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INFLUENCE OF *PSEUDOMONAS AERUGINOSA* PRESENCE IN THE BIODEGRADABILITY STUDY OF SOLVENT-BASED AND WATER-BASED DISPERSANT IN OIL SPILL HANDLING

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ABSTRACT

Oil-Spill Dispersant (OSD) reduces interfacial tensions of oil and water turning oil spill into droplets that makes crude oil easier to be degraded by hydrocarbonoclastic bacteria such as *Pseudomonas aeruginosa*. The purpose of this study is to assess the effect of dispersant utilization (solvent-based and water-based) related its performance efficiency in the presence of *Pseudomonas aeruginosa*. The research was carrried out in laboratory, varying Dispersant-Oil Ratio (DOR) into 3 levels (1:8, 1:20, 1:25) and carbon source adaptation into 3 levels (0%, 1%, 2%). The total number of samples prepared was 84, consist of 21 samples without *Pseudomonas aeruginosa* addition and 63 samples with *Pseudomonas aeruginosa* addition. Total petroleum hydrocarbon (TPH) is measured using gravimetric method to determine the biodegradation of crude oil. Also measured are pH of samples with *Pseudomonas aeruginosa* addition and COD (Chemical Oxygen Demand) value of samples with dispersants. Data were evaluated using ANOVA. The result shows *Pseudomonas aeruginosa* has the ability to degrades crude oil despite the presence of dispersant, whereas the use of water-based dispersant showed better biodegradation ability than solvent-based OSD usage. Dispersant effectiveness of solvent-based and water-based is 33% and biodegradation by *Pseudomonas aeruginosa* achieved 25% in 72 hours.

Keywords: Biodegradability; Contaminated Seawater Remediation; Oil-based Dispersant; Pseudomonas aeruginosa; Solvent-based Dispersant

1. INTRODUCTION

In 2010 there was more than 800,000 liters (5000 barrels) of oil spilled each day (Van Bockstaele, 2010). Most cases of oil spill occured in ocean, coastal, and river water, covering water surface and disrupting aquatic ecosystems and the surrounding environment (CETS, 1989). Chemical treatment using dispersant has been widely used because it is deemed more cost and time effective than bioremediation, biotechnology, and other treatments (Zolfaghari-Baghbaderani et al. 2012) in which longer time to disperse oil required, whereas the handling of oil spill must be done promptly so as not to futher pollute and damage the surrounding ecosystem in reference to the Indonesian Presidential Decree No. 109 Year 2006 on Marine Oil Spill Emergency Response in stating that the oil spill handling must be rapid and appropriate.

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Dispersant is designed to binds with oil surface, reducing the surface tension between oil and water, as of dissociating oil from water that makes oil easily degraded microorganisms, especially hydrocarbonoclastic microorganisms. One of them is the bacterium *Pseudomonas aeruginosa* (Canevari, 1987; Lessard & Demarco, 2000). Dispersant usage has been controverted because of its chemical base material, solvent, is considered to have more toxicity (increases toxicity level) than the *oil spill* (Lindgren et al., 2001). A study in the Gulf of Mexico reported that the core element of dispersant injected into deep water on oil-well runaway of British Petroleum remained trapped in an undersea plume of oil, methane, and other hydrocarbons, resisting decay even as it became diluted (Voosen, 2011). This motivates the development of water-based dispersant that expected to be no less effective than solvent-based dispersant, but more environment friendly. Further study is needed to evaluate whether it is accurate that solvent-based and water-based dispersant promote the performance of hydrocarbonoclastic microorganisms in lowering the level of oil with similar effectiveness and whether the presence of dispersant which contain a lot of chemicals do not obstruct the biodegradation of oil by hydrocarbonoclastic microorganisms. The purpose of this research is to study the performance efficiency of *Pseudomonas aeruginosa* in response to the use of solvent-based and water-based dispersant as auxiliary agents for oil in oil spill handling.

2. METHODOLOGY/ EXPERIMENTAL

2.1. Samples

Samples investigated in this study consists of 2 major experiments: first is the mixing of crude oil and dispersant (solvent-based and water-based) to discover the reaction or effect of dispersant usage on oil spill. second is the addition of *Pseudomonas aeruginosa* culture to the water column derived from the first experiment. Oil, sea water, and bacteria isolates are obtained from Laboratory of Biotechnology, Research and Development Centre for Oil and Gas Technology. The dispersants used are Motto 3025-S (*solvent-based*) and Motto 3025-W (water-based).

The research of this study consist of 5 treatments. First treatment is the mixing of solvent-based dispersant and sea water-crude oil. Second is the mixing of water-based dispersant and sea water-crude oil. Third is mixture of solvent-based dispersant and sea water-crude oil added with culture of *Pseudomonas aeruginosa*. Fourth is mixture of water-based dispersant and sea water-crude oil added with culture of *Pseudomonas aeruginosa*. And fifth is the controlling treatment with no dispersant or bacteria culture added.

Each dispersant treatment consists of 3 levels Dispersant-Oil Ratio (DOR) (1:8, 1:20, 1:25). Treatment with addition of *Pseudomonas aeruginosa* culture consists of 3 levels bacterium adaptation (0%, 1%, 2%). Factorial experiment is used with 3 replications. The total sample studied is 84.

2.2. OSD Concentration Determination

The concentration of dispersant used is determined by finding Critical Micelle Concentration (CMC) using Surface Tension (SFT) method. The surface tension is measured using the Processor Tensiometer (Kruss).

2.3. OSD Biodegradation Test

This test is done using Test Method 12: Biodegradability Determination by Research and Development Centre for Oil and Gas Technology LEMIGAS that is based on OECD Guideline for Testing Chemicals - 306 and BOD5 (MU.P.4-2.1) Test Method.

2.4. OSD Effectiveness Test

This test is done based on EPA/600/R-04/119 (Weaver, 2004) using UV/VIS spectrophotometry.

2.5. Adaptation of *Pseudomonas aeruginosa*

Isolate of *Pseudomonas aeruginosa* was restored on Nutrient Agar (NA) slant and incubated for 24 hours and then cultivated in Nutrient Broth (NB) for 72 hours on shaker at 150 rpm. *Pseudomonas aeruginosa* culture was adapted on a Mineral Salt Medium (MSM) (Zhang, et al. 2005) (concentration in (g/litre)): $NaNO_3$ 4g; NaCl 1g; KCl 1g; $CaCl_2.2H_2O$ 0.1g; KH_2PO_4 3g; $Na_2HPO_4.12H_2O$ 3g; $MgSO_4.7H_2O$ 0.4g; $FeSO_4.7H_2O$ 0.001g and trace element (g/100ml): $FeCl_3.6H_2O$ 0.008; $ZnSO_4.7H_2O$ 0.075; $CoCl_2.6H_2O$ 0.008; $CuSO_4.5H_2O$ 0.0075; $MnSO_4.H_2O$ 0.075; H_3BO_3 0.015; $Na_2MoO_4.2H_2O$ 0.005. The initial pH was adjusted to 6.8.

Bacterium adaptation with 0% carbon source concentration was performed by mixing 50 ml of cultivation with 50 ml MSM in 250 ml flask then kept on shaker for 72 hours at 150 rpm. 1% bacterium adaptation was done in three stages. The first stage (Adaptation I) was done by mixing 50 ml of cultivation with 50 ml of MSM in 250 flask. Then the mixture was put on shaker for 48 hours at 150 rpm. The second stage (Adaptation II) was done by mixing 25 ml of bacterium culture that was derived from Adaptation I and 75 ml of MSM and the addition of 0,5 ml of carbon source (oil) then kept on shaker for 48 hours at 150 rpm. The third stage (Adaptation III) was done by mixing 100 ml of culture derived from Adaptation II and 300 ml of MSM and the addition of 1 ml of oil, then kept on shaker for 72 hours at 150 rpm. 2% bacterium adaptation was conducted in three stages similar to 1% bacterium adaptation, but with the addition of 1 ml oil on Adaptation II and 2 ml oil on Adaptation III.

2.6. Biodegradation Test of Dispersant-Dispersed Oil

This test is done as in previous study by Minoui et al. (2009).

2.7. Natural Degradation Test

120 ml sea water in 250 ml flask is added with 2 ml of crude oil then placed on shaker for 72 hours. Total Petroleum Hydrocarbon (TPH) is measured as in previous study by Minoui et al. (2009).

3. RESULTS AND DISCUSSION

3.1. OSD Concentration

Data obtained from the measurement were plotted in a linear fashion which was the concentration of surface tension value where the deflection of declining part can be more visible and the transition measurement value were not too sharp (Mukerjee & Mysels, 1971).

Based on the National Standard Reference Data System of the CMC of the aqueous surfactant system, the characteristic values of surface tension of a surfactant at room temperature is about 35 dyn/cm or 35 mN/m. When compared to the result of surface tension measurement for each of solvent-based and water-based dispersant, Motto dispersant have the standard characteristic for a surfactant compound with a surface tension value of 30 mN/m. Based on these results, the concentration of 1000 ppm of OSD is used in this study, where the Dispersant-Oil Ratio (DOR) of 1:8 to 2 ml of oil is based on the following calculation:

$$\begin{aligned} V_1 M_1 &= V_2 M_2 \\ V_1 \times 1{,}000{,}000 \text{ ppm} &= 250 \text{ ml} \times 1000 \text{ ppm} \\ V_1 &= 0.25 \text{ ml} \end{aligned}$$

thus,

$$\begin{aligned} V_{OSD} : V_{\text{crude oil}} &= 0.25 \text{ ml} : 2 \text{ ml} \\ \text{DOR} &= 1:8 \end{aligned}$$

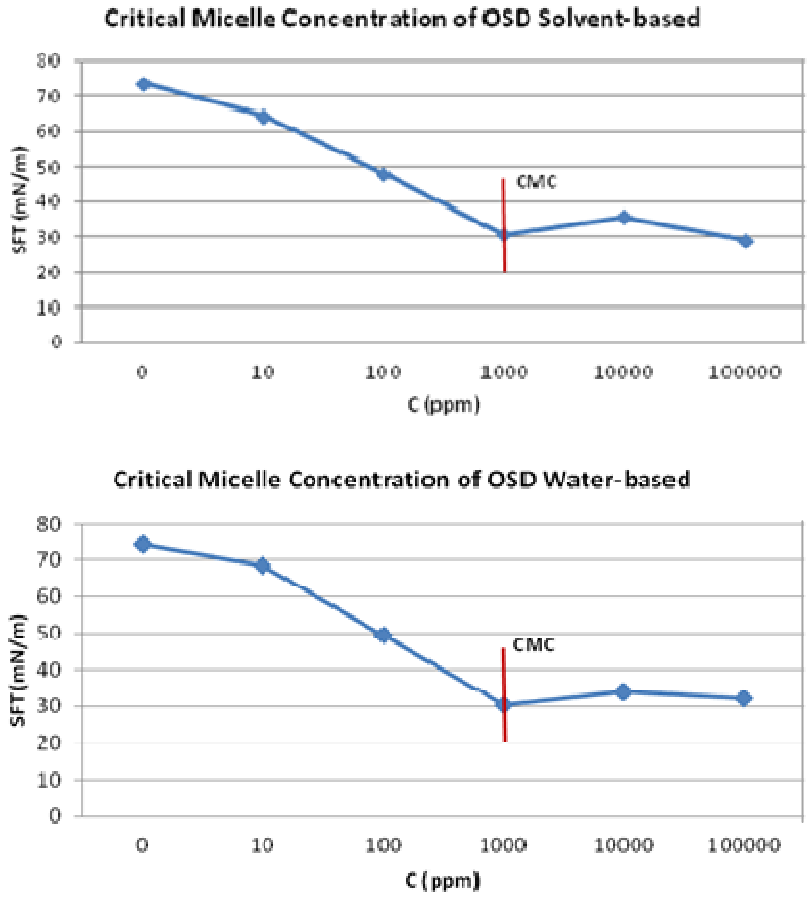

Figure 1 Critical micelle concentration graph of solvent-based dispersant and water-based dispersant

It appeared that the lowest value of surface tension which indicated the best OSD performance was at a concentration of 1000 ppm with the surface tension of 30.71 mN/m for solvent-based dispersant and 30.44 mN/m for water-based dispersant.

In the measurement of surface tension using Kruss Processor Tensiometer, the temperature of OSD solution was within the range of 26-29^{o}C. The difference in temperature did not have a significant effect on the results of measurement of surface tension after repeated measurements were performed using dispersant solution with the same concentration in the varied temperature. This is consistent with previous studies which showed that the effect of temperature generally showed little effect (Mukerjee & Mysels, 1971).

The use of dispersant in this study was varied into 3 DOR. The used DOR were 1:8 based on the CMC result, 1:20 as a reference from the Motto dispersant company, and 1:25 as was referred to the widely used DOR in field applications as well as in the previous researches.

3.2. Biodegradability of OSD

The test result of dispersant biodegradability of Motto solvent-based and water-based dispersant showed that the two types of dispersant have good bioadaptability with the degradation percentage greater than 60%, which was 63.364% for the solvent-based dispersant and 66.160% for the water-based dispersant. In both types of dispersant, the percentage of degradation on the 2nd day looked good, recorded at greater than 60%. Then on the 5th to the 28th day, there had been significant degradation percentage for solvent-based dispersant. In the water-based dispersant, a decrease in the degradation percentage is also found after the 15th day. On the 5th

day, the degradation percentage of water based dispersant looked well, recorded at 75.675%. This suggests that the water-based dispersant is proven to be more easily degraded than solvent-based dispersant, which means water-based dispersant is more environmentally friendly.

Table 1 Biodegradation of OSD

Day	Biodegradation (%)	
	OSD SB	OSD WB
0	0.000	0.000
2	63.364	66.160
5	31.968	75.675
15	29.246	34.700
28	2.108	9.654

Table 2 COD test result

Sample	COD (mg/l)
CO	1376.09
SB1	2145.77
SB2	1554.91
SB3	1275.02
WB1	1951.41
WB2	1368.32
WB3	2169.10

Chemical Oxygen Demand (COD) is the amount of oxygen required to oxidize a number of organic substances chemically. The need of COD determines the amount of organic components in the sample that have the ability to be chemically oxidized by an oxidizing agent (Clesceri et al., 2005). Thus, the organic substances being oxidized do not only come in the form of stable component of the biological reaction or the biodegradable component, but also non-biodegradable component. From the test results for the samples using dispersant, the COD values obtained are presented in Table 2. Furthermore, the test result of the COD parameter for solvent-based dispersant showed that the COD value decreased in line with the decrease in the concentration of dispersant use.

Meanwhile, for the water-based dispersant, it was observed that there was also a tendency for the COD value to fall along with the decrease in the concentration of OSD use. However, there was a surge in the value of COD at DOR of 1:25 which was an anomaly where in 1:20 DOR with the concentration of dispersant use that was not considerably different with 1:25 DOR, COD value was impaired significantly. The result, i.e COD value of water-based dispersant tends to be larger than the solvent-based dispersant. This is in accordance with the study by Zolfaghari-Baghdaberani et al. (2012) claiming that OSD with higher biodegradability has greater COD value.

3.3. Effectiveness of OSD

Qualitative assessment of OSD effectiveness was observed visually using dispersibility category by SINTEF Field Effectiveness Test (Clesceri et al., 2005).

The result from the addition of Motto 3025-S solvent-based dispersant showed that the dispersion formed was quite good. The ability to reduce the surface tension between the oil and water was best demonstrated by the solvent-based OSD with DOR of 1:8. The oil droplets created were small, spread evenly, and last longer in water column so as to facilitate the process of biodegradation. With the addition of solvent-based OSD at 1:20 DOR, the oil droplets were seen to be greater. The same result was also shown with the addition of solvent-based OSD at 1:25 DOR where the dispersions did not completely occur. Although the addition of solvent-based OSD with DOR of 1:20 and 1:25 are not significantly different in terms of ratio, however it appeared that the dispersions formed were different from DOR of 1:25. Oil pattern with different surface tension could still be seen with the oil droplets created due to the reduced surface tension between oil and water.

Meanwhile, the result from addition of Motto 3025-W water-based OSD showed that the dispersion formed was quite good. The capacity to lower the surface tension between the oil and water was also best achieved by the water-based OSD with DOR of 1:8. However, in comparison with the addition of solvent-based OSD with 1:8 DOR, it was shown that the solvent-based OSD decreased the surface tension between the oil and water better than the addition of water-based OSD with the same ratio in which the colour of oil spill still found to be black. With the addition of water-based OSD with DOR of 1:20, larger pattern of oil dispersed showed the surface tension between the oil and water varied at different points. The same result occured with the addition of water-based OSD with DOR of 1:25.

The measurement of UV-spectrophotometry visibility on the use of OSDs indicated that the variation of DOR did not present different results in the calculation of the OSDs effectiveness. Although through visual observaions the different performance of OSDs could be clearly seen, however in the calculation the difference was not visible. The test result of dipersant effectiveness showed the effectiveness value of the use of Motto OSD at a value of 33%. The low OSD effectiveness is largely influenced by the characteristics of the oil used. As advocated by Resby et al. (2007), the physical properties of the oil is an important factor in controlling the effectiveness of OSD.

3.4. Degradation of Crude Oil in Droplets by *Pseudomonas aeruginosa*

The result of total petroleum measured in the biodegradation test with varied adaptation of carbon source on *Pseudomonas aeruginosa* indicates that the adaptation increases the ability of *Pseudomonas aeruginosa* in degrading crude oil. Total petroleum contents and adaptation of each samples is presented in the Table 3.

Table 3 Total petroleum contents measured on each adaptation

Sample Code	Adaptation 0%				Adaptation 1%				Adaptation 2%			
	D-0 (mg)	D-3 (mg)	Δ (mg)	%	D-0 (mg)	D-3 (mg)	Δ (mg)	%	D-0 (mg)	D-3 (mg)	Δ (mg)	%
CO	0.000	0.000	0.000	-	0.000	0.000	0.000	-	0.000	0.000	0.000	-
	0.000	0.000	0.000	-	0.001	0.000	0.001	100	0.000	0.000	0.000	-
	0.000	0.000	0.000	-	0.000	0.000	0.000	-	0.000	0.000	0.000	-
SB1	0.044	0.014	0.030	67.9	0.040	0.003	0.037	92.5	0.041	0.000	0.041	100
	0.043	0.011	0.032	74.1	0.040	0.003	0.037	92.4	0.040	0.000	0.040	100
	0.042	0.011	0.031	73.8	0.039	0.004	0.035	89.8	0.040	0.000	0.040	100
SB2	0.036	0.010	0.026	71.9	0.037	0.003	0.034	91.8	0.037	0.000	0.037	100
	0.036	0.008	0.028	77.9	0.037	0.003	0.034	91.9	0.036	0.000	0.036	100
	0.036	0.010	0.026	72.6	0.037	0.003	0.034	91.8	0.037	0.000	0.037	100
SB3	0.035	0.002	0.033	94.3	0.036	0.018	0.018	49.8	0.036	0.000	0.036	100
	0.036	0.006	0.030	83.2	0.036	0.003	0.033	91.7	0.035	0.000	0.035	100
	0.035	0.004	0.031	88.7	0.036	0.003	0.033	91.7	0.036	0.000	0.036	100
WB1	0.040	0.012	0.028	69.9	0.040	0.000	0.040	100	0.040	0.000	0.040	100
	0.040	0.009	0.031	77.6	0.040	0.000	0.040	100	0.040	0.000	0.040	100
	0.040	0.011	0.029	72.5	0.040	0.000	0.040	100	0.040	0.000	0.040	100
WB2	0.035	0.003	0.032	91.5	0.035	0.001	0.034	97.1	0.036	0.000	0.036	100
	0.035	0.002	0.033	94.2	0.036	0.004	0.032	88.7	0.036	0.000	0.036	100
	0.035	0.000	0.035	100	0.035	0.003	0.032	91.5	0.035	0.000	0.035	100
WB3	0.035	0.003	0.032	91.3	0.035	0.003	0.032	91.4	0.034	0.000	0.034	100
	0.035	0.002	0.033	94.2	0.034	0.000	0.034	100	0.035	0.000	0.035	100
	0.034	0.001	0.033	97.0	0.035	0.000	0.035	100	0.035	0.000	0.035	100

The comparison of biodegradation value of each results with varied DOR of OSD usage is presented on the following chart.

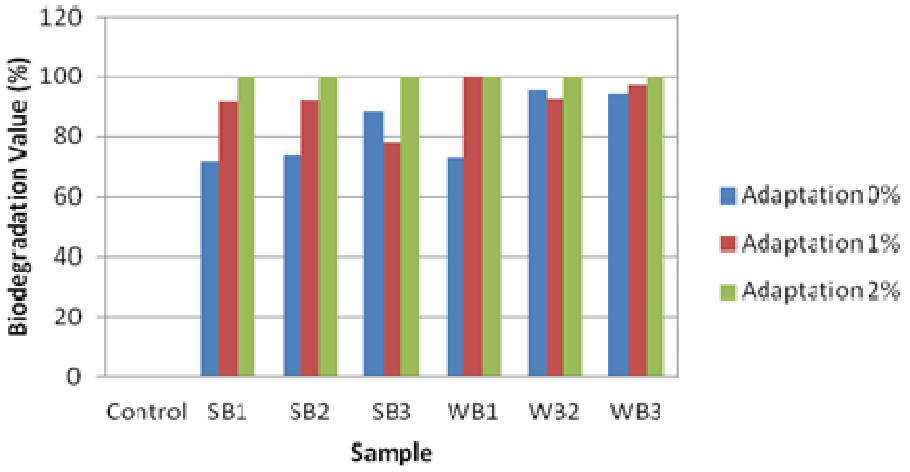

Figure 2 Comparison of biodegradation percentage with varied adaptation

The biodegradation percentage of crude oil by *Pseudomonas aeruginosa* shows a good ability of the bacterium in degrading light crude oil at aerobic state with or without adaptation of the carbon source beforehand. However, with an adaptation on *Pseudomonas aeruginosa* the bioegradation percentage increases along with the adaptation. The presence of dispersant did not seem to have an influence as a pollutant or inhibitor disrupting biodegradation process by *Pseudomonas aeruginosa*. Nevertheless, the type of dispersant used shows an effect wherein addition of water-based OSD usage the biodegradation percentage is greater than addition of solvent-based OSD usage.

3.5. Analysis of Interaction between Dispersant Usage and Biodegradation of Crude Oil

This study demonstrates the interaction between the use of OSD and crude oil biodegradation by bacterium; Solvent-based OSD and *Pseudomonas aeruginosa* and Water-based OSD and *Pseudomonas aeruginosa*. Predetermined significant level (α) in the calculation was 1% to minimize the deviation of the analysis of the interaction between the two factors so that the results obtained demonstrates the significant relationships of one factor to the other factor. The hypothesis is as the following:

H_o : All treatments (DOR variation, bacterium adaptation) provide similar crude oil biodegradation ability by *Pseudomonas aeruginosa*

H_1 : There is a treatment (DOR variation or bacterium adaptation) that provides different crude oil biodegradation ability by *Pseudomonas aeruginosa*.

3.5.1. Solvent-based oil-spill dispersant

The calculation result of line variation source indicates $F_{count} > F_{table}$ showing that H_0 is rejected. It means that there is a treatment (DOR variation, bacteria adaptation) providing different biodegradation ability of *Pseudomonas aeruginosa*, where in line with the variation source the treatment was an adaptation of *Pseudomonas aeruginosa.* The column variation source indicated a $F_{count} < F_{table}$ showing that H_0 was accepted.

Table 4 ANOVA test of solvent-based OSD

Source of Variation	Degree of Freedom	Sum of Squares	Middle Squares	Count Factor	Table Factor
Line (bacterium)	2	2295.311	1147.656	15.963	α = 1%, f=5.614
Column (OSD)	2	18.155	9.078	0.126	α = 1%, f=5.614
LC (interaction)	4	976.699	244.175	3.396	α = 1%, f=3.895
Error	18	1294.108	71.895		
Total	26	4584.273			

This suggests that there is no different response of biodegradation ability of *Pseudomonas aeruginosa* towards varied DOR of OSD usage. Interaction variation source indicates $F_{count} < F_{table}$ showing that H_0 is accepted. It means that treatments conducted (DOR and adaptation variation) provide similar crude oil biodegradation ability of *Pseudomonas aeruginosa*. This demonstrates the interaction between dispersant and bacterium has no effect on the ability to degrade crude oil which is implies that the use of solvent-based dispersant do not influence *Pseudomonas aeruginosa's* performance. However, it is possible that Motto 3025-S solvent-based OSD promotes the bacterium's performance because the difference between count factor and table factor is less than 1 with value of 0.5.

3.5.2. Water-based oil-spill dispersant

The result of the calculations of line variation source indicates $F_{count} > F_{table}$ showing H_0 is unaccepted. It means that there is a treatment (DOR variation, bacteria adaptation) providing different biodegradation ability of *Pseudomonas aeruginosa*, where in line variation source of the treatment is adaptation of *Pseudomonas aeruginosa*. Column variation source indicates $F_{count} > F_{table}$ showing H_0 is unaccepted. It indicates that the concentration of water-based dispersant used affects the biodegradation ability of *Pseudomonas aeruginosa*. Interaction variation source indicates $F_{count} > F_{table}$ showing H_0 is rejected which implies that the interaction between dispersant usage and *Pseudomonas aeruginosa* influences the bacteria's ability in degrading crude oil. This proves that the use of water-based OSD affects the biodegradation by *Pseudomonas aeruginosa* as auxiliary agent by expanding accessible oil surface.

Table 5 ANOVA test of water-based OSD

Source of Variation	Degree of Freedom	Sum of Squares	Middle Squares	Count Factor	Table Factor
Line (bacterium)	2	758.843	379.421	41.330	α = 1%, f=5.614
Column (osd)	2	193.795	96.898	10.555	α = 1%, f=5.614
LC (interaction)	4	857.739	214.435	23.358	α = 1%, f=3.895
Error	18	165.244	9.180		
Total	26	1975.622			

3.6. Degradation of Crude Oil in Droplets by *Pseudomonas aeruginosa*

The result shows that both OSD, Motto 3025-S (solvent-based) and Motto 3025-W (water-based) have good bioadaptability and show no significant indication as pollutant or toxic agent against *Pseudomonas aeruginosa*. In environmental field, this finding can be considered for saltwater remediation in oil spill handling, showing that chemical and biological remediation can be done simultaneously using water-based OSD, where in this study the use of Motto 3025-W (water-based) shows higher biodegradation percentage than Motto 3025-S (solvent-based).

4. CONCLUSION

This study shows that Oil-Spill Dispersant (OSD) MOTTOCHEM 3025S (solvent-based) and 3025W (water-based) have the same effectiveness, which is 33%, when applied to light medium crude oil with physical characteristic tendency towards asphaltic/aromatic. Biodegradability of both dispersants indicates good bioadaptability that shows no effect in disrupting biodegradation process by *Pseudomonas aeruginosa*. The use of water-based OSD increases biodegradation is an exemplify that dispersant usage take effect on oil biodegradation by *Pseudomonas aeruginosa*.

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