Concentration and Distribution of Polycyclic Aromatic Hidrocarbons (PAHS) During Bioremediation Processes of Oil-contaminated Beach Sediments in Karang Song Beach, Indramayu

Konsentrasi dan Distribusi Hidrokarbon Aromatik Polisiklik dalam Proses Bioremediasi Minyakbumi pada Sedimen Gisikpantai yang terkontaminasi di Pantai Karang Song, Indramayu

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ABSTRACT: Bioremediation research was conducted from June to September, 2010 in oil-contaminated beach sediment in Karang Song Beach, Indramayu. The objective of this paper is to determine concentration and distribution of polycyclic aromatic hydrocarbon (PAH) during bioremediation processes. Upon 90 days biodegradation treatment, PAH concentration was reduced in several mesocosms. Concentration of PAH before treatment and after 90 day incubation were follows, Mesocosm A (110.499 mg/kg to 16.125 mg/kg), Mesocosm B (217.067 mg/kg to 12.239 mg/kg), Mesocosm C (102.016 mg/kg to 16.695 mg/kg), Mesocosm D (124.256 mg/kg to 60.869 mg/kg), Mesocosm E (117.723 mg/kg to 50.663 mg/kg), Mesocosm F (143.495 mg/kg to 38.310 mg/kg), and Mesocosm G (9.838 mg/kg to 4.248 mg/kg). Generally, all mesocosms treatment was reduced of PAH concentration, but there are no stable degraded after 60, and 90 days incubation. Fertilizer application has showed good influence for increasing degradation rate of bacteria to degraded oil contaminant.

Keywords: polycyclic aromatic hydrocarbons (PAH), bioremediation, mesocosm, Karang Song, Indramayu

ABSTRAK: *Penelitian bioremediasi dilakukan dari Juni hingga September, 2010 di pantai Karang Song, Indramayu yang sedimen telah terkontaminasi minyak. Tujuan penelitian dari makalah ini adalah untuk mengamati konsentrasi dan distribusi Polisklik Aromatik Hidrokarbon (PAH) selama proses bioremediasi. Setelah 90 hari pengamatan biodegradasi, konsentrasi PAH dalam beberapa mesokosme menjadi menurun.. Konsentrasi PAH saat awal perlakuan dan setelah 90 hari inkubasi adalah sebagai berikut:, Mesokosme A (110,499 mg / kg menjadi 16,125 mg / kg), Mesokosme B (217,067 mg / kg menjadi 12,239 mg / kg), Mesokosme C (102,016 mg / kg menjadi 16,695 mg / kg), Mesokosme D (124,256 mg / kg menjadi sampai 60,869 mg / kg), Mesokosme E (117,723 mg / kg menjadi 50,663 mg / kg), Mesokosme F (143,495 mg / kg menjadi 38,310 mg / kg), dan Mesokosme G (9,838 mg / kg menjadi 4,248 mg / kg). Umumnya, semua pengobatan mesokosme menyebabkan berkurang konsentrasi PAH, tetapi tidak ada yang stabil terdegradasi setelah 60, dan 90 hari inkubasi. Pemupukan telah menunjukkan pengaruh yang baik untuk meningkatkan tingkat degradasi bakteri kontaminan minyak terdegradasi.*

Kata kunci: *polycyclic aromatic hydrocarbons (PAH), bioremediasi, mesocosm, Karang Song, Indramayu*

INTRODUCTION

Indramayu coastal waters have a potential marine pollution by crude oil, e.g., from oil and gas production, tanker oil transportation, or fishing activity. On 14 September 2008 there was accident of oil spill (3000 m3) by broken pipeline which connected a tanker with a single buoy mooring (SBM). This condition has influenced coastal waters ecosystem such as mangrove, coral, sea grass ecosystem, beaches, activity of fishermans and shrimps marine culture (Hartoko, *et al.,* 2010). Oil pollutants pose a serious threat to fishery, marine habitats of wildlife, halobios and human health, and destroy the ecological balance which may take years or even decades to recover (Zhang, *et al.,* 2011).

Crude oil causes a variety of risk for environmental because physically, chemically, and biologically harmful with the presence of many organic compounds having a toxic, persistent, bioaccumulative, mutagenic and carcinogenic activity, e.g., polycyclic aromatic hydrocarbons (PAHs) (Mohajeri *et al.,* 2011 ; Nora *et al,.* 2007). PAHs compounds combined from two or more fused benzene rings (Zakaria *et al.*, 2009).

PAHs characteristic are colorless, white/pale yellow solids with solubility in water is low, melting and boiling point were high (Haritsash & Kaushik, 2009; Eisler & Widlife, 1987).

Residue of pollutant organic like PAH in sediments might be minimalism contamination by several techniques degradation, such as physical, chemical or biological degradation techniques (Haritsash & Kaushik, 2009; Piskonen *et al.* 2004). However, biological degradation or bioremediation by microbial organism is the major degradation process (Phillips *et al.* 2000). Biological degradation processes depend on several factors 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 (Cortes *et al.* 2009, Lin *et. al.* 2009). Final result of this degradation processes are organic minerals, H_2O, CO_2, and CH_4 (Haritsash & Kaushik 2009, Steliga *et al.* 2009). (Figure 1).

for improvement of degradative capacity in several studies bioremediation usually by nutrient supplementation (biostimulation) and introduction of specific competent strains or consortia of microorganisms (bioaugmentation) (Lin et al. 2010; Delille et al. 2008; Chen & Aitken 1999; Mrozika & Seget 2010; Xu et al. 2010).

Furthermore, effectiveness of bioremediation processes was monitored by determination of PAH concentration during bioremediation processes. This study was conducted at Karangsong beach from June to September, 2010. This paper explains about degradation PAH processes, PAH concentration status, and behavior of PAH degradation as soon as before bioremediation, after 30 day, 60 day, and 90 day bioremediation processes.

Regional Geology

The coastal area of weastern part of Java are covered by large alluvium deposits. The present sedimentation proceses is still being continuited and deposited by the river around the river mounth. The

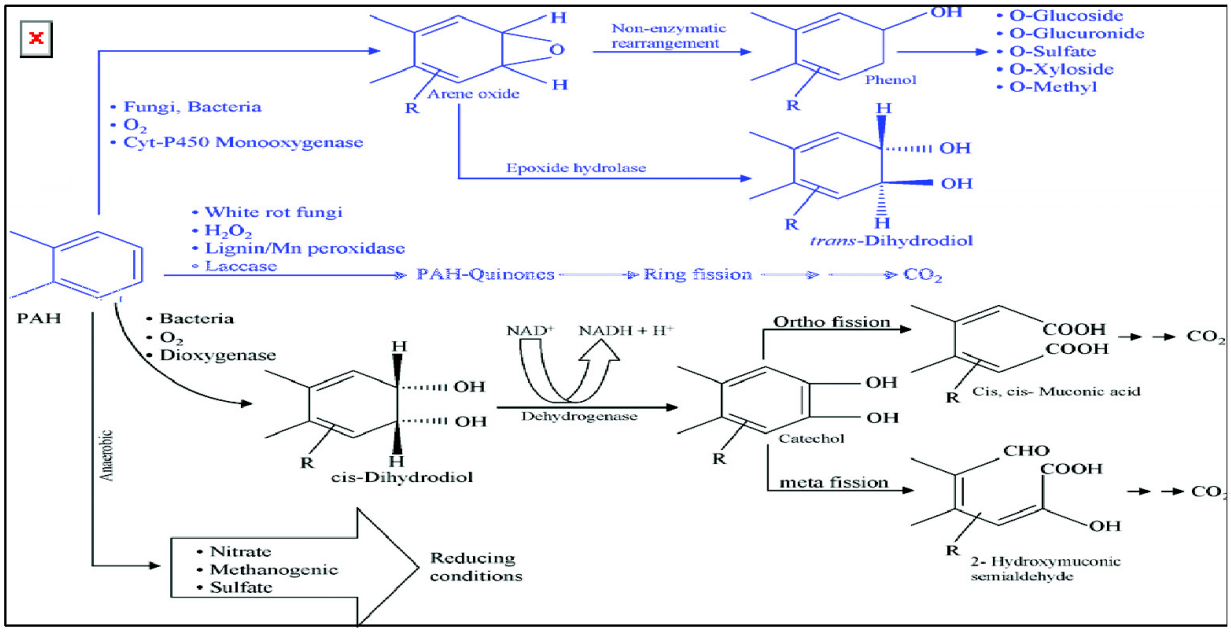

Figure. 1. Proposed pathway of PAH degradation by microbial (Haritsash & Kaushik, 2009)

On the other hand, bioremediation techniques will transform of compounds toxicities to less hazardous/ nonhazardous forms with less input of chemicals, energy, and time (Lin et al. 2010; Delille et al. 2008). Bioremediation become as an effective, economical, and environmentally friendly technology, which is considered a feasible method for treating petroleum hydrocarbon-contaminated beach sediments (Lin et al. 2010 ; Das et al. 2008). Modification of bioremediation

large amount of sediments material is brought by the river and distributed around the near shore of Java Sea. The near shore deposit some time brought back to the coastal area by wave action and deposited around the coastal plane. Consequently the most coast line of West Java Coast is progading.

Based on the Geological map of Indramayu which was made by Sudana and Achdan (1992), the lithology of the study area are characterized by Quaternary

sedimentary rock. The lithology which can be found around the study area as follows :

The oldest lithology is Pleistocene of land deposits which is consists of conglomerate and tuffaceous sandstone. Conglomerate is grayish yellow coloured, loose, the stratification is un clear and have cross bedding structure. The thickness of this lithologi is about 125 meters. This lithology is overlain by Holocene of flood plain deposits, coastal deposits, deltaic deposits and river deposits (Figure 2).

Flood plain deposits is characterized by clayey sand, organic clay, brownish grey and black coloured. To the southern part this lithology changes to be red tufaceous clay.

Coastal deposits is characterized by silt, clay and sand which consists of molusc remains.

Most of beach ridges deposits is dominated by coarse sediment which consists of large amount of mollusc.

METHODS

Field study location of bioremediation processes is conducted in Karangsong Beach, Indramayu (Figure 3). Sediment samples were taken from six mesocosm locations with different treatment (Table 1). The sediment was scooped from three points, where each location in the around mesocosm. After that, the sediment is put into glass bottles with an aluminum spoon, and it is covered with aluminum foil and then stored at 4 ^{o}C until analysis in laboratory.

PAH concentration in sediment samples from several mesocosms is determinated by the method of US EPA (1986), Holden & Marsden (1969), Greve & Grevenstuk (1975), and Duinker & Hillebrand (1978) methods and it is calibrated by QTM PAH standard mixture (QTM PAH mix 47930-U Supelco). By this method, 15 PAH compounds were determined such as naphthalene (Naph), acenaphthylene (Acethy), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr),

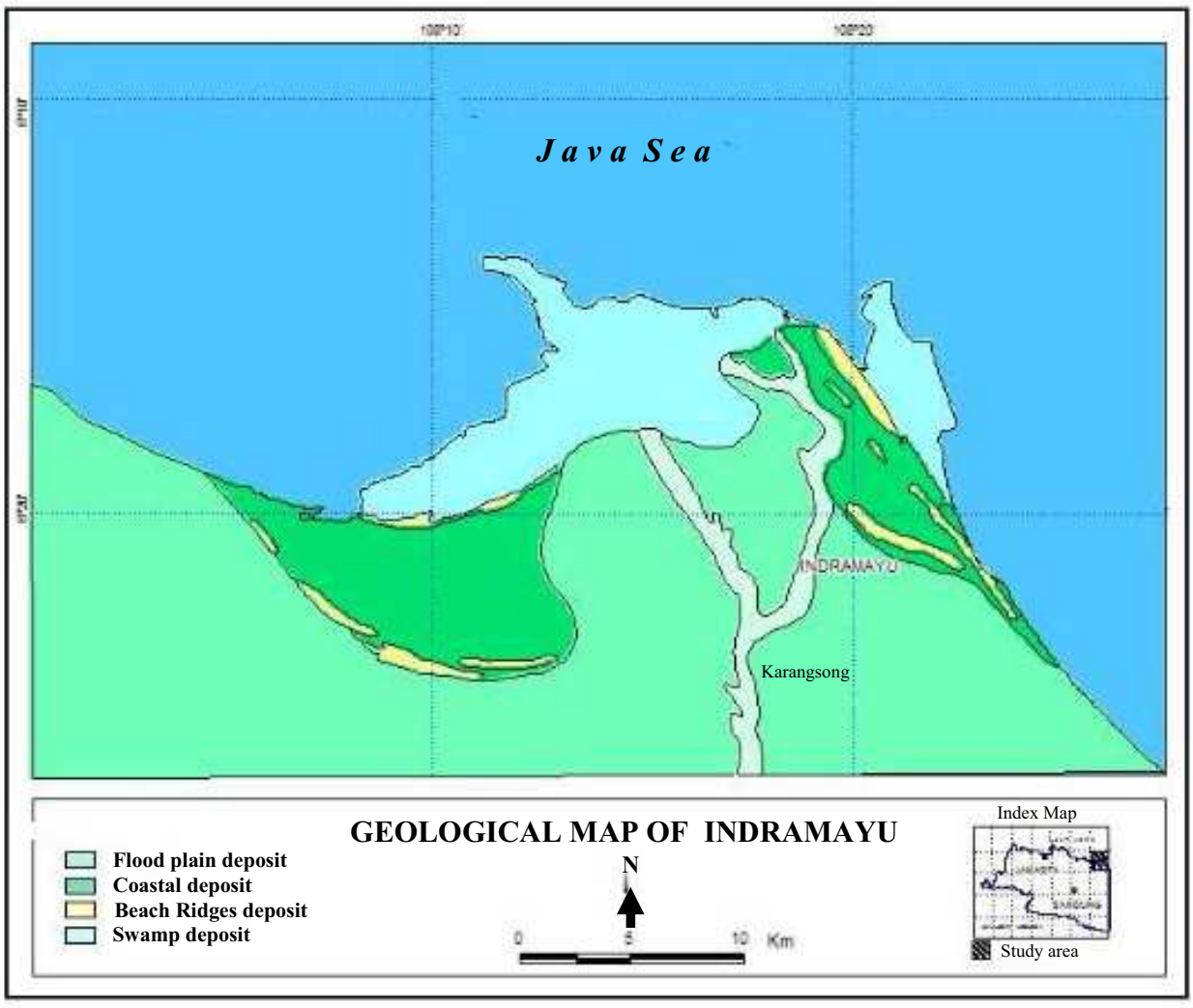

Figure 2. Geological map of Indramayu (Sudana and Achdan 1992)

Figure 3. Field location of bioremediation study in oil-contaminated beach sediments in Karang Song Beach, Indramayu

Table 1. Mesocosm set-up of bioremediation field study in Karang Song Beach, Indramayu, June to September 2010

Mesocosm	Treatment
A (control)	Oil-contaminated beach sediments (OCBS)
B	Alcanovorax sp. TE-9 (10L) + OCBS
C	Consortium A (10L) + OCBS
D	slow release fertilizer Osmocote (2500 g) + OCBS
E	slow release fertilizer Osmocote (1250 g) + Alc. Sp TE-9 (10L) + OCBS
F	slow release fertilizer Osmocote (1250 g) + Consortium A (10L) + OCBS
G/ Out location of mesocosm	-

benzo(a) anthracene (BaA), chrysene (Chr), benzo(b) fluoranthene (BbF), benzo(k) fluoranthene (BkF), benzo(a) pyrene (BaP), indeno (123-cd) pyrene (InP), dibenzo(ah) anthracene (DBA), and benzo(ghi) pyrylene (BghiP).

Before extraction, sediment samples (40 grams) were dried in the oven at 50^{o}C temperature overnight, pure musher in a mortar with addition of Na_2SO_4 to remove residues of water. Each dry sediments sample was extracted with 120 mL dichloromethane (DCM) for 8 hours in soxhlet extractor. After that, it evaporated until 1 ml and clean up using alumina chromatography column. Alumina chromatographic column cleanup techniques was prepared with enter glass wool and 4 grams aluminum oxide WB 5 basic SIGMA and eluted with 10 mL DCM and hexane. After that, a concentrated extract was transferred into column and elute with 4% diethyl ether in hexane and evaporated again until 1 ml. After clean up, the samples were separated and fractionated using silica gel chromatographic column technique. Silica gel column prepared with enter silica gel as much as 4 grams and conditioned before with 10 mL DCM and hexane. Non-polar fraction (F1) was eluted by hexane for analysis pesticide, saturated hydrocarbon; and polar fraction (F2) by 10% diethyl ether in hexane for analysis PAHs. The polar fraction (F2) was analyzed using GC/FID (gas chromatography-flame ionization detector) analyses. PAH concentration results publish is in µg/g or mg/kg (ppm) unit.

RESULT

Monitoring of bioremediation processes was conducted by measurement of PAH concentration in several times. Concentration of PAH before incubation during bioremediation processes are 110.499 mg/kg (Mesocosm A), 217.067 mg/kg (Mesocosm B), 102.016 mg/kg (Mesocosm C), 124.256 mg/kg (Mesocosm D), 117.723 mg/kg (Mesocosm E), 143.495 mg/kg (Mesocosm F), and 9.838 mg/kg (Mesocosm G/ out of mesocosm); (Table 2 and 3). There was no significant different first level of PAH concentration for degraded with bacteria. Besides the beach sediment from out of mesocosm system as a control of research has a PAH concentration below mesocosms treatment concentration.

Characteristic of PAH compounds in contaminated beach sediment of several mesocosms are as follows, mesocosm A has low molecular weight (LMW) PAH (24%) and high molecular weight (HMW) PAH (76%), mesocosm B has LMW (28 %) and HMW (72%), mosocosm C has LMW (47%) and HMW (53%), mesocosm D has LMW and HMW similar presentation are 50%, mesocosm E has LMW (48%) and HMW (52%), mesocosm F has LMW (46%) and HMW (54%), and the last samples is sediment from the location out of system mesocosm has LMW (30%) and HMW (70%). In general, composition of low molecular

Table 2. PAH concentration in several oil-contaminated beach sediments in Karang Song Beach, Indramayu, June to September 2010

No.	PAH	Abbreviation	Mesocosm A				Mesocosm B				Mesocosm C			
			0	30	60	90	0	30	60	90	0	30	60	90
1	Naphthalene	Naph	Nd	Nd	Nd	Nd	0.122	Nd	Nd	Nd	0.102	Nd	Nd	Nd
2	Acenaphthylene	Acthy	3.244	0.836	0.803	1.076	5.668	0.764	0.268	0.665	5.851	2.153	0.563	2.452
3	Acenaphthene	Ace	6.363	0.723	0.714	0.818	7.885	0.531	0.254	0.516	2.377	1.886	1.131	1.98
4	Fluorene	Fl	2.007	0.629	1.685	1.118	3.773	0.41	0.623	0.879	9.864	1.706	2.727	4.162
5	Dibenzothione	Dbenth	2.493	1.315	1.216	0.634	4.636	0.979	0.591	0.51	7.804	3.458	1.221	1.875
6	Phenanthrene	Phe	1.719	0.261	0.568	0.321	3.197	0.133	0.277	0.31	2.444	0.683	0.089	0.643
7	Anthracene	Ant	1.207	0.47	Nd	0.153	2.33	0.585	Nd	Nd	1.565	0.931	0.18	0.547
8	Fluoranthene	Flu	3.603	0.829	0.868	Nd	7.512	0.749	1.061	Nd	3.95	2.531	1.819	0.571
9	Pyrene	Pyr	3.274	0.374	0.791	0.52	7.408	0.387	0.729	Nd	7.688	1.403	1.164	0.734
10	Benzo(a) Anthracene	BaA	7.679	0.898	3.357	3.378	19.257	0.93	16.001	3.252	6.697	0.722	15.026	Nd
11	Chrysene	Chr	5.276	0.31	1.791	0.939	14.167	0.72	5.548	1.78	10.098	0.471	6.39	Nd
12	Benzo(b) Fluoranthene	BbF	17.378	4.037	1.366	0.701	25.237	2.955	4.327	1.634	16.651	1.93	4.977	0.675
13	Benzo(k) Fluoranthene	BkF	12.966	2.54	Nd	Nd	13.603	1.923	Nd	Nd	9.68	1.242	4.376	1.594
14	Benzo(a) Pyrene	BaP	19.643.	4.277	1.639	6.467	20.491	3.193	6.712	2.693	3.94	1.877	8.712	1.462
15	Indeno(123-cd) Pyrene	InP	32.268	4.266	6.112	Nd	60.868	6.57	14.712	Nd	5.806	7.806	11.275	Nd
16	DiBenzo(ah) antracene	DBA	3.548	Nd	Nd	Nd	12.077	Nd	5.134	Nd	1.954	2.582	6.189	Nd
17	Benzo(ghi) Pyrylene	BghiP	7.474	Nd	Nd	Nd	8.836	Nd	Nd	Nd	5.545	Nd	6.115	Nd
TOTAL PAH			**110.499**	**21.866**	**20.91**	**16.125**	**217.067**	**20.834**	**56.237**	**12.239**	**102.016**	**31.382**	**71.954**	**16.695**

Note : Nd= no detected

Table 3. (Continued)

No.	PAH	Mesocosm D				Mesocosm E				Mesocosm F				Out of Mesocom system			
		0	**30**	**60**	**90**	**0**	**30**	**60**	**90**	**0**	**30**	**60**	**90**	**0**	**30**	**60**	**90**
1	Naphthalene	0.125	Nd	Nd	Nd	0.071	Nd	Nd	Nd	0.139	Nd	Nd	Nd	0.012	Nd	Nd	Nd
2	Acenaphthylene	8.523	2.213	0.577	3.921	8.155	1.546	1.011	3.47	9.647	0.939	0.706	1.486	0.558	0.152	Nd	0.02
3	Acenaphthene	5.246	2.04	1.081	3.125	5.656	1.507	1.678	2.736	5.951	0.827	0.636	1.207	0.155	1.961	0.189	0.026
4	Fluorene	13.909	2.17	2.792	6.769	14.118	1.435	4.582	5.123	16.749	0.676	1.447	2.198	0.904	0.167	Nd	0.677
5	Dibenzothione	11.938	3.052	1.826	3.442	8.887	3.216	2.684	1.992	8.679	1.694	1.005	1.081	0.085	0.323	0.201	1.418
6	Phenanthrene	2.969	0.951	0.381	1.265	2.957	0.647	0.875	0.598	3.43	0.274	0.155	0.193	0.066	0.107	Nd	0.465
7	Anthracene	1.712	0.735	0.321	1.208	1.173	0.956	0.876	0.491	1.413	0.744	Nd	0.131	0.055	0.097	Nd	0.242
8	Fluoranthene	3.293	2.493	1.847	2.608	3.099	2.624	2.534	1.199	6.02	1.163	0.837	0.828	0.074	0.256	Nd	0.176
9	Pyrene	6.406	1.251	1.183	1.332	4.707	1.974	0.869	0.977	4.755	0.591	0.785	0.836	0.114	0.104	Nd	0.152
10	Benzo(a) Anthracene	8.182	0.453	4.543	7.514	7.501	1.697	3.005	6.697	9.62	0.975	7.245	6.649	0.915	0.029	Nd	0.499
11	Chrysene	12.444	0.571	3.097	2.317	11.905	2.188	1.541	2.393	15.858	0.75	3.046	3.075	1.182	0.851	0.963	0.249
12	Benzo(b) Fluoranthene	19.617	2.993	2.546	2.102	18.929	5.763	1.13	2.026	26.723	3.331	2.289	2.519	1.942	1.518	0.596	0.14
13	Benzo(k) Fluoranthene	11.462	1.299	5.479	7.111	10.806	3.904	3.33	4.953	15.127	1.908	Nd	4.2	1.261	1.026	Nd	Nd
14	Benzo(a) Pyrene	3.948	2.615	5.43	3.673	2.835	5.683	2.577	2.1	5.029	5.501	3.738	2.663	0.824	Nd	0.922	0.184
15	Indeno(123-cd) Pyrene	7.675	10.508	12.072	8.241	7.686	3.572	8.839	9.138	4.203	6.292	7.156	11.244	0.664	Nd	Nd	Nd
16	DiBenzo(ah) antracene	4.145	3.377	7.042	6.241	3.44	3.414	Nd	6.77	4.404	2.657	Nd	Nd	0.335	Nd	Nd	Nd
17	Benzo(ghi) Pyrylene	2.662	Nd	Nd	Nd	5.798	Nd	Nd	Nd	5.748	Nd	Nd	Nd	0.692	Nd	Nd	Nd
	TOTAL PAH	**124.256**	**36.72**	**50.217**	**60.869**	**117.723**	**45.125**	**35.531**	**50.663**	**143.495**	**28.319**	**29.045**	**38.31**	**9.838**	**6.591**	**2.871**	**4.248**

Note : Nd= no detected

weight and high molecular weight in several samples at first condition of PAH are similar.

The result of first incubation of bioremediation processes are showed in Table 4, during the 30 days incubation in several mesocosm has removed of the total amount of PAH, such as mesocosm A (80%) with release fertilizer and bioaugmentation in mesocosm D and mesocosm E not show significant improvement in the PAH removal efficiency degradation processes compared with mesocosm A. This condition means, that biodegradation processes was inhibited by high concentrations of nutrients where nutrients may inhibit

Table 4. Biodegradation processes during 30 day incubation

Mesococm	Treatment	Day 0	Day 30	Removal	% Removal	Removal rate
		Total PAH concentration (mg/kg dry sediment)				Total PAH concentration (mg/kg dry sediment-day)
A	Oil-contaminated beach sediments (OCBS)	110.499	21.866	88.633	80	2.95
B	Alcanovorax sp. TE-9 (10L) + OCBS	217.067	20.834	196.233	90	6.54
C	Consortium A (10L) + OCBS	102.016	31.382	70.634	69	2.35
D	slow release fertilizer Osmocote (2500 g) + OCBS	124.256	36.72	87.536	70	2.92
E	slow release fertilizer Osmocote (1250 g) + Alc. Sp TE-9 (10L) + OCBS	117.723	45.125	72.598	62	2.42
F	slow release fertilizer Osmocote (1250 g) + Consortium A (10L) + OCBS	143.495	28.319	115.176	80	3.84
Outside system		9.838	6.591	3.247	33	0.11

removal rate approximately 2.95 mg PAH/kg dry sediment per day incubation, respectively. The PAH removal rate of the mesocosm B is approximately 6.54 mg PAH/kg dry sediment per day during the 30 days bioremediation incubation when approximately 90% of the total amount of PAH was removed. Mesocosm C has removed of PAH approximately 69% with removal rate 2.35 mg PAH/kg dry sediment per day incubation. Although the degradation efficiencies of *Alcanovorax* sp. TE-9 is better than consortium A, the PAH removal of consortium A in mesocosm F was higher than in mesocosm E after bio-stimulation application. This might be due that high fraction of HMW of PAH in mesocosm B has low rate in bio-degradation and possible to microbial attack (Mohajer *et al.* 2010). Besides that, biological factor such as the presence of enzyme at significant levels, and different bacteria was a limited factor for degrading HMW PAH, because some compounds of it not served as growth substrates of bacteria, so the induction by degradation enzyme synthesis is very important (Chen & Aitken 1999).

The PAH removal rate of mesocosm D was approximately 2.92 mg PAH/kg dry sediment per day incubation and was removed 70%. In contrast, both bioaugmentation and biostimulation are addition for mesocosm E and F. The PAH removal rate in mesocosm E approximately 42 mg/kg dry sediment per day incubation and has removed 62% PAH in sediment samples (Table 4). Addition biostimulation by slow microorganisms that are adapted to an originally oligotrophic soil environment (Tahhan &Abu 2009).

Besides, several studies were reported by (Braddock *et al.,* 1997) and (Schiewer 2006) indicated that the optimal processes of bioremediation influence by low levels of nutrient (Sanscartier e*t al.* 2009).

DISCUSSION

Positive correlation between biostimulation and bioaugmentation application for degradation processes was showed by mesocosm F. Application both biostimulation with slow release fertilizer and bioaugmentation with consortium A bacteria is removed amount of PAH approximately 80%. Biostimulation induced a clear increase of the number of hydrocarbon-degrading microbes (Delille 2008). PAH biodegradation during the bioaugmentation stage in mesocosm F is higher than during basic bioremediation in mesocosm C by 2.35 mg/kg dry sediment per day incubation removal rate to 3.84 mg/kg dry sediment per day incubation in mesocosm F (Steliga *et al* 2009). The location samples out of system mesocosm is had removal rate of 0.11 mg/kg dry sediment per day incubation and removal percentage is 33%. This result showed that the beach sediment has indigenous organism as bioremediation agent in natural environmental. As noted by (Steliga *et al.,* 2009), the basic bioremediation meaning only activation of the natural microflora in the contaminated area, which was

used in order to decrease the concentration of petroleum pollutants. Fertilizer application has showed good influence for increasing degradation rate of bacteria to degraded oil contaminant. Nutrient deficiency might be a limiting factor in the biodegradation process. (Admon *et al.* 2001) found that hydrocarbon loss was observed only after nutrients were amended to oily sludge contaminated soil at C:N:P ratio equivalent to 50:10:1 (Tahhan &Abu 2009). However, only consortium A bacteria has good activity with addition osmocote fertilizer (slow release) not with other. So, this result must be studied again in future research to know that phenomena. The intensity of hydrocarbon biodegradation in sediment is influenced by a number of site-specific factors (e.g. low temperature, low nutrient availability, low oxygen levels, soil structure, etc.). Among them, nutrients are one of the major factors limiting hydrocarbon metabolization in sediments. Inputs of large quantities of carbon sources (i.e., hydrocarbon contamination) tend to result in rapid depletion of the available pools of major inorganic nutrients, such as nitrogen and phosphorus (Delille 2008).

The degradation efficiency after 30 day incubation was higher and the degradation after 60 and 90 day became flat curve condition (Figure 4). Despite an increase in the concentration of PAH in mesocosm B which is high for 16.31% from the percentage of 30 day incubation. This is due with characteristic of contaminated beach sediments that low molecular weight compounds being easily biodegraded after 30 day incubation. This condition showed with data result that after 30 day incubation, low molecular weight of PAH more decrease on concentration. Besides that, data result showed that high molecular weight of PAH compounds difficult to degrade after 60 and 90 day incubation. As the theoretical, high molecular weight of PAH will became as low molecular weight of PAH after degradation processes. This hypothesis strongly with data results that after 60 and 90 day incubation, several mesocosms showed that PAH total concentration was increased trend. Therefore, it is reasonable to assume that the biodegradation time and degree were affected by the fraction of PAH and TPH components and concentration (Lin *et al.* 2010).

Total PAH concentration after incubation 90 day in all mesocosm were follows, 16.125 mg/kg (Mesocosm A), 12.239 mg/kg (Mesocosm B), 16.695 mg/kg (Mesocosm C), 60.869 mg/kg (Mesocosm D), 50.663 mg/kg (Mesocosm E), 38.310 mg/kg (Mesocosm F), and 4.248 mg/kg (Mesocosm G). Total removal efficiencies of total PAH after 90 day incubation were 85 %, 94 %, 84 %, 51 %, 57 %, 73 %, and 55 % for treatments in mesocosm A, B, C, D, E, F, and G, respectively. Compared with biodegradation processes after 30 day, some bacteria in several mesocosm after incubation 90 day showed increase activity to degrade PAH (mesocosm A, B, and C), while other mesocosm

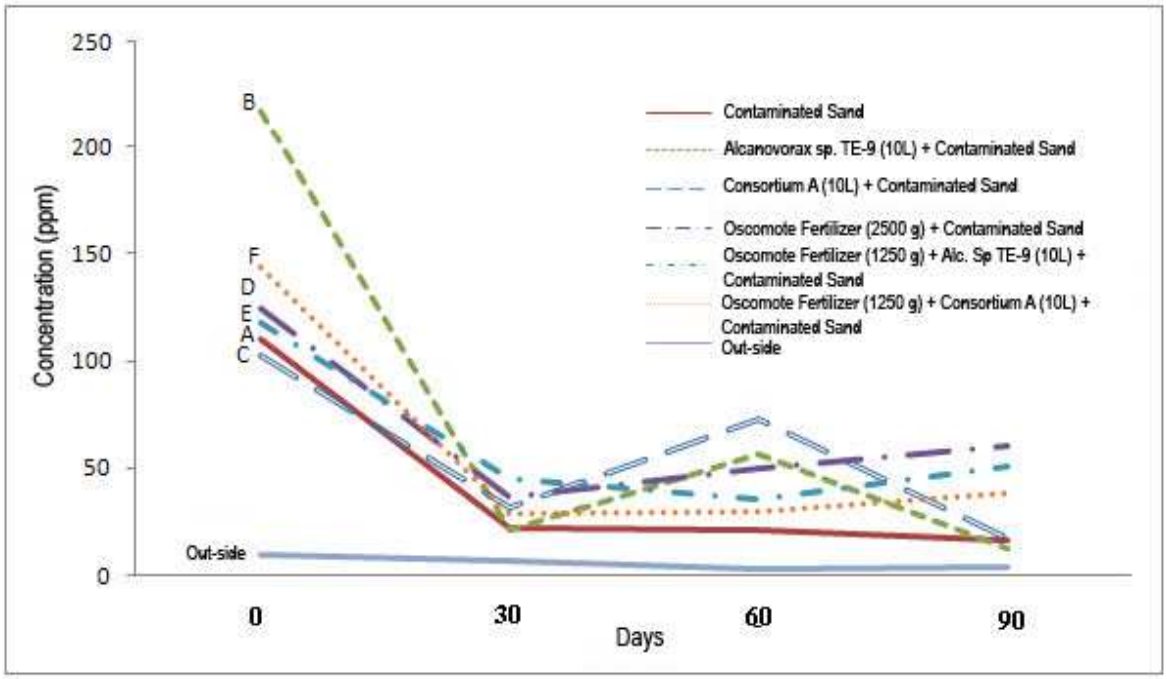

Figure 4. Degradation rate of bioremediation processes after 0, 30, 60, and 90 day incubation with several treatments in Karang Song Beach, Indramayu.

has decrease bacteria activity (mesocosm D, E, and F). This phenomena cause removal rate in all mesocosm was decreased until average of 64 %. However, compared with other study, removal efficiencies of present study were high than Xu (2010) study that removal efficiencies after 12 week biodegradation between 26% to 61% but was less than Makadia *et al.* (2011) study was final result between 85 to 92 % degradation removal efficiencies.

Specific interaction between substrate in biodegradation processes maybe is important in understanding the fate of PAHs biodegradation. Sometimes, high molecular weight PAHs after low molecular weight PAHs have been utilized/degraded, while high concentration of naphthalene may have inhibited degradation of other PAHs due to toxicity (Pumphrey & Madsen 2007). Stringfellow and Aitken (1995) found competitive inhibition of phenanthrene degradation by naphthalene, methylnaphthalene, and fluorene in binary mixtures using two pure cultures. However, Al Saleh (2005) was presented that lead content in sediment samples may possible inhibitory effect on biodegradation processes because dehydrogenase activities also declined. This study showed that the number of hydrocarbon-degrading bacteria decreased with increased levels of lead nitrate added to soil samples, whether oil polluted or not. Other studies reported that microbiological metabolism in bioremediation processes influence by chemical stressor from surrounding area. Therefore, nutrient stock and energy resources was not used only for growth but used for stress management and protection (Ray and Peters 2008).

CONCLUSIONS

PAH concentration was reduced in several all mesocosms after finally treatment (90 days). Concentration of PAH before treatment and after 90 day incubation were follows, Mesocosm A (110.499 mg/kg to 16.125 mg/kg), Mesocosm B (217.067 mg/kg to 12.239 mg/kg), Mesocosm C (102.016 mg/kg to 16.695 mg/kg), Mesocosm D (124.256 mg/kg to 60.869 mg/kg), Mesocosm E (117.723 mg/kg to 50.663 mg/kg), Mesocosm F (143.495 mg/kg to 38.310 mg/kg), and Mesocosm G (9.838 mg/kg to 4.248 mg/kg). Generally, all mesocosm treatment was reduced of PAH concentration, but there are no stable degraded after 60, and 90 day incubation. Fertilizer application has showed good influence for increasing degradation rate of bacteria to degraded oil contaminant.

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