INVESTIGATION OF MARINE SPONGE SYMBIOSIS BACTERIA AS A DEGRADATION MATERIAL FOR HYDROCARBON COMPONENTS

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A. Preliminary

Three types of pollution components are most common in today's global era: first, aliphatic and aromatic hydrocarbons. Second, several types of heavy metals such as Lead (Pb), Mercury (Hg), Arsenic (As), Chromium (Cr) and other heavy metals. Third, waste microplastics fragment type, fibre and movies. These three types of contaminant components can come from natural dynamics such as eruptions and eruptions of Mount, landslides and human activities in the form of mining industry waste, manufacturing industry, health, agriculture and household activities [1,2,3]

The sea area is a giant space for many liquids and reliable components that are undercurrent. The three components of pollution are difficult to decompose. Some of them not degraded, so the sea is the most vulnerable area to experience contamination with these three types of waste components. This pollution's components are increasingly entering the sea bodies in terms of concentration, volume, type, and quantity over time [4,5,6].

Microplastic, hydrocarbon and heavy metal pollution is a severe problem for the sustainability of marine ecosystems that is difficult to overcome. These contaminants' negative impact on the marine ecosystem touches all systems and cycles in the sea, especially on marine life, coral reefs, including disturbance of fishing activities by fishers.

Marine areas, especially in the petroleum and mineral mining industry area, crude oil distribution pipelines and ship transportation routes are very susceptible to contamination with Polycyclic Aromatic Hydrocarbons (PAH). This condition is a severe threat to marine biota, especially fish populations. It concerned about exposure to carcinogenic PAH components and some of them mutagenic. Toxic, carcinogenic and mutagenic properties in PAH, potentially accumulate in the human body. Consumption of PAH contaminated fish within a specific time range can cause severe human health problems [7,8].

Alternative solutions to the dangers of PAH are available in nature. Microorganisms, especially bacteria, are potential as biomaterials that can reduce the toxic properties of PAH through biodegradation methods. Various researches show that some types of bacteria can utilise carbon as nutrient and energy sources to grow and click double-up cells to defend themselves. That is an indication for reducing the toxicity of hydrocarbon contaminants [9,10].

Sponges and bacteria in the marine environment can form a symbiotic mutualism. Bacteria make the sponge's body as a host so as not to be swayed by ocean currents and waves so that bacteria can grow and reproduce. Conversely, sponges function bacteria for self-defence from various predators of life. Bacteria adhere to the sponge body's surface due to the sponge feeder filter's nature, causing bacteria to reside in large part of the sponge body [11].

The population of sponges in the sea is quite large. There is a symbiosis with various types of bacteria that can potentially utilize in the degradation of hydrocarbon components and the function of heavy metal bio-adsorption. In Indonesia, the sponge population is large and of various types, including the sponge found in the Spermonde Archipelago Cluster conservation area, South Sulawesi. Conservation of sponges is carrying out because it has many pharmacy, environment and marine tourism objects, even one of the marine biota that has an ancient and unique civilization [12].

This reviews search method symbiotic bacteria decompose sea sponge has the potential contaminant biodegradation of the hydrocarbons by analysing the dynamics of the relationship sea sponge, symbiotic bacteria, and the characteristics of the phenotype and genotype of the capacity of degradation component hydrocarbons. Thus it is crucial and needed to solve the problem of PAH type hydrocarbon pollution in marine areas, which tends to increase. Viewing biodegradation mechanisms of symbiotic bacteria marine sponges against PAH and analysis contamination dynamics of symbiotic bacteria in the sponge is an exciting study that many in the respective fields by researchers today as well as a part in enriching the science [13,14]

This study's sampling locations were selected several sponges originating from small islands included in the Marine Tourism Region (MTR) of Makassar City. These islands included in the Spermonde Archipelago Cluster in which there are ± 2,500 species of sea sponges that need to be protected by the existing population. They are one of the objects of future research with various benefits and functions, especially in pharmacy and pollution management. Sponges in the MTR area are very susceptible to damage or death due to pressure from household waste pollution in the Center Point of

Indonesia (CPI) area, penetration of human activities in the MTR area and loading and unloading activities and ship transportation. It feared that such conditions would harm the sponge's growing population [15,16].

Sponge population in the region is still pitch observed grow and develop, which confirms worry several parties over the fate and future of future growth in the sponge. Another fact of this sponge is one icon of marine tourism by local authorities. This condition is interesting to study more deeply. It hoped that this study's results are to contribute and as a reference to provide input in the management of the Spermonde Islands Cluster conservation area. The sponge population can be maintained and ran parallel to the government's program to build the area as an exclusive residential area for CPI, MTR destination for Makassar City, and remain a zone of various ship transportation routes still friendly for fishing activities by fishers [17,18].

The investigation of sea sponge symbiotic bacteria as biomaterials in the degradation process of hydrocarbon components is carried out in several stages, namely: determination of sponge sampling points, observation of sponge living habitat, sponge morphological analysis, isolation of symbiotic bacteria, phenotype analysis (determination of gram groups and bacterial biochemical tests), genotype analysis, biodegradation process of symbiont isolates, measurement of biodegradation rates based on chromatogram measurements from Gas Chromatography-Mass Spectrometry (GC-MS) and data on infrared biodegradation products [7, 11].

PAH components were determined based on the analysis of optical density curves, gas bubbles, pH changes, and the smell of fermentation of the biodegradation medium. The biodegradation ability is carried out coherently and entirely according to the stages to see the relationship between sponges and bacteria's symbiotic dynamics in their habitat, on PAH-type biodegradation performance hydrocarbon contaminants.

This review also presents the potential and effort to improve the performance and rate of biodegradation of marine sponge symbiotic bacteria to PAHs by performing several treatments in the media degradation, such as the addition of nutrients giving oxygen supply agitation, temperature and pH regulation of media degradation. The resulting data expected complete and comprehensive. It can use as a reference in the management of tourist areas of marine, residential, zone transportation going well and not going on the degradation of the population lives a sponge, so that marine life remains stable to perform its functions and the cycle of life in the ocean [7,17].

B. Discussion

Data and information regarding the point of sampling sponge on a small island area Marine Tourism Region (MTR) City Makassar, conservation areas Spermonde Barrier Islands presented in Table 1.

Table 1. Data on the condition of the sponge sampling point in the Bahari Tourism Area of Makassar City

Observation parameters	*Sponges Sample*			
	Sp1	*Sp2*	*Sp3*	*Sp4*
Island name	Lae-lae	Samalona	Kodingareng Keke	Barang Caddi
coordinate point	5°11'14.823" S 119°23'31.230" E	5°2'31.776" S 119°22'31.124" E	5° 6'21.487" S 119°17'19.376" E	5° 4'52.259" S 119°19'14.298" E
pH	6,8	6,7	6,8	6,7
The depth of the sampling (m)	4,6	3,7	5,2	5,6
Temperature (°C)	25	24	24	26
Salinity (‰)	29,5	29,4	30,0	29,6

The parameters measured at the sponge sampling locations are shown in Table 1, indicating that the habitat for sponge growth under normal conditions is relatively the same as other sponge populations' locations in the Indonesian region. However, it cannot say that the area is free from contaminants such as hydrocarbons, heavy metals and microplastics. The sponge samples obtained from four different islands in the MTR area of Makassar City. The samples were each given the code: Sp1 sponge body surface covered with mucus and dark, Sp2 dark surface but not covered with mucus, Sp3 dark surface and covered in mud and partly mucus Sp4 light and non-mucus surface [12,18].

The marine sponge morphological analysis was carried out using a Haemocytometer type microscope and obtained data on sponge species, namely Sp1 = *Neopetrosia* sp. Petrosinidae family, Sp2 = *Coelocarteria singaporensis* family Chalinidae, Sp3 = *Callyspongia* sp. family Callyspongiiade, and Sp4 = *Clathria (Thalysias) reinwardti* family Microcionidae.

The next stage is the isolation of symbiotic sponge bacteria using the method of scratching the media to tilt using ose, followed by dilution and purification of the isolates using the direct plating method. Dilution and purification carried out until a single isolate obtained. The number of single isolates obtained from the four samples was ten types with details of the sample code Sp1 were three isolates (Sp1.A, Sp1.B, Sp1.C), sample code Sp2 is two isolates (Sp2.A, Sp2.B), Sp3 samples, obtained two isolates (Sp3.A, Sp3.B), and samples of Sp4 were three samples (Sp4.A, Sp.4.B, Sp4.C) [11,19].

Phenotype analysis of each isolate that was isolated from a sponge carried out in two methods, namely: first, determination of the gram group of symbiont bacteria isolates using the Gram staining method, test with Safranin reagent and reaction with an alkaline solution (1% KOH). Gram staining results on ten symbiotic sponge isolates concluded that all the analyzed isolates were a group of Gram-positive bacteria. Second, biochemical test with 15 reagents to see the tendency of isolate reactions concerning the biodegradability of PAH. In the biochemical test, one isolate selected for each type of sponge—biochemical test simulations of isolates-illustrates the characteristics of 4 isolates presented in Table 2.

Table 2. Biochemical Test and Characterization of Marine Sponges Symbiotic Isolate

Biochemical test parameters	*Media*	*Reaction results*			
		Sp1.A	*Sp2.A*	*Sp3.A*	*Sp4.A*
Hydr. of Starch	Order for starch	+	+	+	+
Casein Hydrolysis	Milk Order	nr	-	nr	-
Indole	Tryptone broth	+	-	+	-
Nitrate Reduction	Nitrate Broth	nr	nr	nr	nr
Glucose ferm.	Glucose Broth	-	+	-	+
Lactose ferm.	Lactose Broth	-	-	-	+
Sucrose ferm.	Sucrose Broth	+	-	+	+
Mannitol ferm.	Mannitol broth	+	-	+	+
Citric	Order S. Citrate	+	+	+	+
Catalase	Nutrient Agar	+	+	-	-
Urease	Urea Broth	-	+	-	+
H_2S	H_2S order	+	+	+	+
Methyl Red	MR broth	+	+	+	-
Voges Proskauer	R-VP broth	+	+	+	+
Gelatin Hydr.	Gelatin Nutrients	+	-	-	+

note: nr = didn't react

Potential bacterial isolates as degradation materials for hydrocarbon components if they reacted positively with MR-VR, citrate, lactose, H_2S and indole reagents in biochemical tests. If the biochemical test results are significantly positive for the MR-VR reagent, indicating that the isolate can produce acid, positive for indole reagent, means that the bacterial isolate can produce indole from tryptophan or the ability to make carbon a source of nutrients for energy. A positive reaction with citric shows that bacteria have enzymes that break molecule cyclic and positive reaction to the reagent H_2S, indicating that bacterial isolates can run the fermentation reaction with acids. Observing Table 2, the results of the isolate reactions through biochemical tests, show that the bacterial isolates obtained from the sponges Sp1 sample are the most potential as biomaterials for the degradation of hydrocarbon components bacterial isolates from samples Sp2 and Sp3. In contrast, sponge bacterial isolates Sp4 have no potential as biodegradation material to hydrocarbon components. Other information that can describe in this review according to Table 2 is that an Sp1 bacterial isolate is a *Bacillus* group of bacteria and the Sp2 and Sp3 bacterial isolates are the *Pseudomonas* group [6,10].

A more comprehensive investigation of the potential of bacterial isolates in the biodegradation of PAH components can carry out through genotypic analysis of each isolate using Polymerase Chain Reaction (PCR) which considered to have the potential for biodegradation of hydrocarbon components to ensure the type of bacteria including the strain of each isolate, to ascertain the bacterial species, the source of the type sponge. In this description, the bacterial isolates used in the biodegradation of hydrocarbons not subjected to RT-PCR analysis, so the description of the number of nitrogen base pairs, species and strains of the isolates could not be presented ultimately. However, it has estimated that these bacterial isolates are bacteria of the Bacillus group and Pseudomonas. These results understood that the bacterial isolates originating from sponges with bright surfaces without being covered in mud or slime matter have no potential as biomaterials for degrading hydrocarbon components [10,19].

The results of the phenotype analysis of the sponge symbiotic bacterial isolate narrowed to the choice of potential isolates in biodegradation, so that among the ten bacterial isolates isolated from 4 types of sea sponges, only seven potential isolates were selected, namely three bacterial isolates sample Sp1 and 2 isolates each as sample Sp2. and Sp3.

Ensuring that the isolate selected as a biodegradation material, a preliminary test is carried out, which is carried out by pouring petroleum into a petri dish until the entire surface covered with liquid, then dripping the bacterial suspension at three points per petri dish, incubating for 2 x 24 hours. Preliminary test seven isolates selected on petroleum showed that all test isolates showed activity growth in petroleum media [6,11].

The next stage is the culture of all selected bacterial isolates to increase the number of cells, the results of the culture are then made in the form of bacterial suspensions, and put in various pials or biodegradation reactors, adapted for 1 x 24 hours in the reactor (new environment). All treatments aerated on a Shaker incubator at 150 rpm. Every three days, observation and recording of biodegradation parameters (OD, media pH, temperature, gas bubbles, and fermentation odour) carried out until ten observations obtained or the interaction period lasted 30 days [6,10].

Biodegradation of bacterial isolate to PAH components runs if it meets at least four of the five biodegradation parameters observed. If there is an increase in turbidity or Optical Density (OD), it indicates that the bacteria can adapt to the extreme environment contaminated by PAH. Several previous studies showed that the increase in OD values occurred after bacteria passed the adaptation phase, or at an interaction period of more than five days. Biodegradation of PAH components by bacteria follows the fermentation reaction mechanism, meaning that if the bacteria in the media threatened, they immediately produce enzymes to survive in an environment that contaminated with PAH. The biodegradation process is said to run if gas bubbles form in the degradation medium, followed by the smell of fermentation, a decrease in the pH value in a narrow range and usually followed by an increase in media temperature [21].

Enzymes carry out biodegradation of hydrocarbon components in enzymatic reactions or fermentation reaction mechanisms. Bacteria produce enzymes in response to extreme environments contaminated with PAH components. The PAH degradation process by bacteria causes the PAH molecules to experience degradation or damage, preceded by an oxidation reaction in one of the b PAH bugs, forming keto-enol. If the biodegradation process continues, these molecules break down to form simple organic compounds in alcohols, aldehydes and possibly carboxylic acids. If a carboxylic compound formed, it characterized by a change in the degradation medium's pH from neutral to acidic pH. At this stage, the biodegradation process generally stops, because the bacterial cells die massively due to the media turning into acid, although it may have that certain types of bacteria can survive. This stage called limiting biodegradation [12,15].

Determination of the biodegradation rate of bacterial isolates carried out by measuring the concentration of the remaining PAH component of the biodegradation using the GC-MS instrument, which previously extracted using a suitable solvent, n-hexane. The chromatogram results from running GC-MS will show the abundance of PAH components. Abundance compared with an abundance of GC-MS results before the interaction between bacterial suspension with PAH. The GC-MS chromatogram also showed that new peaks formed which indicated that the new peaks were biodegradation products in the form of simple organic compounds in alcohols, aldehydes, and carboxylic acids and possibly ketone, CO_2, and other components.

The types of organic compounds that are biodegradable bacteria products depend on hydrocarbons as the contaminants degraded. The following figure shows the abundance of PAH components and their new peaks as biodegradation products.

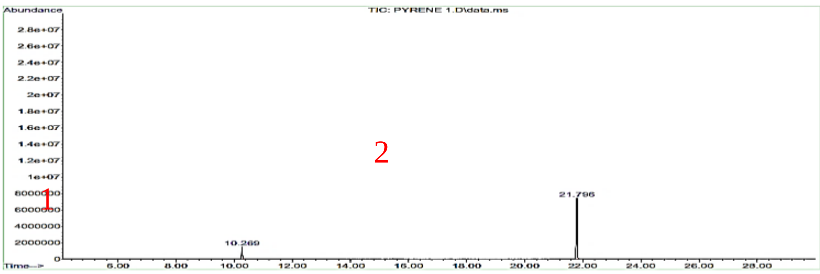

Figure 1. GC-MS chromatogram between symbiotic spongy bacterial suspension against naphthalene and anthracene PAH interaction period of 0 days

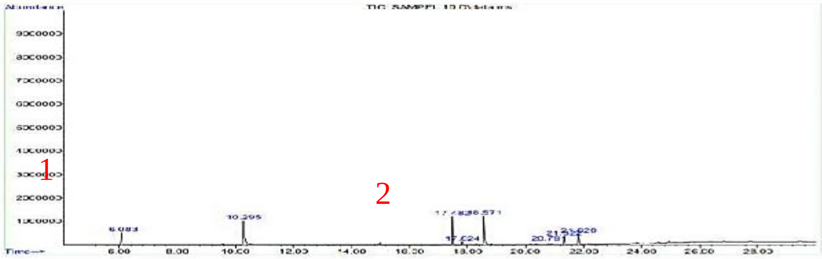

Figure 2. GC-MS chromatogram between symbiotic spongy bacterial suspension against naphthalene and anthracene PAH
interaction period of 24 days

The GC-MS chromatogram Figure 1 shows two peaks, each representing an abundance of naphthalene and anthracene. Figure 2 shows the chromatogram of GC-MS measurement results after the interaction between the spongy symbiotic bacterial suspension and two PAH types lasting 24 days. It appears ± seven peaks, or there are five new peaks thought to be simple organic compounds of biodegradation products by bacteria. The simple organic compounds obtained from the two components of PAH (naphthalene, anthracene) degraded. It can be seen that the abundance of both types of PAH (peak 1,2) has decreased sharply.

To ensure that the new peaks formed as biodegradation products in the form of simple organic compounds, IR measurements are carried out, aiming to determine biodegradation products' organic compounds.

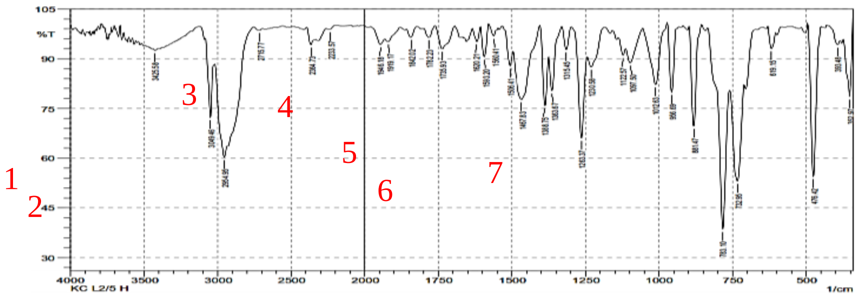

Figure 3. Infrared chromatogram (-IR) between the symbiotic spongy bacterial suspension against naphthalene PAH and anthracene interaction period of 24 days

In the form of components or simple organic compounds using -IR, identifying bacterial degradation products shows that there are many visible compounds, but the most important is seven peaks. Figures in Figure 3 show organic compounds that thought to be degradation products, numbers 1 and 2 are groups of alcohol compounds, number 3 is ketones, number 4 and 5 thought to be an aldehyde group, and number 6 and 7 thought to be an aromatic compound [10].

The post-degradation molecular structure changes for the saturated and aromatic hydrocarbon components undergo different mechanisms depending on the characteristics of the bacteria used, the types and characteristics of the hydrocarbons that degrade and the external factors treatments that applied during the interaction period [11,13].

The level of degradation of anthracene and naphthalene by the seven types of sponge symbiont bacteria isolates used different, but the highest was the Sp1 sample isolate followed by the Sp3 sample then the Sp2 sample. In general, the isolates' rate of degradation, including categories low, only reduces the concentration of PAHs to naphthalene = 38.32% and anthracene = 32.17%. It can be understood that the toxicity of anthracene (3 rings) is more substantial than naphthalene (2 rings). Bacterial biodegradation performance can improve using several bacteria that can hydrocarbon component degradation by bacteria or bacteria hydrocarbon-clastic.

C. Closing

1. The seven types of isolates from marine sponges used had the biodegradability of PAH naphthalene and anthracene;
2. Isolates samples used in isolation from the sponge Sp1 = *Neopetrosia* sp., Sp2 = *Coelocarteria singaporensis*, and Sp3 = *Callyspongia* sp.;
3. The seven types of isolates used were the Bacillus and Pseudomonas bacteria, and all of them were Gram-positive bacteria;
4. There is a relationship between bacteria isolated from sponges whose body surface covered with mucus, phenotypic characteristics, genotypes to the ability of hydrocarbon degradation;

5. The products of bacterial degradation are simple organic compounds such as alcohol, aldehyde, a small proportion of ketones and carboxylic acids;
6. The biodegradation mechanism of hydrocarbon components by bacteria follows fermentation's work pattern using the enzymatic reaction method.
7. The degree of bacterial degradation of marine sponge isolates is possible at a rate with the addition of nutrients and aeration and the potential use of bacterial consortia or hydrocarbon-clastic.

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Glossary

1. *Aliphatic:* organic compounds do not have a group phenyl
2. *Aromatic hydrocarbons:* hydrocarbons with double-bonded bonds in a cyclic form and fulfill Huckel rule among their carbon atoms
3. *Bacterial consortium:* a collection of several bacteria which have relatively the same characteristics in degradation
4. *Bio-adsorption:* absorption carried out by a microorganism, for example, bacteria based on differences in caps
5. *Biodegradation:* the process of breaking down organic matter by enzymes produced by living organisms (bacteria)
6. *Carcinogenic:* organic substances contain cancer- causing
7. *Genotype:* the genetic state of a locus or the entire genetic material carried by chromosomes or genomes
8. *Hydrocarbono-clastic:* a collection of bacteria that can degrade hydrocarbon components
9. *Morphology:* studying the form of organisms, in this case sponges
10. *Mutagenic:* changes that occur at both the level and level of genes or chromosome level
11. *Phenotype:* both structural, biochemical, physiological and behavioral characteristics observed in organisms
12. *Symbiosis:* long-term and close biological interaction between two different biological organisms
13. *Toxic:* a substance which has destructive power when exposed to a living organism

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Curriculum Vitae

Dr. Ismail Marzuki, M.Si, born in Kabere, July 3, 1973. Education followed SD Negeri 19 Kabere 1986, SMP Negeri Kabere Year 1989 and SMA Negeri 1 Enrekang 1989. Holds a Bachelor of Science (chemistry) was in 1999, in the Department of Chemistry F.MIPA UNHAS, and Master of Science (M.Si) of 2003. Completing the program Doctor of the Month in January known 2016, UNHAS Graduate program.

His career as an academic began in 2000 until now. The status of PNS (Lecturer) was obtained in 2005, at the **Kopertis** work unit (L2dikti) Wil. IX Sulawesi. Structural position ever in clothing, namely: Director Academic Chemical Analysis **Yapika** Makassar (Years 2002-2008), Chairman of the I

Academic Affairs College science Health (STIKES) Yapika Makassar (Years 2008-2012). Head of **Stikes Bina Mandiri** Gorontalo (2014-2015), Transfer to Fajar University (UNIFA) year 2015, study program Chemical Engineering home base. Additional tasks mandated by UNIFA editor in chief Journal Techno Entrepreneur Acta (2016-present), Chairman of the Central Unit Career UNIFA (2016-2018) and Chairman of the Institute of Internal Quality Assurance UNIFA, (2019- 2020), as well as Dean Faculty Postgraduate Fajar University, (2020-present). Since the Coviv-19 pandemic and a period of recovery with new habits of life, he has joined the **Yayasan Kita Menulis** community, which until now has written 15 chapters on different book titles.