serta kondisi optimum jumlah bakteri *B. megaterium* (8.35 log CFU g^{-1}) tercapai pada DOR 0.86:1 dan waktu inkubasi 7 hari. *Oil spill dispersant* (OSD) meningkatkan COD larutan tanah baik pada tanah tidak tercemar maupun tanah tercemar minyak berat.

Kata Kunci: Bioremediasi, *bioslurry*, minyak berat, *oil spill dispersant*, tanah tercemar

INTRODUCTION

Oil industry activities such as exploration, exploitation, processing, and transportation have been increasing since the last decade. This is in line with the increase of human need on petroleum as the main energy source in developing industry, transportation, and households. Data from Central Bureau of Statistics Indonesia (2016) showed that oil consumption in Indonesia in 2015 reached 1.63 million barrels per day (bpd), which increased 15.9% in comparison to that in 2010. The greater the oil production, the greater the potential for environmental contaminations if the oil is spilled or discharged into the environment (soil and water). Oil contamination decreases the quality of soil, such as soil fertility, water holding capacity (WHC), permeability, as well as soil aggregate. This petroleum contamination also causes contamination of ground and surface water (Chithra *et al.* 2014).

Naturally, the environment is able to degrade the contaminants through physical, biological, and chemical processes. However, sometimes the level of contamination in the environment exceeds the ability of soil to naturally degrade these contaminants. As a result, contaminants will accumulate so that human intervention is needed to overcome the problem by using technology (Nugroho 2007). In order to avoid environmental contamination, petroleum waste management can be done with three approaches, namely physical, chemical, and biological approaches. Physical management is a direct waste management, such as filtering and absorption of the contaminants at locations of petroleum contaminated soil. However, this method looks like unable to overcome the petroleum contamination that enter into the soil. Chemical waste management is done by using chemical materials, so that in it will lead to contamination of others, due to the chemicals use. One alternative to overcome the contaminated environment is use of bioremediation techniques. The techniques are environmentally friendly, effective, and economical (Margesin *et al.* 2001; Liu *et al.* 2011; Dindar *et al.* 2013) by application of microbes. This techniques can also reduce the waste petroleum and produces by product of microbes (Jun *et al.* 2015).

Bioremediation techniques can be implemented at both in-situ and ex-situ ways. In-situ bioremediation techniques are generally use to on low contaminated environment, locations that cannot be moved, or on the environment which have characteristics of volatile contaminants. Ex-situ bioremediation is a technique of contaminated soil and water are removed, then treated and processed at special location. This management is save for the environment because of use of microbes to decompose it naturally (Budianto 2008). One of the ex-situ bioremediation management is by applying the technique of bio-slurry.

Bio-slurry use bioreactor such as container or reactors that be used for treatment of liquid or slurry. The slurry bioreactor is not only used to degrade liquid phase forms, but also solid phase wastes such as soil. Advantages of the bioremediation process by using slurry bioreactors are to accelerate mass transfer process between solid and liquid phases; to control parameters of environment, such as: soil nutrients, pH, and temperature to be going well; to maintain level of acceptance of electrons in the reactor easily; and to prevent contamination of intruder microbes.

Oil Spill Dispersant (OSD) is a product that can break down waste oil into small parts so that it can be dispersed naturally (Elvina *et al.* 2016). This product consists of surfactants and several chemicals that were specially formulated to enhance the bioremediation process. Surfactant is an active component that decreases surface tension in the area between oil and water so that it can accelerate oil dispersion (Lidgren *et al.* 2001).

Surfactant and Bioenergy Research Center of Bogor Agricultural University (SBRC-IPB) has successfully developed OSD products derived from palm oil. Oil spill dispersant (OSD) is a combination of anionic surfactant/methyl ester sulfone (MES) and nonionic surfactant/dietanolamide (DEA). The results of previous studies indicated that nonionic surfactants and Linear Alkilbenzena Sulphonate (LAS) reduced oil content in solid phase waste (Charlena 2010) and liquid phase (Charlena *et al.* 2009). Adlina (2016) also stated that OSD derived from palm oil reduced total petroleum hydrocarbon

(TPH) by 91.1% for 6 weeks of the bioremediation process.

Based on above mention, this study aimed to find out optimize and performance test of OSD in the process of bioremediation with using bio-slurry technique on contaminated soil with heavy oil.

MATERIALS AND METHODS

Preparation of Contaminated Soil with Heavy Oil

Petroleum contaminated soil samples were taken from petroleum companies in Sumatra. Soil sampling used randomized sampling method. Initial analysis of petroleum contaminated soils included: soil pH, organic matter (C and N), P and K, exchangeable cation (Ca, Mg, K, Na, cation exchange capacity, and basis saturation), interchangeable Al and H, interchangeable heavy metals (Pb, As, Co, Cd, Cr, Ag, and Sn), oxalate Fe, Al, and Si, and TPH levels. The initial analysis of contaminated soil samples are presented at Table 1.

Preparation of Oil Spill Dispersant

The experiment used OSD produced by SBRC-IPB with materials from anionic and nonionic surfactants derived from palm oil. The anionic surfactant used was 1.5% DEA and non-ionic surfactant was 0.9% MES with a ratio of 7:3 formulation (Adlina *et al*. 2017).

Preparation of Bacterial Inoculum

The experiment used bacteria of *Bacillus megaterium* BM-PFFP (Syakti *et al.* 2013).

Table 1. Initial analysis of soil contaminated with heavy oil samples used in this study.

Parameter	Method/ extraction	Average of contaminated soil
pH (H_2O)	pH meter	5.23
pH (KCl)	pH meter	4.02
Organic matters		
Org-C (%)	Walkley and Black	33.33
Tot-N (%)	Kjehldahl	0.42
C/N		83
P_2O_5 (mg Kg^{-1})	Bray 1	1.93
P_2O_5 (mg Kg^{-1})	HCl 25%	50
K_2O (mg Kg^{-1})	HCL 25%	23.3
K_2O (mg Kg^{-1})	Morgan	16.3
Exchangeable cation	NH_4-OAc pH 7	
Ca (cmol Kg^{-1})		0.21
Mg (cmol Kg^{-1})		0.10
K (cmol Kg^{-1})		0.03
Na (cmol Kg^{-1})		0.08
CEC (cmol Kg^{-1})		6.49
BS (%)		10.67
Exc.-Al (cmol Kg^{-1})	KCl 1N	0.1
Exc.-H (cmol Kg^{-1})	KCl 1N	0.23
Heavy metals		
Pb (mg Kg^{-1})	HNO_3	10.38
Cd (mg Kg^{-1})	HNO_3	0.33
Co (mg Kg^{-1})	HNO_3	6.82
Cr (mg Kg^{-1})	HNO_3	18.35
Ag (mg Kg^{-1})	HNO_3	0.48
Sn (mg Kg^{-1})	HNO_3	0.66
As (mg Kg^{-1})	HNO_3	18.35
Fe (%)	Oxalate	0.11
Al (%)	Oxalate	0.04
Si (%)	Oxalate	0.01
TPH (mg Kg^{-1})	Gravimetry	105000

Table 2. Range and extent of variable test of optimization.

Treatment	Low level (-1)	Center level (0)	High level (+1)
Incubation time (day) (X1)	3	5	7
Dispersant to Oil Ratio (DOR) (X2)	0.5:1	1:1	1.5:1

Bacteria were multiplied firstly in marine broth liquid media and then adapted to mineral media (Zhang *et al.* 2005). The one week grown inoculum was inoculated in soil contaminated with oil samples at a dose of 10% v/w.

Experimental Design

OSD Optimization

Optimization on variable of TPH degradation, pH, and total microbial used response surface method (RSM). Data processing used *Design Expert* of 10.01 with individual variables test which consisted of 3 levels. The details of it are presented at Table 2.

This experiment used an incomplete factorial design with 3 replications so that it met the quadratic model (Montgomerry 1997). The first factor was incubation time, namely: 3, 5, and 7 days; while the second factor was DOR, namely: 0.5:1, 1:1, and 1.5:1. With this procedure, there were 11 experimental units in this experiment. The center value of treatment was the incubation time of 5 day and DOR 1:1. Matrix of unit of experiments on bioremediation optimization of fractional composite designs are presented at Table 3.

Based on both variables test, the quadratic model followed this equation:

$$Y= b_0+b_1x_{1i}+b_2x_{2i}+b_{11}x_{1i}^2+b_{22}x_{1i}+ri$$

Note:

Y : response of each treatment

X : (X1: Incubation time ; X2: DOR)

r : error

b : coefficient

Test of OSD Performance with COD in Soil Solution

Test of OSD performance was carried out using uncontaminated soil without OSD (blank-); uncontaminated soil with OSD (blank+); contaminated soil without OSD (TTM-); and contaminated soil with OSD (TTM+). Furthermore, the measurement of COD was carried out on dissolved oil in soil solution (Clesceri *et al.* 2005).

Bioremediation Application

This experiment used reactor of 500 ml (flask of 500 ml with a working volume of 200 ml) and response surface method (RSM). Contaminated soil samples of 40% (w/v) were treated with OSD, microbial consortium, and a combination of OSD and microbial consortium as shown at Table 3. Cultivation was carried out on a shaker at a speed of 180 rpm, at room temperature, for 7 days.

Table 3. Matrix of unit of experiments on bioremediation optimization of fractional composite designs.

No	Code factor I	Code factor II	Factor I Incubation time (day)	Factor II DOR
1	-1	-1	3	0.5:1
2	-1	+1	3	1.5:1
3	+1	-1	7	0.5:1
4	+1	+1	7	1.5:1
5	0	0	5	1:1
6	0	0	5	1:1
7	0	0	5	1:1
8	1.414	0	7.8	1:1
9	-1.414	0	2.2	1:1
10	0	1.414	5	1.7:1
11	0	-1.414	5	0.3:1

Table 6. Analysis of variance of total population *B. megaterium.*

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	3.25	5	0.65	28.29	0.0002
A-Time (day)	3.10	1	3.10	135.18	< 0.0001
B-DOR	2.481E-003	1	2.481E-003	0.11	0.7519

Bacterial Population Growth

Analysis of RSM on response of total microbes with incubation time and DOR factors followed this equation:

$$Y=786+0.62X1+0.018X2-0.054X1X2-0.13X1^2-0.036X2^2$$

Note :
Y : Total microbes
X1 : Incubation time (days)
X2 : DOR

Model analysis result showed that determinant coefficient value of R^2 was 0.9528. This indicated that 95.28% of total microbe variance results were due to treatment variables. F-value of the model from analysis of variance showed significant results at probability of 0.0002. Likewise, F-value of incubation time showed a significant result at probability of <0.0001 (Table 6). This indicated that incubation time treatment and its combination with DOR (model) significantly affected the level of total population of *B. megaterium*.

Total microbe of *B. megaterium* response surface of optimization results both DOR and incubation time are presented in Figure 3. It showed that optimum conditions were reached at 0.86:1 DOR and 7 day of incubation time with a total microbe of *B. megaterium* value of 8.35 log CFU g^{-1}. This indicated that there was an increase in the population of *B. megaterium* bacteria due to incubation time and its combination with DOR. The increase in the number of bacteria was an indication that the bacteria grow well by consuming hydrocarbons as a carbon source for the purposes of their growth and development. This was explained by Liado *et al*. (2012) and Benedek *et al.* (2013) which stated that hydrocarbon decomposition bacteria utilize the contaminant of petroleum as their carbon source.

In addition, experiment results of Eun-Hee *et al.* (2011) also showed that the *Bacillus megaterium* could grow well up to 50% (v/v) of oil sludge concentration. Both indigenous and exogenous bacteria were important factors in the process of oil bio-decomposition (Aler *et al.* 2014). All showed that *B. megaterium* was a great potential to decompose petroleum contaminants.

Chemical Oxygen Demand (COD) in Soil Solution

F-value models of analysis of variance showed that the results were not significant (data not shown). Nevertheless, value of COD in soil solution of contaminated soil with OSD treatment was 2,732 mg L^{-1}, higher 3.8 fold compared with contaminated soil without OSD treatment (720 mg L^{-1}) even 6.36 fold as much compared to the uncontaminated soil with OSD (430 mg L^{-1}) (Figure 4). This showed that addition of OSD could dissolve oil into the soil solution. Dissolved oil in soil solution could increase soil COD value. It means that there was an increase level of hydrocarbons in soil solution, so amount of oxygen needed to oxidize hydrocarbons to CO_2 and H_2O increased. Chemical oxygen demand was amount of oxygen required to oxidize organic materials chemically (APHA 1992). The high content of COD indicated the high number of organic matters in a solution.

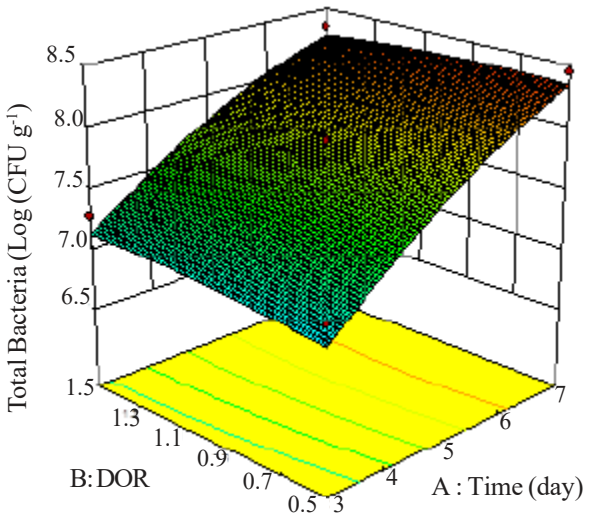

Figure 3. Total microbe response surface.

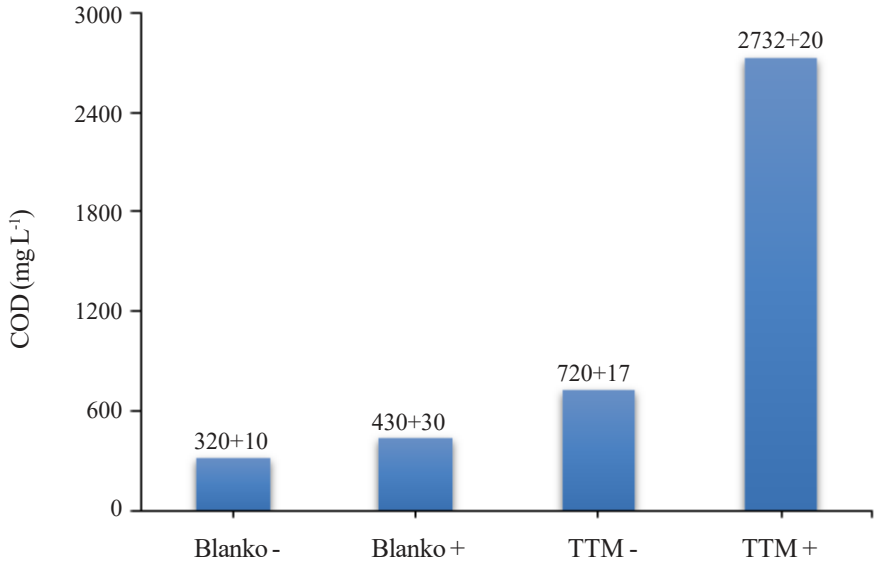

Figure 4. Chemical oxygen demand (COD) in solution of contaminated soil treated with OSD. * Blank- : Uncontaminated soil without OSD; Blank+: Uncontaminated soil with OSD; TTM-: Contaminated soil without OSD; TTM+: Contaminated soil with OSD.

Charlena *et al.* (2009) believed that solubility of oil in water increased COD value of petroleum waste. Suardana *et al.* (2002) also reported that waste of petroleum treated with surfactant improved solubility of oil in water due to dispersion of oil into smaller particle. The oil from pores of contaminated soil could be released and moved into soil solution so that oil content in the soil solution increased and soil COD value also increased.

In addition, application of OSD into the soil also increased soil COD value (Figure 4). The COD value of uncontaminated soils without OSD (320 mg L^{-1}) was much lower than those of uncontaminated soils with OSD (430 mg L^{-1}). The application of OSD increased soil COD because OSD SBRC-IPB itself was an organic compound derived from palm oil, so that number of organic compounds to be oxidized (COD) increased. Elvina *et al.* (2016) stated that application of OSD increased value of COD in solution due to their dispersion of OSD in seawater.

CONCLUSIONS

The treatment of incubation time and its combination with DOR significantly reduced soil TPH, increased soil acidity, and increased soil total *B. megaterium*, but did not significantly affect on COD in soil solutions. Optimization of OSD with RSM showed that the higher DOR of OSD and the longer of incubation time, the higher also the rate of biodegradation of TPH. The optimum conditions were reached at DOR of 1.16:1 and incubation time of 7 days that were able to degrade soil TPH of 54.30%. The optimum conditions of soil pH (8.825) was reached at DOR of 1:1 and incubation time of 5 days, as well as the optimum conditions of *B. megaterium* (8.35 log CFU/g) was reached at DOR of 0.86:1 and incubation time of 7 days. Oil spill dispersant (OSD) increased COD in soil solution in both uncontaminated and contaminated soils with heavy oil.

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