
We send our revised article

1 message

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Sat, Mar 21, 2020 at 10:17 AM

To: IcoBiodiv FST Universitas Airlangga <icobiodiv@fst.unair.ac.id>

Cc: Nadia Safira <safirageez@gmail.com>, aziz hakim <azizph06@gmail.com>, Wiwit Yulia <wivit.sy15@gmail.com>

Dear committee Icobiodiv

We hereby submit a revision of 3 articles that have been revised. Titles of the articles are as follows :

1. Oil sludge biodegradation using biopile reactor with additions of microbial consortium, bulking agent, and inorganic nutrient
2. Oil Sludge Bioremediation Using Biopile With Addition of Sawdust
3. The effect of biostimulation of molase in the pertamina balongan oil sludge biodegradation using bioslurry reactor

We apologize for the delay in sending this revised article. Thank you very much for your help and attention.

Best regards

Dr. Ni'matuzahroh and team

3 attachments



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Wed, Jun 17, 2020 at 6:38 AM

To: nimatuzahroh nimatuzahroh <nimatuzahroh@fst.unair.ac.id>

Your final paper

Guest Editor

Special Issue

Prof. Agoes Soegianto

Department of Biology

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Universitas Airlangga

Surabaya Indonesia

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To: Enviro Unair <enviro.unair@gmail.com>

Sat, Jun 6, 2020 at 9:35 AM

Dear editorial team
Ecology, Environment and Conservation

Thank you for the opportunity given to us to recheck our article. I hereby send the results of our revised article entitles: Oil sludge bioremediation using biopile with addition of sawdust” and also the proof of transfer of payment for our article.

Best regards

Dr. Ni'matuzahroh et al.
[Quoted text hidden]

2 attachments



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Oil sludge bioremediation using biopile with addition of Sawdust

Aziz Purnomo Hakim¹, Wiwit Sri Yuliasuti¹, Nadia Safira¹, Nur Indradewi Oktavetri¹, Agus Supriyanto^{1,2}, Fatimah^{1,2}, Hanif Yuliani³, and Ni'matuzahroh^{1,2*}

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(Received 27 September, 2019; Accepted 10 March, 2020)

ABSTRACT

This research was focused on treating oil sludge from petroleum industry's waste which can cause serious environmental problems. Bioremediation is one of the effective and promising ways to manage oil sludge with biological activity. The ability of the bacterial consortium was used to reduce the value of Total Petroleum Hydrocarbon (TPH) with a biopile system. The pilot scale biopile research design used a mixture of soil, bacterial consortium nutrients, with sawdust variations of 0%, 15%, 30% and 45%. This biopile system was conditioned by the addition of oxygen to maintain aerobic conditions, incubated in atmospheric temperatures and two weekly sampling within 56 days. The combination of treatment at the best for total bacterial count was 7.21×10^7 CFU/g and the percentage of TPH degradation reached 81% at the end of the study period. Based on the results it can be concluded that biopile is able to be an effective, economical and environmentally friendly choice for the management of oil sludge.

Key words : *Biopile, Bioremediation, Sawdust, Oil sludge, TPH*

Introduction

Petroleum waste is classified as B3 (toxic hazardous material) according to Government regulation 2014. Government Regulation Number 101 of 2014 with waste code A307-1, which is sludge from the production process and storage facilities for petroleum or natural gas. One of the oil industry that produces waste oil sludge is PT Pertamina EP Balongan. The company has 14 oil storage tanks that contain petroleum. Each storage tank produces 21.97 m³ of oil sludge per year. According to Fahrudin (2010), the composition of aliphatic hydrocarbons is 57%, aromatic hydrocarbons 29%, and asphaltic hydrocarbons 14%, while the rest are non-

hydrocarbon components. So that the constituents contained in oil sludge are carcinogenic and potentially immunotoxic (Panda *et al.*, 2013).

With the increasing interest in the conservation of the environment, biological treatments have been improved and developed to clean up soils contaminated with hazardous compounds and have become an alternative to physical and chemical treatments (Kufaldi *et al.*, 2006). Bioremediation is a successful procedure for cleaning up polluted sites by petroleum compounds because it is applicable to large areas, leads to the full removal of the contaminants, and cost-effective (Menezes *et al.*, 2003).

Biopile is the process of treating petroleum waste by placing waste in oxygen supply pipes to increase

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aeration and microbiological decomposition of petroleum waste to be more optimal (Minister of the Environment, 2003). Aeration is carried out by an air compressor which drives the oxygen through the perforated tubes that are placed throughout the pile. Compared to composting or land farming, the efficiency of mass transfer of air, nutrients and air in biopiles contributes to the contaminants removal strategy (Motsara, 2008).

Oil sludge has a high clay content, according to the Centre for Environmental Technology (2010) the high clay content in oil mud causes the soil to become more easily expand and sticky when given moisture, resulting in a low percent degradation of 12.56%. This condition is achieved in about 16 weeks. The low rate of degradation of hydrocarbon compounds is due to the reduced oxygen content in the oil sludge (Sudirman *et al.*, 2006).

Various studies from Jie Ma (2016) show that the addition of a microbial consortium, inorganic nutrients (urea and K_2HPO_4) and sawdust can degrade 87% of the 3.5% oil content for 30 days. While Arifudin (2016) with the addition of sand, commercial compost and soil in the biopile system was able to reduce the TPH by a percentage of 80% for 63 days. The aim of this study was to design a small scale biopiles system and to evaluate its efficiency for clean-up of oil sludge from the petroleum industry's waste.

Materials and Methods

The materials mixture in biopile consists of soil, sawdust and oil sludge. The oil sludge used in this research was oil sludge from PT. Pertamina Balongan Indonesia, the soil used was obtained in the Mulyorejo area, Surabaya with a sieve hole size of 2 mm. Soil that escapes the sieve was used in this study. Fertilizers used in this study were commercial urea fertilizer and commercial NPK with CNP ratio of 100: 5: 1 (Zamrud, 2010), distilled water, physiological salt water, spiritus, 70% alcohol, Nutrient Agar (NA) media, Nutrient Broth (Broth) (Both NB), as well as four hydrocarbonoclastic bacteria, including *Bacillus* sp., *Acinetobacter* sp., *Micrococcus* sp., *Pseudomonas* sp. which comes from the collection of Airlangga University Microbiology Laboratory, organic solvent (n-hexana).

Isolates preparation

Four types of bacterial isolation were streaked on

NA media then incubated at room temperature (28 ± 2) °C for 24 hours. One or two full loops of isolates were also inoculated on NB media, then incubated at room temperature for 24 hours

Making of consortium bacteria

The method of making a consortium of hydrocarbonoclastic bacteria is based on STRANAS research by Ni'matuzahroh *et al.*, (2013). The volume of suspension is calculated from each stock of bacterial suspension tested with $A_{600} = 0.5$. The first step used was known that the bacterial consortium 400 mL (5% x 8000 g-soil). So, the bacterial suspension taken was 100 mL for each type of bacteria. The making of microbial consortium was done by mixing microbial suspensions consisting of *Micrococcus* sp., *Pseudomonas* sp., *Acinetobacter* sp., and *Bacillus* sp. (Darisa, 2014).

Biopile system design

As many as 40 biopile reactors used were tubular and made of plastic material. Having a volume of 615.44 cm^3 with a diameter of 7 cm and a height of 16 cm in a reactor equipped with an aerator. Aeration is carried out for 12 hours to prevent the evaporation of water in the reactor as research by Jie Ma (2016) using the aeration rate of 12 hours on and 12 hours of aeration is turned off within 30 days of bioremediation. This research uses a sampling time for 2 weeks based on Indah's research (2017). Taking samples every two weeks is considered easier in observing bacterial growth and weight reduction in TPH oil mud residues. In this study was conducted for 56 days.

Biopile experiment

The main component in the biopile experiment, each tube was filled with as much as 10 grams of oil sludge. To identify the effect of variations in sawdust addition to the percentage of TPH residual weight reduction, four biopile designs were prepared including (1) Biopile reactors without adding sawdust 0% (V0) + oil sludge + microbial consortium + soil + inorganic nutrients (urea + NPK) (2) Biopile reactor with adding 15% sawdust (V1) + oil sludge + microbial consortium + soil + inorganic nutrient (urea + NPK) (3) Biopile reactor with adding 30% sawdust (V2) + oil sludge + microbial consortium + soil + inorganic nutrient (urea + NPK) (4) Biopile reactor with adding 45% sawdust (V3) + oil sludge + microbial consortium + soil + inorganic

nutrient (urea + NPK), 10 mL microbial consortium and 9.5 mL nutrient inorganic were inoculated in each tubes.

Measurement of pH and moisture

During the biopiling process, the pH of the soil was measured using a soil tester by sticking the tip of the soil tester into the soil. The button was then pressed long to measure the pH of the soil. The value on the upper part shows the soil pH value from 1 to 14 and the bottom value shows the soil moisture value expressed in percent (%).

Total Plate Count (TPC)

Cultures that incubated for 0, 14, 28, 42 and 56 days were diluted as needed. Every three last dilutions, the suspensions were inoculated into a Petri dish using the pourplate method, then added 15 mL NA, and homogenized. The culture was incubated for 24 hours at room temperature (28 ± 2)°C. The number of eligible microbial cells (30-300 colonies) is stated in CFU / g (Ibuot and Bajhaiya, 2013).

Total Petroleum Hydrocarbon (TPH)

5 grams of soil sample was taken from each tube and dried in an oven at 70°C for 6 hours. N-hexane was added as much as 20 mL in 2 times the extraction. Samples were homogenized for 10 minutes with vortex and deposited for 10 minutes. The extraction results were evaporated and weighed with analytical scales. TPH calculation was done gravimetrically with the APHA formula (2005):

$$OR = \frac{W_1 - W_0}{W} \quad \dots (1)$$

In the formula, OR is Oil residue (g/g) W_1 is pumpkin weight + residue, and W_0 is pumpkin weight. TPH value obtained can be calculated to obtain the percentage of degradation using the formula:

$$DE (\%) = \frac{W_0 - W_1}{W_0} \times 100 \% \quad \dots (2)$$

In the formula, DE is degradation efficiency (%), W_0 is initial weight, and W_1 is final weight.

Analytical method

All data obtained such as Total Plate Count (CFU/g), Total Petroleum Hydrocarbon (g/g), pH, and humidity were analyzed descriptively.

Results and Discussion

Total Plate Count (TPC)

The total bacterial count was at an abundance of 5.22×10^5 CFU / g (H0V1) to 6.48×10^6 CFU / g (H4V3) during the 56-day incubation time. This insignificant difference also occurred in Lusiana's study (2012) on the addition of *Pseudomonas aeruginosa* concentration of 3% with an abundance of bacteria around 9.31×10^4 CFU/mL to 2.28×10^7 CFU/mL within 21 days. Low bacterial counts and low contaminants levels can indicate that biodegradation is successful and that bacteria are declining because the contamination (food source) is decreasing. These results matched with Obiakalaje and Makinde (2015) who studied the biostimulation effect on the degradation of crude oil-contaminated soil. The number of hydrocarbon degrading bacteria was increased from 2.51×10^5 to 1.74×10^6 CFU / g. The result are presented in Fig. 1.

It can be seen from all treatments from H0 to H1 that an exponential phase occurred here at this time the bacteria can adapt and utilize nutrients on the substrate to metabolize, without a lag phase. This lag phase is expected to last briefly as reported in Suthar and Singh (2008) research that the growth of *Bacillus subtilis* MUV4 only experienced a lag phase in the first 6 hours. This is also due to the initial environment of the bacterial consortium that was added as well as microbial indigenous suitable to the environment contained in this study were isolated and found from environments polluted by petroleum waste. Setyati *et al.*, (2015) stated that if the isolates used in the treatment and research have often been used, then these isolates require an adaptation phase in an effort to adapt to the environment that has only been running for several hour

Furthermore, the results of observations at the

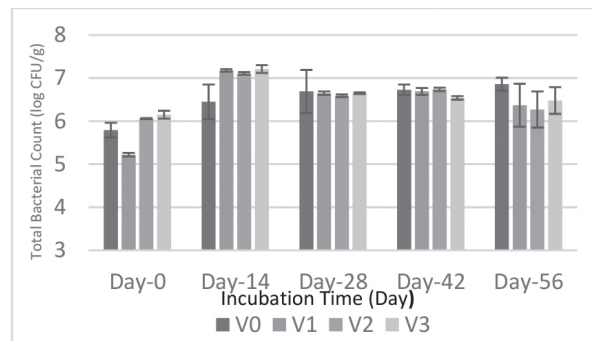


Fig 1. Bacterial growth during incubation time

time of H2 (28th day) incubation decreased bacterial abundance, this result is influenced by the start of decreasing the content of the substrate in the reactor in line with the decline in TPH value of oil mud. According to Nugroho (2006) high bacterial population density can affect the decrease in the number of bacteria due to the presence of acid metabolites produced by the bacteria themselves. Thus, bacteria are able to utilize hydrocarbons as an energy source but due to acid metabolites that are produced become toxic to the bacterial environment and reduce their abundance. On H3 (42nd day) and H4 (56th day) bacterial growth begins to enter the stationary phase until death is estimated to decrease the substrate content.

TPH removal efficiencies

The initial TPH concentration in the tube was 0.069 g/g until 0.071 g/g (Table 1) and after 56 days of bioremediation process, there is increasing of TPH up to 28.58%, 63.78%, 66.25% and 81.01% (Fig. 2) in biopile's tube H4V0, H4V1, H4V2 and H4V3 respectively. The final TPH concentration in the tube H4V0, H4V1, H4V2 and H4V3 were 0.049 g/g, 0.026 g/g, 0.023 g/g, and 0.012 g/g respectively (Table 1.)

Table 1. Calculation results of the TPH degradation value

Treatments	TPH (g/g)	
	0 th day	56 th day
V0	0.069	0.049
V1	0.071	0.026
V2	0.068	0.023
V3	0.063	0.012

Adding sawdust as bulking agents can significantly enhance the contaminant removal efficiency in biopile system. The TPH removal efficiency of the tube H4V1 (63.78%) H4V2 (66.25%) and H4V3 (81.01%), were higher than that of the tube H4V0 (28.58%). Enhancements in contaminant removal by adding bulking agents (sawdust) were also reported in other biopile system by Jie Ma (2016) whom his TPH removal efficiency was 26.6% higher than non adding bulking agents. Oxygen content is known to be a key factor for biopiling process. Additional of bulking agent enhanced the diffusion of oxygen into the pile, thus facilitating microbial metabolism and aerobic biodegradation of contaminants (Marín *et al.*, 2006). Since in nature, the bioremediation process depends on the cooperative metabolic activities

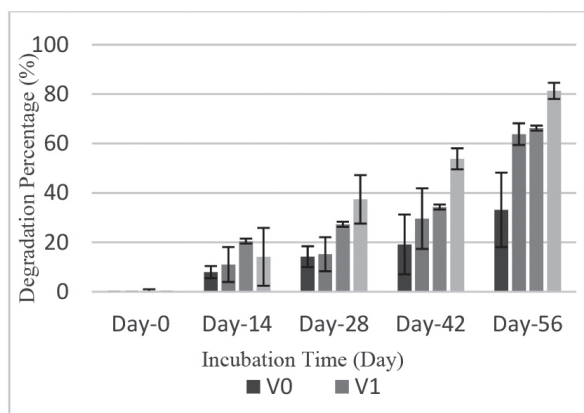


Fig 2. Percentage of TPH degradation

of organisms (Cerquiera *et al.*, 2011). The result agrees with those of (Shabir *et al.*, 2008), who found that the biodegradation rate of kerosene-contaminated soil was significantly enhanced by the addition of inorganic amendments along with the addition of mixed bacterial culture through 6 weeks and reached to 65 %.

The highest final result of TPH percentage degradation was 81.01 and if compared to other studies the percentage was not far behind. The experiment by Arifudin (2016) using a biopile system from crude oil with adding sand, compost and soil reached percentage of degradation in 80% for 63 days. Jie Ma (2016) was also succeeded to degrade crude oil in number 87% for 30 days. This result can be caused by differences in the characteristics of the oil sludge used, the type of bacterial consortium, and the added inorganic nutrients that affect the biodegradation results of hydrocarbon compounds. Although it has different results from this research comparison, it can be seen that sawdust can be used as a potential bulking agent in the process of biodegradation of oil sludge. According to Gustan (2002) sawmills in Indonesia reached 1.4 millions m³/ year, in which sawdust was produced around 15% in total, so there were 210,000 m³/year. Therefore using sawdust as bulking agents in biopile can be reduced the number of sawmills in Indonesia.

Change in pH and moisture during biopile experiment

Variations in the pH of biopile tubes can be related to metabolic processes. However, the pH range observed in this study was within the pH range suitable for microbial growth which showed that this isolate showed optimal growth in the pH range

from 6.8 to 7. Riskuwa and Ijah (2016) reported that the growth of most microorganisms normally the largest in the pH range is 6 to 8. Moisture content is an important and measured factor to ensure that the air injected through the biopile system pipe does not dry the soil and thus determines bacterial growth. Moisture measurements range from 60% to 80%. Suardana *et al.* (2002) state that the ideal groundwater content is 50%, if the groundwater content of the mixture is below 60% then the structural integrity is not good, the increased moisture value does not increase optimally in bacterial growth and constant hydrocarbon degradation happen. The results of pH and water content are listed in Table 2.

Table 2. pH, and moisture content of biopile reactor

Times (days)	pH value	Moisture content (%)
0 th day	7.0	58.7
14 th day	6.8	6.46
28 th day	6.7	7.56
42 nd day	7.0	7.75
56 th day	7.0	7.87

Conclusions

Results show that small design biopile system with adding sawdust 45% was effective to degrade TPH removal as much as 81.01% for 56 days. Based on the results, sawdust have potential as bulking agent in the process of biodegradation of oil sludge

Acknowledgement

We would like to thank Kemenristek Dikti and Universitas Airlangga through the "Penelitian Strategis Nasional, 2017" for funding and facility support and Mrs. Nastiti Trikurniadewi, S.Si., M.Si, Mr. Zainal Abidin S.Pd., M.Si and Mr. Suwarni, S.Sos. who helped us during the research.





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Sat, May 30, 2020 at 8:30 PM

To: nimatuzahroh nimatuzahroh <nimatuzahroh@fst.unair.ac.id>

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Special Issue

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