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Assessing the effectiveness of different bacterial consortium types and varying incubation times in the bioremediation of oil sludge waste

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ABSTRACT

Aims: The aim of this study was to determine the effect of the addition of the type of bacterial consortium, incubation time, interaction between the types of bacterial consortium and incubation time on the number of bacteria (CFU/g-soil), oil sludge content (g/g-soil), percentage degradation of oil sludge in the best treatment, the ratio of C/N at week 6, as well as hydrocarbon components.
Methodology and results: This research is a laboratory experimental study that used a completely randomized design with an 8 × 4 factorial pattern with two replications. For data analysis, logarithmic data of total bacterial plate count (CFU/g-soil), gravimetric results of oil sludge (g/g-soil) and degradation percentage were analyzed descriptively and statistically. The results showed that the percentage of degradation in the best treatment, namely the treatment of a consortium of biosurfactant-producing bacteria (B) was 77.8% and the time was after six weeks of incubation period. The C/N ratio at the end of the 6-week incubation period ranged from 70.5-102.1. While the hydrocarbon components that can be degraded in the treatment of a consortium of biosurfactant producing bacteria (B) are Naphthalene, 2,3,6-trimethyl, Naphthalene, 1,4,6-trimethyl and Naphthalene, 1,6,7-trimethyl.
Conclusion, significance and impact of study: The type B consortium treatment produced the best results in decomposing oil sludge-contaminated soil. Research findings indicate that the kind of consortia B has the potential to be employed as a consortium to degrade oil sludge or other waste with similar characteristics.

Keywords: Bacterial consortium type, composting, incubation time, oil sludge

INTRODUCTION

The phenomenon of oil pollution is still an emerging issue in Indonesia. Oil pollution is mainly caused by the activities of the mining industry as well as oil and gas processing companies. The result of oil mining and processing activities is oil sludge. The main content in oil sludge, which is the most difficult to decompose in nature, is hydrocarbon compounds. When these compounds contaminate the soil surface, these substances can evaporate, be washed away by rainwater, or enter the soil and then precipitate as toxic substances, causing disruption of ecosystems and the water cycle (Larasati and Mulyana, 2013).

Several physicochemical treatment efforts have been used to overcome the oil sludge problem, but there are still some shortcomings in terms of safety, duration and cost. Therefore, bioremediation technology is widely used to speed up the recovery process of oil-contaminated land. There are limiting factors in bioremediation, namely microbial population, environmental factors and nutrition (de Oliveira Santos *et al*., 2018).

Additionally, there are several factors that can affect the process of biodegradation, which include biosurfactants produced by microbes, pH, nutrients, salinity, oxygen, temperature, pressure, humidity and increased genetic mechanisms. The factor is the length of incubation time, or the time required for a microbe

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(microorganism) to adapt and multiply until it reaches an exponential phase, which has high activity in remediating pollutants. The length of incubation time shows that the microbial growth process is increasing with the addition of the number of microbial cells so that the bioremediation process can run quickly (Fitri *et al*., 2017). The bioremediation process can be carried out through regulating environmental conditions, adding nutrients (biostimulation) and adding microbes from the outside (bioaugmentation) (Ławniczak *et al*., 2020). Bioaugmentation techniques can be applied adding microbes that are foreign (exogenous) or native (indigenous) populations (Salim *et al*., 2018). The addition of microbes is not only focused on their ability to degrade the main pollutant compounds but also their ability to survive in complex conditions of contaminants, such as the presence of heavy metals as co-contaminants (Azubuike *et al*., 2016).

The techniques of providing competent microbes at the right time can help the degradation process so that it can occur continuously (Prayitno, 2017). Some of the advantages offered by this bioaugmentation technique are that the removal time of contaminants in the soil will be faster and more efficient compared to bioremediation technology that is carried out without the addition of microbes at all. Several genera of microorganisms that can decompose or change the presence of petroleum and its derivatives, among others, are from the bacterial group (*Pseudomonas*, *Rhodococcus*, *Arthrobacter*, *Alcaligenes*, *Acinetobacter*, *Alteromonas*, *Moraxella*, *Bacillus*, *Flavobacterium*, *Vibrio, Micrococcus*, *Gluconobacter* and *Mycobacterium*), from the *Trichoderma* and *Candida* (Ławniczak *et al*., 2020).

Larasati and Mulyana (2013) stated that the addition of a microbial consortium, such as indigenous fungi (*Aspergillus niger* and *Trichoderma zeanum*) as well as non-indigenous bacteria (*Bacillus sphaericus*, *Bacillus cereus* and *Pseudomonas aeruginosa*) based on irradiation in 30% sawdust at 50% soil concentration could optimally degrade TPH (Total Petroleum Hydrocarbon) by 81.32% on land contaminated with oil sludge waste.

Pseudomonas fluorescens, *P. cepacea*, *P. pseudomallei* and *P. putida* are species of the genus *Pseudomonas* sp., which are capable of degrading hydrocarbon compounds (aliphatic and aromatic) and also have a bioabsorption and bioaccumulation mechanism for heavy metals for one week (Handrianto, 2018). Bioaugmentation techniques can be used in various applications of *ex-situ* bioremediation techniques, such as composting, which is an effective and economical way to treat oil sludge (Melati, 2020).

The composting process includes the addition of nutrients, irrigation, improvement of soil structure, bioaugmentation (addition of microbes to polluted areas) and biostimulation using bulking agents (rice husks or sawdust), which are determined as effective options to increase bioremediation of soil contaminated with oil sludge (Prakash *et al.*, 2015). These bulking agents function as regulators of porosity, humidity and a source of nutrients (Fitri *et al*., 2017).

Based on the facts stated above, it turns out that during this time, the combination of incubation time and the addition of a consortium of bacteria consisting of indigenous oil sludge isolated from oil sludge waste (*Pseudomonas aeruginosa* strain HF1 and isolate 2, hydrocarbonoclastic (*P. pseudomallei*, *P. cepacea*, *P. tzutzeri* and *P. fluorescens*) and biosurfactants (*Micrococcus* sp., *Bacillus* sp., *Acinitobacter* sp. and *P. putida*) with composting have never been carried out on a larger scale in the laboratory, to see their effectiveness against soil bioremediation polluted with oil sludge. Therefore, this study was aimed to determine the effect of adding the type of bacterial consortium and the length of incubation time in the bioremediation of soil contaminated with oil sludge in the hope that it can help eliminate oil sludge contamination in the soil.

MATERIALS AND METHODS

Place and time of research

This research was carried out at the Integrated Laboratory, Department of Biology, Faculty of Science and Technology, Universitas Airlangga Surabaya. The C/N ratio analysis was carried out at the Soil Science Laboratory, Universitas Brawijaya Malang and GC-MS (gas chromatography - mass spectrometry) analysis was performed in PT. Gelora Djaja Surabaya.

Research tools and materials

The tools used in this research are as follows: autoclave (type MC-30 L), laminar air flow (LAF) (Faster), GC-MS (gas chromatography-mass spectrometry) Hewlett-Packard 5890 Seri II, soil tester, spectrophotometer (Scientific type Enesys), rotary evaporator (type RV05-ST), separating funnel volume 100 mL and 500 mL, shaker (Wina Instrument type 109).

The research material included isolates of hydrocarbonoclastic bacteria, namely *Pseudomonas fluorescens*-25, *Pseudomonas pseudomallei*, *Pseudomonas cepacea* and *Pseudomonas stutzeri*. Biosurfactant-producing bacteria such as *Micrococcus* sp., *Pseudomonas putida*, *Bacillus* sp. and *Acenitobacter* sp. Meanwhile, the indigenous bacteria are *Pseudomonas aeruginosa* strain HF1 and isolate 2. The bacterial growth medium used was nutrient agar (NA) and nutrient broth (NB); bioremediation test substrates are fertile soil (garden soil) and beach sand. The bulking agent used is sawdust and oil mud obtained from the VICO company's oil well drilling site.

Making a bacterial consortium for treatment

Ten culture bottles of 250 mL volume were prepared. The volume of bacterial suspension that must be taken from each stock of the tested bacterial suspension with an OD

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A600 nm = 1, equal to 3.0 × 10^8 cells/mL. The concentration used was 5% = 50 mL.

Oil sludge bioremediation test treatment

Ten per cent (10%) of oil sludge (100 g) was weighed in the treatment container. Then, fertile soil, sand, sawdust, a consortium of bacteria and oil sludge were mixed in a tub for each treatment with a total media of 1000 g. The treatments tested included control (K), addition of a consortium of hydrocarbonoclastic bacteria (H), addition of a consortium of biosurfactant bacteria (B), addition of a consortium of indigenous bacteria (I), a combination of a consortium of indigenous bacteria and biosurfactants (IB), combination of a consortium of indigenous bacteria and hydrocarbonoclastic (IH), a combination of a consortium of hydrocarbonoclastic bacteria and biosurfactant (HB), as well as a combination of a consortium of hydrocarbonoclastic, biosurfactant and indigenous bacteria (HBI).

The pH measurement was carried out using pH paper which was dipped in a solution consisting of 1 g of sample in 9 mL of sterile distilled water. The pH paper is used because it can easily and quickly determine the pH from a small sample. The pH measurements were conducted at the beginning of the treatment (week 0), the second (week 2), the fourth (week 4) and the sixth week (week 6). Based on research by Ni'Matuzahroh *et al*. (2017) where the optimal degradation process is 5 weeks. We tried for up to 6 weeks to find out if there was a significant difference between the degradation process and previous studies. The 60% humidity setting was done by spraying 7 mL of sterile aquadest in the treatment every 2 days.

Biodegradation ability measurement

For the measurement of total plate count (TPC), with dilution series which pour plate technique. Bacteria growing on nutrient agar media in Petri dishes were counted and then multiplied by 1/dilution factor.

The TPH calculation from oil sludge is done gravimetrically. Gravimetric calculation formula (Harvey, 2005):

Oil residue (g/g) =
(Total bottle weight and residue - Bottle)/Initial weight (g)

Biodegradation (%) =
[(Residue on control - Residue on treatment)/Residue on control] × 100

C/N ratio determination analysis

The analysis of C level determination followed the Pangestuti method (2008). The calculation of N content followed the Kjeldahl method.

Gas chromatography analysis

Gas chromatography analysis was performed on the oil residue from the gravimetric results at the end of the incubation period. The residual oil obtained was then dissolved in 7 mL of hexane. The solution was then analyzed using GC-MS.

Statistical analysis

The logarithmic data of total plate count bacteria (CFU/g-soil), gravimetric results of oil sludge (g/g-soil) and the percentage of degradation were then analyzed descriptively and statistically. The analysis used was Brown-Forsythe, followed by the Games-Howell test (p=0.05).

RESULTS AND DISCUSSION

The total of bacteria (CFU/g-soil)

All types of bacterial consortium treatment at week 0 experienced an increase in growth until week 4. Then, in the 6th week, there was a decrease in growth, except for the type B consortium treatment, which continued to experience an increase in growth until the 6th week (Figure 1).

Along as the incubation time increased, the consortium treatments K, H, B, I, IB, IH, HB and HBI gave different growth responses, which were observed from the increase and decrease in the number of TPC (total plate count) cells in the 2nd week to 6th week (Figure 1).

The total number of bacteria (TPC) value is used to determine the presence of heterotrophic bacteria, with the growth medium used as nutrient agar. The total plate count (TPC) is not a determinant of the level of pollutant degradation ability because even though the degradation process was taking place during the study, maybe the TPC for hydrocarbonoclastic bacteria was not completely captured. The appearance of the TPC log results in almost all treatments decreasing, but the degradation process continues.

The cause of the decrease in the number of bacterial cells is the availability of oil in the oil sludge. The small amount of oil that can be released from oil sludge causes a decrease in growth. In addition, aeration factors, temperature, pH and metabolite products that can inhibit bacterial cell growth can also be triggers (Pratiwi, 2012).

Rahayu *et al*. (2019) stated that growth in bacteria can be caused by increased concentrations of hydrocarbons. This increase in concentration is in line with the increase in metabolic functions for degradation by organisms. In addition, this is often due to the accumulation of hydrophobic compounds in the bacterial membrane, which can cause damage to the membrane structure.

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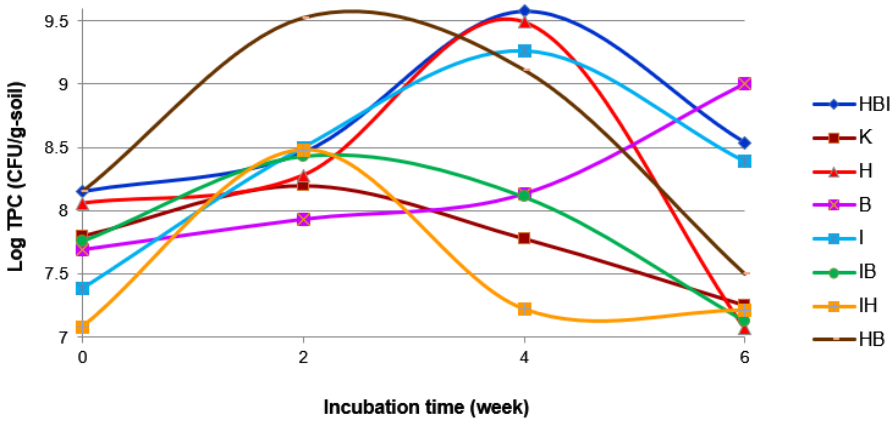

Figure 1: Bacterial growth (Log TPC CFU/g-soil). HBI: Hydrocarbonoclastic + Biosurfactant + Indigenous, K: Control, H: Hydrocarbonoclastic, B: Biosurfactant, I: Indigenous, IB: Indigenous + Biosurfactant, IH: Indigenous + Hydrocarbonoclastic, HB: Hydrocarbonoclastic + Biosurfactant.

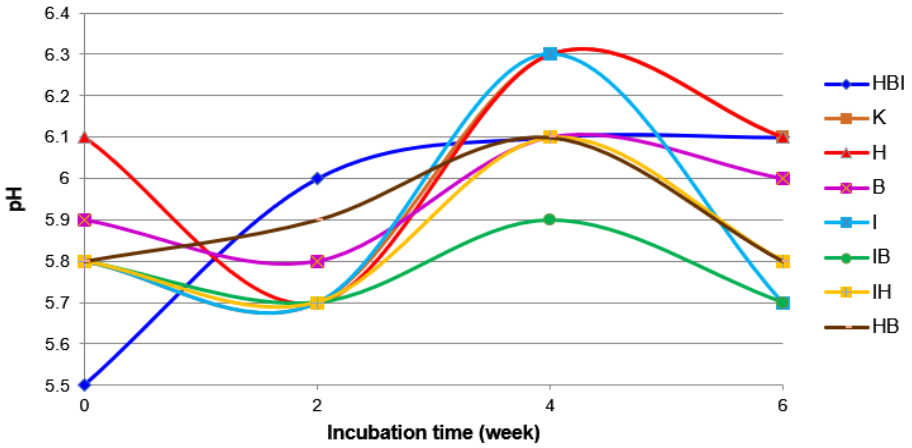

Figure 2: Soil pH in the treatment, due to the addition of different types of bacterial consortium and incubation time (weeks). HBI: Hydrocarbonoclastic + Biosurfactant + Indigenous, K: Control, H: Hydrocarbonoclastic, B: Biosurfactant, I: Indigenous, IB: Indigenous + Biosurfactant, IH: Indigenous + Hydrocarbonoclastic, HB: Hydrocarbonoclastic + Biosurfactant.

Bacterial growth is also influenced by genetic factors. The genetic factor that influences the speed of division is the bacterial genome. The longer the genomic DNA that is owned by a bacterial cell, the longer the cell division time because the replication process takes longer (Murtagh *et al*., 2020).

Effect of bacterial consortium and incubation time on pH

Meanwhile, data analysis of changes in pH due to the addition of bacterial consortium types and the length of incubation time showed that there was a change in pH (which increased and decreased during the incubation time) (Figure 2).

There are intermediate products during the oil sludge degradation process by bacteria that contain lots of OH groups so that the pH rises. An increase in the number of microbes along with a decrease in pH, indicates using a carbon substrate as a carbon source for growth and producing metabolite products in the form of organic acids, which cause a reduction in pH (Pratiwi, 2012). According to Alexander *et al.* (2014), the decrease in pH is suspected to have a carbonate fraction that is acidic and dissolves in the medium. One of the results of carbon degradation is carboxylic acids, which are soluble in air, causing a decrease in pH.

Residual oil content (oil sludge) (g/g-soil)

There was a decrease in the oil residue content (oil sludge) and an increase in the bacterial consortium type and incubation time. This indicates that the initially high oil content is reduced by the degradation activity of bacteria.

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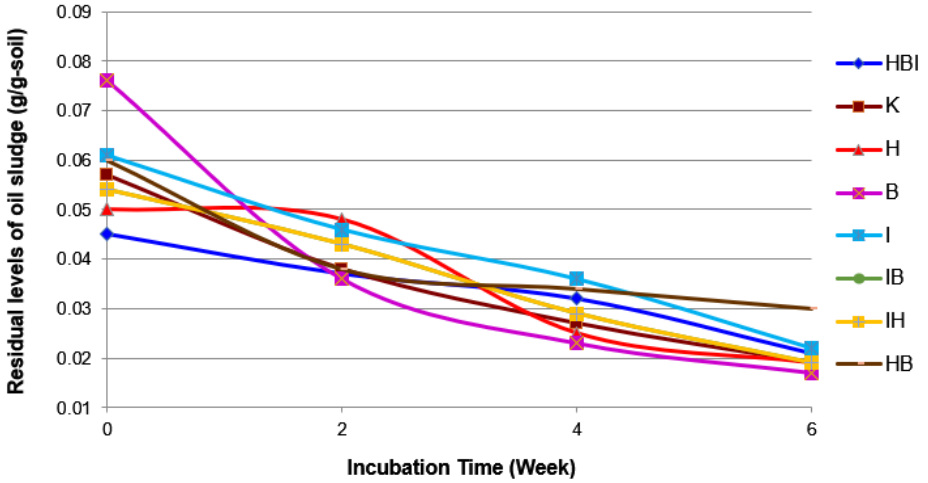

Figure 3: Residual levels of oil sludge (g/g-soil) due to the addition of different types of bacterial consortium treatment and incubation period. HBI: Hydrocarbonoclastic + Biosurfactant + Indigenous, K: Control, H: Hydrocarbonoclastic, B: Biosurfactant, I: Indigenous, IB: Indigenous + Biosurfactant, IH: Indigenous + Hydrocarbonoclastic, HB: Hydrocarbonoclastic + Biosurfactant.

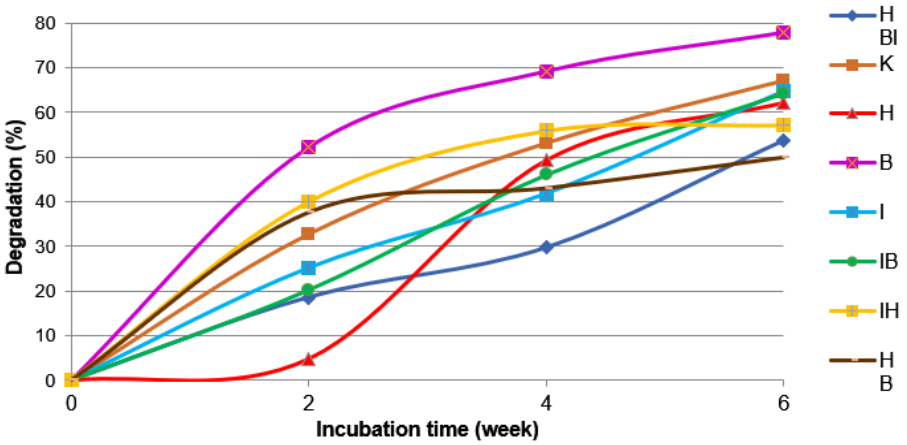

Figure 4: Percentage of oil sludge degradation (%) due to the addition of different types of bacterial consortium and incubation time. HBI: Hydrocarbonoclastic + Biosurfactant + Indigenous, K: Control, H: Hydrocarbonoclastic, B: Biosurfactant, I: Indigenous, IB: Indigenous + Biosurfactant, IH: Indigenous + Hydrocarbonoclastic, HB: Hydrocarbonoclastic + Biosurfactant.

This analysis shows that the type of bacterial consortium used could live and degrade petroleum that contaminates the soil media (Figure 3).

Based on the Brown-Forsythe test, a significance value of $0.00 < \alpha = 0.05$ indicated that the interaction between the type of bacterial consortium and the length of incubation time affected the levels of oil residue (oil sludge).

The decrease in residual oil sludge content (g/g-soil) was followed by an increase in the percentage of oil sludge degradation (%). So, the data between the residual content and the percentage of degradation are inversely related. The percentage of oil sludge degradation (%) from all the additional types of bacteria consortium treatment and incubation time was increased (Figure 4). This is because as the incubation time increases, the percentage of oil sludge degradation increases. Based on the Brown-Forsythe statistical test, a significant value of $0.0 < \alpha = 0.05$ indicates that there is an effect of long incubation time on oil sludge degradation. The K treatment is in the second position because indigenous bacteria play a role in increasing the biodegradation of oil sludge-contaminated soil. Combining will create complexity because each microorganism has a different ability to degrade substrate components than using only 1 single isolate. This indicated that the treatment given was effective in degrading the oil sludge during an incubation period of 6 weeks.

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Table 1: The ∆ value of oil sludge degradation percentage at the treatments compared with the control in the 6th week.

Treatments	Oil sludge degradation percentage (%) in the 6th week	∆ Percentage (%) (Treatment degradation percentage - Control degradation percentage)
Control	67.1	
H (Hydrocarbonoclastic)	62.2	-4.9
B (Biosurfactant)	77.8	10.7
I (Indigenous)	64.6	-2.5
IB (Indigenous + Biosurfactant)	64.3	-2.8
IH (Indigenous + Hydrocarbonoclastic)	57.2	-9.9
HB (Hydrocarbonoclastic + Biosurfactant)	49.9	-17.2
HBI (Hydrocarbonoclastic + Biosurfactant + Indigenous)	53.7	-13.4

Table 2: Calculation of C/N ratio results before and at the end of incubation.

Levels before treatment				Levels after treatment at the end of the incubation period				
Treatment	C Organic (%)	N Total (%)	C/N ratio	Treatment	C Organic (%)	N Total (%)	C/N ratio	Organic material (%)
Control	28.51	1.33	21.40	Control	11.29	0.12	91.5	19.53
				H	10.35	0.12	85.3	17.90
				B	8.47	0.12	70.5	14.66
				I	11.51	0.11	100.3	19.91
				IB	12.39	0.12	102.1	21.43
				IH	8.92	0.12	73.1	15.43
				HB	12.61	0.13	98.9	21.81
				HBI	10.00	0.12	84.3	17.29

HBI: Hydrocarbonoclastic + Biosurfactant + Indigenous, K: Control, H: Hydrocarbonoclastic, B: Biosurfactant, I: Indigenous, IB: Indigenous + Biosurfactant, IH: Indigenous + Hydrocarbonoclastic, HB: Hydrocarbonoclastic + Biosurfactant.

It can be seen that the percentage of degradation and the highest percentage difference is found in treatment B with the result of the percentage comparison of 10.7% and the percentage value of degradation of 77.8% (Table 1). After getting the percentage of oil sludge degradation, we tested the linear regression equation to find out the length of incubation time needed to degrade 100%.

Other treatments are still successful if seen from the results, no one has reached 100%. This is because each bacteria that interacts with each other requires a different time to optimize the degradation process by utilizing oil sludge as a substrate. The results of achieving a 100% percentage of oil sludge degradation from the results of the best linear regression function were in treatment B, namely 7 weeks, followed by K, H, IB, I, IH, HBI and HB with respective times of 8.6 weeks, 9 weeks, 9.16 weeks, 9.4 weeks, 9.6 weeks, 11.6 weeks and 11.7 weeks.

C/N ratio level

A difference was shown before and after treatment at the end of the incubation period with the addition of the type of bacterial consortium and the incubation time (Table 2). The addition of the type of a bacterial consortium and incubation time, it showed that the results of organic carbon content, total nitrogen and organic matter were decreased. The lowest reduced level occurred in the type of bacterial consortium in treatment B. This proved the presence of bacterial activity in the oil sludge degradation process. From these data, it can also be seen that there were differences in the source of N in the hydrocarbon degradation process by bacteria.

Gas chromatography (GC-MS)

The results of the analysis of gas chromatography test data (GC-MS) in treatment B (the best treatment) at week 6 showed a loss of aliphatic hydrocarbon compounds (n-dodecane, 2,6,10,14-tetramethyl-hexadecane and 2,6,10,15,19,23-hexamethyl tetracosame) at retention time 8.87. (Figure 5). While polyaromatic hydrocarbons (2,3,6-trimethyl-Naphthalene and 1,4,6-trimethyl-Naphthalene). The retention time was 7.64 while the retention time was 7.93 for polyaromatic 1,4,6-trimethyl-Naphthalene and 1,6,7-trimethyl-Naphthalene). This shows the effectiveness of Type B consortium compared to other treatments. Based on the results of the GC-MS test, it showed that the constituent compounds of petroleum in the control, which were shown at important peaks in week 0, were dominated by paraffinic aliphatic hydrocarbons (dodecane, hexadecane, tetracosane, pentadecane) and polyaromatics (Naphthalene, 2,3,6-trimethyl, Naphthalene, 1,4,6-trimethyl and Naphthalene, 1,6,7-trimethyl).

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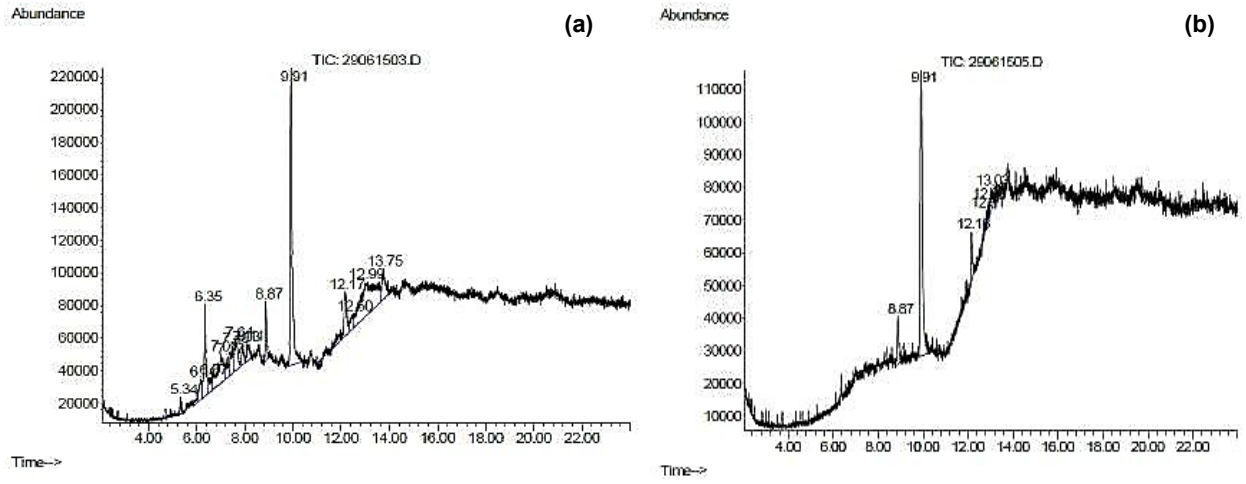

Figure 5: GC-MS profile in the control treatment (a) and the best treatment (b) at the 6th week.

Total bacteria (CFU/g-soil)

At week 6 there was a decrease in bacterial growth and only in treatment B did it continue to increase. Decreased growth in bacteria can be caused by increasing the concentration of hydrocarbons. In addition, this can be caused by the accumulation of hydrophobic compounds in the bacterial membrane, which can cause damage to the membrane structure (Sikkema *et al*., 1995; Murínová and Dercová, 2014). In treatment B, there was an increase in the concentration of bacteria. This means that in treatment B, there was an increase in the metabolic function of the organism to carry out degradation until the 6th week.

Meanwhile, the analysis of treatment data for the addition of bacterial consortium species and the length of incubation time on pH showed an increase and decrease in pH during the incubation time. The increase in the pH value indicates that the crude oil pollutants in the wastewater samples have been degraded into non-toxic products, and the organic acids were decreasing (Murínová and Dercová, 2014). An increase in the number of microbes along with a decrease in pH indicates that bacteria used hydrocarbon substrates as a carbon source for growth and produced metabolite products that can lower the pH (Nikolova and Gutierrez, 2020). Biodegradation of hydrocarbons by bacteria will produce products in the form of organic acids, which can reduce pH (Nikolova and Gutierrez, 2020).

Residual oil content (oil sludge) (g/g-soil)

The results of the data analysis showed a decrease with increasing incubation time of oil sludge (g/g-soil) due to the addition of the type of bacterial consortium and incubation time. Based on these results, it can be indicated that all types of bacterial consortiums have the ability to degrade soil contaminated with oil sludge. The treatment of the type B consortium showed good results in degrading soil contaminated with oil sludge. Hence, it can be said that a type B consortium has the potential to be used as a consortium to degrade oil sludge or other waste with the same characteristics.

Data analysis of the percentage of oil sludge degradation (%) in the control compared with the treatment in the 6th week showed that the highest percentage occurred in treatment B. The process of complete biodegradation of hydrocarbon compounds is not possible to be carried out only by one type of microbe; it is always conducted by a group of microbes that interact synergistically in the form of a consortium. A consortium is a mixture of microbial populations in the form of a community that has a cooperative, commensal, and mutualistic relationship. Community members who are related will associate so that they are more successful in degrading hydrocarbons than if they do the process individually (Patowary *et al*., 2016).

The results of the degradation of hydrocarbon compounds that contaminate the soil are better when obtained from a mixed culture of bacteria compared to single bacterial isolates (Alsayegh *et al*., 2021). The average maximum degradation yield (µg/h) in mixed culture for n-C8 was 4.1; n-C9 2.0; n-C10 0.5; and n-C11 0.1, while for single species such as *P. aeruginosa*, the maximum average degradation (µg/h) for n-C8, n-C9, n-C10 and n-C11 was 0.8, 0.5, 0.2 and 0.1, respectively. In *Rhodococcus globerulus*, the average maximum degradation (µg/h) for n-C8, n-C9, n-C10 and n-C11 was 0.6, 0.8, 0.4 and 0.2, consecutively (Ward *et al*., 2003).

The results of the data analysis of the estimated attainment of the percentage of oil sludge degradation based on the linear regression function (%) of all treatments resulted in varying percentage values. This means that each bacterium has a different way of transporting hydrocarbons into its cells. There are three ways of transporting hydrocarbons in bacterial cells, namely: (1) Cell interactions with hydrocarbons dissolved in the water phase, where generally the average solubility of hydrocarbons by physical processes is very low so that it cannot support; (2) Direct contact (adhesion) of cells

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with a larger hydrocarbon droplet surface than microbial cells. In this second case, adhesion can occur because the bacterial cells are hydrophobic. The cell will be attached to the surface of the hydrocarbon droplet, which is larger than the cell and then the substrate will be taken up by diffusion or active transport. This adhesion occurs due to the presence of biosurfactants on the bacterial cell membrane; (3) Cell interactions with droplets of hydrocarbons that have been emulsified or stabilized by bacteria. In this case, microbial cells will interact with hydrocarbon particles that are smaller than the cells. Hydrocarbons can be emulsified and solubilized due to the presence of biosurfactants released by bacteria into the medium (Hua and Wang, 2014).

The addition of a type of consortium B treatment, which is a producer of biosurfactants consisting of bacteria *Micrococcus* sp., *Acinetobacter*, *Bacillus* sp. and *Pseudomonas putida*. While the type of consortium bacteria H is hydrocarbonoclastic bacteria consisting of *Pseudomonas pseudomallei*, *P. tzutseri*, *P. cepacea* and *P. fluorocens*. Consortium type I are 2 isolates of indigenous bacteria isolated from oil sludge from East Borneo. Bacterial species that have a high potential for hydrocarbon biodegradation and biosurfactant production can be identified as bacteria *Bacillus subtilis*, *Bacillus licheniformis*, *Pseudomonas aeruginosa*, *P. pseudomallei* and *Bacillus polimyxa* (Patowary *et al*., 2016).

The ability of bacteria from the *Bacillus* genera to produce biosurfactants has been reported by several researchers, including *Bacillus subtilis*, which is capable of producing lipopeptide biosurfactants (Patowary *et al*., 2016). PCR studies and DNA hybridization of the bacterium *Pseudomonas putida* IR1 have shown that the enzyme that plays a role in the metabolism of PAH is naphthalene dioxygenase (Mishra and Das, 2017). *Micrococcus* sp. is able to produce biosurfactants and degrade aliphatic, aromatic and polyaromatic hydrocarbons. *Micrococcus* sp. is also capable of degrading hydrocarbon components of hexadecane, toluene, naphthalene, phenanthrene and oily sludge above 90% after seven days of incubation (Partila, 2013).

The level of ratio C/N

Treatments with the addition of the type of bacterial consortium and incubation time showed different results in each treatment on organic carbon content, total nitrogen and organic matter content. The lowest decrease in the C/N ratio occurred in the B bacteria consortium type treatment, which means that in B treatment, there was bacterial activity in degrading oil sludge. This is thought to be related to the use of N sources for the synthesis of enzymes owned by bacteria. Karigar and Rao (2011) stated that native bacteria from the soil have dioxygenase enzymes, which have the ability to oxidize aromatic compounds and are applied in environmental improvements. In addition, another enzyme produced by bacteria is laccase (p-diphenol:dioxygen oxidoreductase) which functions in catalyzing the oxidation of various phenolic substrates and aromatic reduction. Bacteria that have lots of enzymes in both quality and quantity will need more sources of N than bacteria that have few enzymes (Moat *et al*., 2002).

Gas chromatography (GC-MS)

Gas chromatography was performed only for the best treatment at the end of the incubation period. The results of GC-MS in the control and the best treatment were in treatment B. Based on the results of GC-MS, the peak that came out at retention times of 5.34 to 6.35 in the control treatment was no longer found in the type B consortium treatment. This indicated that the biosurfactant-producing bacterial consortium (treatment B) was able to degrade the hydrocarbon compounds that appeared during the retention time. These compounds are known as aliphatic and polyaromatic hydrocarbons.

Based on the results of the GC-MS test, the compounds that make up crude oil in the control shown at important peaks in week 0 are dominated by paraffinic aliphatic hydrocarbons. Consortium type B treatment in the 6th week showed the loss of aliphatic and polyaromatic hydrocarbon compounds (Naphthalene, 2,3,6-trimethyl, Naphthalene, 1,4,6-trimethyl and Naphthalene, 1,6,7-trimethyl). This indicates that the type of bacterial consortium B is capable of degrading aliphatic and polyaromatic hydrocarbons, although the degradation process that occurs has not been able to remove the hydrocarbon components completely.

The inability of the bacterial consortium B to completely degrade aliphatic paraffin hydrocarbon compounds during the degradation process may be due to insufficient biosurfactant production to dissolve the long carbon chain of the paraffin type, as well as the short degradation time. Thus, to improve the degradation process, it can be carried out by extending the incubation time, adding biosurfactant-producing bacteria and adding nutrients. In addition, the abundance of the compound before treatment was indicated to be very high, so it took time to completely degrade.

CONCLUSION

The type B consortium treatment showed the best results in degrading soil contaminated with oil sludge. Thus, it can be said that the type of consortium B has the potential to be used as a consortium to degrade oil sludge or other waste with the same characteristics. The inability of the B bacteria consortium to completely degrade the aliphatic paraffin group of hydrocarbons during the degradation process is probably caused by insufficient biosurfactant production to dissolve the long carbon chain of the paraffin type and the short degradation time.

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AUTHOR CONTRIBUTION STATEMENT

Dita Artanti and Ni'matuzahroh designed the study and carried out the laboratory work. Mulya Fitrah Juniawan analyzed the data. Dita Artanti, Ni'matuzahroh, Vella Rohmayani and Anindita Riesti Retno Arimurti wrote the manuscript. All authors read and approved the final version of the manuscript.

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