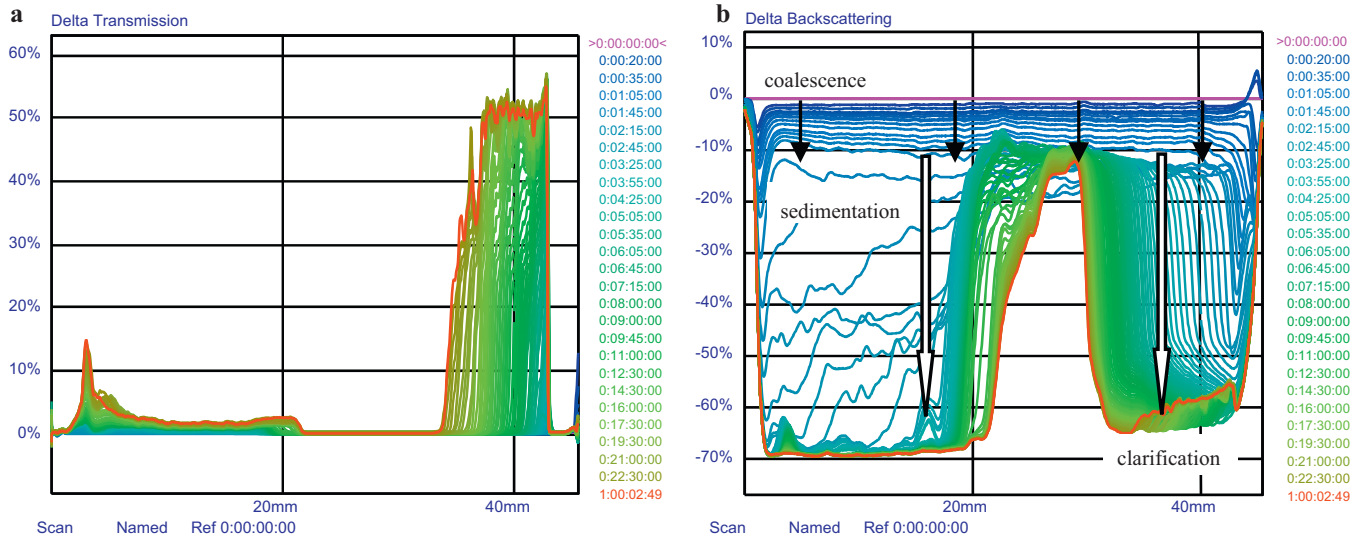

Fig. 6. Transmission (a) and backscattering (b) profiles of the demulsification process with biodemulsifier I evaluated by the Turbiscan Lab® Expert. Data are reported as a function of time (0–24 h) and sample height of the biodemulsifier (0–45 mm).

the emulsion) than in the separated water (lower phase of the emulsion). This was because the separated water turned cloudy as residual biodemulsifier (in solid form) settled to the bottom after demulsification.

The delta backscattering signals decreased as the demulsification progressed. By analyzing the decrease of delta backscattering signals at 24 h (red curve, Fig. 6), 29–45 mm of the cylindrical sample cell was identified as the height of upper separated oil phase and 0–27 mm as the height of lower separated water phase. We analyzed the changes of backscattering profile according to the principle reported by Mengual et al. [14], and found the demulsification process was induced by coalescence and sedimentation of the dispersed droplets. In the initial period, coalescence was dominated in the emulsion, which was evidenced by a progressive decrease in backscattering. As the coalescing droplets became bigger, they migrated from the top to the bottom of the emulsion. This corresponds to $\Delta BS = -10\%$, a decease in BS at the top and bottom of the sample. Finally, the droplets formed the water phase at the bottom of the sample cell (sedimentation) while the oil phase migrated towards the top of the sample (clarification), which could be detected by the naked eye.

In the emulsion added with biodemulsifier II (Fig. 7a), the backscattering intensity was not as strong as that biodemulsifier I (Fig. 6a), which indicates that the separated oil and separated water phases in the former were cloudier. By analyzing the decrease in delta backscattering signals at 24 h (red curve, Fig. 7b), 30.1–45 mm of the cylindrical sample cell was identified as separated oil and 0–17.3 mm as separated water. The demulsification ratio of biodemulsifier II obtained using the Turbiscan was 71%, which was lower than that of biodemulsifier I. Furthermore, the initial coalescence of the droplets in the emulsion with biodemulsifier II took longer time, suggesting that the demulsification process is less efficient.

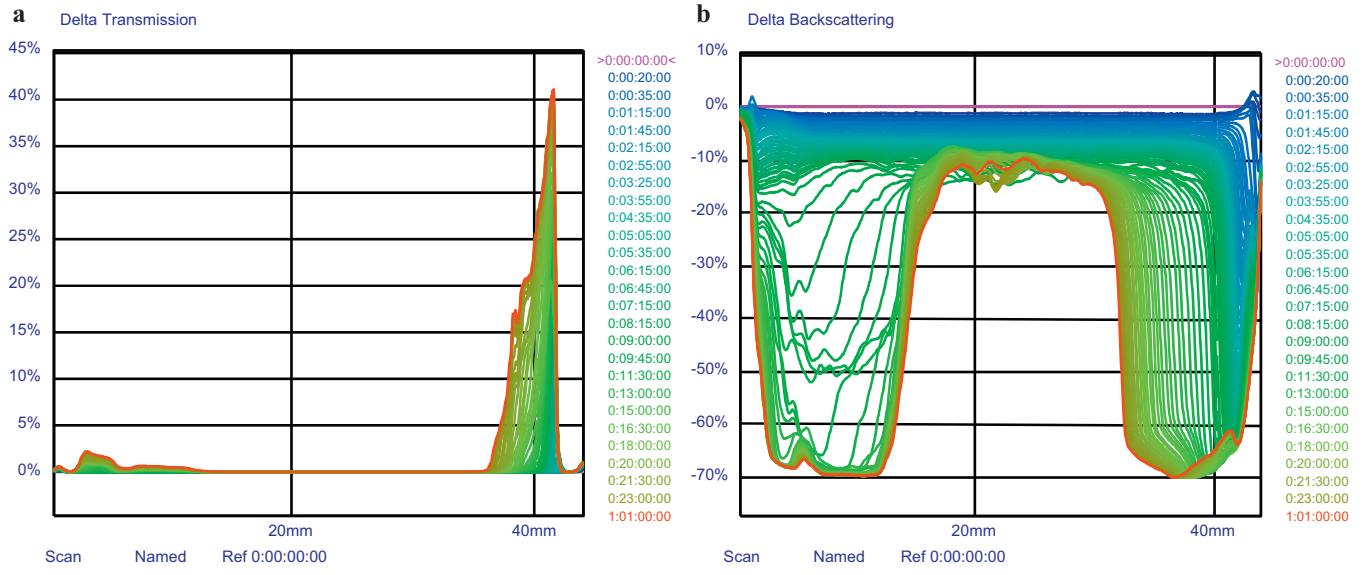

Fig. 7. Transmission (a) and backscattering (b) profiles of the demulsification process with biodemulsifier II evaluated by the Turbiscan Lab® Expert. Data are reported as a function of time (0–24 h) and sample height of the biodemulsifier (0–45 mm).

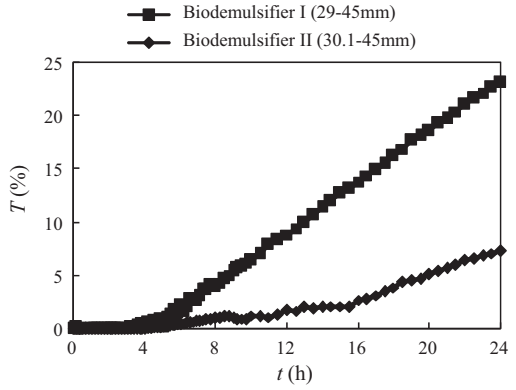

Fig. 8. Mean transmission intensity of the upper oil phase separated by the two demulsifiers (I and II). Data are reported as a function of time (0–24 h) at sample heights of I = 29 to 45 mm and II = 30.1 to 45 mm.

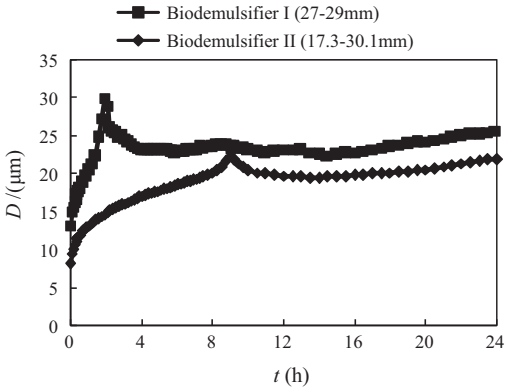

Fig. 10. Mean droplet size in the middle residual emulsion with the two demulsifiers (I and II). Data are reported as a function of time (0–24 h) at sample heights of I = 27–29 mm and II = 17.3–30.1 mm.

3.5.2. Comparison of upper separated oil phase based on T data

Upper separated oil phases obtained with the two biodemulsifiers were compared by calculating the mean transmission intensity of the separated oil (Fig. 8). The mean transmission intensity increased as a function of time, indicating that clarification occurred in the oil phase. The transmission intensity of emulsion dosed with biodemulsifier I was much higher and increased much faster than that with biodemulsifier II. These results show that biodemulsifier I was a more efficient demulsifier than biodemulsifier II.

3.5.3. Comparison of bottom separated water based on backscattering data

Lemarchand et al. believed that the backscattering signal could only be analyzed if the transmission signal is nil, because the reflection light from the walls of the measurement cell would interfere with the backscattering signal [16]. In this study, the transmission signals of the lower and middle phases were close to zero (as shown in Figs. 6 and 7), and thus backscattering can be analyzed for the comparison of two biodemulsifiers. The mean backscattering intensity of biodemulsifier I was weaker than that of biodemulsifier II (Fig. 9), which indicates that the water separated by biodemulsifier I was more transparent than that produced by biodemulsifier II. There were two inflection points in the two curves of Fig. 9, at hour 3 for biodemulsifier I and at hour 9 for biodemulsifier II. For biodemulsifier I, the backscattering intensity decreased sharply during the initial 3 h, indicating that the coalescence of droplets was faster than that in biodemulsifier II. The inflection point is a very important indicator for the demulsification process, and can be used in advance identification of the demulsification efficiency of biodemulsifiers. Hence, the time required for destabilization phenomena can be shortened dramatically by detecting changes in *T* and/or *BS* profiles before the appearance of macroscopic-scale physical modification of the emulsion.

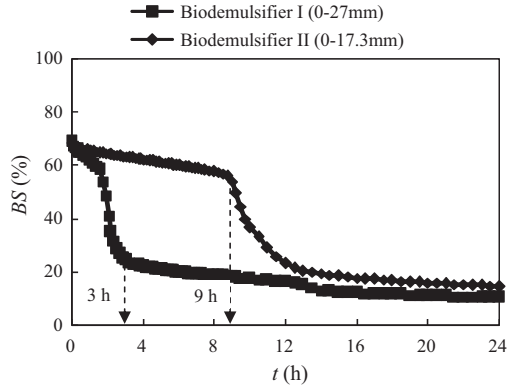

Fig. 9. Mean backscattering intensity of the lower water phase separated by the two demulsifiers (I and II). Data are reported as a function of time (0–24 h) at sample heights of I = 0–27 mm and II = 0–17.3 mm.

3.5.4. Comparison of mean droplet size in the middle residual emulsion based on BS data

The change in droplet size plays an important role in the destabilization of the emulsion. Some methods have been used to measure the droplet size [26]. In this paper, the mean droplet size in residual emulsion, which contained biodemulsifiers, was calculated by using the backscattering data. The mean droplet size firstly increased, and then decreased a little and leveled off over the 24 h of the demulsification process (Fig. 10). This can be explained in such a way that the process of droplet coalescence slowed down as the biodemulsifiers moved into the separated water phase. For biodemulsifier I, the mean droplet size was 30 μm at maximum at 3 h, and for biodemulsifier II, the maximum mean size was 22 μm at 9 h. The time that maximum droplet size occurred coincided with that of the inflection point in backscattering data analysis (as discussed in Section 3.5.3). According to Stokes formula, the settling speed of a droplet is proportional to the square of its diameter. Moreover, the settling of water droplets is vital to the phase separation of demulsification. As a result, it is easy to understand why the mean size of the droplet will influence the demulsification efficiency of a biodemulsifier. In addition, it also found that a large variance in droplet size would improve boundary coalescence of the droplets and accelerate phase separation [27].

Based on the above analysis, we suggest that the biological demulsification process occurs in the following sequence. The biodemulsifier first contacts and adsorbs onto the water–oil interface because of its amphiphilic nature. It then reacts with the emulsifiers because of its interfacial activity, which results in the removal of thin liquid film between the droplets and the coalescence of droplets occurs. As the droplets become large and start to settle, phase separation occurs and two separate layers become visible. The residual biodemulsifiers settle into the lower water phase together with the droplets and then the demulsification process finally ends.

4. Conclusion

The demulsification abilities of biodemulsifiers were greatly influenced by their cultivation conditions. The demulsification performance of two biodemulsifiers was firstly examined by bottle test. Their performance difference was further studied by the analysis of their surface and interfacial activity, microscope and

Turbiscan. It was found that their ability to decrease the water–oil interfacial tension was crucial to their demulsification performance. The coalescence of the droplet was a very important step in the destabilization of emulsion. The Turbiscan optical analyzer was proved to be a non-invasive, efficient and convenient tool for analyzing the demulsification process. It is much easier to be performed than the bottle test and generates more quantitative observations than microscopy. Thus Turbiscan Lab® Expert can be used to evaluate the demulsification efficiency and to analyze the destabilization process of different biodemulsifiers.

Acknowledgments

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