The Efficacy of Bioaugmentation on Remediating Oil Contaminated Sandy Beach Using Mesocosm Approach

Yeti Darmayati[1,4*], Harpasis S. Sanusi[2], Tri Prartono[2], Dwi Andreas Santosa[3] dan Ruyitno Nuchsin[1]

[1]Research Center for Oceanography, Indonesian Institute of Sciences, Jakarta
Jln. Jend. Gatot Subroto 10, Jakarta 12710 Indonesia
[2] Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, Bogor, Indonesia
Jln. Agatis, Kampus IPB Dramaga Bogor 16680 Indonesia
[3]Faculty of Agriculture, Bogor Agricultural University , Bogor, Indonesia
Jln. Agatis, Kampus IPB Dramaga Bogor 16680 Indonesia
[4]School of Post Graduate Studies, Bogor Agricultural University, Bogor, Indonesia
Jln. Agatis, Kampus IPB Dramaga Bogor 16680 Indonesia
Email: yeti.darmayati@yahoo.com

Abstract

Bioremediation is basically consists of two approaches, biostimulation and bioaugmentation. The efficacy of bioaugmentation for combating oil pollution in field application is still argued. The purpose of study was to evaluate the efficacy of bioaugmentation and to compare the affectivity of single strain and consortium application in remediating oil polluted sandy beach. Experimental study in a field has been conducted with two (2) treatments and one (1) control in three different plots. The treatmens were introduction of a single strain (Alcanivorax sp TE-9) and a consortium (Alcanivorax sp. TE-9, Pseudomonas balearica st 101 and RCO/B/08-015) cultures into oil contaminated sediment. The experiment in mesocosm approach was taken place in Cilacap coast. Arabian light crude oil was used in the concentration of 100.000 mg.kg^{-1} sediment. Changes of oil concentration, bacterial density and pore water quality have been monitored periodically for 3 months. The result showed that oil degradation percentage and bacterial growth in both treatments were higher than in control. After 3 months, the percentage of oil degradation experiment in control, single strain and formulated consortium treatments were observed at 60.4%, 74.5% and 73.5%. It proves that bioaugmentation tehnique can enhance significantly oil biodegradation in sandy beach. The applications of bacteria in single or consortium culture give no different impact on their affectivity for bioremediation in Cilacap sandy beach. By data extrapolation it can be predicted that both of treatments able to reduce remediation time from 210 days into 135–137 days. Bioaugmentation can be proposed as a good solution for finalizing oil removing in Cilacap sandy beach when oil spilled occurred in this environment.

Keywords: Bioremediation, bioaugmentation, oil, sandy beach, Alcanivorax, mesocosm, Cilacap

Abstrak

Efikasi Tehnik Bioaugmentasi dalam Memulihkan Pantai Berpasir Tercemar Minyak Menggunakan Pendekatan Mesokosm

Bioremediasi pada dasarnya terdiri dari dua pendekatan yaitu biostimulasi dan bioaugmentasi. Teknik bioaugmentasi dalam menanggulangi pencemaran minyak di lapangan masih diperdebatkan efektivitasnya. Penelitian ini bertujuan untuk mengevaluasi efikasi tehnik bioaugmentasi serta membandingkan efektivitas kultur tunggal dan konsorsium dalam memulihkan pantai berpasir tercemar minyak. Studi eksperimental di lapangan telah dilakukan dengan menggunakan dua perlakuan dan satu kontrol di tiga plot berbeda, Perlakuannya adalah penambahan bakteri kultur tunggal (Alcanivorax sp TE-9) dan bakteri konsorsium (Alcanivorax sp. TE-9, Pseudomonas balearica st 101 dan RCO/B/08-015) ke dalam sedimen yang tercemar minyak. Eksperimen dengan pendekatan mesokosm dilakukan di pantai Cilacap. Minyak mentah ringan Arabia dengan konsentrasi 100.000 mg.kg^{-1} sedimen digunakan sebagai bahan cemaran. Perubahan konsentrasi minyak, kepadatan bakteri dan parameter lingkungan diamati secara periodik selama 3 bulan percobaan. Hasil penelitian menunjukkan bahwa persentase degradasi minyak dan densitas bakteri di sedimen perlakuan lebih tinggi daripada kontrolnya. Setelah 3 bulan eksperimen, persentase degradasi minyak pada kontrol, perlakuan kultur tunggal dan konsorsium masing-masing teramati 60.4%, 74.5% and 73.5%. Hal ini membuktikan bahwa

tehnik bioaugmentasi secara signifikan mampu meningkatkan biodegradasi minyak di pantai berpasir. Pemberian mikroba dalam bentuk kultur tunggal ataupun konsorsium mempunyai efektivitas yang tidak berbeda untuk proses bioremediasi di pantai berpasir Cilacap. Dengan ekstrapolasi data dapat diduga bahwa kedua perlakuan bioaugmentasi ini mampu mempercepat waktu pemulihan lingkungan dari 210 hari ke 135-137 hari. Bioaugmentasi dapat diusulkan sebagai solusi yang cukup baik untuk menghilangkan minyak pada tahap akhir pembersihan pantai berpasir Cilacap, jika terjadi tumpahan minyak di lingkungan ini.

Kata kunci: *single strain, consortium, bioaugmentation, oil, bioremediation, sandy beach, Alcanivorax, Cilacap*

Introduction

Indonesian marine waters is highly risk on oil spill disaster. It is due to marine Indonesian waters in one of prime routes for world oil transportation and here was located 82 sites of oil exploration which was located in the near shore and offshore. Cilacap coastal is one of the areas most often spilled by oil. Rely on data from various sources, there were 16 cases of oil spills occurred in Cilacap area during the period 1989-2011. The reasons were 12 times due to tanker accidents and 4 cases due to leaked pipes (Mauludiyah, 2012). Oil pollutants pose a serious and wide range threat to fishery, marine habitats of wildlife, human health, and destroy the ecological balance which may take years or even decades to recover (Zhang *et al.,* 2011). These environmental risks are due to organic compounds mixture in oil with different characteristic and properties, e.g. polycyclic aromatic hydrocarbons (PAHs). PAHs compounds have characteristic as toxic, persistent, bioaccumulative, mutagenic and carcinogenic for biota and human (Zakaria *et al.,* 2009). Therefore, it is very urgent to develop an effective clean-up method for reducing the impact of oil spills on marine life. Greater understanding of microbial biodiversity and development of bioengineering, bioremediation is one of the best, effective and economist solutions in an integrated environmental restoration effort (Philp *et al.,* 2005).

Oil degrading bacteria in Indonesian marine environment was ubiquitous (Harwati *et al.,* 2007, Darmayati, 2009a). However, bioremediation study on coastal and marine environment in Indonesian is very limited. It is still in the stage of research in laboratory level and very limited in controlled field experiment (Syakti *et al.,* 2008, Darmayati, 2009b; Murniasih *et al.,* 2010, Darmayati *et al.,* 2015).

Bioremediation can be applied basically in two approaches: biostimulation and bioaugmentation. In biostimulation, the growth of indigenous hydrocarbon degraders is stimulated by the addition of nutrients to increase biodegradation rates. In bioaugmentation, degradation rate is boost by the addition of single strains or consortia of hydrocarbon–degraders bacteria. Several hydrocarbon degraders from Cilacap, which is oil chronic pollution area has been isolated and characterized, also their capability has been studied. Bioaugmentation has been reported expressed a good result from some laboratory works (Syakti *et al.,* 2008, Murniasih *et al.,* 2010). However, there is no information on the efficacy of this method neither for Indonesian marine environment nor Cilacap coastal area on the field. Therefore, the purpose of study is to evaluate the effectiveness of bioaugmentation and to compare the efficacy of single strain and consortium bacteria for oil bioremediation in Indonesian tropical sandy beach in the field. This research has been done by mesocosm approach as bridging to provide practical information about its efficacy.

Materials and Methods

Site description

This research was conducted at a sandy beach in the area between high and low tide in Cilacap coastal shoreline, along the mouth of Donan river estuary in Cilacap region .Cilacap region lies on the south of Java In a downstream area was located Cilacap industrial estate, harbor for oil refinery and other industries which potentially contribute oil to the environment. The experimental site is estuarine sheltered area which protected by Nusakambangan island. It has semi diurnal tidal pattern.

Sediment preparation and spiking

Seashore sand uncontaminated by oil was collected (surface layer: 0–15 cm) from the north coast of Nusakambangan Island. Prior to spiking, the sandy sediment was air-dried for 24 hr and then homogenized by sieving to 8 mm. Moisture content was determined through oven drying at 105°C for 24h. Total oil concentration was determined gravimetrically (US EPA, 1999).

For experimental work, certain volume of Arabian Light crude oil (ALCO) was applied by sprayer into sediment to provide 100,000 ppm (w/dw) oil polluted sediment and then homogenized thoroughly. ALCO is a major crude oil used as raw material for Cilacap Refinery, so that it is the most

potential to be spilled in this environment. The characteristic of ALCO was presented in Table 1. Oil polluted sediment was put outdoor for 5 days to allow weathering. Oiled sediment was prepared in the volume of 0.3375 m^3 for 9 mesocosm box. Each box was filled by 0.0375 m^3 oil-polluted sediment, was 50 x 50 x 15 cm in dimension. Tilling up to 15 cm depth in each plot was conducted once a week throughout the experiment (Darmayati *et al.,* 2015).

Bacterial culture preparation

Three selected strain cultures were obtained from Research Center for Oceanography Culture Collection, Indonesian Institute of Sciences (LIPI), which were *Alcanivorax* sp. TE-9, *Pseudomonas balearica* st 101 and RCO/B/08-015. These strains were collected from Jakarta ports. They have capability to degrade Poly Aromatic Hydrocarbon (PAH) and crude oil (Darmayati, 2009b). According to preliminary observation using microcosm approach on seven (7) different strains, *Alcanivorax* sp. TE-9 and consortium B (mixture of the three strains) showed the best performer on oil degradation activity (unpublish data). Thus, we selected those strain for this study. Prior treatment in field experiment, acclimation to 100.000 ppm of oil has been conducted (Darmayati *et al.,* 2015).

Experimental design

A controlled application of petroleum hydrocarbon on a sandy beach was used to test the efficacy of bioaugmentation and to compare two biaugmentation strategies for enhancing oil-biodegradation process. The strategies were addition of Single Strains (SS) and Formulated Consortium (SC). Intrinsic remediation was used as a control. Each treatment was conducted in triplicate. Tilling were applied once a week to add oxygen and to homogenized the sediment. A randomized complete block design with three blocks was established, with three plots assigned per block. Plot elevation and location relative to Donan river served as blocking criteria. Treatments were assigned randomly to plots within a block such that each block would have all the three potential treatments. The plots assigned were presented in Figure 1. Three blocks were established, the distance between blocks was 6 meter and between plots (treatments) was 2 meter (Xu *et al.,* 2005)

Basic design and construction of mesocosm

Mesocosms were constructed with 4 unit bamboo walls coated by transparent plastic in the size of 50x50x170 cm. The top and bottom were opened to allow seawater in and out from the bottom, also sunlight in for photo oxidation occurred. To protect the mesocosm from disturbance, the outside of enclosure structure was covered by thick plastic and in the surrounding was build bamboo edge which supported by sand sacks.

The enclosure structures (plots) were inserted 30 cm to the bottom. The upper part constructions were 140 cm in height to protect the oil to spill the environment when high tide period occurred .A clean strip of 25 cm between the sediment treated and outer of enclosures and also clean base of 40 cm was provided to eliminate the impact of oil background concentration from surrounding area. It was conducted by removing of 100 cm x 100 cm x 55 cm sediment insitu, then it was replaced by Nusa Kambangan clean sandy sediment and oil polluted sediment. Treated oil polluted sediment was spiked into enclosure plots in the volume of 50 x 50 x 15 cm mesocosm box was lied on 1 x 1 x 0.4 m oil uncontaminated sand base from Nusakambangan Island (Figure 2). A sediment characteristic of the base was similar with the experimental used. It was collected and treated similarly, except no oil addition.

In the middle of each plot was constructed a pore water sampler. It was made from a hose protected by PVC paralon with small holes in 5–10 cm of the end part which allowed filtered pore seawater from sediment treated collected. It was immersed in 10 cm of depth during experiment.

Sampling strategy

Periodical sampling was conducted on 0, 3, 8, 16, 23, 60 and 90 days after treatments. Sampling were conducted before high tide (water height was about 5 cm inside plot). The samples were sediment and pore water. Composite sediment sample for oil and microbial analysis were collected from 5 sampling points in each plots. The sample then separated into an oil-free glass (100 gram) for oil analysis and 50 ml sterile falcon tube for microbial analysis. All samples were transported into laboratory in a cool box.

Pore water sample was collected for nutrient and environment analysis by syringe cautiously to avoid oxygen contamination and transferred into 2 x 50 ml airtight bottle. The sample in the first bottle was filtered and prepared for total nitrogen (N) and phosphate (P) analysis. The second was used for environmental analysis. All samples were transported into laboratory in a cool box.

Table 1. Phycicochemical Characteristics of Arabian Light Crude oil used

| No	Parameters (Units, Analysis Methode)	Crude Oil (ALCO)	Naphtha Fraction	
			Light	Heavy
1	API Gravity at 60/60°F (-, ASTM D 1298)	33.3		
2	Sulphur content (mass %, ASTM D 1552)	2.24		
3	Nitrogen content (mass ppm, ASTM D 3228)	ND		
4	Nickel (Ni) (mass ppm, IP 470)	2		
5	Vanadium (mass ppm, IP 470)	<1		
6	Hydrocarbon Types			
	Saturates (vol. %, ASTM D 5134)		99.3	83.9
	Aromatics (vol. %, ASTM D 1319)		0.6	16
	Olefins (vol. %, ASTM D 1319)		0.1	0.1
	Resin & Asphaltenes (-,-)		NA	NA
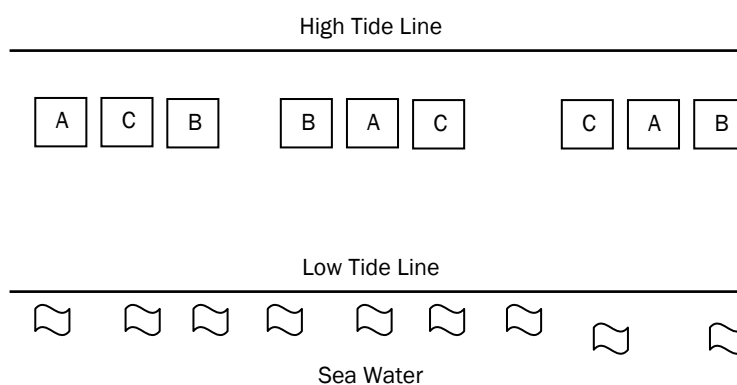
Figure 1. Plot layout on inter-tidal foreshore of Donan River bank on a randomized complete block design
A = Control, B = Single strain and C = Consortium

Sample analysis

Total Petroleum Hydrocarbon (TPH) was determined gravimetrically (US EPA, 1999). Sample was prepared by putting a 2 g of subsample homogenized sediment into glass tube. Extraction was conducted by using maceration method with the mixture of Dichloromethane: n-hexana (1:1) in pro-analysis grade as a solvent. Na_2SO_4 was used to absorb remained water in samples.

Subsample sediments from each plot were processed for enumeration of total cell bacteria. It was prepared by mixing 1 g wet weight of sediment into dilution water containing 9 ml seawater sterile. Then, this solution was placed in vortex at 300 rpm for 15 minutes to detach bacteria from sediment. Dilution of this subsample was used for enumerating total cell bacteria by using Acridine orange direct count (Hobbie *et al.,* 1977). To monitor environmental condition changes in sediment during experiment, measurement of pore water salinity, pH, Dissolved Oxygen (DO) and Oxidation-reduction potential (orp) was carried out immediately after sampling by using hand-refractometer, pH meter, DO meter and ORP meter, respectively.

Experiment was conducted using three independent replicates. Data were subjected to analysis of variance and the averages were compared by Duncan multiple range test at $P \leq 0.05$.

Results and Discussion

Pore water quality inside enclosed plots and the surroundings were different during experiment (Table 2). This showed that oil pollution and treatment applied showed an impact on pore water quality, beside the construction of mesocosm itself. Pore water quality in surrounding plots during experiment was relatively constant in the average 6.93, 30.2 ppt, 4.16 mg.L^{-1} and 65.25 mV for pH, salinity, DO and ORP, correspondingly. The impact of construction was detected. The occurrence of oil spill and process of oil degradation have changed
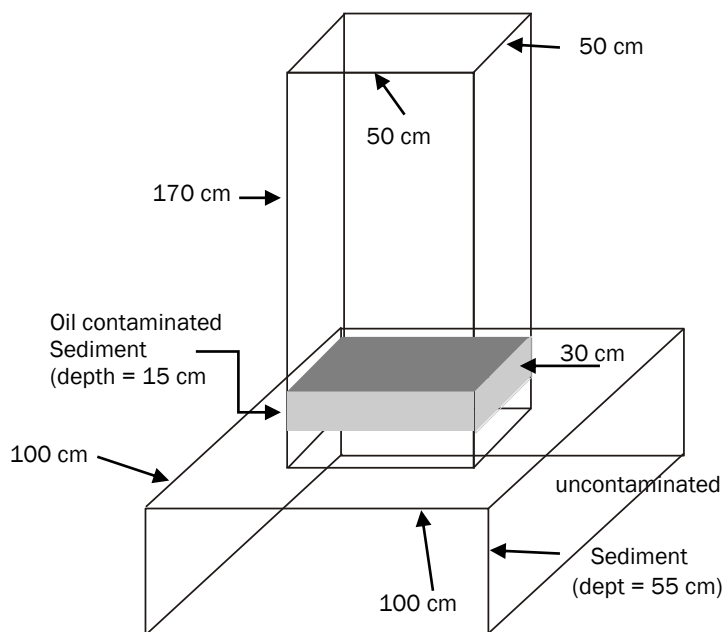
Figure 2. Schematic of mesocosm

Table 2. Pore water quality during 90 days experiment (Mean ± SD, range)

Environment Parameters	Enclosured			Outside enclosure
	Control	Single strain	Consortium	
Dissolved Oxygen (mg.L^{-1})	1.58 ± 0.96 (0.57–2.96)	1.41 ± 0.78 (0.68 – 2.97)	1.58±1.00 (0.46–3.14)	4.16 ± 1.11
Oxidation- Reduction Potensial (mV)	(-35) ± 241 [(-518) – 151]	(-12) ± 191 [(-446) – 155]	(-6) ± 208 (-484) – 174)	63± 36 (22 – 120)
pH	6.77 ± 0.18 (6.55 – 7.30)	6.61 ± 0.13 (6.30 – 6.75)	6.62 ± 0.09 (6.40 – 6.81)	6.93 ± 0.32 (6.61 – 7.39)
Salinity (ppt)	33.62 ± 7.53 (13 – 38)	33.29 ± 4.92 (16 – 38)	33.92 ± 5.42 (15 - 36)	30.20 ± 2.59 (27 - 34)
Total Nitrogen (mg.L^{-1})	2.43 ± 0.83 (1.50 – 4.60)	3.43 ± 1.62 (0.60 – 6.77)	2.71 ± 1.12 (0.70 – 4.60)	1.54 ± 0.96 (0.40 – 3.00)
Total Phosphate (mg.L^{-1})	0.25 ± 0.19 (0.03 – 0.75)	0.15 ± 0.08 (0.02) – 0.25)	0.15 ± 0.14 (0.02 – 0.62)	0.21 ± 0.16 (0.1 – 0.49)

the environment condition. Salinity was slightly increased from the beginning to 23 days after treatment (30.33–36.5 ppt), then stable until the 60th days. In the last observation, the salinity was dropped due to 3 days rain before sampling time (16.00–29.00 ppt).

Experiment has been conducted in a dynamic condition. Pore water quality in all treatment during experiment were relatively have similar pattern, but it was in different level of concentration. The value of pH during experiment was relatively constant between 6.34 to 6.81, except in the 8th days after treatment in the control plots (7.30). This was still in

an optimal condition for biodegradation occurred. Bioremediation can be occurred in wide range of pH value which is 5–9, however the optimal condition was 5.6–8.5.

Oxygen has an important role as an acceptor electron to activate substrate in oxygenase reaction in aerobic condition (Widdel and Rabus, 2001). In this study, DO and ORP has a good correlation (r= 0.68) and fluctuated between 0–23 day after treatment (DO= 1.03–2.97 mg.L^{-1}; ORP= 77.67–148 mV). DO was long-standing at 0.57-0.83 ppm since 60th days to the end of experiment. However, ORP was radically dropped into negative value since

the 60th days observation. ORP value in the 60th and 90th days after treatment was in the range of -55 to -160 mV and - 378.67 to - 499.33 mV, respectively. DO and ORP value was detected in low value at mesocoms plots since the beginning of the experiment, it caused the measurement was conducted at 9 hours after oil polluted sediment inserted into mesocosm.

Nutrient in the form of N and P total was varied in concentration. N and P total during experiment was observed in the range of 0.60–6.77 mg.L^{-1} (2.89$\pm$1.31, n= 51) and 0.02–0.75 mg.L^{-1} (0.19$\pm$0.14, n= 50), correspondingly. Nutrient concentration trend in control and treatment was slightly increased simultaneously with time, except P total concentration in single strains treatment. Although, correlation between N total and P total concentration was not significant with time. Coefficient correlation (r) between N total concentration with time in control, single strains and consortium treatment was 0.19, 0.17 and 0.25, respectively. Whereas, the r value for P total concentration was even lower which were 0.08, 0.15 and 0.06 mg.L^{-1} (Table 2).

The difference of growth pattern and biomass of bacterial cells in control and bioaugmentation application were observed (Figure 3.). In control, the growth period was longer (up to 60th days after test initiation) but the cells number was lower than bioaugmentation treatments (0.2–1.6x10^8 cells.mg^{-1}). In bioaugmentation (single strain and consortium treatment), the growth was only observed between 0–23 days after treatment. Bacterial cells was

observed in higher number (0.4 - 4.41 x 10^8 cells.mg^{-1}) in single strains and 0.5 – 3.06 x 10^8 cells.mg^{-1} in consortium treatment, respectively. The increase of oil concentration and biodegradation activities creates nutrient limitation and environmental changes. The increased of C source (by oil spilled) will enhanced the growth of bacteria. Then, the DO value in the environment will dropped caused by bacterial oxygen consumption. The growth will not be long when the environment is unfavourable.

Oil degradation experiment was occurred in different rate in term of time and treatment. Rapid depletion occurred between 0–23 days. The depletion slightly decreased between 23-60 days. After that, there were almost no changes on oil concentration (Figure 4). It was concomitant with bacterial growth observed. There was a strong correlation between oil degradation and bacterial number (r=0.55). This phenomenon was also occurred in biostimulation-only or combination of biostimulation bioaugmentation treatments (Chang and Miles, 2004; Darmayati *et al.,* 2015). The first month (0-23 days), almost 70% oil was degraded in treatment and 50% in control plots. However, after that oil was degraded very slowly (about 10%) until the end of experiment (3 months). The level of reduction was higher in treatment than control. It may caused by the availability of carbon and energy source, also environmental conditions (namely temperature, salinity, oxygen and nutrients) was optimum at 0-23 days after initiation of experiment. Nikolopoulou *et al.*, (2013) mentioned that the
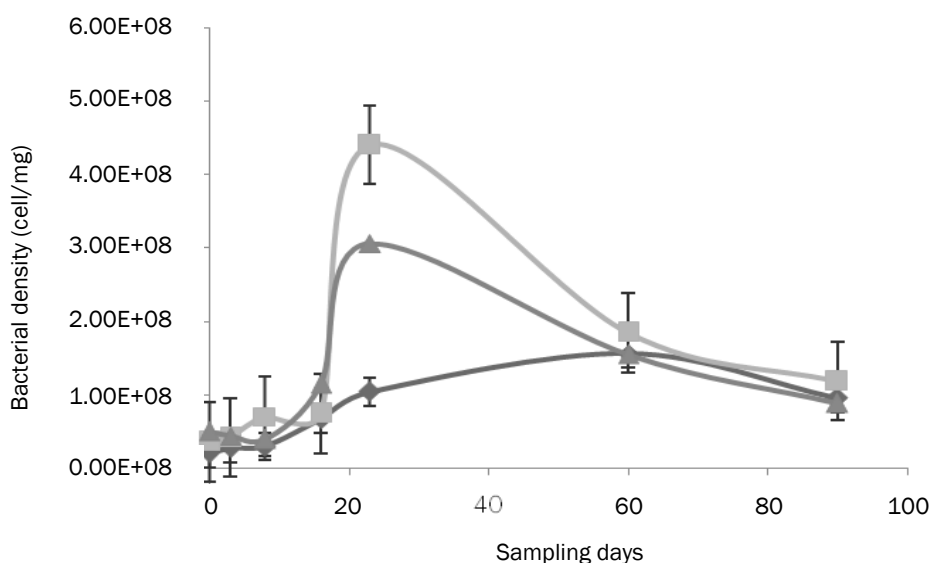

Figure 3. Bacterial Growth during 90 days experiment. Standard deviation of the replicates (n-3) are denoted by the error bar

Note : ◆ = Control ▲ = consortium ■ = Single strain

success of oil spill bioremediation depends on the establishment and maintenance of physical, chemical and biological conditions that favor enhanced oil biodegradation rates in the marine environment.

Constant degradation rate (k) during 90 days experiment was varied between treatments and periodical times (Table 3). k value in control was lower than the two treatments. k value in control was significantly different with single strain and consortium with P= 0.005 and 0.031, respectively. However, the k value was no significant different between Single Strain and Consortium application. k value was also varied between periodical times (Table 4). It was concomitant with specific growth rate (μ) of bacteria observed. In general, the highest value was observed at the period of 16-23 days in Single Strain treatment (k= 0.111). The highest value in control, consortium and single strains treatment, were observed at 0–3 days, 8-16 days and 16–23 days after after experiment initiation, respectively. This strong correlation was shown in the coefficient correlation between k and μ value is 0.56.

Natural oil degradation in Cilacap sandy beach was considered as high if we compared with other previous studies in California and Hongkong coastal (Bento *et al.,* 2005). This high intrinsic capability may be caused by several factors, such as environmental conditions, oil characteristics and the availability of oil-degrading microbes in this coastal area. Arabian light crude oil was predominant oil

used by Cilacap oil refinery unit. Therefore, the site exposed prior to this oil and the native microbes might have capability to degrade the contaminant. Biological degradation processes depends on several factor such as environmental conditions (pH, temperature, oxygen, degree of acclimation, accessibility of nutrients), number of microorganism, type of microorganism, cellular transport properties, chemical partitioning in growth medium, and chemical structure of compounds degraded (Coartes *et al*., 2009; Lin *et al.,* 2010).

This field evaluation using mesocosm approach showed that bioaugmentation in oil polluted sandy beach was enhanced affectively oil degradation. Although the impact of differences on the amendment in single strain or formulated consortium was not significantly different. Total oil depletion was observed in all plots, control and treatments throughout experiment (Figure 4). However, Oil degradation in bioaugmentation treatments showed a significantly faster rate than the control. When 100,000 mg.kg^{-1} oil artificially spilled into Cilacap sandy beach, oil degraded naturally about 60% (60.31± 3.93%) over 90 days. The degradation percentage of oil in single isolates (74.47±6.24%) and in consortium formulated amendment (73.91±0.62%) was significantly higher than in control. This showed that exogenous bacteria added were synergistically metabolized Arabian light crude oil with indigenous bacteria from this environment. This study confirmed that bioremediation technology can be applied to make

Table 3. First order rate constant k (day^{-1}) in certain periodical time (±= Standard deviation of the replicates (n-3))

Periode	Control (Bioattenuation)	Single strains	Consortium
0 - 3 d	0.024 ± 0.008	0.029 ± 0.016	0.036 ± 0.016
3 - 8 d	0.009 ± 0.007	0.049 ± 0.031	0.015 ± 0.011
8 - 16 d	0.037 ± 0.017	0.013 ± 0.010	0.031 ± 0.021
16 - 23 d	0.037 ± 0.11	0.111 ± 0.019	0.1 ± 0.21
23 - 60 d	0.006 ± 0.003	0.004 ± 0.002	0.003 ± 0.002
60 - 90 d	0.001 ± 0.001	0.0004 ± .001	0.002 ± 0.001
0 – 90 days	0.0104 ± 0.001	0.0154 ± 0.003	0.0148 ± 0.0003

Table 4. Specific Growth Rate of bacteria (μ) over experiment in Different Treatment

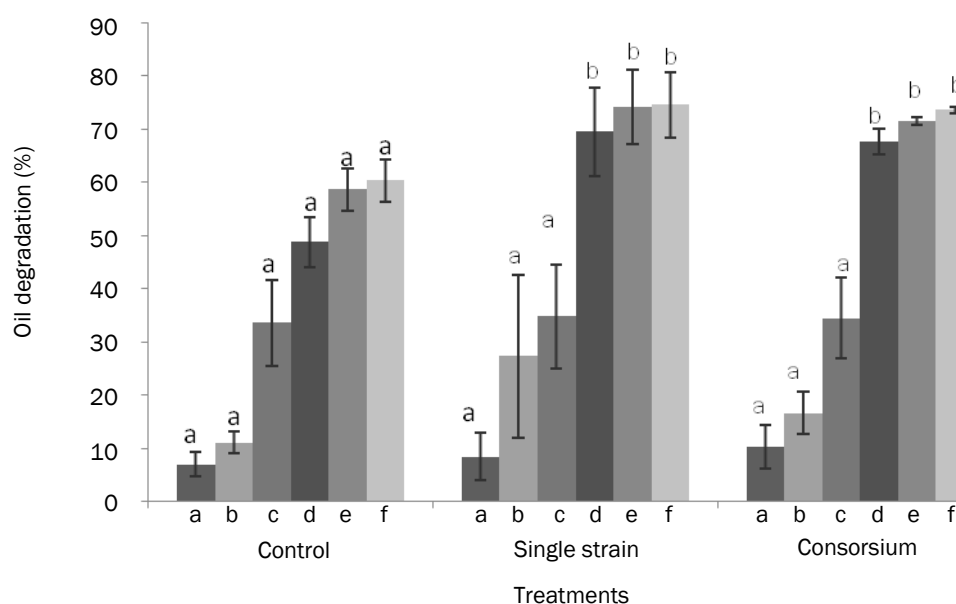| Treatments	Sampling Periodical Time (days)					
	0-3	3-8	8-16	16-23	23-60	60-90
Control	0.101	0.023	0.102	0.026	0.008	-0.012
Single Strain	0.069	0.095	0.009	0.103	-0.022	-0.016
Consortium	0.013	-0.018	0.132	0.057	-0.018	-0.018

Figure 4. Oil Degradation Percentage during 90 days Experiment indifferent Treatments. Standard deviation of the replicates (n-3) are denoted by the error bar. **Note :** a = 3d, b = 8d, c = 16d, d = 23d, e = 60d, f = 90d

oil-clean up faster in Cilacap coastal. Although, previous study proved that the addition of nutrient only and nutrient plus microbes has a better result in increasing oil biodegradation rate (Darmayati *et al.,* 2015).

Alcanivorax and Consortium B (mix of *Alcanivorax*, Pseudomonas and RCO/B/08-015 cultures) have been showed the best result among others in our previous laboratory experiment. Microcosm experiment in laboratory over 28 days showed that *Alcanivorax* sp. TE-9 degraded ALCO until 65% and Consortium B was 44.3%. Microcosm was conducted by using sediment and seawater from Cilacap (unpublished data). Kasai *et al.* (2002) explained that *Alcanivorax* has ability to use a wide number of compounds of the oil as preferred energetic and nutritional source and produced lipidic biosurfactant which increases the availability of hydrocarbons of the oil for the organism. Therefore, *Alcanivorax* not only degrades oil hydrocarbons in vitro, but seems to play a crucial role in the natural cleaning of oil-polluted marine systems. The unusual physiology and metabolic capability for hydrocarbon substrates, and the potential for biotechnological applications, make bacteria related to the *Alcanivorax* genus an interesting promise for bioremediation and lead to the basis of novel biotechnology strategies to accelerate oil degradation (Capello *et al.*, 2007).

By data extrapolation, we can predict the time to achieve the oil concentration into less than 1% (Indonesian government regulation, 2008). By natural process, it will be needed 210 days (7 months). Whereas, by application of single strain *Alcanivorax sp TE-9* culture and consortium B culture will reduce the remedial time into 137 days (4.6 months) and 135 days (4.5 months), respectively. Subaphol *et al.* (2006) by using microcosm approach found a slightly different result that selected consortium more effective than single isolates at degrading lubricant oil in both liquid media and in contaminated sand. It may caused by the variability of indigenous oil degrading bacterial community in Cilacap sandy beach was high and effective enough. Syakti *et al.* (2008) has mentioned the availability of PAHs degrading bacteria from sediment of mangrove swamps in Cilacap such as *Flexibacteraceae bacterium, Bacillus aquamaris, B. megaterium, B. pumilis,* etc. Lisdiyanti (2008) also isolated some oil degrading bacteria such as *Thalassospira profundamaris* Wp0211, *Rhodospirillaceae bacterium* PHp3 and *Sphingomonas bacterium* E4A9 from Cilacap waters. Therefore, the addition of bacterial number was more important than bacterial diversity.

Conclusion

Bioaugmentation in oil polluted sandy beach enhanced effectively oil degradation. The impact of differences on the amendment in single strain or formulated consortium was not significantly different on their efficacy. The condition of bioremediation site will influence strongly on the selection of bioremediation strategy. Bioaugmentation can be

proposed as a good solution for finalizing oil removing in Cilacap sandy beach when oil spilled occurred in this environment.

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