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I Marzuki[1*], K Nisaa[2], R Asaf[3], R Armus[4], M Kamaruddin[5], A Sapar[6], A Emelda[7]

[1] *Department of Chemical Engineering*, Fajar University, Panakkukang-Makassar, South Sulawesi, PO 90231, Indonesia
[2] *Fishery Faculty*, Cokroaminoto University of Makassar, Tamalanrea-Makassar, South Sulawesi, PO 90245, Indonesia
[3] *Research Center for Brackish Aquaculture Fisheries and Extension Fisheries*, Maros District, South Sulawesi, PO 90512, Indonesia
[4] *Department of Environment Engineering*, Nusantara Indonesia of College Technology, Makassar, South Sulawesi, PO 90234, Indosnesia
[5] *Department of Medical Laboratory Science*, Faculty of Nursing and Health Science, Muhammadiyah Semarang University, Semarang Central Java, 50273
[6] *Chemistry Dept. Faculty of Mathematics and Natural Sciences*, Tanjungpura University, Jl. Prof. Dr. Hadari Nawawi, Pontianak, 78124, Indonesia
[7] *Department of Pharmacy*, Universitas Muslim Indonesia, Urip Sumoharjo-Makassar, South Sulawesi, PO 90321, Indonesia

*E-mail: ismailmz@unifa.ac.id, ismailmz3773@gmail.com

Abstract: Generally, all petroleum processing industries produce oil sludge or sludge. Polycyclic Aromatic Hydrocarbons (PAH), one of the components contained in sludge, are hazardous and toxic waste material with toxic, carcinogenic and mutagenic properties. The research objective was to understand the biodegradation mechanism of naphthalene by utilizing a marine sponge symbiotic bacterial isolate. Partial bacteria *Bacillus* Sp strain AB353f (BC), sponge isolate Neopetrosia sp and *Acinetobacter Calcoaceticus* strain PHCDB14 (AC) isolate sponge Callyspongia (Aerizusa) as biomaterial for PAH degradation. Biodegradation method integrates bacterial suspension with 10,000 ppm naphthalene for 25 days. Every 5 days, the biodegradation indicators were observed and the products of the destruction of naphthalene components were measured using FTIR and GC-MS. The results showed that BC isolates and AC isolates from sponge symbionts could degrade naphthalene. The biodegradation performance of BC bacteria tended to be more dominant than AC against naphthalene. Based on the functional groups resulting from FTIR, three types of biodegradation products were identified, namely: alcohol, aldehyde and carboxylic acid and one transition product in the form of a catechol. Maximum naphthalene bio-degradation occurs at an interaction period of 20 - 25 days.

1. Introduction

The search for potential natural materials in the biodegradation of polycyclic aromatic hydrocarbons (PAHs) is important to be carried out continuously, so that the benefits of natural materials as Indonesia's wealth can function optimally to create a balance in the environment in the biogeochemical cycle [1]. Naphthalene is one of the PAH components formed by the combination of carbon atoms and hy-

drogen in such a way as to form a molecule with the chemical formula $C_{10}H_8$. Naphthalene is a class of hazardous and toxic substances with moderate toxicity and low category of carcinogenic properties [2], [3]. Naphthalene is found in several industrial products such as camphor, magic chalk, both of which are insect poisons [4]. Handling and managing PAH type waste is very important because of the negative impact it causes. PAH exposure to humans has the potential to trigger cancer types or some types of body cells can mutate and eventually become deadly cancer cells, while contamination in the environment is most susceptible to occur in the waters or marine environment, thus threatening the lives of various marine biota, including the potential for exposure to humans [5]. The other side of marine life is that the environment is very vital to the life of many living things without except humans, because the sea is not only a medium for the life of various biota, but also a means of transportation, more than that the sea is an area that contains various types of mining materials which is very much needed [6], [7].

Natural materials that have the potential to degrade hydrocarbon components are quite a lot. Research on hydrocarbon degradation has been carried out for a long time using various treatments such as physical, chemical and biological methods, but it is considered less effective and efficient because of its benefits which only slow down the distribution, are expensive and the scale of the degradation is carried out in a limited environment [8]. Screening of potential natural materials as degradation materials for hydrocarbon components, especially PAH. Currently, there is an intense search for natural materials that have the potential for degradation of hydrocarbon components, especially tracing the use of microorganisms such as mangroves, fungi, fungi and bacteria [9,10]. Isolation of bacteria from seawater, sediment in the sea and soil that is suspected of being contaminated by PAH is carried out for the purpose of looking for potential microorganisms in biodegradation as well as tracing the biodegradation mechanism pursued by bacteria [11], [12]. This study aims to find new materials for PAH degradation, especially the type of naphthalene using microorganisms isolated from sponge-type marine life [13]. The selection of sponges as a source of new materials in screening for potential bacteria for bio-degradation of naphthalene is based on several considerations, including (1) sponges are one of the oldest marine biota with a large enough population to date, (2) The uniqueness of the life pattern of the filter feeder sponge, that is, having a way of looking for nutrients by sucking the sludge, then spraying it again, (3) almost every sponge species found in its habitat has a mutualism symbiosis with microorganisms, (4) special consideration of potential sponge symbion bacteria as biodegradation material of PAH is a sponge that the surface of its body is covered with mucus or substances that behave like microorganisms in biodegradation as well as tracing the biodegradation mechanism pursued by bacteria [2], [14], [15].

Characterization of sponges and bacterial symbionts used in the biodegradation of naphthalene was carried out to ascertain sponge species, sponge microsymbiont species and the mechanism of biodegradation of naphthalene by bacteria. Some of the study results referred to include sponge morphological analysis, phenotypic analysis of sponge symbiont bacteria, biochemical tests and gram staining of isolates and genotype analysis of sponge isolates using PCR instruments all of which lead to the ability of bacteria to biodegradation hydrocarbon components, especially PAH types, making it interesting to carry out tracing associated with the biodegradation mechanism by spongy symbiotic bacteria to naphthalene [11], [16], [17]. Exploration of sponges and benefits in the biodegradation of hydrocarbon components, including the mechanism of biodegradation that occurs or the process of destruction of hydrocarbon components by bacteria, needs to be done in more detail, comprehensively, and structured by Indonesian scientists. The exploration of potential new materials for hydrocarbon biodegradation can be carried out by cooperating with parties who have the competence, capacity, facilities, infrastructure and capabilities in this field to obtain significant results. PAH compounds need attention given their wide use and high release in nature due to careless handling [18]. Several previous researchers stated that the concentration of petroleum in the waters of 400 ppm was polluting the environment [19], [20]. The problem raised in this study is the analysis of the ability of symbiotic microorganisms with marine sponges in the biodegradation of polycyclic aromatic hydrocarbons of the type

of naphthalene and tracing the mechanism of hydrocarbon destruction by bacteria based on biodegradation products [21], [22].

2. Research methods

The material consists of two types of bacterial isolates that have been phenotypically and genotypically characterized. These two types of bacteria, namely partial Bacillus Sp strain AB353f (BC) and Acinetobacter Calcoaceticus strain PHCDB14 (AC), naphthalene (Merck Sigma-Supelco), HCl pa, 0.9% physiological NaCl, anhydrous Na_2SO_4 pa, N-hexane GR (Brand], sterile sea water, nutrient agar, cotton, plastic wrap, aluminum foil, alcohol, gauze, tissue, filter paper. Equipment includes: Shaker, incubator, universal pH paper, glass set, incubator, laminar air flow, micropipette, Mortar, round loop, oven, tweezers, syringe, test tube, deck glass, Erlenmeyer, pH indicator paper, microscope, and some equipment other. The instrument used was the GC-MS type Agilent 7890. Operating conditions GC-MS: maximum temperature 350 ^{0}C, temperature rose 10 ^{0}C every 5 minutes, pressure 18.406 psi, Helium carrier gas speed 150 mL/min, capillary column type Agilent 19019S -436HP-5 ms, dimensions 60 mx 250 μm x 0.25 μm, maximum retention time of 30 minutes, FTIR Nicolet IS 10 FTIR Spectrometer standard detector with type Deuterated Triglycine Sulfate (DTGS), Spektronik-20 D^+ Shimadzu [23].

Type of BC as bacteria samples, isolated from sponge Neopetrosia sp and AC bacteria, were isolated from Callyspongia Aerizusa. Two types of marine sponges source BC and AC bacteria were obtained from Kodingareng Keke Island, the administrative area of the Makassar City Government. Neopetrosia sp sponge obtained at the coordinate point S = 050 07 '05.58"; E = 1190 18' 11.74", the characteristics of the sampling point are ± 4.8 m above sea level, salinity = 29.6 ‰, pH 7, and temperature 29.6 ^{0}C, while the sponge Callyspongia Aerizusa, was obtained at the coordinate point S = 050 06 '07.94"; E = 1190 18' 10.87", the characteristics of the sampling point were ± 5.3 m asl salinity = 29.7 ‰, pH 7 and a temperature of 29.4 ^{0}C [24], [25].

The isolates of BC and AC were reproduced each by enriching the direct plating method on nutrient agar media, then the isolates were converted into suspensions by adding 2 ml of physiological 0.9% NaCl and shaking. The suspension was put into a 100 mL Erlenmeyer, the volume was made up using physiological 0.9% NaCl. Pipette 5 ml of each suspension into 2 x 6 vials, then incubate for 1 x 24 hours. Enter 5 ml of 10,000 ppm Naphthalene PAH solution, then in the incubator shaker with each contact time (1; 5; 10; 15; 20; 25) days for each vial. Uring the interaction period between the symbiotic bacterial isolate suspension and naphthalene, biodegradation parameters were observed, namely the pH of the biodegradation medium, optical density, temperature, smell of fermentation and gas bubbles formed to visually see the biodegradation process that occurred. After the contact time was reached, each sample in the vial was extracted with n-hexane [6], [26].

Sample extraction was carried out to separate the isolate suspension from Naphthalene PAH contaminants. Extraction was carried out by adding 5 mL of n-hexane to each sample in the vial, then transferred to a separating funnel, shaken for 5 minutes, let stand ±1 minute until two layers of polar and non-polar liquid were formed. The non-polar extract that has been separated from the isolate (polar) suspension, added 0.11 g of anhydrous Na_2SO_4 solids into each vial to remove water content, the samples were ready to be measured by GC-MS. The procedure is the same for measurement with FTIR [27].

3. Results and discussion

The turbidity of the biodegradation media was measured using Spectronics 20 D^+ carried out every 2 days of the interaction period, the measurement data was compared with the absorbance of the media at the beginning of contact, which was zero days. The absorbance of the biodegradation media measured in NTU units, which shows the growth of bacteria [16]. The turbidity of the biodegradation medium began to appear at the 5 day contact period, and continued to increase until the 17th day of contact. The increase in turbidity indicated that there was an increase in the number and size of the degrading bacterial cells. This also indicates that bacteria can live and carry out breeding activities in an

environment contaminated with naphthalene [28]. The decrease in optical density began to appear on the 19th day of the interaction. This decrease in optical density continued until the end of the interaction on the 25th day, is presented in Figure 1.

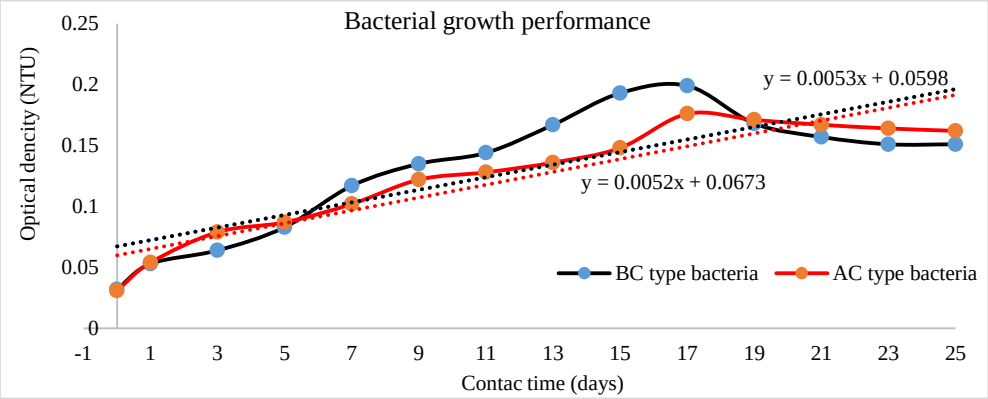

Figure 1. Optical density of interaction medium between BC bacteria and symbiotic sea sponge With AC PAH of naphthalene by contact time (days)

The experimental data can be assumed that the proliferation of bacterial cells occurs in several phases, namely the adaptation phase is estimated to occur during the contact period until the first 5 days, the exponential phase is thought to occur on contact after 5 - 17 days, the stationary phase is estimated at the contact period 17 - 20 days and the death phase occurred over 20 days of contact [29], [30], [31]. Comparing the biodegradation performance of the two types of sponge symbiotic bacteria to the naphthalene component showed that BC bacteria tended to be more dominant than AC bacteria. This condition can be caused by several factors, including: *first*, the adaptation period of AC bacteria is shorter than BC; *second*, AC bacterial cells were less able to survive long in the condition of the biodegradation medium which was slightly more acidic than BC bacteria, where it was known that the biodegradation products of hydrocarbons were simple organic compounds that could increase the degree of acidity of the media; Third, it is assumed that AC bacteria require faster supply of oxygen and nutrients than BC to survive for a long time under conditions of interaction media exposed to naphthalene contaminants [1], [32].

The increase in the number and increase in the size of the sponge symbiont bacteria cell as a biomaterial for degrading naphthalene was followed by several changes in the degradation medium which were the parameters for the biodegradation of bacteria to hydrocarbon components, shown in Table 1.

The temperature of the degradation medium began to show a tendency for changes in the contact period after 5 days to 20 days with an increase in temperature from 28 ^{0}C - 32 ^{0}C. During the contact period, it is suspected that the maximum degradation of naphthalene components into simple components by the work of spongy symbiotic bacteria occurs. Changes in the pH of the degradation medium also occurred during the contact period after 5-20 days. Theoretically, it is stated that the biodegradation products of hydrocarbon components are simple compounds in the form of alcohols, aldehydes, ketones, and carboxylic acid components, as well as CO_2 gas and other types of gases [16], [32]. This component of the biodegradation product triggers an increase in the acidity of the degradation medium which is thought to be due to the presence of an acidic component of the biodegradation product such as carboxylic acid which contributes to increasing the acidity of the media. The process of biodegradation of hydrocarbon components by bacteria through an enzymatic reaction mechanism, so that it is suspected that if the degradation occurs maximally, other degradation indicators will appear in the form of fermentation odors and gas bubbles, so that the presence of CO_2 components, carboxylic acids and other components in the degradation medium is very likely to occur. Observing Table 1, shows that the fermentation odor was identified during the contact period of 10 - 20 days as the biodegrada-

tion performance of BC bacteria, while the contact period with a shorter range, namely 10 - 15 days was the biodegradation performance of AC bacteria. This difference is an indication of the different degradation strength of the two types of bacteria with respect to naphthalene. Gas bubbles are a consequence of the formation of gas in the form of CO_2 or other types of gas in the degradation medium [33], [34], [35]. The value of the biodegradation parameters as shown in Table 1 and the existing assumptions are strengthened by the results of FTIR measurements, to identify the bio-degradation production functional groups as shown in Figure 2.

Table 1. Characterization Results of Several Indicators of biodegradation based on contact time (days)

Isolate	Time-measured biodegradation indicator contact (day)					
	0	5	10	15	20	25
1. Temperature (^{0}C)						
BC	28	28	30	31	32	29
AC	28	28	30	32	32	29
2. pH						
BC	7	7	6	6	6	7
AC	7	7	6	6	7	7
3. Fermented Smell						
BC	TT	TT	T	T	T	TT
AC	TT	TT	T	T	TT	TT
4. Air bubble						
BC	TA	TA	A	A	A	TA
AC	TA	A	A	A	TA	TA

Trannformation:
TT = No fermentation odor detected T = Detected fermentation odor
TA = no bubbles A = there are bubbles

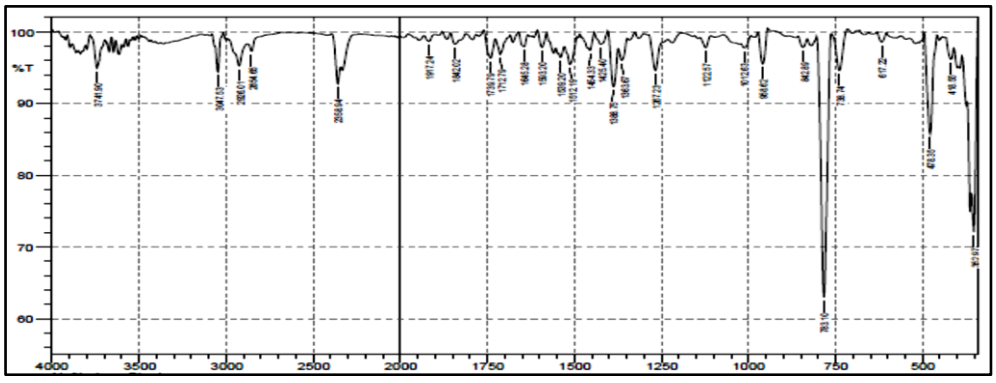

Figure 2. FTIR Chromatogram Interactions between BC microsymbiont with Naphthalene Contact period of 5 days

FTIR measurement results according to Figure 2, shows that there has been a bio-degradation process marked by the identification of the OH group at the absorption wavenumber 3750-3300 cm^{-1}, although the degradation is not yet perfect, meaning that there is still an aromatic component in the absorption of wave number 1675- 1500 cm^{-1}. This result is consistent with the observation of biodegradation parameters, in which an increase in turbidity has not occurred significantly [36], [37]. Likewise there has also been no change in pH and also the formation of air bubbles and the smell of fermentation has not been significant, as shown in Table 1. This condition indicates that during the contact pe-

riod of 5 days the biodegradation process of the BC symbionts indicates that during the contact period of 5 days the biodegradation process of BC symbionts to naphthalene has just entered the initial stage, where during the contact period, Bacillus Sp strain AB353f partial (BC) symbionts sponge Neopetrosia Sp bacteria is in the adaptation phase towards the growth and cell division phase shown in Figure 3.

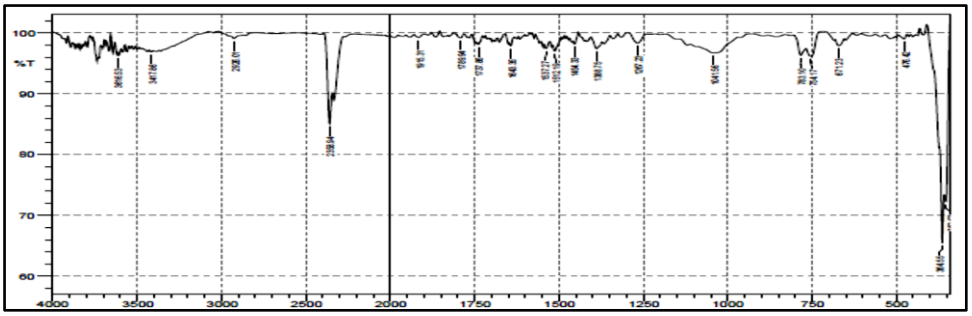

Figure 3. FTIR Chromatogram Interactions between BC microsymbiont and Naphthalene Contact period of 25 days

The chromatogram shown in figure 2 that uptake occurred more frequently at the contact period of 25 days. The absorption that occurs in wave numbers is identified in the range 3000-2700 cm^{-1}, indicating the presence of an aldehyde functional group, 2400-2100 cm^{-1}. This absorption is thought to occur in the formation of compounds with ketone, ester and carboxyl groups. Another absorption at wavenumber 1740 cm^{-1} is the functional group C=O which is thought to be a component of CO_2. This result is the biodegradation work of the two types of sponge symbiotic bacteria in degrading the naphthalene component. Figure 2 also shows that there is still an absorption that shows the hydroxyl functional group, although there is still an absorption that indicates the presence of an aromatic functional group. This shows that the components of naphthalene are not completely degraded to produce several components of simple organic compounds [1], [38].

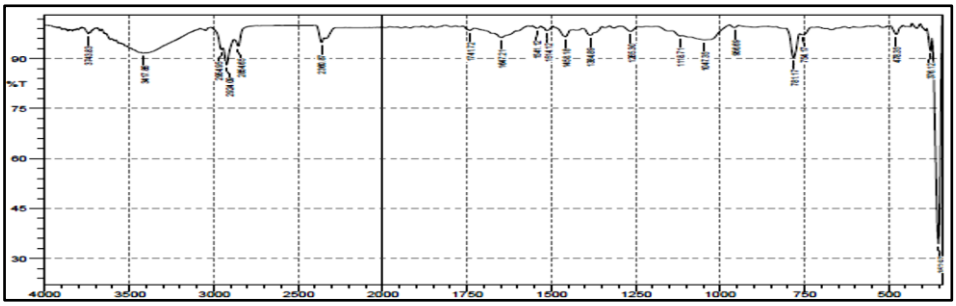

Figure 4. FT-IR Chromatogram Interaction between AC microsymbiones with Naphthalene Contact period of 5 days

The functional groups identified as a result of biodegradation using the microsim-bion isolate of Acinetobacter Calcoaceticus strain PHCDB14 (AC), the sponge isolate Callyspongia Aerizusa Neopetrosia sp on the contact period of 5 days were hydroxyl (-OH) at 3450 cm^{-1} absorption and aromatic (C=C) at 3600 cm-1 absorption. The -OH functional group identified indicated that the biodegradation process of the naphthalene component had been carried out by the AC microsimbion sponge bacteria.

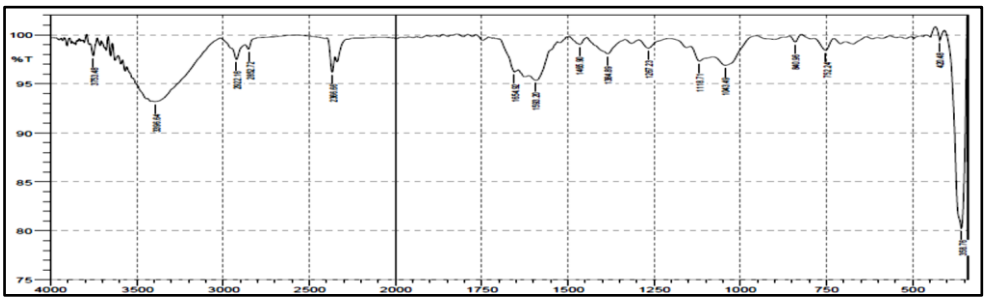

Figure 5. FTIR Chromatogram Interaction between AC microsymbiones with Naphthalene Contact period of 25 days

The absorption identified in the use of AC isolate, according to Figure 5, is not too different from the absorption found in the use of BC isolate biodegradator (Fig. 2), indicating that the components of organic compounds resulting from the biodegradation of isolate Bacillus Sp strain AB353f partial (BC) are relatively the same as the component. The results of biodegradation using the isolation of Acinetobacter Calcoaceticus strain PHCDB14 (AC), namely compounds with aldehyde, ketone, carboxylate functional groups and the suspected presence of CO_2 gas [6], [31]. These results indicate that the mechanism of reaction of the naphthalene molecule breakdown by the two types of sponge micro symbionts is relatively the same, even though they were isolated from different sponges, namely BC microsymbionts was isolated from the sponge Neopetrosia Sp, while AC microsymbionts were isolated from the sponge Callyspongia Aerizusa Neopetrosia Sp. Comparing the biodegradation performance of the two types of sea sponge symbiotic bacteria, based on the data in Table 1 and figures 1-4 it can be stated that the biodegradation performance of BC bacteria is more dominant than AC bacteria in the degradation of naphthalene components [12].

Matching and confirming the biodegradation product data of the two types of sponge microsymbionts (BC and AC) can be seen in the GC-MS chromatogram data from the measurement results of the n-hexane extract of the degradation medium for the interaction period of 5, 15 and 25 days, respectively.

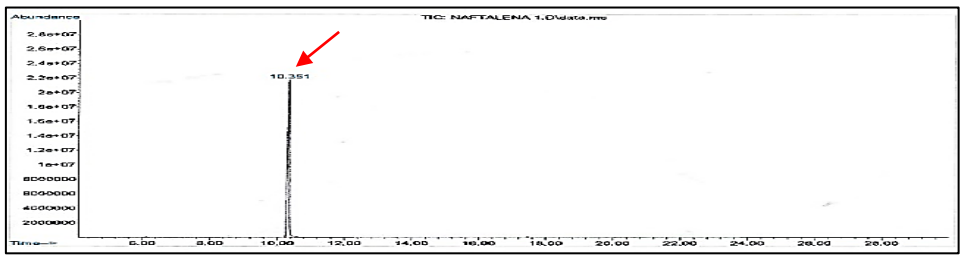

Figure 6. GC-MS Chromatograms Interaction between BC microsymbiones with Naphthalene Contact period of 5 days

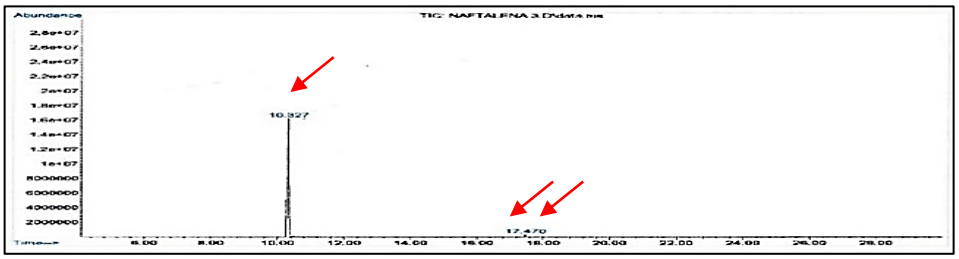

Figure 7. GC-MS Chromatogram Interactions between BC microsymbiones with Naphthalene Contact period of 15 days

The chromatogram resulting from the interaction between BC microsymbionts and naphthalene with a contact period of 5 days (Fig. 6), shows that one peak with a retention time (RT) of 10.280 seconds is a component of naphthalene, indicating that organic compounds have not been formed as a result of the biodegradation of BC microsymbionts, even though the FTIR chromatogram (Fig. 1), identifies the presence of the –OH group which means that the biodegradation that occurs is only at the oxidation reaction stage, in other words no organic compound component has yet been formed.

Figure 7, the contact period of 15 days began to appear with 3 (three) peaks, each with RT 10.280 seconds (naphthalene), 17.467 seconds (alcohol component) and 19.563 seconds (aldehid component). The abundance of naphthalene seemed to decrease significantly compared to the abundance at the 5 days interaction period (Fig.5), meaning that some of the naphthalene components had been degraded to produce organic compounds such as alcohol and aldehyde [39, 40]. The formation of organic compounds in the type of alcohol and aldehyde and a decrease in the abundance of naphthalene components at the contact period of 15 days as a result of the biodegradation process by the sponge microsymbionts Bacillus Sp partial strain AB353f (BC). Theoretically, BC bacterial cells with a contact period of 15 days have gone through an adaptation period which generally lasts in the 2-7 days range and then enters the breeding phase on days 7-15. So it can be concluded that there is a correspondence between the contact time of 15 days, namely the phase of bacterial development with the results of biodegradation of the formation of organic compounds in the alcohol and aldehyde groups (Fig. 7) after going through the oxidation reaction mechanism (Fig. 6).

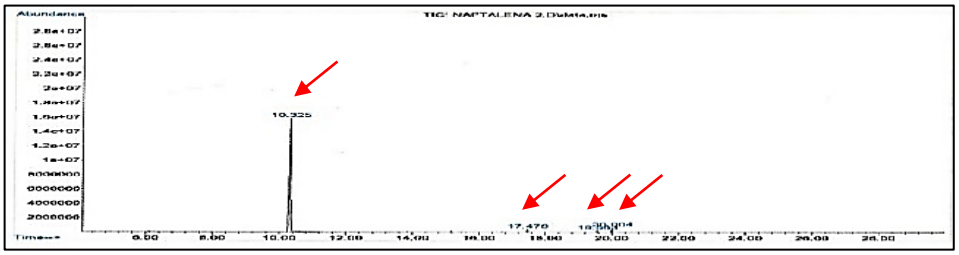

Figure 8. GC-MS Chromatogram Interactions between BC microsymbiones with Naphthalene Contact period of 25 days

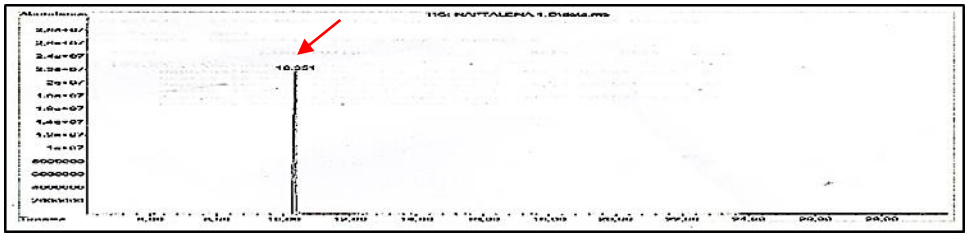

Figure 9. GC-MS Chromatogram Interaction between AC microsymbiones with Naphthalene Contact period of 5 days

The GC-MS chromatogram as shown in Figure 8, shows that the 4 peaks identified are non degraded components of naphthalene, alcohol, aldehyde and carboxylate compounds. The new components that appear are cyclopentyl, cyclopentane, 1, 2, 4-Triazole-4 amine and 2-propyl-benzyl carboxylate. The identification of the carboxylate components showed that the organic compounds resulting from the biodegradation of micro symbiosis BC against naphthalene produced 3 types of organic compound groups, namely: alcohol, aldehyde and carboxylate [2,41]. The decrease in the abundance of naphthalene with increasing contact period and followed by the formation of organic compounds shows that at 15 - 25 days of contact interaction, the naphthalene ring structure breaks to form simple organic molecules. The molecular structure of naphthalene breaks down gradually, starting from the oxidation reaction which continues until the alcohol, aldehyde and some ketones are formed, including carboxylic acids. The formation of carboxylic acid components resulting from the biodegradation of

BC and AC bacteria to naphthalene is the stage to stop the biodegradation reaction. This is because the interaction medium becomes acidic which causes the bacterial cells to not survive and eventually experience mass death.

The biodegradation process that occurs for naphthalene in the use of the biodegradator Acinetobacter Calcoaceticus strain PHCDB14 (AC) as a naphthalene biodegradator is not much different from the biodegradation process using BC bacteria (Fig. 8-10). The difference that appears in the product of biodegradation, wherein compounds are formed between catechols (1, 2, 4-Triazole-4-amine and 2-profil-1-methyl benzyl-alcohol) at 5 days of contact, alcohol and aldehyde type products (methyl-ethyl- cyclo-2-hexene), on contact 10-15 days. Additional product type carboxylic acid (as.trimethyl pentanoate) at the contact period of 25 days [9]. Changes naphthalene molecular structure material due to the work of spongy symbiont bacteria biodegraders following the enzymatic reaction mechanism, where BC and AC isolates behave as enzymes and naphthalene as substrates [42].

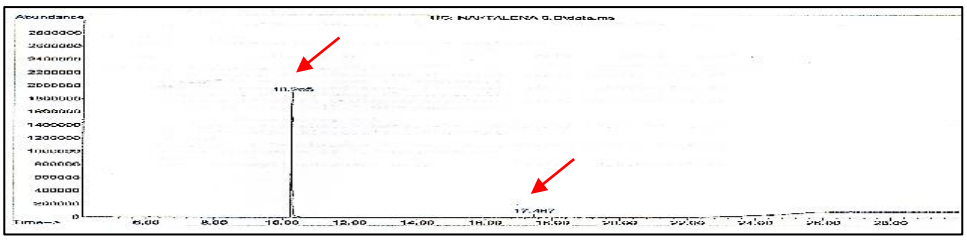

Figure 10. GC-MS Chromatogram Interaction between AC microsymbiones with Naphthalene Contact period of 15 days

Figure 10, the contact period of 15 days, also shows 3 (three) peaks, each with a retention time (RT) of 10.256 seconds (naphthalene), 17.321 seconds (identical to the alcohol component) and 19.412 seconds (identical to the aldehyde component). The formation of organic compounds in the alcohol and aldehyde groups and a decrease in the abundance of naphthalene components at the contact period of 15 days, is understood to be a result of the biodegradation process by the sponge microsymbiont Acinetobacter Calcoaceticus strain PHCDB14 (AC) against the naphthalene component [34, 43]. The biodegradation process of naphthalene by AC microsymbionts, resembles an enzymatic reaction mechanism, where the naphthalene component is the substrate and the biodegradator of AC isolates is an enzyme.

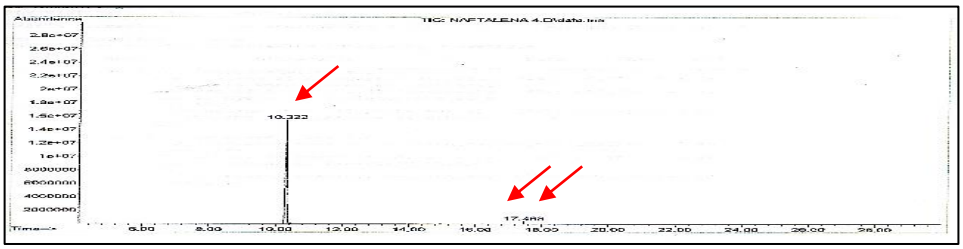

Figure 11. GC-MS Chromatogram Interaction between AC microsymbiones with Naphthalene Contact period 25 days

The results of biodegradation of sponge symbiont bacteria by enzymatic reaction mechanisms confirmed by data on several biodegradation indicators (Table 1) and identification of several types of functional groups, especially hydroxyl (-OH), aldehyde (-OCH) and carboxylate (-COOH) types on the FTIR chromatogram. (Fig. 1-4). Comparing the biodegradation performance of the two types of sponge micro symbionts above, it can be concluded that the performance of the partial Bacillus Sp strain AB353f (BC) micro symbionts, the Neopetrosia Sp sponge isolate tends to be stronger in degrading naphthalene when compared to the Acinetobacter Calcoaceticus strain PHCDB14 (AC)

sponge isolate Callyspongia Aerizusa.

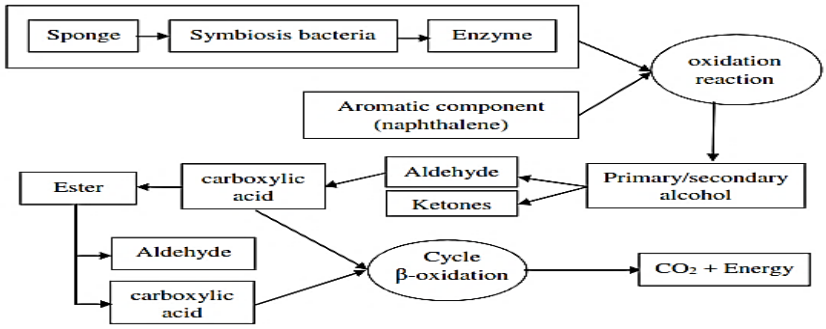

Figure 12. Mechanism of biodegradation of naphthalene by microbial biodegradator BC sponge of Neopetrosia sp and AC symbiont sponge Callyspongia Aerizusa.

The combination of biodegradation parameter data, namely: optical density, biodegradator indicators (pH, air bubbles, fermentation odor), functional groups shown on the FTIR chromatogram and organic components shown on the GC-MS chromatogram and the stages of bacterial development (sponge micro symbionts) in accordance with incubation time, a simple pathway for the biodegradation process or mechanism of naphthalene biodegradation is made by the BC and AC micro symbionts biodegradator, according to Figure 12.

Figure 12, shows the alleged biodegradation process or pathway of naphthalene by BC and AC micro symbionts with a mechanism initiated by an oxidation reaction to produce primary alcohols and possibly secondary alcohols. Along with the interaction period between the substrate (naphthalene) and biodegradation bacteria, the components of naphthalene undergo further oxidation, as well as the components in the form of further re-oxidation alcohols, producing aldehydes or ketones and finally turning into carboxylate and ester compounds, then the organic components resulting from biodegradation enter into the krebs cycle, eventually producing several types of end products including CO_2, and energy products. The energy resulting from the destruction and subsequent degradation of the bacteria by the bacteria is reused as nutrients and energy to extend their life time as well as to defend themselves against naphthalene toxicity [21, 31].

Several findings regarding the performance and reaction mechanisms that occur between sponge microsymbionts and naphthalene substrates in the biodegradation process of hydrocarbon components, especially PAH types, include: (1) the potential for the formation of intermediate products, as a result of degradation in the form of carboxylic acids, has a strong effect on the performance of micro symbionts in carrying out molecular destruction. Advanced PAH; (2) the toxicity of several types of PAH is very high, giving an effect that allows the failure of the adaptation of bacterial cells and (3) available or not available nutrients that can initiate bacteria into the growth phase. This factor is an inhibitor of the performance of bacteria to carry out the maximum biodegradation of PAH components, as a result of which PAH has never been found to be completely degraded or it is said that if the biodegradation of hydrocarbon components has reached the stage of forming organic acids, then this stage is a limiting performance of biodegradation and then enters [33]. The expiration period of the biodegradation process due to the mass of bacteria in the media has died.

4. Conclusion

Partial Bacillus SP strain AB353f (BC) bacteria, sponge isolate Neopetrosia Sp and Acinetobacter Calcoaceticus strain PHCDB14 (AC) sponge isolate Callyspongia Aerizusa can degrade naphthalene. There are three types of naphthalene biodegradation products, namely the type of alcohol, aldehid, carboxylic acid and one transition product in the form of a catechol. There are 5 indicators of biodeg-

radation of hydrocarbons by bacteria, namely: increased optical density, increased pH, temperature changes, a fermentation odor is detected and gas bubbles are formed on the biodegradation medium. The maximum biodegradation of naphthalene occurs at a contact period of 20-25 days. The components of the biodegradation of naphthalene by BC bacteria and sponge AC symbionts were recorded in the GC-MS chromatogram and amplified by the functional group data of organic compounds in the FTIR chromatogram. The biodegradation performance of BC bacteria against naphthalene components tends to be more dominant than the biodegradation performance of AC bacteria. The biodegradation products of BC bacteria are cyclopentyl, cyclopentane, 1, 2, 4-Triazole-4 amine and 2-propyl-benzyl carboxylate, while the bio-degradation products of AC bacteria include catechols (1, 2, 4-Triazole-4-amine and 2-profil-1-methyl benzyl-alcohol) at 5 days of contact, alcohol and aldehyde type products (methyl-ethyl-cyclo-2-hexene).

References

[1] 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
[2] 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
[3] Abraham N A, Offiong N-A O, Ukafia O P and Akpan P E 2018 Source Apportionment of Polycyclic Aromatic Hydrocarbons (PAHs) in a Tropical Estuarine Epipelic Sediment and Its Associated Bacterial Degrading Potentials *Curr. J. Appl. Sci. Technol.* **32** 1–11
[4] Srivastava S, Singh M, George J, Bhui K, Murari Saxena A and Shukla Y 2010 Genotoxic and carcinogenic risks associated with the dietary consumption of repeatedly heated coconut oil *Br. J. Nutr.* **104** 1343–52
[5] Vijay R, Sharmila K P, Bekal M P and N S K 2015 Evaluation of Phytochemical Constituents and Fatty acid Content in Sesamum indicum L . *J. Pharm. Chem. Biol.* **3** 84–90
[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] Murniasih T, Yopi Y and Budiawan B 2010 Biodegradasi Fenantren oleh Bakteri Laut Pseudomonas sp KalP3b22 Asal Kumai Kalimantan Tengah *MAKARA Sci. Ser.* **13** 77–80
[8] Adriyani R 2006 Control of Environmental Pollution caused by Pesticide in Agricultural Process *Kesehat. Lingkung.* **3** 95–106
[9] 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 Conference Series: Earth and Environmental Science* vol **584** p 012013
[10] Syakti A D, Yani M, Hidayati N V, Siregar A S, Doumenq P and Made Sudiana I M 2013 The bioremediation potential of hydrocarbonoclastic bacteria isolated from a mangrove contaminated by petroleum hydrocarbons on the cilacap coast, Indonesia *Bioremediat. J.* **17** 11–20
[11] 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
[12] Akinde S B and Iwuozor C C 2012 Alkane Degradative Potentials of Bacteria Isolated From the Deep Atlantic Ocean of the Gulf of Guinea *J. Bioremediation Biodegrad.* **03** 1–6
[13] Najafi A, Moradinasab M and Nabipour I 2018 First record of microbiomes of sponges collected from the Persian Gulf, using tag pyrosequencing *Front. Microbiol.* **9** 1–18
[14] 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
[15] 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
[16] Marzuki I, Kamaruddin M and Ahmad R 2021 Identification of marine sponges-symbiotic bacteria and their application in degrading polycyclic aromatic hydrocarbons *Biodiversitas* **22** 1481–8
[17] Kadhim R, Maajeed H and Munim G 2013 Sequence similarity for identification of locally isolate Geobacillus stearothermophilus according to lipase gene sequence . Full Length Research Paper Sequence similarity for identification of locally isolate Geobacillus stearothermophilus according to l *Sci. Park Journals* **2** 42–50
[18] 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
[19] 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
[20] 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
[21] Maldonado M, López-Acosta M, Busch K, Slaby B M, Bayer K, Beazley L, Hentschel U, Kenchington E and Rapp H T 2021 A Microbial Nitrogen Engine Modulated by Bacteriosyncytia in Hexactinellid Sponges: Ecological Implications for Deep-Sea Communities *Front. Mar. Sci.* **8** 1–35
[22] Costantini S, Guerriero E, Teta R, Capone F, Caso A, Sorice A, Romano G, Ianora A, Ruocco N, Budillon A, Costantino V and Costantini M 2017 Evaluating the effects of an organic extract from the mediterranean sponge Geodia cydonium on human breast cancer cell lines *Int. J. Mol. Sci.* **18**
[23] Orani A M, Barats A, Vassileva E and Thomas O P 2018 Marine sponges as a powerful tool for trace elements biomonitoring studies in coastal environment *Mar. Pollut. Bull.* **131** 633–45
[24] 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
[25] Parama Cita Y, Kamal Muzaki F, Radjasa O K and Sudarmono P 2017 Screening of Antimicrobial Activity of Sponges Extract from Pasir Putih, East Java (Indonesia) *J. Mar. Sci. Res. Dev.* **07**
[26] Marzuki I 2021 *Eksplorasi Spons Indonesia: Seputar Kepulauan Spermonde* ed A Noor and M Kamaruddin (Medan: Yayasan Kita Menulis)
[27] Marzuki I and Erniati 2017 Skrining Spons Potensial Sebagai Biodegradator Hidrokarbon Berdasarkan Data Morfologi *Pros. Semin. Nas. Has. Penelit.* **1** 43–8
[28] Meutia Samira Ismet, Soedharma D and Effendi H 2011 Morfologi Dan Biomassa Sel Spons Aaptos aaptos Dan Petrosia sp. *Ilmu dan Teknol. Kelaut. Trop.* **3** 153–61
[29] Marzuki I, Noor A, Nafie N La and Djide M N 2014 Isolasi dan identifikasi bakteri shimbion spons penghasil enzim amilase asal pantai melawai balikpapan *dr. Aloei Saboe* **1** 11–8
[30] 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
[31] Marzuki I, Sinardi S, Pratama I, Chaerul M, Paserangi I, Mudyawati M and Asaf R 2021 Performance of sea sponges micro symbionts as a biomaterial in biodegradation naphthalene

waste of modified *The 5th International Seminar on Sustainable Urban Development; IOP Conference Series: Earth and Environmental Science* vol **737** p 012016
[32] Marzuki I, Pratama I, Ismail H E, Paserangi I, Kamaruddin M, Chaerul M and Ahmad R 2021 The Identification and Distribution Components of Polycyclic Aromatic Hydrocarbon Contaminants at the Port of Paotere , Makassar , South Sulawesi *The 1st International Conference on Biotechnology and Food Sciences IOP Conf. Series: Earth and Environmental Science IOP Publishing* vol **679** p 012017
[33] Kamaruddin M, Marzuki I, Burhan A and Ahmad R 2021 Screening acetylcholinesterase inhibitors from marine-derived actinomycetes by simple chromatography *The 1st International Conference on Biotechnology and Food Sciences IOP Conf. Series: Earth and Environmental Science* vol **679** p 012011
[34] Marzuki I, Ali M Y, Syarif H U, Erniati, Gusty S, Ritnawati, Daris L and Nisaa K 2021 Investigation of Biodegradable Bacteria as Bio indicators of the Presence of PAHs Contaminants in Marine Waters in the Marine Tourism Area of Makassar City *The 6th International Conference on Tropical and Coastal Region Eco* p 012006
[35] Armus R, Noor A, Ahmad A, Lukman M, Zakir M, Nafie N L and Riani E 2020 Dimethyl Sulfide and Dimethylsulfoniopropionate Production Along The Southwest Coastal of Makassar Strait Indonesia *Mar. Chim. Acta* **22** 20–6
[36] Armus R, Selry C, Marzuki I, Hasan H, Syamsia and Sapar A 2021 Investigation of Potential Marine Bacterial Isolates in Biodegradation Methods on Hydrocarbon Contamination *J. Phys. Conf. Ser.* **1899** 012006
[37] Marzuki1 I, Noor A, Nafie N La and Djide M N 2015 The Potential Biodegradation Hydrocarbons Of Petroleum Sludge Waste By Cell Biomass Sponge Callysppongia sp. *Mar. Chim. Acta* **16** 11–20
[38] Marzuki I, Sinardi S and Asmeati A 2018 Karakteristik fenotip dan genotip mikrosimbion spons niphates sp sebagai biomaterial potensial pendegradasi poli aromatik hidrokarbon *Prosiding Seminar Hasil Penelitian (SNP2M* vol 2018 pp 16–21
[39] Ahmad F 2012 Kandungan Senyawa Polisiklik Aromatik Hidrokarbon (PAH) di Teluk Jakarta **17** 199–208
[40] Araújo S C da S, Silva-portela R C B, Lima D C de, Fonsêca M M B da, Araújo W J, Silva U B da, Napp A P, Pereira E, Vainstein M H and Agnez-Lima L F 2020 MBSP1 : a biosurfactant protein derived from a metagenomic library with activity in oil degradation *Sci. Reports Natureresearch* **10** 1340–52
[41] 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
[42] 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
[43] Pawar R P, Rahman A and Said M 2017 Community structure of macrobenthos with reference to biomass to study effects of Jawaharlal Nehru Port on coastal biodiversity along Uran coast, Navi Mumbai *Adv. Environ. Biol.* **11** 20–33

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