Biodegradation of aliphatic waste components of oil sludge used micro symbiont of Sponge *Niphates* sp.

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I Marzuki[1*], M Chaerul[2], Erniati[3], Asmeati[4] and I Paserangi[4]

[1]Chemical Engineering, Fajar University, Jalan Prof. Dr. Abdurrahman Basalamah No. 101, Makassar, South Sulawesi, 90231, Indonesia
[2]Infrastructure and Environmental Engineering Program, Fajar University, Jalan Prof. Dr. Abdurrahman Basalamah No. 101, Makassar, South Sulawesi, 90231, Indonesia
[3]Civil Engineering, Fajar University, Jalan Prof. Dr. Abdurrahman Basalamah No. 101, Makassar, South Sulawesi, 90231, Indonesia
[4]Mechanical Engineering, Fajar University, Jalan Prof. Dr. Abdurrahman Basalamah No. 101, Makassar, South Sulawesi, 90231, Indonesia

[*]e-mail: ismailmz@unifa.ac.id, ismailmz3773@gmail.com

Abstract. Investigation has been carried out using a sponge *Niphates* sp. as biodegradation agent of the aliphatic hydrocarbon component of crude oil sludge. The experiment was carried out by colonizing and making suspension of *Bacillus pumilus* strains GLB197 and *Bacillus cohnii* strains of DSM 6307, isolated from sponge *Niphates* sp. Both types of micro symbiont suspension were mixed with sludge waste for 30 days. There are five biodegradation parameters observed, carried out every five days of incubation. Parameters that observed during incubation were pH, gas bubbles and fermentation odor. Meanwhile, the optical density of the media was measured using spectrometer-D20+, Aliphatic components degradation was measured using GC-MS. Observation of biodegradation parameters showed there was a change in pH from 7 to 6, formed gas bubbles, resulting in fermentation odors and showed an increased optical density by an average of 35%. These four parameters occur during the interaction of 10-20 days of interaction in the media. There were 20 types of aliphatic components identified in the crude oil sludge waste. Analysis of four aliphatic components, namely Tridecane, Octadecane, Tricosane, and Nonacosane showed a decrease in the average concentration of 48.11 %.

1. Introduction

Generally processed petroleum sludge waste contains several hazardous and toxic materials, especially hydrocarbon components and some heavy metals (Pb, As, Hg, Cr, Zn, Cd, Ni) [1][2][3][4]. Most hydrocarbon components are aliphatic groups and few polycyclic aromatic components [5][6][7]. The hydrocarbon component of processed sludge oil waste consists of saturated components of alkane and cycloalkane, alicyclic and aromatic rings, which reaches total amount of 85-91%. The composition of these hydrocarbon components, in the form of paraffin in the range of 15-60%, naptene 30-60%, asphaltene, and about 6% resin and aromatic components 3-30% [8][9][10]. The short-term impacts of oil sludge waste pollution in the sea include damage to marine biota cell membranes, penetration of hydrocarbon components into cells and discharge of biota cell fluids and the death of several species of fish due to lack of oxygen, carbon dioxide poisoning and poisoning of petroleum sludge hydrocarbon components [11][12]. The hydrocarbon toxic components of oil sludge waste also affect reproductive ability, growth, development, and behavior of marine biota, especially plankton [13][14].

The long-term effect of oil waste pollution in the sea is that hydrocarbon components can be nourished by marine of biota and some hydrocarbon components can accumulate to form complex compounds with fats and proteins [15]. The accumulation of hydrocarbon components in marine biota could influence other higher organisms through marine food web, whereas the accumulation of toxic compound in small organisms for example plankton, could be exposed into higher trophic level like fish or even human [16]. Hydrocarbon waste in the sea is partially trapped and settles into the sediments, by aggregating with sea mud and could contributes into the increasing of toxic persistence power for up to 20 years after the pollution occurs. For example, Rhizophora species that are exposed to oil waste take about 8 years to restore their original conditions, other ecosystems in the sea even could be perished when being exposed to the oil waste pollution, for example coral reefs that are in direct contact with petroleum waste hydrocarbon components [17]. The decomposition process of sludge waste into simple non-toxic components is generally in the form of metabolic molecule of hydroxylates [18]. The degradation velocity of oil sludge waste subtracting by the microorganism is influenced by many factors, such as: (1) Amount, type, volume and toxicity of substrate, (2) Type, number of cells, source of isolates and microorganism consortia of degradation agent, (3) Effect of temperature, salt content, nitrate and phosphate content, (4) Other technical and non-technical factors, such as the interaction time of substrate with biodegradation agents, pH, exposure to sunlight, oxygen adequacy. The number of influential factors in the degradation process of oil sludge wastes causes the hydrocarbon molecules velocity of destruction to run very slowly and vary in the range of an average of 0.02 - 2.0 g / m2 / day at 24 ° - 30 ° C [5][6][19].

Research findings on microorganisms that are able to degrade petroleum sludge waste are divided into three groups, namely the fungi group: *Cunningham* spp.; yeast groups: *Torulopsis*, *Trichosporon*, *Rhodotorula*, *Rhodosporidium*, *Pichia*, *Debaryomces*, *Candida parapsilosis*, *C. lipolytica* [11][12], and the groups of bacteria; *Brevibacterum* spp., *Bacillus* sp., *Arthrobacter* spp., and *Pseudomonas* sp. [1][10][13]. These microorganisms are generally isolated from various sources, such as hydrocarbon waste contaminated mud, oil-exposed seawater, marine sediments, mangroves, lignin-type wood. Other sources of bacteria, such as microsymbiont from sponges as a biodegradation agent for petroleum sludge, have not received many scientific reports [2][8]. The purpose of this research is to trace and find variations in the types of microbes that can degrading hydrocarbon components, increasing the rate of degradation by sing consortium of several carbon plastic bacteria [14][15][16]. This is important as an effort to improve the quality of the marine environment and reduce the volume, concentration, and toxicity of sludge waste pollutants for the biodiversity of marine biota.

2. Materials and methods

2.1. Material

Isolate samples of *Bacillus pumilus* strain GLB197 (BP) and *Bacillus cohnii* strain DSM 6307 (BC), from *Niphates* sp. sponge, in comparison with *Pseudomonas* sp. (SP) bacteria [22][23], dichloromethane GR [Brand], n-hexane PA, Na2SO4 anhydrous PA, NPK [Norwegian Hydro US Norway], Isooctane standard [CA540-84-1], aquabides, 0.9% physiological sodium chloride, Marine Agar (MA), Nitrogen (N_2) gas, Processed petroleum Sludge Waste from PT. Indonesian Chevron Pacific, Dumai-Riau, in reservoir tank number B.05, type of chemical sludge (processed petroleum sediment sludge which has undergone coagulation) [24].

2.2. Analysis instruments

Analysis instruments consist of: (1) GC-MS [Agilent 7890], the operating conditions for GC-MS (maximum temperature of 350 0C, the increase in temperature of 10 ^{0}C every 5 minutes, pressure 18406 psi, a Helium gas carrier, a speed of 150 mL/min, capillary column [Agilent 19019S-436HP-5 m s], dimensions of 60 m x 250 µ m x 0.25 µ m, the pressure 18,406 psi, separation 26,128 cm/sec., A retention time of maximum 30 minutes), (2) Spectrophotometer 20 D * [Thermo E. Corp]. a wavelength of 600 nm, (3) Filter of 0.2 µm [Millex-LH], (4) Shaker incubator [Enviro-Genie], (5) Micro pipette [Dragon Onemed], (6) Set analytic sheet [PM-Mettler 200], (7) injector, (8) Ultrasonic, (9) Universal pH paper Brands.

2.3. Method

2.3.1. Making suspension of isolates and determining the total plate count of bacterial colonies. Three types of isolates, namely SP, BP and BC were cultured into a petri dish containing MA media, incubated for 2 x 24 hours. The isolate was made into suspension by procedure of: 5 mL 0.9% physiological sodium chloride solution was put into a test tube containing *Pseudomonas* sp. (SP) culture, shaken, obtained concentrated SP suspension, diluted ratio of 1:100, (5 mL suspension in 500 mL 0.9% physiological sodium chloride l) in a 500 mL, the flask is measured and homogenized. The same procedure was carried out to make BP and BC bacterial suspensions. Determination of the number of colony cells per suspension was carried out by the procedure: the number of SP suspension colonies was determined by preparing 6 sterile test tubes labeled I. P.10-1; II. P.10-2; III. P.10-3; IV. P.10-4; V. P.10-5; and VI. P.10-6. Test tube was filled with 900 µL of 0.9% sodium chloride solution physiologically. The SP suspension is diluted by taking 100 µL of a 250 mL measuring flask, then put into a test tube. Then I prepared 6 sterile petri dishes each labeled with dilution I; II; III; IV; V, and VI. The SP suspension for each dilution tube is piped as much as 100 µL, and spread on a petri dish evenly according to the dilution label, each petri dish containing 100 µL of SP suspension each, added by each of the 15 mL media Marine Agar (MA), closed and allowed to solidify, then wrapped in paper and incubated 2x24 hours [25]. The number of colonies is calculated using the formula:

$$N = \Sigma C / (V \times 1.1 \times d) \quad (1)$$

This procedure was carried out together to make a suspension and determine the number of BP and BC bacterial cells used in the biodegradation of the aliphatic hydrocarbon components of processed oil sludge waste.

2.3.2. Procedure for degradation of sludge waste by bacterial suspension. 5 Erlenmeyer (100 mL) were prepared and labeled with code of SP.5; SP.10; SP.15; SP.20 and SP.25. Each Erlenmeyer (reactor) was filled with ± 500 mg sludge, sterilized for 15 minutes. Each Erlenmeyer then filled with 10 mL of SP suspension, and they were to homogenized, the bacterial was also isolated. The same procedure was carried out using BP and BC suspension, so that the total reactor was 15 (3 types of bacterial suspension and 5 variations in contact time). All reactors were shaken at 150 rpm speed, at room temperature. During the interaction period, 2 times injection of nutrient NPK was carried out with a concentration of 1% at the contact period of 8 days and 15 days using injector and injection volume of 1 mL each [26].

2.3.3. Determination of degraded aliphatic concentration. Non-degraded sludge extracted using 5 mL dichloromethane, 3 extractions were carried out so that all sludge residues could be withdrawn from the degradation medium. Extractions were conducted using Ultrasonic. The organic phase was taken, together with anhydrous Na2SO4 to remove the water content. The organic phase was concentrated with nitrogen gas up to a final volume of ± 5 mL, then extracted is streaming using GC-MS. The sludge waste concentration and biodegradation rate are determined using the formula:

$$\text{Conc. Comp. n} = \frac{Peak\ area\ component\ n}{Standard\ peak\ area}\ x\ Conc.standart\ x\ multifactorial \quad (2)$$

2.3.4. Degradation parameters. Parameters analyzed as a reference to determine whether the three bacterial isolates can degrade aliphatic hydrocarbon components in processed petroleum sludge waste, namely: (1) Optical density, (2) Gas bubble formation, (3) Identification of fermentation odor (4) PH value of media depravity, (5) Abundance and composition of aliphatic components of processed oil sludge waste, (6). The rate of degradation of the aliphatic component of sludge waste, in percentage unit, and (7) Identification of components resulting from degradation [10][16][27].

3. Results and discussion

3.1. Characteristics of samples

The characteristics of processed- oil sludge waste samples as biodegradation substrate, that were obtained from PT. Chevron Pacific Indonesia. They determined the composition of hydrocarbon components, especially the aliphatic types. The characteristics of sludge are shown in Table 1, as follows:

Table 1. Characteristics of waste samples of processed oil sludge.

Analysis description	Measurement results	Reference
Color	black	PT. Chevron Pacific Indonesia
Embodiment	shape of pasta	PT. Chevron Pacific Indonesia
Melting point	45 ^{o}C	PT. Chevron Pacific Indonesia
Water content	4,9769 %	Max 5% (Pertamina)
Insoluble material	0,7845 %	Max 1% (Pertamina)
Comp. Hydro. Aliphatic	85,98 %	Min. 80% (Pertamina)
Comp. Hydro. Aromatic	3,03 %	Max 5% (Pertamina)
Other Components	10,99 %	Max. 15% (Pertamina)

Data in Table 1, in accordance with the requirements of PT. Pertamina and PT. The CPI for the by-products produced by refined petroleum fuels is based on the characteristics of processed oil production, as contained in the reference column. In general, processed oil sludge waste as a side product of processing contains at least 80% of aliphatic hydrocarbon components, with maximum 5% of aromatic hydrocarbon component, 1% of maximum insoluble component, (heavy metal component) and other components not exceeding 15% of the total sludge weight [24].

Characteristics of bacterial isolates used in the biodegradation of the aliphatic components of sludge waste, two of which are bacteria isolated from marine sponges *Niphates* sp. obtained around Kodingareng Keke Island, Spermonde Archipelago, part of the administrative area of the Makassar City Government. Primary data sampling point of sponge *Niphates* sp., family Niphatidae, ie coordinate points S = 05^{0} 06 '06.76", and E = 119^{0} 17' 10.66" ", depth of 2.6 m sl, salinity 29.6 ‰, pH 7 at temperature 29 ^{0}C. The process to get the sponge symbiont strain was sponge morphology analysis, isolation of symbionts, isolate phenotypic analysis consisted of biochemical test and Gram determination of isolates and genotype analysis using PCR, thus 2 symbiont isolates were identified as *Bacillus pumilus* strains GLB197 (BP) and *Bacillus cohnii* strain DSM 6307 (BC), and pure culture of bacterial have have known to be able to degrade hydrocarbon components, namely Pseudomonas sp. (SP), as control. Previous researches have used sponge micro symbiont as a biodegradation agent for hydrocarbon components [22][23][28]. The number of cells used to degrade sludge waste was BP 4, 1. 104 cells/mL isolates; BC 3, 8. 104 cell/mL isolates and comparative isolates SP 3, 9. 104 cells/ml.

3.2. Biodegradation parameters

Parameter of biodegradation of processed fuel oil sludge waste from PT. CPI, Dumai-Riau-Indonesia used BP isolates and BC isolates of symbiotic bacteria *Niphates* sp. and control bacteria SP. The measurement of biodegradation parameters consisted of optical density, measured using spectrophotometer 20 D *, formation of air bubbles and fermentation odors by visual observation, and pH values of the media, using pH Indicators Universal, presented in Table 2, below:

Table 2. Parameters of biodegradation of sludge waste of process oil during the incubation of time contact (days).

Num.	Biodegradation parameters	Time of contact (days)	Types of bacterial isolates		
			BP	BC	SP
1	Optical Density (OD)	0	0.056	0.116	0.136
		5	0.612	0.787	0.483
		10	0.581	0.623	0.535
		15	0.575	0.592	0.372
		20	0.542	0.552	0.364
		25	0.441	0.431	0.312
2	Formation of gas bubbles	0	-	-	-
		5	+	-	+
		10	++	++	++
		15	++	++	++
		20	++	++	+
		25	+	+	+
3	Fermentation smell	0	-	-	-
		5	-	-	-
		10	√	√	√
		15	√√	√√	√√
		20	√√	√	√
		25	√	√	√
4	Value of pH	0	7	7	7
		5	7	7	7
		10	6	6	6
		15	6	6	7
		20	7	6	7
		25	7	7	7

\- *There is no visible formation of gas bubbles and no fermentation odors*
\+ *There is no significant gas bubble formation*
++ *There is a significant formation of gas bubbles*
√ *Weak smell of fermented smell*
√√ *Strong smell of fermented smell*

According to Table 2, it shows that the optical density value (OD) increases significantly for all isolates. This increasing occurs during the 5-day of incubation time and gradually decreases as the contact period of the isolates increases with processed petroleum sludge waste. Adaptation and growth and the development of isolate cells characterized by increasing cell total and cell size, thus during the contact period, bacterial cells worked to degrade the components of processed petroleum sludge waste. The stationary phase of the degradation isolate was identified during the 10-20 days contact period marked by a decrease in the OD value of the media, there was a strong suspect on this matter due to the effect of poison oil sludge as a form of resistance to the degraded disturbance which carried out the function of oil sludge destruction and decomposition. The production of enzyme from bacterial isolates as a response to the presence of toxic substances processed refined sludge oil, eventually, degradation performance is lowered to zero points of degradation along with the increase in contact period [29][30]. The formation of gas bubbles of degradation media in the reactor was shown during the contact period of 10-20 days (Table 2 number 2). This situation has been predicted that the performance of maximum bacterial isolates in degrading hydrocarbon components of fuel sludge waste occurs in the stationary phase or after passing through the phase of adaptation and growth. The increasing in gas bubbles in the degradation reactor appears as a physical change due to a large number of oxidation reactions of long

carbon chain nodes by enzymes produced by bacteria BP, PC and SP as biodegradation materials for the compilation of processed oil sludge waste. The gas formed also shows that in the reactor there is CO2 gas, one of the biodegradation results. Oxidation reactions of sludge waste by bacteria isolates could cause simultaneous physical and chemical changes, namely gas bubble formation, fermentation odor and decrease in pH value. These results are in line with previous studies that have been carried out by looking at biodegradation parameters [19][21]. The degradation media fermentation process creates a distinctive odor due to the formation of simple organic substances in the form of alcohol, aldehyde, carboxylic acid and the presence of surfactants and other organic components. This organic compound is a biodegradable product of processed petroleum sludge waste by bacterial isolates, which contributes to the increased value of media optical density. Acidic properties occur during the contact period of 10-20 days in the degradation medium with a pH value of 7 to 6 due to the alleged contribution of several factors, namely the component of carboxylic acid and surfactant resulting from degradation and an increase in the number of bacterial cells in the degradation medium. Enhancement acidic nature of degradation media is thought to be one of the factors causing bacterial cells to die faster. The performance analysis on sludge oil waste degradation using sponge bacterial symbiont showing that NPK addition was significantly contribute to the interaction periods, the 8^{th} and the 15^{th} days. The effect of NPK nutrients addition causes a longer time in stationary phase of the bacterial isolates in the degradation media, before entering the death phase of the contact 25 days and above. Thus, the degradation period was expected to be longer so that also increasing the degradation rate. In previous studies, it was stated that bacteria can survive for a maximum of 20 days and the degradation period can be extended if modifications are made, such as the addition of nutrients and agitation [5][18].

3.3. Degradation rate of subtracted processed petroleum sludge waste

In measuring the degradation rate of biodegradable petroleum sludge waste as the performance of BP isolates and BC isolated from the microspheres of sponge *Niphates* sp as well as the pure culture of SP bacteria can be identified by analyzing several variables seen in the chromatogram of GC-MS measurements, including abundance, composition, concentrations changing components which were devoted to aliphatic components and degradation compounds formed in the biodegradation process of sludge waste using the degradation of micro symbiont isolated of sponge Niphates sp.

3.3.1. Abundance and composition of aliphatic components that are compiled in waste processed petroleum sludge. Retention time, number of peaks and height of peaks that appear as a result of GC-MS measurements of processed oil sludge waste, it shows that the hydrocarbon component is composed in sludge waste. Changes in the peak height of each component for the same retention time after treatment, indicate that the composition and aliphatic sludge hydrocarbon components were undergone changes. These changes are thought to be the result of the work of bacterial isolates degradation to construct the aliphatic component of sludge waste through a biodegradation mechanism.

The chromatogram results of GC-MS measurements have identified 26 hydrocarbon components, consisting of 21 aliphatic components forming a homologous series of 10-30 carbon chains following the formula CnH2n + 2 and the other 5 components are aromatic compounds. The visible chromatogram of the abundance of processed hydrocarbon components has a shift in the height of new peaks and peaks formed (Figure 2, 3, 4) compared with Figure 1, it is strongly assumed that the new peak formed is a component resulting from degradation. The identification of new peaks as an indication of the components of previous biodegradation results has been carried out on biodegradation of diesel oil and research using bacteria isolated from *Callysspongia* sp. [9][31].

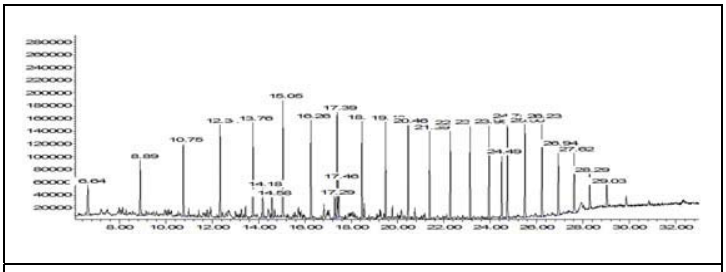

Figure 1. The abundance and quantity of hydrocarbon components seen in the waste oil waste before the degradation process.

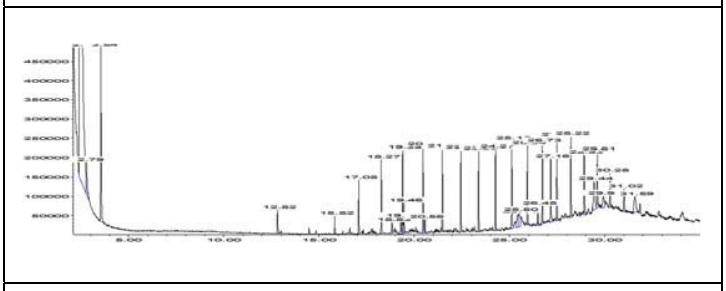

Figure 2. The trend of decreasing abundance and the number of hydrocarbon components in petroleum sludge after the degradation process using BP bacterial isolates.

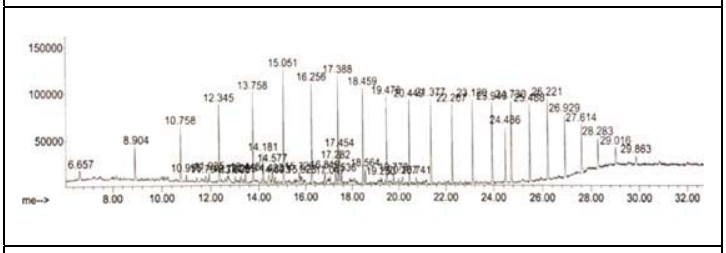

Figure 3. The chromatogram trend will decrease the hydrocarbon component when using BC bacterial isolates.

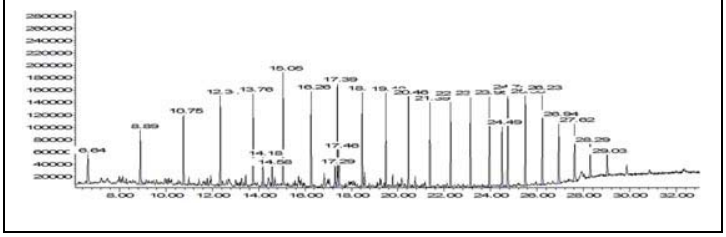

Figure 4. The chromatogram trend will decrease the hydrocarbon component when using SP bacterial isolates.

3.3.2. Changes in aliphatic component concentrations of processed petroleum sludge waste.
The biodegradation level of the aliphatic component of processed petroleum sludge waste by sponge micro-symbiont was determined by calculating the change in concentration of 4 aliphatic components representing 21 components identified in processed petroleum sludge waste.

Tables 3, 4 and 5, show that BP bacterial isolate more strongly degrades the aliphatic components of processed petroleum sludge waste compared to BC bacterial isolates, although both isolates are *Niphates* sp. sponge symbiont. The degradation ability of pure bacterial culture of SP was weaker than the two test isolates. These results are in line with previous studies in biodegradation methods of petroleum sludge and polycyclic aromatic hydrocarbons [26][32][33]. Other information presented in accordance with Table 3-5, above, is that there is a tendency that long-chain carbon aliphatic components are more degraded than short carbon chains for the use of a single degradation, presumably because long carbon chains have more potential vertices for oxidation reactions. As results, more bacterial cells and active work induce carbon chains derived from the rate of biodegradation increases. These results are in accordance with previous studies on biodegradation of petroleum sludge using sponges and mangroves [10][18], although there are contradictions with previous studies [5][8]. The development of further research can be carried out on aspects of increasing the rate and rate of biodegradation by modifying biodegradation using bacteria consortium which have the ability to degrading the aliphatic component of hydrocarbons. Another modification is increasing the surface area of the substrate sludge waste with

the addition of quartz sand to form pores that allow the bacterial suspension to seep between the sludge between slit, so that more contact will be occurred between sludge and the degradation bacteria [33].

Table 3. Biodegradation rate of aliphatic components waste of processed petroleum sludge using *Bacillus pumilus* strains GLB197 symbiont of Sponge *Niphates* sp. in contact period of 25 days.

Types of aliphatic components	Aliphatic component concentration (1. 10^4 ppm)			Degradation level (%)
	Before	After	Changes	
Tridecane ($C_{13}H_{28}$)	3,16	1,54	1,52	51,21
Octadecane ($C_{18}H_{38}$)	6,56	3,12	3,44	52,42
Tricosane ($C_{23}H_{48}$)	4,95	2,23	2,72	54,99
Nonacosane ($C_{29}H_{60}$)	3,85	2,71	2,14	55,53

Table 4. Using *Bacillus cohnii* strains of DSM 6307.

Types of aliphatic components	Aliphatic component concentration (1. 10^4 ppm)			Degradation level (%)
	Before	After	Changes	
Tridecane ($C_{13}H_{28}$)	3,16	1,82	1,34	42,34
Octadecane ($C_{18}H_{38}$)	6,56	3,36	3,20	48,76
Tricosane ($C_{23}H_{48}$)	4,95	2,36	2,59	52.36
Nonacosane ($C_{29}H_{60}$)	3,85	1,74	2,19	54,76

Table 5. Using pure culture of *Pseudomonas* sp.

Types of aliphatic components	Aliphatic component concentration (1. 10^4 ppm)			Degradation level (%)
	Before	After	Changes	
Tridecane ($C_{13}H_{28}$)	3,16	2,12	1,04	32,84
Octadecane ($C_{18}H_{38}$)	6,56	3,99	2,57	39,15
Tricosane ($C_{23}H_{48}$)	4,95	2,71	2,24	45,30
Nonacosane ($C_{29}H_{60}$)	3,85	2,01	1,84	47,72

The degradation of aliphatic components based on the interaction time between petroleum sludge and bacterial isolate as shown in Figure 5-7 confirms that the bacterial isolates were able to degrade aliphatic components of petroleum sludge as a performance of Niphates sp. micro symbiont and BP bacterial isolation deprivation as the highest aliphatic component achieved during the contact period 10-15 days, moderate performance during the contact period of 5 days and 20 days and low performance during the contact period of 25 days. This result is relevant and in accordance with the phase of bacterial development, namely the first 5 days is the adaptation phase, so that degradation performance is also low. Day 10-15, was the phase of cell growth and division. The number of bacterial cells in this phase was estimated to have a maximal level with a performance of isolate degradation against aliphatic components. The performance of bacteria in 20 days of contact, was still quite good and tends to decrease until 25 days of contact. Theoretically, the bacteria at the time of contact should have entered the stationary phase of the bacteria towards the mortality phase bacterial cells, in fact it did not occur due to the positive effect of NPK nutrients addition, hence the duration of bacterial cell growth and development took longer. The stages of degradation and decreasing in the concentration of aliphatic components of petroleum sludge waste using BP isolates, were relatively identical when using BC bacterial isolates or bacterial SP isolate, (see Figure 6 and Figure 7). Significant differences occurred in

the rate of degradation depend on the type of isolate and the degradation ability of the bacteria isolate. These results corroborate the results of previous studies [5][18].

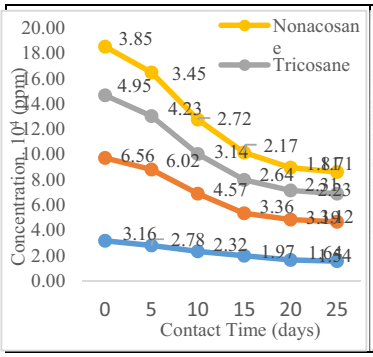

Figure 5. The increasing trend of aliphatic concentration components in petroleum sludge waste using BP bacterial isolate degradation (*Bacillus pumilus* strains GLB197) based on the contact time.

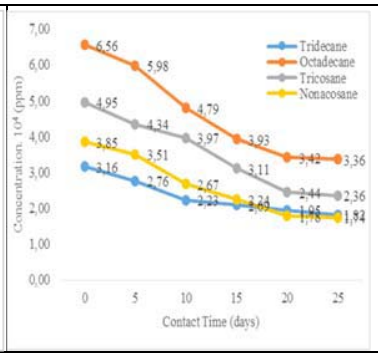

Figure 6. The increasing trend of aliphatic concentration components in petroleum sludge waste using bacterial isolate degradation BC (*Bacillus cohnii* strains of DSM 6307).

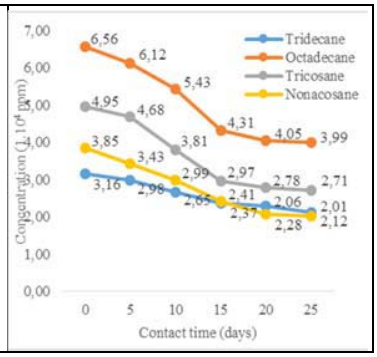

Figure 7. The increasing trend of aliphatic concentration components in petroleum sludge waste using SP Culture Pure degradation (Pseudomonas sp.).

3.3.3. Biodegradation components of processed petroleum sludge waste. Some types of simple organic compounds are thought to be the result of biodegradation of aliphatic components in identified petroleum sludge wastes, namely groups of alcohols, aldehydes, carboxylic acids, esters and ethers. Looking at the degradation products showed that the biodegradation pathway taken by the test bacteria was primary and secondary destruction of the aliphatic component of sludge waste.

Table 6. Biodegradation products processed petroleum sludge waste using bacterial isolates, contact period 25 days.

Retention time	Components	Type of bacterial biodegradation
8,17	Methanol	BP, BC, SP
8,34	Propanol	BP, BC
9,18	Pentanol	BP, BC
9,21	Butiraldehyde	BP, SP
9,28	Ethyl Methyl Ether	BP, BC
11,22	Methyl-ethyl-ketone	BP, BC, SP
11,84	1-methyl hexanol	SP
24,12	Methyl ester	BP, BC, SP
24,40	6-octadecanoat acid	BP, BC, SP

Table 6 shows that there were different biodegradation products of aliphatic components in the use of different bacterial degradation. This indicates that the isolates of bacteria took a specific path that was identical to the isolate's strain, and different from the biodegradation mechanism that was traversed in the use of other types of bacterial isolates. The mechanism of biodegradation taken by a bacterium in the biodegradation method is the basis for the selection and preparation of a combination of carbon clastic bacteria in the method of a bacterial consortium, for modification of degrading bacteria. Looking at the results obtained above and paying attention to several results of biodegradation studies of hydrocarbon components using bacteria, it is recommended to use several types of bacteria (consortium)

in biodegradation and expansion of sludge waste surface using quartz sand as an effort to increase the biodegradation rate of hydrocarbon components in petroleum sludge waste [14][17].

4. Conclusion

According to the research data and analysis results, several conclusions were drawn, namely: Three types of test isolates were used (*Bacillus pumilus* strains GLB197, *Bacillus cohnii* strains of DSM 6307, *Pseudomonas sp.*) capable of degrading aliphatic components of processed oil sludge waste with an average level of 48 , 11%; The order of degradation strength of bacterial isolates against the aliphatic sludge component is BP > BC > SP; Tested isolates tend to degrade long carbon chain aliphatic components than short chains; The addition of NPK nutrients has the benefit of increasing the duration of development and growth of bacterial cells; The level of degradation of the aliphatic component can be increased by modifying the joint use (consortium) of bacteria or expanding the surface of sludge with the addition of quartz sand or a combination of both, so that there is an increase in the rate and rate of biodegradation of hydrocarbon aliphatic sludge waste.

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