Effects of the Combination between Bio-surfactant Product Types and Washing Times on the Removal of Crude Oil in Nonwoven Fabric

by Ni'matuzahroh Effects Of The Combination Between Bio-surfactant

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Effects of the Combination between Bio-surfactant Product Types and Washing Times on the Removal of Crude Oil in Nonwoven Fabric

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Abstract. This research aimed to characterize bio-surfactants produced by *Bacillus subtilis* 3KP, *Pseudomonas putida* T1-8, *Micrococcus* sp. L II 61 and *Acinetobacter* sp. P 2(1) and to investigate its combination's effects on the removal of crude oil in nonwoven fabric with different washing times vary from 12, 24 to 36 hours. The production of bio-surfactants was done on Synthetic Mineral Water mixed with molasses 4% within four days. The bio-surfactant products were characterized by measuring the Surface Tension (ST) (mN/m) and Emulsion Activity (EA) (%). Oil removal experiment was done by mixing 10 mL bio-surfactant with nonwoven fabric that contains crude oil into 50 mL bottle inside a shaker. The removed crude oil was extracted with n-hexane and measured gravimetrically. The results were then being analyzed with two ways ANOVA and Duncan test. Bio-surfactant produced by four bacteria has variations of Surface Tension and Emulsion Activity values. Bio-surfactant produced by *Bacillus subtilis* 3KP and *Pseudomonas putida* T1-8 showed the increasing of crude oil removal as washing times increase, while bio-surfactant produced by *Micrococcus* sp. L II 61 and *Acinetobacter* sp. P2(1) showed the decreasing result at 36 hours. However, the combination that showed the best result was *Acinetobacter* sp. P 2(1) at 24 hours valued 65,3%.

Keywords: Bio-surfactant, Crude Oil, Nonwoven Fabric, Surface Tension, Emulsion Activity.

INTRODUCTION

According to the data of Group of Expert on Scientific Aspects of Marine Pollution (GESAMP) 6,44 million tons of hydrocarbon compounds from crude oil have been contaminating the ocean every year. Crude oil waste contains complex compounds such as benzene, toluene, ethyl benzene, and xylene isomer (BTEX) that are harmful for the marine ecosystem including corals, mangroves, and water organisms. Various methods have been developed to overcome the oil spillage problems, including the usage of oil sorbent, polypropylene nonwoven fabric. However, most of the nonwoven fabrics were eventually wasted and caused another source of pollutant. Thus, another solution is needed to reduce the nonwoven fabric waste. The used nonwoven fabric can be re-used through separating process between the oil content and the fabric itself.

One of the effective alternatives to remove oil content from nonwoven fabric is by using bio-surfactant. Bio-surfactants, biologically produced, have been increasingly used in soil washing and oil removal from contaminated areas (Mulligan *et al*., 1999, 2001). Furthermore, bio-surfactant has low toxicity and highly bio-degradable (Batista *et al*., 2006). Hence, hydrocarbon bacteria that have the ability to produce bio-surfactant are highly recommended in crude oil degradation process in a short time (Kumar *et al*., 2006). Result of Integrated Research (Ni'matuzahroh*et al*., 2004) and National Grant Research (Ni'matuzahroh*et al*., 2009) have successfully identified potential bacteria in

producing bio-surfactant such as *Bacillus subtilis* 3KP, *Pseudomonas putida* T1-8, *Micrococcus* sp. L II 61 and *Acinetobacter* sp. P 2(1).

This research aims to characterize bio-surfactants produced by *Bacillus subtilis* 3KP, *Pseudomonas putida* T1-8, *Micrococcus* sp. L II 61 and *Acinetobacter* sp. P 2(1) and to investigate its combination's effects on the removal of crude oil in nonwoven fabric with different washing times vary from 12, 24 to 36 hours.

MATERIALS

1. Bacteria Cultures
   *Bacillus subtilis*3KP, *Pseudomonas putida*T1-8, *Micrococcus* sp.L II 61dan*Acinetobacter*sp. P 2(1)are owned by the Microbiology Laboratory of Biology Department, Faculty of Science and Technology, Airlangga University.
2. Crude Oil
   Crude oil is owned by the Microbiology Laboratory of Biology Department, Faculty of Science and Technology, Airlangga University which was taken from oil plant in Bojonegoro regency, Indonesia.
3. Media and Other Materials
   The media that was used is synthetic mineral water and molasses (PruthidanComeotra, 1997). Other materials that were used are n-hexane, Tween-20,spirituous, alcohol 70 %, aluminum foil, and cotton
4. Nonwoven Fabric

TABLE 1. Specifications of Nonwoven Fabric

Specification	Detail
Color	White
Raw Material	Meltblown Polypropylene
Basic Weight (g/m^2)	346.3 – 353.8
Thickness (mm)	2.35 – 2.45
Tensile Strength (N/50 mm)	MD: 26.4 – 35.7
Elongation (%)	16 – 22

METHODS

Bio-surfactant Production Media

Synthetic Mineral Water according to Pruthi and Comeotra (1997) contains (NH4)2SO4 (3 g), MgSO4.7H2O (0,2 g), NaCl (10 g), CaCl2 (0,01 g), MnSO4.H2O (0,001 g), H3BO3 (0,001 g), ZnSO4.7H2O (0,001 g), CuSO4.5H2O (0,001 g), CoCl2.6H2O (0,005 g), and Na2MoO4.2H2O (0,001 g) diluted in 1 L of water with pH 7. Furthermore, 4% of substrate molasses (v/v) were then added. After being sterilized, 4.8 mL of nutrient stock of KH2PO4 (1 g), K2HPO4 (1 g) and 4.8 mL of FeSO4.7H2O (1 g),were added aseptically.

Bio-surfactant Production

4% starter of *Bacillus subtilis* 3KP, *Pseudomonas putida* T1-8, *Micrococcus* sp.L II 61, and *Acinetobacter* sp. P 2(1) with OD= 0,5 at λ= 650 nm were added into 100 mL of media in each bottle. Incubate in a shaker with 90 rpm speed for 4 days.Centrifuge for 20 minutes with 450 rpm speed to produce supernatant without cells. This supernatant considered as bio-surfactant product and then used in the treatment.

Characterization of Bio-Surfactant

Characterization of bio-surfactant products was done by measuring the surface tension (ST) (mN/m) and emulsion activity (EA) (%). Surface tension measurement was using Tensiometer Du-Nouy. Pour 10 mL of supernatant into a clean and fat-free petri dish then set to the Tensiometer Du-Nouy. The measured surface tension value is the scale value when the ring detaches from the sample (Ni'matuzahroh*et al.*, 2009). Distilled water and molasses were used as control.Each sample uses 3 replications, and the result was determined using following formula:

$$r = r_o \times \frac{\theta}{\theta_o}$$

where:

r	=	sample surface tension
r_o	=	aquadest surface tension at t°C
θ	=	sample surface tension read from tensiometer
θ_o	=	aquadest surface tension read from tensiometer

Emulsion activity (%) was measured using a method based on Suryatmana*et al.* (2006). Pour 1 mL of supernatant and 1 mL of diesel fuel into a tube, the vortex for 2 minutes.The value of emulsion activity (%) was measured after 1 hour and 24 hours by measuring the emulsion phase layer (cm) compared to the total solution (cm) (PruthidanCameotra, 1997) as follows:

$$\frac{\text{Emulsion layer (cm)}}{\text{Total layer (cm)}} x100\%$$

Preparation of Nonwoven Fabric and Crude Oil

Nonwoven fabric was cut into 3 cm x 1 cm then submerged into crude oil. The weight of crude oil absorbed by nonwoven fabric was measured by detracting the weight after submergence to the weight before submergence. Nonwoven fabric containing crude oil was then put into 50 mL sample bottles with 10 mL of supernatant. All of the sample bottles were put into a shaker according to the treatment of washing time.

Treatment

Tween-20 was used as positive control and distilled water as negative control. Washing time treatments were 12 hours, 24 hours, and 36 hours. During the washing process inside a shaker, samples were kept in an aseptic condition to avoid contamination that could possibly affect the result of crude oil removal.

Crude Oil Removal

Extraction

Extraction was done by using n-hexane which functioned to bind the water phase crude oil. N-hexane was added into the bottle samples. After that, the sample solutions were moved into sample tubes before being vortex-ed for 1 minute. The vortex process resulted 3 layers. Upper layer is n-hexane mixed with crude oil and lower layer is water while emulsion layer in between. Upper layer which contains crude oil was then moved to another tube for the next process, evaporation.

Evaporation

Rotary evaporator was used to separate the n-hexane and crude oil. During the evaporation process at 65° C, n-hexane evaporated while the crude oil that was bound by the n-hexane remained inside the tube. Finally, the volume of crude oil removed from the washing process could be calculated gravimetrically.

Data Analysis

The data of crude oil removal (%) was analyzed statistically using Two Ways Analysis of Varian (ANOVA) (significance degree 5%) and continued with Duncan test to know which group of treatment that has significantly different result among all group of treatments.

RESULT AND DISCUSSIONS

Surface Tension Measurement

Distilled water, molasses, and tween-20 were used as the control group. Based on the Table 2 below, the samples that have the highest result were*Acinetobacter* sp. P 2(1) and *Bacillus subtilis* 3KPvalued 55,8 ± 2,79 mN/m and 55.8 ± 0.87 respectively, while the sample that has the lowest result was *Pseudomonas putida* T1-8 valued 43,4 ± 0,46 mN/m. This means that bio-surfactant produced by *Pseudomonas putida* T1-8 has the highest value of surface tension reduction towards molasses and distilled water valued 16,8 mN/m and 27,56 mN/m respectively, comparable with synthetic surfactant tween-20's surface tension reduction value which are 18.47mN/m and 30.23mN/m, respectively.

Willumsen and Karlson (1997)stated that good bio-surfactant products are shown by their ability to decrease the surface tension value towards its growth media. From the data, it could be concluded that there was activity of bio-surfactant that is able to reduce the surface tension value.

TABLE 2. Result of Surface Tension (ST) Measurement

Materials	Average ST Value (mN/m)	Reduction Value of ST to Molasses (mN/m)	Reduction Value of ST to Distilled Water (mN/m)
Distilled Water	71.96 ± 2	-	-
Molasses	60.2 ± 1.35	-	11.76
Tween-20	41,73 ± 0, 17	18,47	30.23
Bacillus subtilis 3KP	55.8 ± 0.87	4.4	16.16
Pseudomonas putida T1-8	43.4 ± 0.46	16.8	27.56
Micrococcus sp. L II 61	55.3 ± 3.11	4.9	16.66
Acinetobacter sp. P 2(1)	55.8 ± 2.79	4.4	16.16

Emulsion Activity Measurement

Emulsion activity was measured by using the method from Suryatmana*et al.* (2006). The result showed that the emulsion activity value decreased at 24 hours (Table 3). However, the result of emulsion activity measurement showed that there were relatively stable emulsion activities. This indicates that those bio-surfactant products could be categorized as bio-emulsifiers. The occurrence of emulsion activity in the samples was indicated by the forming of foam that creates layers inside the tube. The foam layer was then measured to calculate the value of emulsion activity.

TABLE 3. Result of Emulsion Activity (EA) Measurement

Materials	Emulsion Activity (EA) (%)		Reduction Value of Emulsion Activity (%)
	1 Hour	24 Hours	
Tween-20	47.1 ± 13.99	46.3 ± 13.04	0.8
Bacillus subtilis 3KP	30.09 ± 8.02	20.9 ± 3.66	10
Pseudomonas putida T1-8	35.2 ± 10.87	27.1 ± 13.12	8.1
Micrococcus sp. L II 61	23.6 ± 10.05	20.8 ± 6.78	2.8
Acinetobacter sp. P 2(1)	9 ± 2.57	10.1 ± 1.19	-1.1

Crude Oil Removal from Nonwoven Fabric

Based on the Table 4 below, each control group and treatment group showed positive result in oil removal but with different percentages. Negative control using distilled water had the lowest oil removal percentage among other samples valued 5,2 % at 12 hours, 9,5% at 24 hours and 12,2% at 36 hours. This happened because negative control used distilled water which did not have surface active compound or emulsifier and the removed crude oil was estimated as the result of mechanical process while shaking. Positive control using synthetic surfactant tween-20 had

the highest oil removal percentage among other samples valued 51,3% at 12 hours, 67,1% at 24 hours, and 71,2% at 36 hours.

TABLE 4. Percentage of Oil Removal from Nonwoven Fabric using Different Treatments and Washing Times

Materials	Crude Oil Removal (%)		
	12 Hours	24 Hours	36 Hours
Distilled Water	$5,2 \pm 5^{a}$	$9,5 \pm 10,35^{a}$	$12,2 \pm 3,53^{a}$
Tween-20	$51,3 \pm 10,9^{c}$	$67,1 \pm 10,42^{b}$	$71,2 \pm 26,67^{d}$
Bacillus subtilis 3KP	$36,4 \pm 7,5^{b, c}$	$34,36 \pm 9,3^{a, b}$	$43,8 \pm 1,19^{b, c, d}$
Pseudomonas putida T1-8	$33,4 \pm 11,33^{b, c}$	$44,9 \pm 24,14^{b}$	$54,7 \pm 12,84^{c, d}$
Micrococcus sp. L II 61	$19,7 \pm 1,6^{a, b}$	$37,3 \pm 7,97^{a, b}$	$22,8 \pm 8,48^{a, b}$
Acinetobacter sp. P 2(1)	$50,2 \pm 15,15^{c}$	$65,3 \pm 29,79^{b}$	$35,3 \pm 23,95^{a, b, c}$

The result of *Analysis of Varian* (ANOVA) showed that data of each treatment of crude oil removal has real difference, hence H_o is rejected which means the combination between bio-surfactant product types and washing times indeed affected the crude oil removal. The results of Duncan Test are shown by notations of a, b, c, and d. Every different notation indicates significant difference of the result. However, notation of ab means it has no significance to either a or b.

Bio-surfactant from *Acinetobacter* sp. P 2(1) had the ability in oil removal relatively equal with synthetic surfactant tween-20 at 12 hours and 24 hours and also the highest among other bio-surfactant products. This result correlated with previous research by Widodo (2010) that reported the effectiveness of crude oil mobilization by bio-surfactant of *Acinetobacter sp.* P 2(1) is higher than *Pseudomonas putida* T1-8. Another research by Pratiwi (2012) reported that the characteristics of bio-surfactant from *Acinetobacter* sp.P 2(1) are similar with synthetic surfactant *tween-20* at CMC concentration. However, oil removal percentage by bio-surfactant from *Acinetobacter* sp.P 2(1) decreased at 36 hours. This could be correlated with the instability of the bio-surfactant itself as shown at the emulsion activity result in Table 4.3 where emulsion activity at 24 hours increased, while it was supposedly decreased. Bio-surfactant's instability was presumably caused by incubation time that took too long. Changjun (2014) stated that the optimum incubation time for *Acinetobacterbaylyi*is 12 to 36 hours, while in this research the incubation time was 48 hours and might cause the decreasing of product's quality.

Several other researchers reported different results. Ekpo dan Udofia (2008) reported that some species such as *Pseudomonas aeruginosa, Micrococcus varians,* and *Bacillus subtilis* have the ability to produce bio-surfactants that are able to degrade crude oil up to 97,2%, 85,7% dan 72,3%, respectively at 10 incubation time. This explains that each and every different bacteria has differentoptimum incubation time in producing bio-surfactant. Wei *et al.* (2004) reported that the ability of bio-surfactant in crude oil removal increase as the washing time incerases.

CONCLUSIONS

This research had shown that the bio-surfactant produced by *Bacillus subtilis* 3KP, *Pseudomonas putida* T1-8, *Micrococcus* sp. L II 61, and *Acinetobacter* sp. P 2(1) could be used to remove crude oil form nonwoven fabric. The reduction of surface tension and emulsion activity indicated that these bio-surfactants have the potential in emulsifying hydrocarbon compound of crude oil. The combination between bio-surfactant product types and washing times affected the removal of crude oil where oil removal increases along with the increasing of washing time until it reaches maximum limit. However, the combination showed the best result was produced by *Acinetobacter* sp. P 2(1) at 24 hours, valued 65,3% ± 29,79.

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