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Biodegradation of Soils Contaminated with Naphthalene in Petroleum Hydrocarbons Using Bioslurry Reactors

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Abstract. Soil contaminated with naphthalene by the activities of exploration, production and disposal of petroleum waste into the environment causes serious damage to ecosystems environment. This is the target of processing by the bacteria as a model for remediation of soil contaminated with naphthalene. Thus, the study was focused on naphthalene biodegradation in soil contaminated with polyaromatic hydrocarbons with measurement parameters: the bacterial growth and dissolved oxygen. The research was conducted in a slurry bioreactor with soil to water ratio of 20:80 (%wt) with the addition of a consortia of *Bacillus cereus* and *Pseudomonas putida* bacteria with a concentration of 10% (v/v) and 15% (v/v). The slurry bioreactor was aerated, stirred with stirring of 100 rpm and temperature range of 26ºC – 35ºC. Naphthalene residue in petroleum hydrocarbons were measured using GC-MS. The result of identification with naphthalene initial concentration of 115.646 ng/uL, after 49 days of incubation for bacterial consortium 10% (v/v) with a bacteria ratio 3:2; 1:1; 2:3; and control showed the reduction to 33.77 ng/uL; 0.77 ng/uL; 10.28 ng/uL and 23.88 ng/uL, respectively with biodegradation percentages of 70.80%; 99.33%; 91.11%; and 79.35%. As for the bacterial consortium 15% (v/v) with the same ratio and control, the naphthalene concentration was reduced to 0.31 ng/uL; 0.19 ng/uL; 5.12 ng/uL and 23.88 ng/uL, respectively and biodegradation percentages of 99.73%; 99.84%; 95.57%; and 79.35%. This finding shows that percentage of crude low degraded considering the concentration crude oil increased.

Keywords: biodegradation, naphthalene, slurry bioreactor, *Bacillus cereus* and *Pseudomonas putida*.

1. Introduction

Hydrocarbons are widely used as primary energy and fuel resources, due to the energy they produce, therefore a large quantity of hydrocarbons are being released into the environment, accidentally or not [Das & Chandran, 2011; Panda et al., 2013]. The most common environmental contaminants include petroleum, gasoil, solvents (chlorinated solvents and benzene, toluene, ethylbenzene and xylene BETX), polynuclear aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) [Najirad et al., 2012] These contaminants are highly persistent in the environment, toxic and present significant health risks to humans, ingestion of which can affect several body organs such as lungs, liver andkidneys [Akpor et al., 2014; Alrumman et al., 2015]. The amount of petroleum-contaminated soil resulting in the production process thousands of tons of oil has risen each year appears logical [Yudono et al., 2009].

In recent years there has been increasing concern over fate of aromatic hydrocarbons in marine and terrestrial ecosystems since several of these compounds are known to exhibit toxic, mutagenic and carcinogenic properties and the nature of the environment of persistent organic pollutants [Daly et al., 1972; Santos et al., 2008]. The naphthalene metabolism of mesophilic microorganisms under aerobic conditions has been intensely investigated, and detailed information has been presented on degradation rates, metabolic pathways, and the involved enzymes [Cerniglia and Heitkamp, 1989; Cerniglia, 1992; Gibson and Subramanian, 1984; Smith, 1990]. Because naphthalene is a major component in the

water-soluble fraction of crude and certain fuel oils [Winters et al., 1976]. Based on the problems caused by pollution impact naphthalene, conducted by the method approach biodegradation and bioremediation by utilizing a combination of *Bacillus cereus* and *Pseudomonas putida* bacteria in degradation of naphthalene in petroleum contaminated-soil, which play an important role in the restoration of the environment by utilizing microorganisms.

The objectives of this study were to determine naphthalene biodegradation in soil contaminated with polyaromatic hydrocarbons with measurement parameters: the bacterial population and dissolved oxygen.

2.   Materials And Methods

2.1 Collection of Soil and Biodegradation Process

Polluted soil was obtained from oil drilling sites by Pertamina-Petrochina East Java (PPEJ), Tuban, Indonesia. Soil sample were collected from several point of a surface layer area polluted. Polluted soil was then mixed with water at 20:80 (%wt) ratio to form slurry. *Bacillus cereus* and *Pseudomonas putida* bacteria with concentration of 10% and 15%(v/v) (ratio of bacteria, 3:2; 1:1; 2:3) were then added to each bioreactor along with nutrient and aeration process (**Fig. 1**). The operation condition of 6–8 pH, 26°C–30°C and stirring of 100 rpm were used throughout this process. Naphthalene analysis will be done every week, while dissolved oxygen were monitored every day. Biodegradation process occurred for 7 weeks.
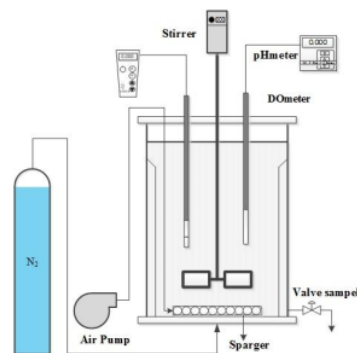

Fig. 1 Slurry Bioreactor

2.2 Preparation of Medium and Bacilus cereus and Pseudomonas putida Bacteri

Liquid medium was prepared by mixing 24 grams of NBA with 1% glucose and 1% yeast extract into 1 liter of distilled water. After sterilizitaion by autoclave for 15 minutes at 121°C, media was cooled until 28°C. *Bacillus cereus* and *Pseudomonas putida* bacteria was moved from agar medium to liquid medium in laminar flow. The new medium were then incubated in incubator shaker at 30°C and 70 rpm. Bacteria were counted by Haemacytometer method and after 24 hours, total bacteria were 1.13×10^7 Cell mL^{-1}.

2.3 Extraction and Analysis of Naphthalene

Analysis of naphthalene degradation was confirmed by monitoring the disappearance of the 16 priority PAHs in sediment slurries. Sediment samples (10 g) were extracted in a Soxhlet apparatus for 16 h with 100 ml of n-hexane and 10 g of sodium sulphate hydrous to remove moisture (EPA Method 3540C) and the organic phase was concentrated to 1 ml by rotary evaporation. Total and individual EPHs/PAHs in sediment samples were analysed with a GCMS at Research Centre for Oceanography

Indonesia Institut of Science, by GCMS Thermo Trace ISQ 1310 LT (Single quadrupole Mass Spectrometer) method Supelco Standard QTM PAH Mix 47 930-U quantitatively. Systems is equipped with a Thermo TR-5 column (30 m length and 0.25 mm diameter). Helium was used as carrier gas with rate of 1.2 ml/min through column. The initial temperature column was 50°C and increased to 300°C with rate of 62.5 C/min.

Biodegradation percentage of naphthalene were calculated according to the following equation:

$$Degradation\ (\%) = \frac{\left[Naphthalene\right]_0 - \left[Naphthalene\right]_n}{\left[Naphthalene\right]_0}\ x\ 100 \tag{1}$$

3. Results and Discussion

3.1 Change of DO during 7-weeks bidegradation period

The supply of oxygen to the bioremediation process intended to increase the recipients of electrons. Microorganisms of need oxygen either in the form of free oxygen obtained from air and the oxygen dissolved in water. In petroleum oil biodegradation, oxygen used to oxidation reaction and respiration. Most microorganisms petroleum oil biodegradation are classified as a part aerobic microorganisms [Jordan and Payne, 1980]. Naphthalene is one of the polluting parameters of a highly harmful polyaromatic hydrocarbons in humans and surrounding environmental ecosystems. Naphthalenen contaminated land is also harmful to groundwater quality. From the **Fig. 2** can be seen that a decrease in the concentration of naphthalene on the day 0 to 7 tending to slowly. Further degradation continue and faster on the day 7 to 49 days. Significant degradation occurred at a bacterial ratio 1:1 for bacteria concentration of 10%(v/v) and 15%(v/v). Leahy and Colwell (1990) explained that biodegradation rates have been shown to be highest to the saturates, followed by the light aromatic, with high molecular weight aromatics and polar compound.
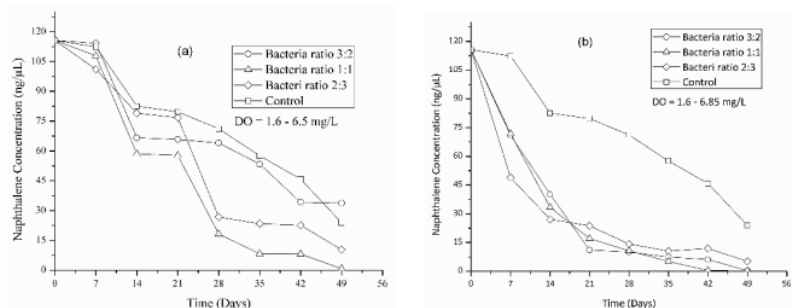

Fig. 2 Effect of Time against Concentration of Naphthalene :
a) Bacteria concentration 10%(v/v)); b) Bacteria concentration 15%(v/v))

In **Fig. 2a** shows that the concentration of naphthalene in the slurry decreases with increasing time. In the addition of 10% bacteria concentration it was seen that the naphthalene concentration decreased in the first week until seventh week (day zero intervals to day zero until the day of 49) was significant for each the ratio bacteria 3:2; 1:1; 2:3, except control (without bacteria addition) was with initial concentration of 115.65 ng/µL, on 49 day to 33,77 ng/µL; 0.77 ng/µL; 10,28 ng/µL and 23,88 ng/µL. The monitoring of dissolved oxygen show good behavior (increasing) every day on each the bacteria ratio during 49 days of remediation period. The average of dissolved oxygen that reads during the remediation period for each the ratio of bacteria and control was 4.82 mg/L; 4.81 mg/L; 4.80 mg/L

and 4.62 mg/L. With the level of dissolved oxygen, led to the process by oxidation aerobic and respiration bacteria grow better led to the process remediation naphthalene for oil contaminated-soil significant. Whereas at 15 % (**Fig. 2b**) bacteria concentration have been very significant biodegradation, one of the causes is microorganisms contained in bacteria 15% more so it can be better in reducing naphthalene waste on contaminated land. The very significant decrease seen in the first week was 115.65 ng/μL to 0.31 ng/μL; 0,19 ng/μL; 5.12 ng/μL and 23,88 ng/μL. This result indicate that the reduced concentration naphthalene very good occurs in a bacteria ratios 1:1 to concentration of bacteria 10% and 15% (v/v). Different things happened in the consortia of bacteria 15% with the same of ratio, during 49 days the remediation period shows that, DO averages for each of bioreactor was 4.98 mg/L; 4.98 mg/L; 4.95 mg/L; and 4.62 mg/L. DO the average that reads, appeared not much different from a consortia of bacteria 10% and tend to nearly the same, during the period of biodegradation. These results indicate that the reduced naphthalene is excellent, occurring in a bacterial ratio 1:1 for 10% and 15% of bacterial concentration.

The supply of dissolved oxygen enough to be provide a positive response to bacteria as recipients an electron to growing out in significant. Although the amount of oxygen given into the system from a source of supply (air pump) not known, the level of dissolved oxygen it noted that oxygen also attended all the time to ensure survival bacteria in the aerobic slurry bioreactor to do soil-contaminated oil degradation. This process showed that microorganisms breakdown structure naphthalene by entering slurry containing naphthalene waste into microorganisms itself body. The waste naphthalene is broken by the combination a bacillus cereus and pseudomonas putida bacteria with using a process metabolism. In the body of microorganisms there is an enzyme that acts as remodel of naphthalene waste, then naphthalene issued again from the body of microorganisms into the form of which is less dangerous.

3.2 Biodegradation Percentage of Naphthalene in the Period of Time

PAHs (Naphthalene) biodegradation depends on the complexity of the chemical structure, type and position in group substitution and the level adaptation enzymatic. To soil contaminated petroleum, a consortium of bacteria demonstrating ability good to degradation naphthalene and increase biodegradation percentage. From this **Fig. 3**, it showed that in the treatment with the addition of bacteria 10% (v/v), 15% (v/v) with a bacteria ratio, 3:2; 1:1; 2:3 dan control (without addition of bacteria), cause the value of naphthalene can be reduced quite well. The greater the value of naphthalene decreases, the greater the biodegradation presentage increases. Although at some time tend to experience fluctuations in the degradation of naphtahelen. On the other hand, percentage of biodegradation naphthalene of soil contaminated oil after 49 days the period remediation between ranged 6.74 to 99.33% (**Fig. 3a**) to concentration 10% in ratio of bacteria 1:1, compared with the ratio of bacteria 3:2; 2:3 and control, eachs percentage of biodegradation was 1.33 to 70.80%; 12.56 to 91.11% and 2.83 to 79.35%. Compared to control, bioreactor with the ratio of bacteria 1:1 is percentage biodegradation 1.25 times as greater.
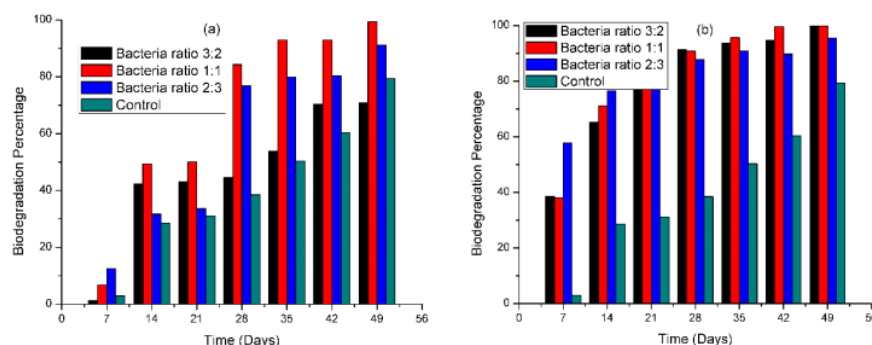
Fig. 3 Relationship between Biodegradation Percentage against Time:
a) bacteria concentration 10%(v/v)); b) bacteria concentration 15%(v/v))

As for bacteria concentration 15% (**Fig. 3b**) with ratio of bacteria 1:1, biodegradation percentage range from 37.97 to 99.84% after 49 days the period remediation, while to the ratio of bacteria 3:2; 2:3 and control, biodegradation percentage was 38.64 to 99.73%; 57.75 to 95.57% and 2.83 to 79.35%, respectively. To the ratio of bacteria 1:1, percentage of biodegradation 1.26 times as great compared to control. It is suspected that this because the group bacteria of polluted soil are a bacteria indigenous that cannot utilization a hydrocarbon oil for growth, so the rate of degradation to be slow [Menzie et al., 1992]. The condition is consistent with the fact that hydrocarbons degradation is strongly influenced by time, where the period remediation a long, reduction concentration naphthalene the greater.

The condition is clearly very consistent with the fact that hydrocarbons degradation is greatly influenced by time, where the long remediation period, the concentration of naphthalene degradation is greater. Overall, point to the different level of degradation between each bioreactor. **Fig. 3b** also shows that 49 days of remediation period, the concentration naphthalene tend to be constant, because reaches the equilibrium stage. A concentration of 10% bacteria, although the pollutant reduction is quite good, but the percentage of biodegradation relatively significant, possibly because the bioavailability naphthalene is more complicated when interacting with non aqueous phase liquid (NAPL) and soil colloids, resulting in less or not at all available for microorganisms. This can occur at a greater rate, so desorption becomes very slow, limits the flux of contaminants into th water phase, and the contaminant is biologically ready. The combination of the above phenomena causes different of distribution and partition the polluter in the soil, they make biologically less susceptible, resistant to biodegradation, and more persistent in the soil [Atlas, 1981].

3.3 Effect of Bacteria Growth in Biodegradation Process

In **Fig. 4**, it shows that, in bioreactors with bacterial concentration of 10% (v/v) and 15%(v/v), for days 0 to 7, bacterial growth changes are not significant and tend to lag phase. It is likely that within this time span the existing microorganisms, still adaptation to their environment. While on the 7th day until the 42th there was a significant increase in bacterial growth, although on 49 days the tendency decreased. This increased bacterial grow, resulting in significantly reduced naphthalene for a bacterial ratio, 3:2 1:1; 2:3 and control reached, 115.65 to 33.77 ng/µL; 115.65 to 0.77 ng/µL; 115.65 to 10.28 ng/µL; 115.65 to 23.88 ng/µL, with bacterial growth were 1.08×10^7 to 7.10×10^7; 1.08×10^7 to 7.30×10^7; 8.25×10^6 to 4.75×10^7; respectively, for bacterial concentration of 10% (v/v) (**Fig. 4a**). When compared with bacterial concentration of 10%, the bacterial concentration of 15%(v/v) (**Fig. 4b**), showed significant bacterial growth which responded positively to naphthalene reduction every weeks, were 115.65 to 0.32 ng/µL; 115.65 to 0.19 ng/µL; 115.65 to 5.11 ng/µL, bacterial growth

were 1.13×10^7 to 8.55×10^7; 1.13×10^7 to 8.75×10^7; 1.13×10^7 to 8.10×10^7 cell/mL, respectively. The different things occurred in the bioreactor control, showed no significant results for bacterial growth or naphthalene reduction during 49 days remediation period. These results indicate that bacteria concentration of 15% gives a more optimal effect in degrading naphthalene.
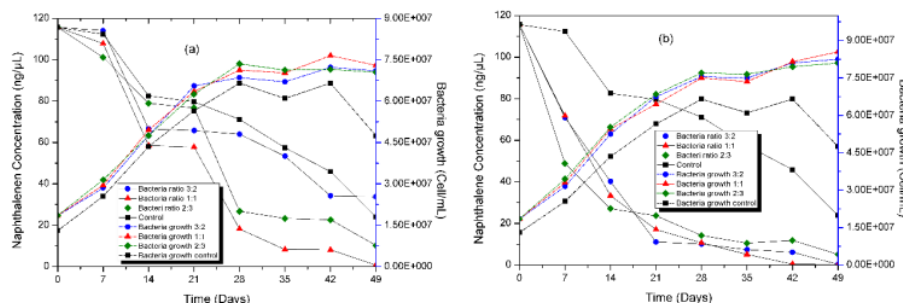

Fig. 4 The Relatioship between the Concentration Naphthalene against Time and Bacteria growth:
a) Concentartion of Bacteria 10%(v/v)); b) Concentartion of Bacteria 10%(v/v))

The growth of microorganisms is strongly influenced by the environmental conditions in which the microorganisms live (Robles-Gonzales et al., 2008). Greater concentrations of bacteria in the mixture cause the naphthalene contained in the mixture is also more rapidly degraded because the microorganisms use naphthalene as its carbon and energy source to maximize its metabolism in treating naphthalene waste in petroleum.

Biodegradation as a process of decomposition by microbial activity, resulting in the transformation of the structure of a compound resulting in changes in molecular integrity. In order for biodegradation to be effective, a suitable environmental condition is required to support microbial growth and development (Leisinger, 1981; Sheehan, 1997 in Nugroho, 2006). The bacterial consortium produced a larger petroleum sludge degradation presentation compared to petroleum sludge free from microorganisms (Nugroho, 2006).

4. Conslusion

The naphthalene waste treatment approach with slurry bioreactor shows good performance in degrading naphthalene in hydrocarbons petroleum contaminated-soil. The effects of the consortium *Bacillus cereus and Pseudomonas putida* bacteria in naphthalene degradation on oil-contaminated soil showed a significant decrease, the bacterial consortium was able to reduce the waste of petroleum well. Naphthalene concentration for variation of bacterial ratio was 1:1, bacterial concentration 10% from 115.65 ng/μL reduced to 0.77 ng/μL and bacterial concentration 15% (v/v) with the same variation of bacteria ratio from 115.65 ng/μL to 0.19 ng/μL, for 49 days remediation period, compared with other bacterial ratios. A larger percentage of Biodegradation was also achieved by a 1:1 bacterial ratio for 10% and 15% (v/v) bacteria concentrations.

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