Biosurfactant and chemical surfactant effectiveness test for oil spills treatment in a saline environment

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ABSTRACT. Toxic and persistent nature of hydrocarbon and its products make it a significant concern for treating oil spills. In addition, hydrocarbons such as crude oil have long and complex carbon chains, making them challenging to remove directly. Emergency response for oil spills generally conducted by spraying dispersant agent into spillage surface. However, the use of chemical dispersants is reported to have a negative impact on the environment. Therefore, an environmentally friendly method for treating oil spills, utilizing biological agents such as biosurfactant or bioemulsifier, is needed. This research focuses on performance tests of more environmentally friendly surfactants as substitute for chemical surfactant which causes toxic effect when used. Surfactant performance was evaluated through three indicators: emulsifying index, dispersion effectiveness, and germination index (G.I.). Performance test was carried out for three types of surfactants: sophorolipid biosurfactant, methyl ester sulfonate (MES), and tween-80. Dispersion effectiveness test showed sophorolipid, MES, and tween-80 enhanced hydrocarbons dispersion in saline water up to 26.59, 38.65, and 48.19%, respectively. Germination index test showed the average G.I. for oil dispersed by sophorolipid, MES, and tween-80 are 153.16%, 143.94%, and 6.69 %, respectively. Research result suggests sophorolipid and MES have the properties to enhance oil dispersion under-examined laboratory conditions.

1. Introduction
Oil spills in the aquatic environment have been recorded as recurring incidents in Indonesia, such as the incident in Balikpapan Bay in 2018 due to the rupture of a seabed pipeline, then in 2019 in the Java Sea due to the leaking of an offshore oil platform. Therefore, we need to develop an emergency response for oil spills treatment in the environment. Oil spill dispersant is one of the critical components in oil spills treatment because of their role in breaking up and dispersing smaller hydrocarbon fractions into a water body. The dispersant is composed of surfactants (active substances) and solvents. Smaller hydrocarbon fractions increase surface area, make it easier to biodegrade in an aquatic environment. Currently, commonly used dispersants are still chemical-based, which may pose some disadvantages over their uses. Gulec and Holdway [1] reported the toxic effects of the dispersants chemical Corexit 9500 and Corexit 9527 on *Palaemon serious* (ghost shrimp) and *Macquarie novemaculeata* (Australian bass). The
toxicity test was carried out using the LC50 test method (Median Lethal Concentration). LC50 test results in Corexit chemical dispersants that use in oil spill treatment are shown in Table 1.

Table 1. LC50 Test result.

| Test Subject                  | *Palaemon serenus* | *Macquaria novemaculeata* |
|-------------------------------|--------------------|---------------------------|
| WSF Crude Oil                 | 258,000 ppm        | 465,000 ppm               |
| Corexit 9500                  | 83.1 ppm           | 19.8 ppm                  |
| Corexit 9527                  | 49.4 ppm           | 14.3 ppm                  |
| Dispersed Oil (Corexit 9500)  | 3.6 ppm            | 14.1 ppm                  |
| Dispersed Oil (Corexit 9527)  | 8.1 ppm            | 28.5 ppm                  |

(Source: Gulec and Holdway, 2000)

Water-soluble fraction (WSF) crude oil is the fraction of oil dissolved in water. The lower concentration in the LC50 test means a higher toxic effect for the environment. Low concentration in LC50 test indicates the small presence of test subject can kill 50% of the animal test population. Based on the test results, chemical dispersants are more toxic than WSF crude oil, making its usage on the oil spill treatment may increase the harmful effect on the environment. This phenomenon is undesirable, and generally use of chemical dispersants is not recommended for continuous use. Based on research conducted by Gulec and Holdway [1], the use of chemical dispersants, which should be an alternative solution for oil spill treatment, is not a preferable option due to the toxic effect. The continuous use of chemical dispersants is feared to cause long-term destructive side effects in the saline environment. However, given the usefulness of dispersants in the emergency response to oil spill incidents, modifications in the active ingredients are needed to reduce the adverse effects. Substituting the active dispersing agent (surfactant or solvent) is a potential modification without reducing the dispersing ability.

This study aims to test the performance of potential natural surfactants for active substances substitution in the dispersants formulation. Potential natural surfactants tested in this study are methyl ester sulfonate (MES) derived from palm oil and biosurfactant lactonic sophorolipid. For comparison, chemical surfactant tween-80 performance was also tested in this study.

2. Methodology

2.1 Materials

Lactonic sophorolipid biosurfactant with a purity of 50.5% purchased from Richest Group Ltd, China. Methyl ester sulfonate used is the product of Wilmar International, and Tween-80 used is the product of Tokyo Chemical Industry Co Ltd. Used lube oil obtained from a local motorcycle workshop, while Pertamina lube oil (SAE20 and SAE40) purchased from Pertamina Gas Station, used as a model for oil spill incidents.

2.2 Methods

2.2.1 Critical micelle concentration (CMC). Critical micelle concentration is the concentration of surfactant at the time micelles begin to form. As more surfactant is added, solution surface tension decreases, so more surfactants bind to the surface. However, the decrease in surface tension will be insignificant after the surface is fully saturated. After the surface is fully saturated, excess surfactant added will form micelles. The CMC value can be determined based on the decrease in surface tension from variations of sample concentration. In determining the sample concentration variation, a literature review was carried out and shown in Table 2.
Table 2. Critical micelle concentration reference.

| Surfactant     | Critical Micelle Concentration | Reference |
|----------------|-------------------------------|-----------|
| Sophorolipid   | 65 mg/L                       | [2]       |
|                | 9.5 mg/L                      | [3]       |
|                | 1.3 x 10^{-4} M               | [4]       |
| C_{14}-MES     | 3.68 mM                       | [5]       |
| C_{10}-MES     | 0.9 mM                        | [5]       |
| C_{10}-MES     | 1.02 mM                       | [6]       |
|                | 13.4 ± 0.6 mg/L to 24.7 ± 1.4 mg/L | [7] |
| Tween80        | 0.015 mM                      | [8]       |
|                | 2 x 10^{-5} g/ml              | [9]       |
| Rhamnolipid    | 20 mg/L                       | [10]      |

Based on Table 2, sample concentration variation was determined for surface tension test as follows:
- Sample blank: distilled water 0 mg/L
- Sophorolipid: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 mg/L
- MES: 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, 2000 mg/L
- Tween80: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 mg/L

At 0 ppm, surface tension shows 72 dyne/cm and decreases as the surfactant concentration increases until amphiphile is reached. Amphiphile is an equilibrium of hydrophilic and lipophilic groups. Surface tension test was carried out using Du Nuoy Tensiometer (Fisher), Platinum Irridium Ring 6 cm (Fisher), and 25°C water bath (Thamson) [11].

2.2.2 Emulsifying Index (E.I.). The measurement of surfactant ability to emulsify oil uses emulsifying index method, which refers to Abouseoud et al., 2007 [12]. Emulsifying index (E24) indicates the ability and stability of surfactants when used to emulsify the oil. 5 ml of emulsifier and 5 ml of oil sample poured into a centrifuge tube and stirred using Thermolyne Maxi Mix II vortex for 2 minutes. Sample observation was carried out 24 hours after the sample was mixed.

E.I. sample variation is based on the CMC value obtained. For each type of surfactant, E.I. was carried out on three types of oil samples at concentration 1-10 times of CMC. Emulsifying Index (E.I.) is obtained using the following equation.

\[ EI(\%) = \left(\frac{Emulsion \ layer \ height_{sample}}{Total \ height_{sample}} - \frac{Emulsion \ layer \ height_{blank}}{Total \ height_{blank}}\right) \times 100\% \]  

(1)

2.2.3 Dispersion Effectiveness (D.E.). The measurement of surfactant ability to disperse oil uses two methods, baffled flask test, which refers to Venosa et al. [13], and USEPA Method 1664 Revision B for N-Hexane Extractable Material. Baffled flask test is a lab-scale simulation method of oil dispersion process using a baffled flask reactor. Synthetic seawater (35 ppt), surfactants, and oil are mixed in the reactor using baffled mixing. After the mixing process, the sample was removed from the shaker and remained stationary to remove influential force during the mixing process. Stable oil dispersion will remain in the water body, and the rest will return to the surface due to density differences. The first 2 ml of sample drained through the bottom valve and discarded. Then, approximately 30 ml sample is transferred into 50 ml graduating cylinder for further analysis. Adjustments were made to the research method are shown in Table 3.

Dispersed oil sample in graduating cylinder was measured for total petroleum hydrocarbon (TPH) levels using USEPA Method 1664 Revision B N-Hexane Extractable Material. Dispersion Effectiveness (E.I.) is obtained using the following equation.

\[ DE(\%) = \left(\frac{TPH_{sample} (g/ml) - TPH_{blank} (g/ml)}{Weight_{oil \ added} (g)}\right) \times Volume_{total} \times 100\% \]  

(2)
Table 3. Baffled flask test modification.

| Indicator                  | Reference | Modification |
|----------------------------|-----------|--------------|
| Synthetic seawater volume  | 120 ml    | 400 ml       |
| Surfactant to oil ratio    | 1:25      | 1:1          |
| Oil volume                 | 0.1 ml    | 10 ml        |
| Surfactant volume          | 0.04 ml   | 10 ml        |
| Mixing type                | Baffled shaker | Magnetic stirrer |
| Mixing time                | 10 minutes| 5 minutes    |
| Settling time              | 10 minutes| 5 minutes    |
| Rotation speed             | 200 rpm   | 1000 rpm     |
| D.E. index                 | Absorbance area | TPH ratio   |

D.E sample variation is based on an emulsifying index. For initial mapping, three surfactant concentrations were tested (best emulsifying index concentration, average emulsifying index concentration, and worst emulsifying index concentration for each surfactant).

2.2.4 Germination Index (G.I.). Germination index is a parameter used to show environmental effects caused by the use of a specific product. The germination index quantifies the effect of surfactant used for green beans (*Phaseolus radiatus*) growth. Research conducted refers to Tam and Tiquia, 1994 [14]. However, the planting phase was carried out without bean hydration because the aqueous sample increased from 10 ml to 25 ml. Green beans sorted have relatively equal size assuming the amount of seed food reserves are relatively identical. Germination index (G.I.) is obtained using the following equation.

\[
\text{Seed germination} (\%) = \frac{\text{Number of seeds germinated in variation}}{\text{Number of seeds germinated in control}} \times 100\%
\]

\[
\text{Root elongation} (\%) = \frac{\text{Mean root length in variation}}{\text{Mean root length in control}} \times 100\%
\]

\[
\text{Germination index} (\%) = \frac{\text{Seed germination} (\%) \times \text{Root elongation} (\%)}{100 \%}
\]

The surfactant concentration used for the G.I. test was at 10 CMC. G.I. test samples consist of blank (distilled water), 10 CMC surfactants, and dispersed oil using 10 CMC surfactants (baffled flask method). Growing media used in G.I. was cotton watered with the sample (water, surfactant, dispersed oil) placed in a 90 mm petri dish. 6 green beans were planted in each petri dish and given equal 5-Watt lighting. Observations were made day by day from the first to the fifth by collecting several germinating seeds and root lengths. G.I. index is suitable for testing the effect of samples with low toxicity (affecting root growth) and high toxicity (affecting the number of germination).

3. Results and discussion

3.1 Critical micelle concentration (CMC)

Critical micelle concentration value was determined based on two analyses, visual analysis (concentration when graph started sloping) and mathematical analysis (surface tension drop below 5%). Critical micelle concentration was determined based on surface tension value. CMC value for sophorolipid, MES, and tween 80 are 60 mg/L, 1600 mg/L, and 90 mg/L, respectively. If compared, the sophorolipid CMC value is the smallest among the three surfactants. A smaller CMC value means less concentration of surfactant is needed to start forming micelles. For the initial indicator, sophorolipid is the most efficient surfactant compared to the other two surfactants.
3.2 Emulsifying Index (E.I.)

Emulsification is the process of mixing two or more liquids that cannot be mixed under normal conditions (without assistance). In oil spill treatment, two emulsification types occur between oil and water: oil in water emulsion (o/w) and water in oil emulsion (w/o). In determining the appropriate emulsifier for oil spill treatment, the expected type of emulsification is oil in water emulsion (o/w). Oil in water emulsion formed when emulsifier covers oil droplets surface with nonpolar tail (hydrophobic).

In selecting an emulsifier, one of the commonly used parameters is the hydrophilic-lipophilic balance (HLB). HLB shows the hydrophobic nature of the emulsifier with a higher HLB value more hydrophobic. Emulsifiers with HLB 3.5 to 6 are better used as w/o emulsifiers, while emulsifiers with HLB 8-18 are better used as o/w emulsifiers [15]. However, the use of the HLB parameter is limited to nonionic surfactants such as tween, Brij, and triton. Tween-80 surfactant used in this research has an HLB value of 15 [16].

The emulsifying index was carried out on three types of surfactants and three types of oil (Table 4). For each series, E.I. was conducted three times for each variation of concentration (1-10 CMC). For each series, concentration with the highest E.I. value sorted and margin of error is determined. The calculated margin of error is the error value for the triple test performed. Margin error is obtained using the following equation.

\[
\text{Margin of error (\%)} = \frac{\text{standard deviation (sample)}}{\text{number of sample}} \times 100 \quad (6)
\]

Table 4. Highest emulsifying index result.

| Sample            | Surfactant Concentration | Emulsifying Index  |
|-------------------|--------------------------|-------------------|
| Tween-80 – Used Oil | 810 mg/L | 89.47 ± 1.32% |
| Sophorolipid – Used Oil | 420 mg/L | 81.66 ± 0.77% |
| MES – SAE20 Oil   | 9600 mg/L | 2.16 ± 0.75% |
| Tween-80 – SAE20 Oil | 90 mg/L  | 1.3 ± 1.3%    |
| Sophorolipid – SAE20 Oil | 420 mg/L | 10.93 ± 2.35% |
| Sophorolipid – SAE40 Oil | 300 mg/L | 67.15 ± 4.75% |

Table 4 shows that Tween-80 give the highest E.I for used oil emulsification, while sophorolipid shows the highest E.I. for SAE20 oil and SAE40 oil. Sophorolipid has the best overall performance with E.I. for three types of oil above average.

3.3 Dispersion Effectiveness (D.E.)

There are at least three conditions for oil dispersion to occur. First, the ratio of dispersant to oil must be adjusted based on dispersant ability. Second, dispersants must be able to mix homogeneously with oil. Third, weathering issue. The addition of dispersant to hydrocarbon compounds will form hydrocarbon micelles then, with the help of water current, oil is dispersed. Dispersion effectiveness was carried out on three types of surfactants and three types of oil. For six series with E.I. value, three concentrations (concentration with best E.I., average E.I., and worst E.I.) are taken each series as reference for initial mapping of D.E profile. Then, research is continued by gradually increase the surfactant until concentration with best D.E discovered. D.E results are shown in Table 5 and it can be explained that sophorolipid, MES, and tween-80 have the properties to disperse the oil. MES shows the highest D.E. for SAE20 oil while tween-80 shows the highest D.E. for used oil and SAE40 oil. For three types of surfactants, sophorolipid is relatively stable. The average D.E value for sophorolipid, MES, and tween-80 are 24.39; 21.01; 33.13%, respectively.

Table 5. Highest dispersion effectiveness result.

| Sample            | Surfactant Concentration | Dispersion Effectiveness |
|-------------------|--------------------------|-------------------------|
| Sophorolipid – Used Oil | 660 mg/L | 23.87%     |
| Sophorolipid – SAE20 Oil | 540 mg/L | 26.59%     |
| Sophorolipid – SAE40 Oil | 540 mg/L | 22.72%     |
Sample Surfactant Concentration Dispersion Effectiveness
MES – Used Oil 17600 mg/L 9.93%
MES – SAE20 Oil 9600 mg/L 38.65%
MES – SAE40 Oil 14400 mg/L 12.67%
Tween-80 – Used Oil 540 mg/L 35.22%
Tween-80 – SAE20 Oil 810 mg/L 15.98%
Tween-80 – SAE40 Oil 990 mg/L 48.19%

3.4 Germination index
Germination index was carried out on 13 variations: 1 blank sample (distilled water), 3 surfactant samples at 10 CMC, and 9 samples of dispersed oil by 10 CMC surfactant. Observations were made day by day from the first to fifth day by measuring germinating seeds and root lengths. G.I. results are shown in Table 6.

| Sample                  | G.I. Day 1 | G.I. Day 2 | G.I. Day 3 | G.I. Day 4 | G.I. Day 5 |
|-------------------------|------------|------------|------------|------------|------------|
| Sophorolipid            | 4%         | 1.79%      | 5.95%      | 5.95%      | 3.79%      |
| Sophorolipid + Used Motor Oil | 10.67%   | 26.79%     | 35.71%     | 35.71%     | 63.64%     |
| Sophorolipid + SAE20 Oil | 36%       | 50%        | 50%        | 104.76%    | 219.70%    |
| Sophorolipid + SAE40 Oil | 93.33%    | 59.52%     | 59.52%     | 59.52%     | 176.14%    |
| MES                     | 2.67%      | 1.79%      | 12.50%     | 12.5%      | 7.95%      |
| MES + Used Motor Oil    | 13.33%     | 71.43%     | 71.43%     | 71.43%     | 79.55%     |
| MES + SAE20 Oil         | 58.67%     | 28.57%     | 62.5%      | 62.5%      | 308.71%    |
| MES + SAE40 Oil         | 4%         | 47.62%     | 68.45%     | 68.45%     | 43.56%     |
| Tween-80                | 1.33%      | 1.19%      | 2.38%      | 2.38%      | 3.79%      |
| Tween-80 + Used Motor Oil | 16%      | 14.29%     | 14.29%     | 14.29%     | 12.88%     |
| Tween-80 + SAE20 Oil    | 1.33%      | 2.98%      | 2.98%      | 2.98%      | 6.44%      |
| Tween-80 + SAE40 Oil    | 0%         | 1.19%      | 1.19%      | 1.19%      | 0.76%      |

Based on Table 6, if three types of surfactants at 10 CMC are directly in contact with green beans, the growth will decrease to 3.79-7.95% compared to blank growth. However, dispersed oil by 10 CMC surfactant sophorolipid and MES showed promising G.I. values. Average G.I. values for oil dispersed by sophorolipid, MES, and tween-80 are 153.16%, 143.94%, and 6.69%, respectively.

4. Conclusion
In terms of CMC value, sophorolipid showed the lowest value (60 mg/L). A low CMC value indicates that low surfactant concentration is needed to saturate the surface and form micelles. In terms of E.I. value, tween 80 showed the highest E.I. value for emulsifying used oil (89.47% ± 1.32%), and sophorolipid showed an above-average E.I. value for emulsifying used oil (81.66 ± 0.77%). Sophorolipid showed the highest E.I. value for emulsifying SAE20 oil (10.93 ± 2.35%) and SAE40 oil (67.15 ± 4.75%). For overall performance in E.I., sophorolipid showed the best performance with high E.I. value for three types of oil. Regarding the D.E. value, tween 80 showed the highest D.E. value for dispersing used oil (35.22%) and SAE40 oil (48.19%). MES showed the highest D.E. value for dispersing SAE20 oil (38.65%). Sophorolipid showed very stable and average performance with D.E. value for dispersing used oil, SAE20 oil, and SAE40 oil are 23.87%, 26.59%, and 22.72%, respectively. In terms of G.I., sophorolipid and MES showed outstanding performance with average day 5 G.I. above 100% (153.16% and 143.94%). G.I. above 100% means green bean growth with dispersed oil by sophorolipid and MES are better than green beans growth in the blank sample. So, based on G.I. value can be concluded that sophorolipid and MES are environmentally friendly. Based on 4 parameters in this research can be concluded that sophorolipid is very potential to substitute active compound in chemical dispersant with low required dosage, high E.I. value, average D.E. value, and environmentally
friendly. MES is the potential to substitute active compounds in specific chemical dispersant treating for SAE20 oil.

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