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Investigation of Biodegradable Bacteria as Bio indicators of the Presence of PAHs Contaminants in Marine Waters in the Marine Tourism Area of Makassar City

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Abstract. The activities of the petroleum processing industry and marine transportation potentially cause of hydrocarbon pollution. It is assumed that every water area exposed to hydrocarbons also found bacterial species that have biodegradation properties against PAH contaminants. This study aims to analyze the relationship between biodegradable bacteria and the status of marine waters exposed to hydrocarbons. The method of analyzing the type of PAH contaminants for each seawater and sponge samples were processed at 4 different points using GC-MS, the same sample was also carried out isolation, characterization and activity test of bacterial isolates against naphthalene and pyrene type PAH. The analysis showed that the four samples of seawater were contaminated with hydrocarbons. The type of hydrocarbons found in each sample is different and the concentration value varies. The types of bacteria identified in seawater and sponge samples also varied. There were 8 types of isolates selected, each one isolate per sample, all of which showed biodegradation activity against hydrocarbon contaminants, while the order of aromatic contamination levels at the four sampling points Sp.1 > Sp.2 > Sp.3 > Sp.4. These results indicate that the presence of biodegradable bacteria in water areas can be a bio-indicator for the presence of PAH pollutants.

1. Introduction

Transportation activities of various types of ships, industrial and household activities are the main sources of pollutants in marine areas. These contaminants are in the form of micro plastics, heavy metals, hydrocarbons, especially polycyclic aromatic hydrocarbons (PAHs) [1]. This type of contaminant is very dangerous and a serious threat to the survival of various marine life, even the danger of these contaminants can target and interfere with human health [2]. This condition needs the attention of many parties, including researchers to contribute in handling and managing marine resources so that they do not suffer further damage due to exposure to these pollutants. Hopefully, by

producing various methods and knowledge that can be applied in the context of recovery to maintain the preservation of the marine environment [3].

Under normal conditions, the marine environment actually has the natural ability to maintain its quality. Among them there are a number of marine bacteria, both those isolated from seawater itself and those isolated from various types of marine life, especially bacteria isolated from sponges which have the ability to be biodegradable against several types of contaminants [4,5]. Several research results indicate that marinesponges are bio-indicators of heavy metal pollution. Another function of sponges have symbiosis with a number of microorganisms, especially bacteria and not a few of these microbes have the function of biodegradation of hydrocarbons [5]. Community activities and technological advances today, massively contribute significantly to increasing the level of pollution in the sea, so it is important to carry out scientific interventions to help maintain the quality of marine resources [6,7].

The status of a number of islands in the administrative area of Makassar City, such as Samalona Island, Barrang Caddi, Langkai, Kodingareng Keke which are part of the Marine Tourism Area, are very close to the CPI Area, Container port, and Paotere Port and in the ship traffic route. The condition of this water area is very susceptible to contaminate with dangerous pollutant components, so it is necessary to analyze the level of pollution, the type of pollutant and determine the status of this area whether there are biodegradable bacteria as bio-indicator against hydrocarbon contaminants and their correlation in a reciprocal relationship with polluting compounds.

2. Materials and Methods

2.1 Materials

There were 8 kinds of bacterial isolate samples used in this study, four sample isolates were isolated from sea water, namely Sp1.a; Sp2.a; Sp3.a Sp4.a, and four sample isolates were isolated from sponges, namely Sp1.b were isolates from sponge *Auleta sp*; Sp2.b isolate of *Clatharia reinwardti* sponge; Sp3.b was isolated from the sponge *Callyspongia sp* and Sp4.b was a sponge *Hyrtios erectus* isolate. Other materials include yeast extract, glucose, Nutrient Agar (NA), pure sea water, Marine Agar (MA), PCA media pa, PBS pa media, SWC media, aquabidies, nitrogen gas, glycerol pa, used diesel oil, naphthalena (sigma), pyrene (Sigma), Na_2SO_4 anhydrous pa, alcohol, ethanol pa, n-hexane pa, glycerol and Bergey's manual of determinative bacteriology standards. Equipment includes GC-MS (Agilent 7890), PCR-Real Time Pocit-Generaach, polyethylene wrab plastic bottles, GPS, digital pH meter, thermometer, microscope, oven, ultrasonic, autoclave, pH paper (brand), separating funnel and a set of glassware.

2.2 Sampling technique

Seawater samples were obtained by dipping a sterile 600 mL polyethylene plastic bottle into the water body up to 45 cm from the surface until full. The bottlethen closed until it was tight, then removed and put immediately in an ice box.

Seawater samples were obtained from 4 different stations with the code Sp 1; Sp 2; Sp 3 and Sp 4. Each station was done with duplicate sampling. Sponge samples were also obtained on the seabed at the seawater sampling point. This is done by taking 1 clump of sponges at each sampling point and immediately put it in a plastic bag in an ice box. Several observations made during sampling, such as coordinate points, pH, salinity and temperature.

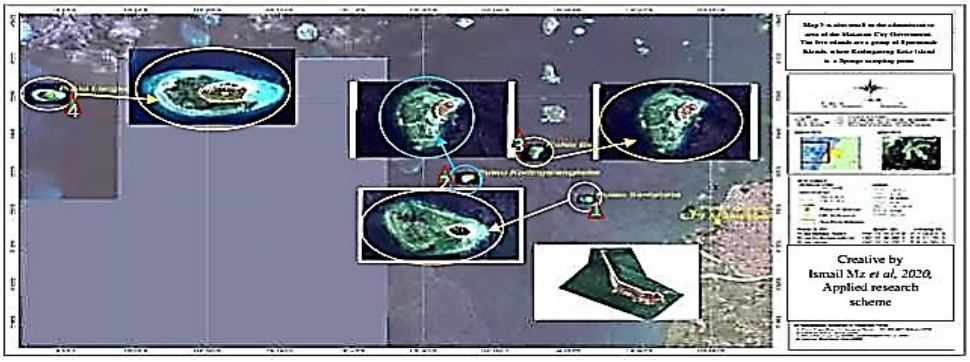

Figure 1. Map of sampling points Seawater and sponges in waters on 4 different islands. Point 1 Samalona Island; point 2 Kodingareng Keke Island; point 3 Barrang Caddi Island; and point 4 Langkai of island. The four islands are part of the Spermonde Archipelago, part of the Administrative area of Makassar City

2.3 Sample preparation

The isolation of bacteria in seawater samples was carried out by means of enrichment in selective media made from 0.4 g peptone and 0.2 g yeast extract, both dissolved in 1000 mL of sterile sea water and homogenized, then poured into a 25 mL Erlenmeyer volume. The enrichment is carried out up to three cycles. Isolation of bacteria was grownby using the pour plate methodon Plate Count Agar (PCA) media. Colony that grew on the surface of the media were purified by the streak method and incubated for 1x24 hours [8,9]. This process is carried out in stages up to 6 cycles until pure culture isolates were obtained through microscopic observation..

Isolation of bacteria from marine sponges was initially bymorphological analysis to determine the species of each sample, then isolation of bacteria by spraying the sponge sample with sterile sea water, then cutting the mesohyl sponge with the size of ± 1x1x1 cm^3, crushed, then diluted with Phosphate Buffer Saline (PBS). Isolation of bacteria on the sponge using the direct plating method using a sterile swab, then put into a test tube that already contains Sea Water Complit (SWC), incubated for 2x24 hours at 26^0C [10,11]. The selection of colonies that grew was using round loop needles and purified with the same media, until pure isolates were obtained. The isolates were stored in 25% glycerol.

2.4 Phenotype Analysis and Isolate Genotype

2.4.1 Isolate Phenotype Analysis.

Using standard biochemical reagents Bergey's manual of determinative bacteriology, especially the TSIA (Triple sugar iron agar) test, MR/VP (Methyl red-Vogeues Praskauer) test, indole test, citrate and sucrose, or specific tests according to the biochemical characters for biodegradable bacteria on hydrocarbons [12]. This test was carried out for all 23 types of isolates, then based on the results of the phenotype analysis, one isolate was selected for each sample, so that 8 test isolates were obtained. Parameters for biodegradable isolates were isolates that had at least a positive reaction with at least 4 kinds of biochemical tests.

2.4.2 Isolate Genotype Analysis.

All isolates are potential as biodegradable hydrocarbons according to the results of phenotype analysis. Genotype analysis was carried out with only 1 potential isolate from each sample so that only 8 isolates were selected, namely 4 isolates isolated from seawater with the code "a", namely Sp1.a; Sp 2.a; Sp3.a and Sp 4.a. The other four isolates were sponge symbiont isolates with the code "b", namely Sp1.b; Sp2.b; Sp3.b and Sp4.b. Genotype analysis of isolates to obtain sequencing was carried out in 3 stages, namely extracting isolate DNA, mixing PCR reagent components and running polymerase Chain Reaction (PCR) and sequencing [13]. Phenotype and genotype analysis data are presented in Table 2 and Table 3.

2.5 Extraction and Measurement of Hydrocarbons Contaminants Seawater Samples

Each seawater sample was vacuum filtered using 2 µm filter paper. The filtrate was extracted using ethanol in the ratio of 50 mL: 50 mL in a 250 mL separating funnel by shaking for ± 15 minutes. A total of 5 mL of seawater ethanol extract and 5 mL of n-hexane were put into a glass tube, then ultrasonic for 15 minutes. The residue was re-extracted with 5 mL n-hexane. The residue wass collected in 1 container, the solvent was evaporated with nitrogen gas, the thick extract was added with Na_2SO_4 to bind water [14]. The prepared sample can then be injected into the GC-MS instrument to see the types and components of the hydrocarbons present as contaminants.

3. Results and Discussion

The presence of biodegradable isolates and the status of sea water contaminated with hydrocarbons, both of which are thought to have a reciprocal relationship such as sponges as bio indicators of heavy metal pollution in a water area. The selection of Samalona Island, Kodingareng Keke, Barrang Caddi and Langkai as sampling locations was because it was strongly suspected that the area had been contaminated with various carcinogenic hydrocarbon components. The preservation of these four islands in the future is very susceptible to contamination by hydrocarbon compounds, and can even become an area for the accumulation of a number of dangerous contaminants, such as heavy metals, micro plastics and hydrocarbons, because these islands are closest to the Center Point of Indonesia (CPI) and also because this island is part of the Marine Tourism Area (KMB) of Makassar City. Table 1, shows the waters on these four islands have not shown any significant physical changes, such as salinity, pH and temperature, but that does not mean they are free from chemical problems with the presence of hazardous hydrocarbon contaminants.

Table 1. Physical Characteristics of Sampling Points

Observation type	Physical-Chemical Characteristics of Sample			
	Sp 1	Sp 2	Sp 3	Sp 4
Coordinate point	119^0 23'37.051" E 5^0 07'31,979" S	119^0 17'19.399" E 5^0 06'21,125" S	119^0 13'14.373" E 5^0 14'52,272" S	119^0 15'12.007" E 5^0 21'55,033" S
Salinity (‰)	31 ‰	30 ‰	31 ‰	31 ‰
Temperature (^{0}C)	30 ^{0}C	31 ^{0}C	30 ^{0}C	29 ^{0}C
Seawater sampling (dpl)	45 cm	45 cm	45 cm	45 cm
The sponge sampling (dpl)	3.65 m	4.20 m	3.50 m	3.85 m
pH	6.8	7.1	6.9	7.2

Salinity, pH and temperature look like other marine waters scattered in various regions in Indonesia. Observation of parameters such as Table 1, above is intended to provide information on the physical conditions of the sampling location, so that a correlation analysis can be carried out between the biodegradable ability of the isolate, its relationship with the status of sea water contaminated with hydrocarbon compounds.

Table 2. Characteristics of Bacterial isolate samples

Analysis Variables	Characteristics of Bacterial Isolates							
	Seawater isolate samples				Sponges symbiont isolate samples			
	Sp 1.a	Sp 2.a	Sp 3.a	Sp 4.a	Sp 1.b	Sp 2.b	Sp 3.b	Sp 4.b
Source of isolates	Seawater	Seawater	Seawater	Seawater	Sponge	Sponge	Sponge	Sponpe
Types of biota	-	-	-	-	Auleeta sp	Clathria reinwardti	Callyspongia sp	Hyrtios erectus
types of bacterial isolates	Alcaligenes faecalis starin Cu4-1	Sphingobac-terium strain 21	Bacillus cereus strain MER_8	Pseudomonas sp strain RCH2	Pseudomonas sp strain Hi1	Bacillus subtilis strain BAB-1684	Pseudomonas stutzeri strain RCH2	Bacillus flexus strain PHCD-20
Group of Gram isolates	Gram -	Gram +	Gram -	Gram +	Gram -	Gram +	Gram +	Gram +

Data Table 2, provides information on 4 species of biodegradable sponge source isolates, the species of all test isolates used, both those isolated from seawater samples and from sponge samples. The eight test isolates used were generally isolates dominated by bacillus and pseudomonas, while the gram

group of 5 tested isolates were Gram + (positive) and 3 Gram - (negative) group. The data above cannot be used as a reference for categorizing biodegradable isolates or not against hydrocarbons.

The phenotypic characteristics of the 8 types of test samples (Table 3) indicate that there is potential for these isolates to be biodegradable bacteria against hydrocarbon contaminants. The phenotypic characteristics of the isolates (Table 3), it appears that 6 kinds of biochemical tests were carried out on the test isolates, there were 2 isolates that only reacted positively with 4 types of test reagents, namely isolates Sp1.a and Sp1.b, although all test isolates were considered potential as a biodegradable isolate against hydrocarbons.

Table 3. Characteristics of phenotypes and genotypes of bacterial isolate samples

Analysis Variables	Characteristics of phenotypes and genotypes of bacterial isolates							
	Sp 1.a	Sp 2.a	Sp 3.a	Sp 4.a	Sp 1.b	Sp 2.b	Sp 3.b	Sp 4.b
Phenotype Analysis								
Indol Test	-	+	+	+	+	-	+	+
Rx Lactose ferm.	+	+	+	+	-	+	+	+
Rx Citrate Test	+	+	+	+	+	+	+	+
Rx Catalase Test	-	+	+	-	+	+	+	+
Rx Methyl red	+	+	+	+	+	+	+	+
Rx Voges Proskauer	+	+	+	+	-	+	+	+
Genotype Analysis								
Number of Sequences	6-933	6-939	1-894	4-927	11-607	27-866	9-949	9-973
Sequence GenBank	18-955	539-1467	512-1408	523-1433	23-654	68-996	78-1043	111-1073
Identity Quality	918/928 99 %	926/937 (99 %)	891/898 (99 %)	903/912 (99 %)	585/610 (96%)	817/913 (89%)	928/956 (97%)	948/952 (99%0
Gabs	0/928 (0%)	3/937 (0%)	5/989 (0%)	3/912 (0)	24/610 (3%)	18/913 (1)	11/966 (1%)	3/952 (0%)
Strain	Cu4-1	21	MER_8	RCH2	Hi1	BAB-1684	RCH2	PHCD-20

This is based on the results of phenotypic analysis which recommends that isolates that are potentially biodegradable to hydrocarbon components are those that have at least a minimum positive reaction to 4 reagents out of a total of 6 biochemical test reagents (Table 3). The positive reaction of a number of biochemical reagents above is one of the requirements for the isolate to be said to be biodegradable to PAHs components according to several previous studies [11, 15].

The genotype data of the test isolates showed that the comparison of the number of isolates sequences to the Gen-Bank sequence resulted in the quality identity of 5 isolates reaching 99% and 3 other isolates (Sp1.b; Sp2.b and Sp3.b) with quality 96% respectively; 89% and 97%. The three types of isolates were isolates from sea sponges. The quality level is below 99%, indicating the potential for finding new types of bacteria. A more detailed investigation of the possibility of new bacteria from the three sponge isolates can be done by registering with NCBI [1,16].

Table 4. Activity of bacterial isolates on hydrocarbon contaminated media

Hydrocarbons contaminated media	Bacterial isolat test							
	Sp 1.a	Sp 2.a	Sp 3.a	Sp 4.a	Sp 1.b	Sp 2.b	Sp 3.b	Sp 4.b
Used diesel oil	+	++	+	++	+	+	++	+
Naphthalene	-	+	+	+	-	+	+	+
Pyrena	-	+	-	+	-	-	+	+

Noted: + = there is isolate growth low activity;
++ = strong isolate growth high activity;
- = No growth activity

Data in Table 4, shows that eight types of test isolates, each of 4 seawater sample isolates and 4 sea sponge symbiont isolates all have growth activity on media contaminated with used diesel oil. There were 6 isolates having activeness in the media exposed to PAH types of naphthalene, while for the media contaminated with pyrene, only 4 isolates were found to show growth activity, namely isolate Sp2.a; Sp4.a; Sp3.b and Sp4.b. These results indicate that there is a tendency to decrease the activity of the isolates against the media exposed to pyrene and naphthalene, it is understood that pyrene type PAH contaminants have very high toxicity to isolates and also have carcinogenic and even

mutagenic properties [1,17]. According to Table 4, it is concluded that the activity of the test isolates against hydrocarbon contaminants with a tendency for used diesel oil > naphthalene > pyrene, or high reactivity to saturated hydrocarbon components and low to PAH contaminants.

The status of seawater samples in the waters of the 4 islands of the sampling location was declared contaminated by hydrocarbon contaminants. This is based on the results of GC-MS measurements (Figures 2 - 5) of 4 seawater samples, each of which was obtained from the waters on 4 islands according to the location of the coordinate points (Table 1).

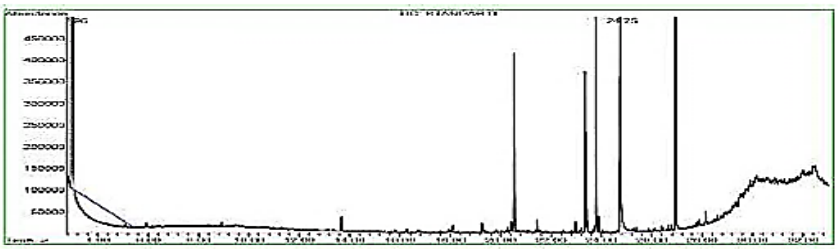

Figure 2. Chromatogram Profile of hydrocarbon components measured by GC-MS At the sampling point Sp.1 (Samalona islands)

Figure 2 shows that Sp 1, the sample obtained in the waters around Samalona Island, was exposed to several types of hydrocarbons. According to the chromatogram, ± 6 types of aromatic types of contaminants were identified with different abundances and about 5 peaks with low abundances which are suspected to be aliphatic hydrocarbons. Types of PAH contaminants, namely naphthalene, acenaphthylene, anthracene, phenanthrene, pyrene and chrycene.

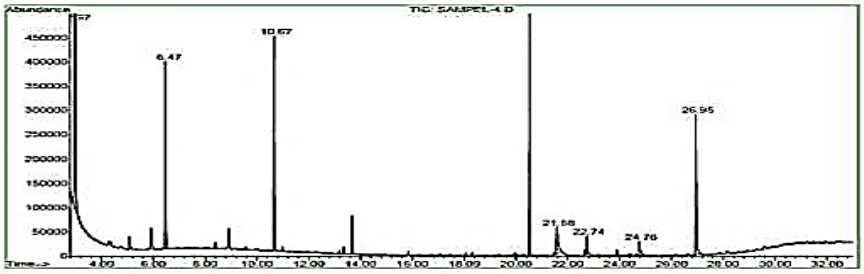

Figure 3. Chromatogram Profile of hydrocarbon components measured by GC-MS At the sampling point Sp.2 (Kodingaren Keke islands)

The sea conditions around Kodingareng Keke Island are also contaminated with several types of aromatic and aliphatic hydrocarbons. The aromatic hydrocarbon contaminants identified according to the GC-MS measurement results (Figure 3), were naphthalene, acenaphthalene, anthracene, phenanthrene, pyrene and benzo(a)anthracene with high abundance, while alipatic type contaminants were also detected ± 6 peaks with low abundance and moderate [18].

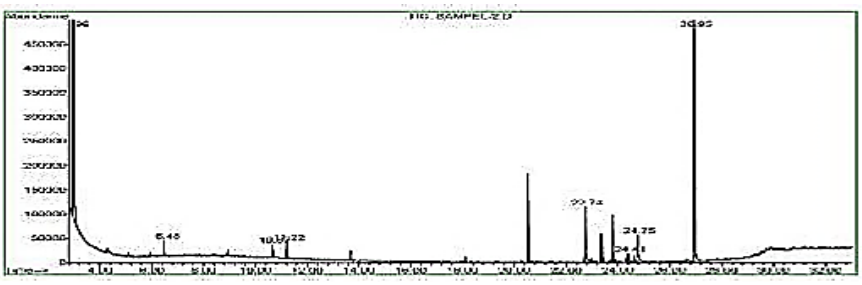

Figure 4. Chromatogram Profile of hydrocarbon components measured by GC-MS At the sampling point Sp.3 (Barrang Caddi islands)

The distribution of PAH hydrocarbons is slightly different in the waters around Barrang Caddi Island, sample code Sp3 (Figure 4. These waters are stated to be exposed to contaminants from aromatic and alipatic hydrocarbons, although the PAH types identified are different and the abundance of each component is classified as low. According to Figure 4, it appears that only 1 component of PAH with high abundance, namely pyrene and several other PAHs including naphthlene, antharacena and chrycena have low and medium abundances [9,19,20].

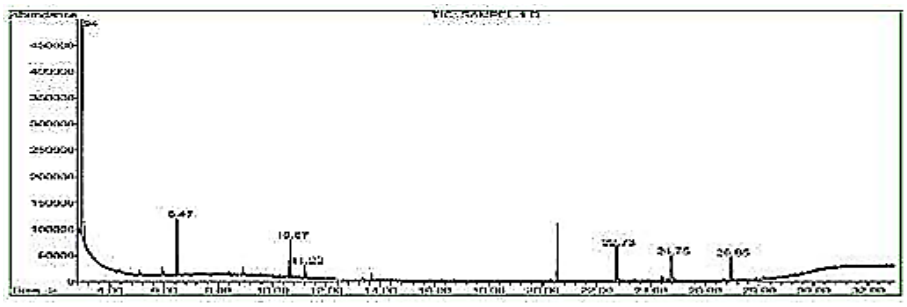

Figure 5. Chromatogram Profile of hydrocarbon components measured by GC-MS At the sampling point Sp.4 (Langkai island)

The chromatogram shown in Figure 5 is the status of hydrocarbon contaminants in the waters around Langkai Island. Based on its abundance, aromatic contaminants naphthalene is the highest pollutant, followed by anthracene and pyrene. The sample Sp.4 also identified ± aliphatic hydrocarbon components. Comparing the level of aromatic contamination of PAHs types at the four sampling points (Figures 2 - 5) can be ordered Sp.1 > Sp.2 > Sp.3 > Sp.4, or it means that the waters around the island of Samalona have the highest and most PAHs pollutants. Low level of pollution is the waters around the island of Langkai. This condition is understandable, because Samalona Island is the island closest to the CPI area, closest to the container port and the Paotere loading port, the most activity and visits of domestic tourists and also the island that is most inhabited by people who are generally fishermen compared to the three another islands [2, 20,21,22] Based on the analysis data as described above, it shows that there is a reciprocal relationship between the biodegradable properties of bacterial isolates to PAH and the status of exposure to aromatic and aliphatic hydrocarbons in an area of marine waters. It can be said that the nature of PAH biodegradable isolates in a water area is a bio monitoring of the presence of aromatic contaminants or vice versa identification of exposure to PAH substances as a bio indicator as well as the potential to find biodegradable PAH bacteria in the area [21,.22]

4. Conclusion

This study concluded that all tested bacterial isolates had growth activity on aliphatic and aromatic hydrocarbon contaminated media. The activity of bacterial isolates on media exposed to hydrocarbons tended to decrease in line with the increase in the toxicity of the contaminants. All seawater samples obtained from marine waters on the four islands of the marine tourism area of Makassar City were contaminated with various aromatic and aliphatic hydrocarbons. The biodegradable nature of marine bacteria isolates obtained in marine waters and sponges as a bioindicator of contamination of hydrocarbon components.

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References

[1] Bendouz M, Dionne J, Tran L H, Coudert L, Mercier G and Blais J F 2017 Polycyclic Aromatic Hydrocarbon Oxidation from Concentrates Issued from an Attrition Process of Polluted Soil Using the Fenton Reagent and Permanganate *Water. Air. Soil Pollut.* **228** 114–27

[2] Bezza F A and Chirwa E M N 2016 Biosurfactant-enhanced bioremediation of aged polycyclic aromatic hydrocarbons (PAHs) in creosote contaminated soil Chemosphere Biosurfactant-enhanced bioremediation of aged polycyclic aromatic hydrocarbons (PAHs) in creosote contaminated soil *Chemosphere* **144** 635–44

[3] Marzuki I, Noor A, Nafie N La and Djide M N 2015 Sponge Role In Alleviating Oil Pollution Through Sludge Reduction , A Preliminary Approach *Int. J. Appl. Chem.* **11** 427–41

[4] Yogaswara D 2017 Concentrations of PAHs (Polycyclicaromatic Hydrocarbons) Pollutant in Sediment of The Banten Bay *Bull. Mar. Geol.* **32** 61–6

[5] Yetti E, Thontowi A and Lisdiyanti P 2015 Screening of Marine bacteria capable of degrading various polyaromatic hydrocarbons *Squalen Bull. Mar. Fish. Postharvest Biotechnol.* **10** 121–7

[6] Marzuki I, Chaerul M, Erniati, Asmeati and Irwan Paserangi 2020 Biodegradation of aliphatic waste components of oil sludge used micro symbiont of Sponge Niphates sp . Biodegradation of aliphatic waste components of oil sludge used micro symbiont of Sponge Niphates sp . *ICMS, IOP Publ.* **429** 012056

[7] Marzuki I, Noor A, Nafie N La and Djide M N 2016 Morphological and phenotype analysis of microsymbiont and biomass marine sponge from melawai beach, Balikpapan, east kalimantan *Int. J. Mar. Chim. Acta* **17** 8–15

[8] Marzuki I, Noor A, Nafie N La and Djide M N 2015 Isolation and Identification On Degradator Bacterium Of Petroleum Waste Which Symbions With Sponge From Melawai Beach *First Int. Conf. Sci.* **1** 493–503

[9] Marzuki I, Noor A, Nafie N La and Djide M N 2015 Moleculer Characterization Of Gene 16S rRNA Microsymbionts In Sponge At Melawai Beach, East Kalimantan *Mar. Chim. Acta* **16** 38–46

[10] Tereza Č, Wiesnerová L, Praus L, Jablonský I, Koudela M and Tlusto P 2018 ScienceDirect Comparing the removal of polycyclic aromatic hydrocarbons in soil after different bioremediation approaches in relation to the extracellular enzyme activities *J. Environ. Sci.* **XX** 1–10

[11] Khabouchi I, Khadhar S, Chaouachi D, Chekirbene A and Doumenq P 2020 Study of organic pollution in superficial sediments of Meliane river catchment area : aliphatic and polycyclic aromatic hydrocarbons Study of organic pollution in superficial sediments of Meliane river catchment area : aliphatic and polycyclic aromatic hy *Enviroment Monit Assess* **April** 282–90

[12] Shareef H K, Muhammaed H J, Hussein H M and Hameed I H 2016 Antibacterial Effect of Ginger (Zingiber officinale) Roscoe and Bioactive Chemical Analysis using Gas Chromatography Mass Spectrum Antibacterial Effect of Ginger (Zingiber officinale) Roscoe and Bioactive Chemical Analysis using Gas Chromatography Mas *Orient. J. Chem.* **32** 817–37

[13] Pawar P R 2017 Assessment of microbial diversity in marine waters along the Uran coastal area of Navi Mumbai , *Adv. Environ. Biol.* **11** 11–24

[14] Obire O, Harcourt P, Wemedo S and Owhonka A 2020 Fungi in Biodegradation of Polycyclic Aromatic Hydrocarbons in Oilfield Wastewater Fungi in Biodegradation of Polycyclic Aromatic Hydrocarbons in Oilfield Wastewater

[15] Muzaki F and Sudarmono P 2017 Screening of Antimicrobial Activity of Sponges Extract from Pasir Putih , East Screening of Antimicrobial Activity of Sponges Extract from Pasir Putih , East Java (Indonesia) Journal of Marine Science : Research

[16] Marzuki I, Enryani H I, Nafie N La and Dali S 2017 Study Biodegradation of Aromatics Pyrene

Using Bacterial Isolates from the Sea and micro symbionts Sponges *Int. J. Appl. Chem.* **13** 707–20

[17] Liu W J, Duan X D, Wu L P and Masakorala K 2018 Biosurfactant Production by Pseudomonas aeruginosa SNP0614 and its Effect on Biodegradation of Petroleum 1 *Appl. Biochem. Microbiol.* **54** 155–62

[18] Lu C, Hong Y, Liu J, Gao Y, Ma Z, Yang B, Ling W and Waigi M G 2019 A PAH-degrading bacterial community enriched with contaminated agricultural soil and its utility for microbial bioremediation ☆ *Environ. Pollut.* **251** 773–82

[19] Medic A, Ljesevic M, Inui H, Stojanovic K, Karadzic I, Beˇskoski V and Koji I 2020 Efficient biodegradation of petroleum n-alkanes and polycyclic aromatic hydrocarbons by polyextremophilic Pseudomonas aeruginosa san ai with multidegradative capacity *R. Soc. Chem. Adv.* **10** 14060–70

[20] Veeramuthu A and Chawalit Ngamcharussrivichai 2020 Potential of Microalgal Biodiesel: Challenges and Applications *Potential of Microalgal Biodiesel: Challenges and Applications* (Nevertheless,) pp 1–14

[21] Marzuki I, Daris L, Nisaa K and Emelda A 2020 The power of biodegradation and bio-adsorption of bacteria symbiont sponges sea on waste contaminated of polycyclic aromatic hydrocarbons and heavy metals *IOP Conf. Ser. Earth Environ. Sci.* **584** 012013

[22] Marzuki I, Daris L, Yunus S and Riana A D 2020 Selection and characterization of potential bacteria for polycyclic aromatic biodegradation of hydrocarbons in sea sponges from Spermonde Islands , Indonesia *AACL Bioflux* **13** 3493–506