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Oil sludge biodegradation using biopile reactor with additions of microbial consortium, bulking agent, and inorganic nutrient

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ABSTRACT

Oil sludge is a form of hydrocarbon sludge that comes from oil refining industry, where sludge is obtained from the result of oil drilling and from the sediment in the tank. Biopile is one of many attempts to prevent oil pollution. This study was to know the effects of variation of adding microbial consortium, bulking agent (rice husk), inorganic nutrient and length of incubation time on total microbial (CFU/g) and reduction of residual weight of hydrocarbon in oil sludge (g/g) on biodegradation using biopile reactor. Parameters measured were total microbial (CFU/g), reduction of residual weight of hydrocarbon in oil sludge which were measured with gravimetric method (g/g), and pH that measured using soil tester. The results showed that variation of treatments and incubation time had an effect on total of microbial count (CFU/g) and residual hydrocarbon in oil sludge (g/g). The variation that reached the highest biodegradation percentage was V3 with value of 44.84% on day 21st with log of total microbial count as much as 8.71 (CFU/g), final TPH (Total Petroleum Hydrocarbon) 0.22 (g/g), and pH 7.36.

Key words: *Biodegradation, Oil sludge, Biopile reactor, Bulking agent, Inorganic nutrient.*

Introduction

Oil sludge is produced since the process of oil drilling until process of storage in the refinery. Oil sludge consists of hydrocarbon, water, ash, tank rust, sand, and other chemical compounds (Bustan, 2008). Oil sludge causes pollution to the surrounding terrestrial and aquatic environment quality, causing the biota that occupies the environment to be disturbed. Besides that, the decreasing of environment quality due to oil sludge contamination can reduce functions of ecological balance as well as functions of the environment for human (Munawar and Zaidan, 2013).

Oil sludge treatment is done physically, chemically and biologically. Biological oil sludge treatment is an effective alternative in terms of cost and is safe for the environment. Treatment with biological method is also called with bioremediation, that is biotechnology which uses living creatures particularly microorganisms to decrease concentration or the toxic power of pollutants (Kurniasari, 2005).

In bioremediation, the more number of specific microorganism population such as bacteria, fungus, or algae, the more contaminant concentration is decreased, which represented generally by concentration of Total Petroleum Hydrocarbon (TPH) (Kurniawan, 2012). Bioremediation for pollution recovery due to oil mining activities must involve microbes which have metabolic capacity to carry out biodegradation on the oil sludge compound. Bacteria are the most important type of microbe and commonly involved in petroleum degradation. That group of bacteria is called hydrocarbonoclastic (Juliani, 2016). The genera of hydracarbon-degraders which commonly reported the most are *Vibrio*, *Acinetobacter*, *Achromobacter*, *Pseudomonas*, and *Nocardia* (Salleh *et al.,* 2003).

In bioremediation of soil, biopile is an ex situ treatment system which consists of contaminated soils that is aerated and remedied by adding mineral, biological, or organic material which depends upon particular needs (Benyahia, 2005). Biopile is a bioremediation technique that involves piling petroleum polluted soil on top of soil that has low permeability or can be put on top of a concrete. To increase the process of petroleum biodegradation, the pile of polluted soil is given aeration and nutrition (Arifudin, 2016). Addition of inorganic nutrient would significantly enhance the contaminant removal efficiency (Ma *et al.,* 2016). On bioremediation process using biopile, bulking agent is added as media to fix permeability, water holding capacity and soil porosity, so that biodegradation would be rapidly increased (Kurniawan, 2012). Bark chips or wood as bulking agents are usually recommended because they have high content of xylogen and high resistance to degradation, but they are not so easy to get. Rice husks, on the other side, are readily available at minimal cost (Li *et al.,* 2002).

Research on bioremediation of hydrocarbon polluted soil using biopile reactor had been done, some of them are research by Ma *et al.* (2016) that used saw dust as bulking agent, research by Effendi (2006) that successfully decreased TPH until achieving environmental quality standards in 15 months, and a study by Chemlal *et al.* (2012) which compared two microcosm systems, indigenous and preadapted consortium, in their biopile. This research was aimed to know the effects of adding microbial consortium, bulking agent (rice husk), and inorganic nutrient on oil sludge biodegradation using biopile reactor. This research was expected to achieve the best consortium concentration on degrading oil sludge in optimal time using biopile reactor.

Materials and Methods

Materials used on this research were oil sludge from PT. Pertamina Balongan Indonesia, aquades, physiological salt water, spiritus, alcohol 70%, Nutrient Agar (NA), Nutrient Broth (NB), also four hidrocarbonoclastics bacteria (*Bacillus* sp., *Acinetobacter* sp., *Micrococcus* sp. and *Pseudomonas* sp). from the collection of Microbiology Laboratory Airlangga University, n-hexane, NPK fertilizer and urea, bulking agent (rice husk), and soil. Also, other materials like aluminum foil, cotton, and plastic wrap.

Biopile Reactor

The biopile reactor is a rectangular container made from polymer. The dimensions of the container were 13 cm in width, 13 cm in length, and 9 cm in height. The reactor was completed with aerator that has 2l/minute air flow capacity. The reactor design was arranged in series. Hose with diameter of 0.5 cm was installed inside the biopile reactor so that it will accomodate in achieving equal air inside the reactor. Wire mesh was cut in the same length as the reactor and then put on the base of the reactor with 1 cm space above the base. Muslin then cut in the same width of the container. The muslin had pore diameter of 200 μm and put on top of wire mesh to hold oil sludge and substrate mix so that it will not fall to the base of the container and clog the aeration. Reactor was given water until humidity of 60-80% achieved, with frequency of 3 times a day by using spray bottle so the soil will stay damp.

Total composition of substrate and oil sludge inside the reactor was as much as 240 g. Substrate composition consisted of fertile soil, microbial consortium, bulking agent which is rice husk, and also

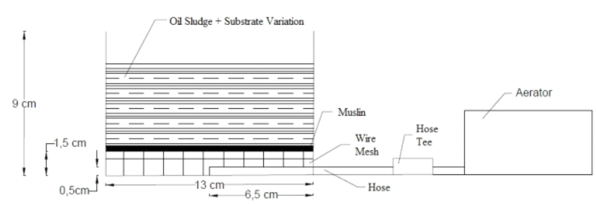

Fig. 1. Biopile Reactor Design

inorganic nutrient in the form of NPK fertilizer and urea. There were 6 different reactor variations, which were V0 (oil sludge + fertile soil), V1 (oil sludge + fertile soil + microbial consortium), V2 (oil sludge + fertile soil + bulking agent), V3 (oil sludge + fertile soil + inorganic nutrient), V4 (oil sludge + fertile soil + bulking agent + microbial consortium), and V5 (oil sludge + fertile soil + bulking agent + microbial consortium + inorganic nutrient).

Gravimetric Method

TPH was measured using gravimetric method. As much as 20 g sample poured inside vortex tube and extraction was done with using n-hexane on the ratio of 1:1 which was 20 g sample and 20 mL n-hexane with two steps of adding (10 mL-10 mL). Substrate then homogenized with a vortex as long as 20 minutes. After that, substrate was left sit to separate liquid phase and organic phase. Supernatant which was solvent that already contained with TPH then transferred into a dark bottle. Then, evaporation was done in gravimetric manner with n-hexane boiling point which was as high as 60-70 °C and then scaled (Munawar, 2013).

Total Plate Count (TPC)

Total bacterial count was done by taking sludge sample as much as 1 mL using measuring pipette. Sample was then diluted until 10-15 times dilution using physiological salt solution. Ratio of sample volume and physiological salt solution inside one test tube was 1:9. Each one of three last dilution were taken as much as 1 mL and poured inside Petri dish using pour plate technique with NA media. Sample then left as long as 15 minutes and incubated on temperature 30 ^{o}C as long as 24-48 hours. Colony was counted using colony counter. Total colony that qualified to be counted was as much as 30-300 colonies.

pH Measurement

pH was measured using soil tester. The tip of soil tester was plugged into the soil. Then the button was pressed for quite a long time to measure the pH of the soil. The value that showed on the upper part indicates the value of the pH from 1 until 14 and the value that showed on the lower part indicates the value of the humidity in % (Hasanah *et al.*, 2014).

Data Analysis

All data obtained such as Total Plate Count (CFU/g), TPH oil sludge (g/g) and pH were analyzed descriptively.

Results and Discussion

Total Plate Count (TPC)

Based on the Figure 2, it can be seen that the average of total microbial (CFU/g) kept increasing during the incubation time. The highest average of total microbial (CFU/g) was on day 21st, and the lowest was on day 0. In all treatments, microbes grew until the 7th day of incubation, after which it slowed to 14th day and increased again until the 21st day. On the 7th and 14th day there was not any real difference. Microbes were in stationary phase where some microbes died because they didn't have the ability to adapt. Then, there was a rise on log of total microbial count from day 14th to day 21st with value of 7.96 (CFU/g) to 8.71 (CFU/g). The rise shows that microbes were in second exponential phase where microbes have the ability to grow and adapt. However, in this research microbes had not entered death phase.

Figure 2 also shows that only on the control variation (V0) bacteria were able to live from nutrition obtained from oil sludge and fertile soil and also supported by enough oxygen supply from the aerator. Arifudin (2016), stated that organic material content inside of soil effected bacterial growth and the soil ability to hold water. Soil with high organic material content has the ability to hold water in longer time. Enough availability of oxygen and nutrition inside the soil can increase bacterial activities on degrading hydrocarbon.

It also can be seen that the highest average of total microbial (CFU/g) in combination between the variation of each reactor and incubation time occurred in variation V3 on the 21st day with value of

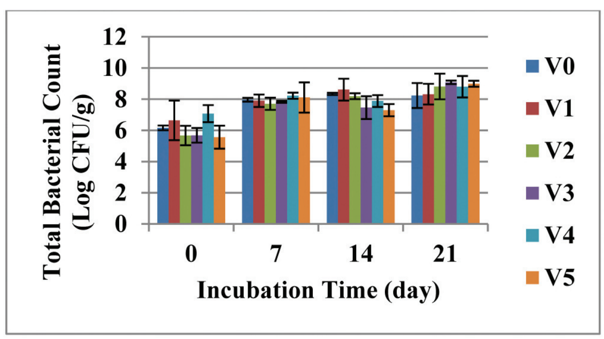

Fig 2. Total of microbial count during incubation time

9.08 CFU/g. Overall, every variation showed an increase in value of total microbial (CFU/g) on every incubation day, but the variation mostly showed fluctuation on the value of total microbial (CFU/g). Variation that indicated increasing value on every incubation time was variation V2, which was oil sludge mixed with fertile soil and bulking agent in the form of rice husk.

Total Petroleum Hydrocarbon (TPH)

The most important factor in hydrocarbon biodegradation is the microbes inside of oil sludge or also known as indigenous microbial. Variations of treatment that had been done in this research and aeration inside each reactor helped those microbes to degrade hydrocarbon faster and more effectively. Bacteria in degrading hydrocarbon need oxygen to produce oxygenase enzyme that will produce carbon dioxide (Arifudin, 2016).

In every reactor, fertile soil was added because organic material existence in the soil have the ability to hold water in longer time than soil with low organic material content such as sandy soil. Because of the soil crumb characteristic, air will flow through soil much easier either from blower or atmosphere (Arifudin, 2016). Bulking agent in the form of rice husk was added to make loose of oil sludge and fertile soil mix so that air will fill the empty space created by crumby soil aggregate which has different shape and size.

Microbial consortium was added to aid the indigenous microbes in degrading hydrocarbon of oil sludge. Those microbes took advantage of carbon from hydrocarbon as source of carbon for their growth. Then, inorganic nutrient in the form of NPK fertilizer and urea was added to maintain nutrition availability. However, the added nutrients can also be toxic if added too much. There is also a possibility that microbes will favor the added nutrients more than hydrocarbon so that hydrocarbon will not be degraded anymore.

The value of TPH residue on day 0 had a difference on starting point and it showed tendency of decreasing value on day 21st. Baldan *et al.* (2015), stated that during the first month the decrease in oil content is the most significant, because all of the variations of the oil content were quite adequate. TPH degradation percentage was counted based on difference of TPH residue value on day 21st and day 0 because in this research sampling was done from one same reactor in different incubation time. Order of treatment variations that had biodegradation percentage from the highest to the lowest were V3, V5, V2, V0, V4, V1, with value of 44.84%, 41.88%, 38.91%, 37.38%, 20.52%, and 12.13%. Variation V3 had the highest value of hydrocarbon degradation because TPH residue value in each incubation time was always constantly decreased, different from any other treatment which values fluctuated. Variation V3 consisted of oil sludge, fertile soil, and inorganic nutrient in the form of NPK fertilizer and urea. Ma *et al.* (2016), stated that inoculation of exogenous microorganism will not add much efficiency in hydrocarbon degradation with biopile. Therefore, rather than adding new species, it's more important to make more appropriate condition so that indigenous bacteria will live.

Adding inorganic nutrient should be able to increase total microbial and optimize reduction in TPH residue. In this research, reactors which had NPK and urea fertilizer added (V3 and V5) had the lowest value of average total microbial, but the highest value of average TPH residue. Concentration and type of inorganic nutrients will affect the process of microbial metabolism. Inorganic nutrient requirements are specific for each microbe.

Initial TPH of oil sludge was as much as 0.43505 g/g, and TPH value in the end of the incubation time at 21st day was 0.21701 g/g. Based on Decree of Ministry of the Environment (2003), the regulation of the final value of TPH for petroleum processing biologically is 10.000 µg/g. Therefore, TPH value in the end of incubation time still has not met the requirement so it will take subsequent treatments and also longer time.

Changes in pH

Figure 4 shows that the average pH value of each variation of treatment is different and has value range of 7.13-7.71, which the value is still in between

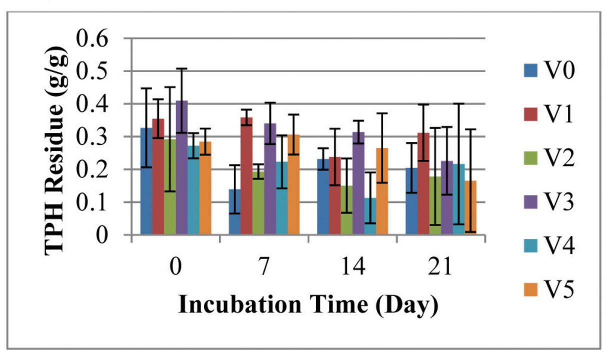

Fig 3. TPH residue during incubation time

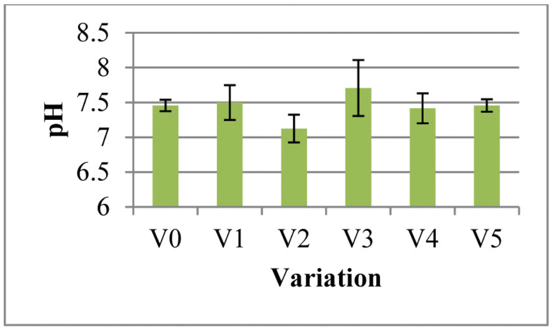

Fig 4. pH value on different variation of treatments

optimal pH value range for hydrocarbon degradation in soil according to Marques *et al.* (2010), which is 6.0-8.0. Based on the figure, it can be seen that there was a variation which had significant difference of average value of pH. V2 treatment had the lowest average value of pH among all variations. V2 which consisted of fertile soil and bulking agent in the form of rice husk, shows that adding bulking agent increase biodegradation activities that can decrease the average value of pH.

The hydrocarbon biodegradation process begins with the release of carboxylic radicals and the consequent formation of organic acids, resulting in acidification of the substrate (Morais *et al.*, 2014). While, the variation of treatments that have the highest average pH is V3 which consists of fertile soil and inorganic nutrients such as fertilizer. The fertilizer is added to neutralize the pH and added as a buffer that will balance the impact of organic acid production in the biodegradation process, so that the pH in this treatment is still relatively stable.

Conclusion

Variation of treatment with the addition of a microbial consortium, bulking agent, and inorganic nutrients affects the biodegradation of oil sludge in the biopile reactor. The treatment by optimizing the ability of indigenous microbial degradation with nutrient stimulation produced the highest percentage of oil degradation, reaching 44.84% at 21 days of incubation with a TPC achievement of 8.71 log CFU/g., TPH residue of 0.22 (g/g) and pH 7.36. The percentage of oil biodegradation in a biopile reactor can be increased by extending the incubation time.

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