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Review

Potential Utilization of Bacterial Consortium of Symbionts Marine Sponges in Removing Pollutants Global Trends, *A Review*

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Abstract: Toxic materials in waste generally contain several components of the global trending pollutant category, especially PAHs and heavy metals. Bioremediation technology for waste management that utilizes microorganisms (bacteria) has not been fully capable of breaking down these toxic materials into simple and environmentally friendly chemical products. This study examines the potential application of a consortium of marine sponge symbionts with high performance and efficiency in removing PAHs and heavy metal contaminants. The method is carried out through a review of several related research articles by the author and published by other researchers. The results of the study concluded that the development of GTP bioremediation technology could be carried out to increase the efficiency of remediation. Several types of marine sponge symbiont bacteria, hydrocarbonoclastic (R-1), metalloclastic (R-2), and metallohydro-carbonoclastic (R-3), have the potential to be applied to improve waste removal performance. A consortium of crystalline bacterial preparations is required to mobilize to GTP-exposed sites rapidly. Bacterial symbionts of marine sponges can be traced mainly to sea sponges whose body surface is covered with mucus.

Keywords: removal; PAHs; heavy metals; marine sponges; bacterial consortium

1. Introduction

Global trending pollutant (GTP) is a term applied to several types of pollutant materials (heavy metals, aromatic hydrocarbons, microplastics, medical waste, pesticide residues) that pose many complex problems to the global environment [1-7]. The problem of environmental quality is especially felt by developing countries [3,8]. The issue of GTP in this decade has been very much discussed, not only by environmental observers and activists as well as scientists in the field of environmental management but also by several world leaders who have voiced the need for real action in reducing fossil fuel consumption [9-11] The global policy of reducing carbon consumption is a tangible manifestation of environmental quality in an emergency [7,12]. It is very reasonable because the rate of increase in global trends of pollutants increases massively, far beyond the natural recovery ability of nature to reduce these pollutants [13-15]. Reducing carbon in the environment is not enough because various types of GTP and the adverse environmental effects are also different [7,16].

The aquatic environment is a giant container that is most vulnerable to being affected by GTP contaminants [17]. It is because the topography of the area is generally low. Almost all types of GTP contaminants are found in particulates and residues and can be dissolved in air, soil and water bodies [4,7]. These materials eventually empty into the aquatic environment of rivers, lakes, swamps and the sea [18,19]. In this environment, these toxic pollutants form parasitic accumulations of almost all marine organisms, especially fish, sponges, algae, plankton, phytoplankton and other types of biota [20,21].

At the same time, much research has been carried out related to the management of toxic pollutants in order to achieve the dream of creating a green environment [22,23]. The results of this research have given birth to many findings, technologies, innovations and methods related to removing the toxic nature of contaminants [24,25]. Degradation, reduction, destruction, and absorption are recommended and can be applied to decompose carcinogenic pollutants in the environment [26-28]. This method can be called bioremediation technology if it involves the role of living organisms, including the contribution of microorganisms as biodegradators that can decompose or may be able to eliminate the toxic properties of GTP contaminants [29-31].

The principle of bioremediation technology is the development of biological methods that can be applied in a gradual combination with physical, chemical, and biological methods, depending on the characteristics of the pollutant being degraded [32,33]. Bioremediation technology that uses the role of microorganisms as a decomposer component is generally good and is more often applied to wastewater treatment [34,35]. Sources of microorganisms that can carry out bioremediation functions can be obtained in an aquatic environment contaminated with pollutants, for example, in port areas, offshore oil processing industries or marine areas around locations that have experienced oil spills [36-38]. Pollutant degrading microorganisms can also be found in the soil, especially on land with a history of contamination due to oil spills and agricultural land exposed to pesticides [3,39]. oxic pollutant-degrading microorganisms can also be found in other or-ganisms that form a symbiotic relationship, for example, in sponges and mangroves [40,41].

Isolation, screening and screening methods can be applied to obtain potential microorganisms as biomaterials for degrading pollutants [42,43]. In general, three microorganisms have the potential as biomaterials for degrading pollutants, namely bacteria, fungi and fungi [44-46]. These three groups of microorganisms have different abilities and degradation mechanisms for pollutants [47]. The application of bacteria in bio-remediation mainly removes hydrocarbon contaminants, especially polyaromatic hydrocarbons (PAHs) and heavy metal pollutants. At the same time, the use of fungi and fungi in pollutant bioremediation has also been carried out but is still limited or not as popular as bacteria [48-50].

The types of bacteria identified have bioremediation capabilities against PAHs, but the level of bioremediation produced is still low, mainly if one type of bacteria is used [51,52]. It is because bioremediation bacteria are generally resistant to acidic environments. At the same time, it is known that one of the hydrocarbon component bioremediation products is simple organic compounds resulting from oxidation reactions in the form of alcohols, aldehydes, ketones and possibly carboxylate group compounds [18,53,54]. Carboxylic compounds of bacterial bioremediation products can change the conditions of bacterial habitat (media) in an acidic environment so that the degradation activity of bacterial cells decreases or may die in bulk [51,55]. This condition is called the limiting factor for the performance of bacterial bioremediation [56].

In the aquatic environment, especially in marine ecosystems, there are several types of biota, such as sponges which are known to be often used as objects for biomonitoring of pollution of hydrocarbon components and heavy metals, even some types of sponges are used as references or bioindicators in analyzing the level of PAHs and heavy metal contaminants [54,55]. Further exploration related to biomonitoring and bioindicators of this type of GTP contamination, it is known that this sponge can perform the degradation of hydrocarbon components and biosorption of heavy metals [7,56-58]. It is based on research results that show the ability of sponges to live and thrive in an environment contaminated with GTP pollutants [7,59]. The development of knowledge about the degradation and adsorption function of marine sponges against pollutants was revealed after discovering that these sponges can have a mutualistic symbiosis with microorganisms, especially bacteria [60-62].

The bacterial-sponge symbiosis model is intensified when the sponge habitat is exposed to hydrocarbon or heavy metal pollutants or perhaps both, whereas the sponge tries to survive in the extreme conditions of its growing environment [63-65]. At the same time, symbiont bacteria also use these conditions to produce a mucus substance that behaves as an enzyme, which is then spread on the surface of the sponge body to avoid the toxic nature of the pollutant [66-68]. Internal sponges also independently stimulate themselves to have immunity against all forms of predators and changes in their habitat by producing metabolic substances [53, 69,70].

The types and populations of sponges are huge, so the right sponge selection is needed by tracing, including symbiont bacteria that have the potential and ability to degrade and adsorb. It can be done by selecting sponges in their habitat, especially those with dark colours or smooth body surfaces because they are coated with mucus [20,21,71-73]. Bacterial symbionts from the selected sponge were then isolated to obtain a single isolate [74,75]. Phenotypic analysis of sponge symbiont bacteria through biochemical tests using standard reagents needs to be carried out to ensure that these symbiotic bacteria can perform the functions of PAH degradation and heavy metal adsorption [6,76]. Bacterial symbionts are potential if they react positively with several biochemical reagents, especially Methyl Red, Voges-Proskauer, citrates, lactose, catalase, nitrate reduction and indole reagents [77,78]. Genotypic analysis of bacterial symbionts is important to obtain complete information related to bacterial species, strain and number of DNA base pairs, and it is also possible to carry out genotypic analysis of these symbiotic bacteria using 16S rRNA sequences [36,79-81].

The degradation function of sponge symbiont bacteria for the target of qualitative analysis can be done by spotting several bacterial cells on media containing hydrocarbon components such as pyrene [82-84]. Bacteria that can adapt to the environment exposed to PAHs are characterized by their activity after being incubated for ± 24 hours. This situation indicates that bacteria can carry out the function of biodegradation of polyaromatic hydrocarbon pollutants [85,86]. A preliminary qualitative test can determine the adsorption function of sponge symbiont bacteria by inserting ± 1 mL of bacterial suspension into a medium containing heavy metal contaminants after being incubated for ± 24 hours, then measuring the optical density (OD600) of the interaction medium. If there is an increase in increased turbidity or absorption indicates that the symbiont bacteria could perform the adsorption function on the heavy metals tested [87,88].

The mechanism of bioremediation of hydrocarbon components is slightly different between aliphatic and aromatic hydrocarbons [89,90]. The mechanism of degradation of hydrocarbon components in general by microorganisms, especially sponge symbiont bacteria, through oxidation reactions or biochemical reactions at the molecular level [69,91]. The entry of metabolic substances or enzymes (dioxygenase) produced by bacteria into the structure of hydrocarbon molecules that act as substrates so that these molecules undergo destruction, causing the molecules to break down into molecules that produce organic compound products containing hydroxyl functional groups, which then turn into keto-enol compounds [92-94].

The oxidation reaction continues for the initial component and the degradation product of the first product of the keto-enol complex, which is further oxidized to produce organic compounds of the aldehyde, carboxylic and possibly ketone groups [95,96]. The oxidation reaction process ideally continues until simple organic molecules are formed in the form of substances that can enter the metabolic cycle [97]. The oxidation reaction of hydrocarbon components can run if the ideal conditions required for the degradation of bacteria are met [98]. Generally, the degradation performance of bacteria decreases when the oxidation results produce a carboxylic acid product [99]. Another factor that can inhibit bacterial degradation of the substrate (reactant) of the hydrocarbon component is the low solubility of the hydrocarbon component, making it difficult for bacteria to penetrate [100,101].

The rate of bacterial biodegradation of hydrocarbon contaminants varies in the range of 35-97%. Several factors cause this, for example, the type of bacteria used and the type of hydrocarbon pollutant (aliphatic or PAHs) [102,103]. Interaction time, degradation method, the concentration of hydrocarbon components as reactants, number of bacterial cells, presence or absence of nutrition, oxygen injection (aeration), the scale of experiments carried out and other factors [104,105]. Variations in the level of bacterial degradation of hydrocarbon components indicate that multiple factors influence bioremediation [106 Two things always occur in the biodegradation of hydrocarbon components (subtracts) by bacteria (degradators), namely: (1) Biodegradation of hydrocarbon pollutants by bacteria through an oxidation reaction pathway involving enzymes produced by bacteria in response to the presence of toxic substances in their growth habitat [107,108]. (2) A decrease in the performance of bacterial degradation when degradation products are formed in the form of carboxylic compounds. These two things cause the degradation of hydrocarbon components, especially PAHs, to be incomplete or unable to degrade the substrate 100% [109]. Pyrene biodegradation generally stops at the stage where the benzene component is formed [110,111]. Under these conditions, it was assumed that all bacterial cells had died [112].

These data make it possible to modify the biodegradation of hydrocarbon components by using a consortium of bacteria with diverse species or a consortium of microorganisms (a mix of bacteria and fungi) [51,92,113]. The first modification is a consortium of certain species of bacteria (X), which has high degradation activity, is expected to work at the beginning of contact and is combined with bacteria (Y) which have a slow adaptation rate to continue the degradation process when the cells of the X-species bacteria have died [114]. The second modification, a consortium of X-species bacteria that performs in degradation combined with fungi of type (Z), is more tolerant of acids that can degrade hydrocarbons in acidic media [115,116]. This method is one of the potential alternatives for developing bioremediation technology. It is hoped that all hydrocarbon components will be wholly or 100% degraded and produce the final product of simple organic compounds, in the form of salicylic acid and the like, which are environmentally friendly [117,118].

2. Global Trends Pollutant Bioremediation Analysis Instrument

Bioremediation of global pollutant trends is important by applying new technologies and innovations to improve remediation efficiency so that the natural balance, especially in marine ecosystems, can be maintained [119]. Bacterial consortia in bioremediation is a new approach and are needed in the future. Analysis of the performance and efficiency of bacterial remediation against PAHs generally uses instruments such as Gas chromatography-mass spectrometry (GC-MS) [3,57], Fourier-transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM), Energy dispersive X-ray spectroscopy (EDS) and X-ray diffraction analysis (XRD). In contrast, remediation of heavy metals by bacteria generally uses the Atomic Absorption Spectroscopy (AAS) analytical instruments; Inductively Coupled Plasma (ICP) can be combined with Optical Emission spectroscopy or mass spectrometry (MS) [120-124]. The combination of analytical instruments in pollutant bioremediation can be carried out, especially for the bioremediation of pollutants containing two or more types [122].

Waste generated in the petroleum processing industry in the form of sludge which generally contains hydrocarbon component pollutants, both aliphatic and aromatic, also contains heavy metal toxic materials, so a combination of analytical instruments is often used to obtain data related to performance, efficiency, mechanism, remediation products, including models and changes to the pollutant material during remediation [125,126]. Researchers often use the combination of analytical instruments in the bioremediation of petroleum sludge waste by a single bacterium/bacterial consortium, such as GC-MS, FTIR and AAS [20,57,121]. SEM, EDS and XRD instruments usually observe changes in surface shape or structure of bacteria-heavy metal complexes through extracellular bonding [120-123].

3. Bacterial performance in pollutant bioremediation

Bioremediation innovation by using a consortium of bacteria in pollutant remediation is a global trend, especially for PAHs and heavy metal pollutants, which aims to improve remediation efficiency and performance so that the final product of remediation is in the form of simple organic compounds, environmentally friendly and no longer causes health effects on all living things around them [51,91,110].

The relationship between single species biodegradator remediation performance on PAH components and bacterial cell growth based on interaction time is presented in Figure 1.

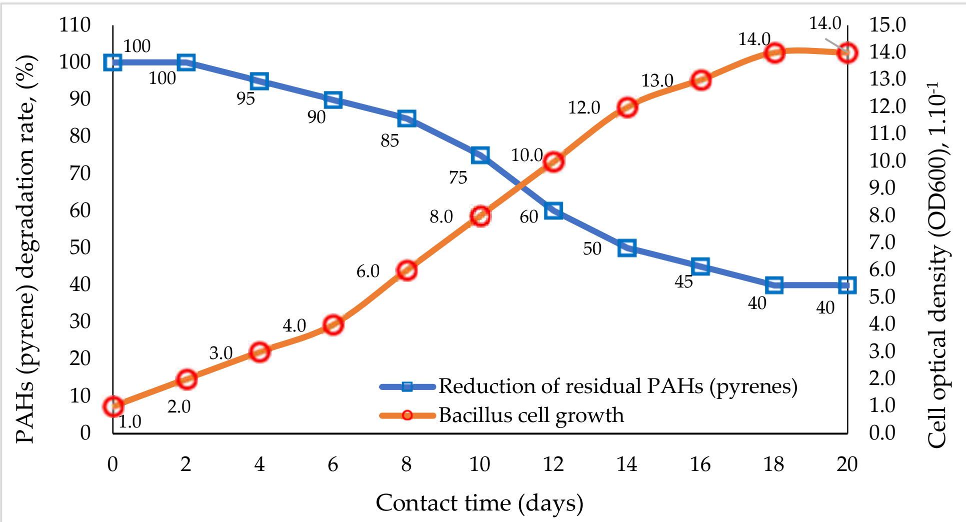

Figure 1. Comparison of the percentage of PAHs (Pyrene) bioremediation with bacterial cell growth rates based on interaction time.

Bioremediation of PAHs using one type of microorganism (bacteria) is often inefficient and has low performance in degrading PAHs substrate [48,106]. It happens because the biodegradation of PAHs by bacteria takes place in several stages, generally starting with an oxidation reaction and then destroying the substrate's molecular structure. The substrate degradation process, both biostimulation and bioaugmentation methods [127,128], using bacteria, continues until transition products are obtained in the form of acidic compounds (carboxylic) [8-11,33,56]. At this stage, the performance of bacteria often drops significantly due to their inability to tolerate an acidic medium [28,77]. As a result of this process, bacterial cells cannot continue the process of cell division, so there is no

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more prolonged regeneration of bacterial cells that grow to continue the degradation process [56,124]. The degradation step is the formation of carboxylic acid compounds, where most bacteria cannot tolerate acidic conditions, so this step in the bioremediation method is often called the rate-limiting step of degradation [11,23,41].

The reduction of the PAHs component (pyrene) by Bacillus bacteria (Fig. 1) increased with increasing interaction time, followed by an increase in the growth of bacillus cells [38,51]. The growth of Bacillus cells appeared to be stagnant on the 18th to 20th day of interaction (Fig.1). In this condition, the growth activity of Bacillus cells was considered non-existent, so the biodegradation process of the pyrene substrate also stopped, while the pyrene residue remained ±40% of the initial amount [30,129].

Figure 1 above illustrates the weaknesses or limitations of using one type of microorganism species (bacteria) in the biodegradation process. This illustration shows the performance of bacillus in pyrene degradation for 18 days [6,109]. Bacteria use this time duration to carry out their degradation mission of pyrene through the adaptation phase of the interaction environment, cell growth and multiplication, and stationary and cell-cell death phases [111,113]. The performance of bacterial degradation results in the gradual destruction of the pyrene molecular structure following the growth and development of bacterial cells, resulting in transitional organic compound products until the bacteria reach the degradation stage of the production of carboxylic acid components [44,129]. The concentration of ±40% remaining pyrene at the end of the biodegradation process, if it enters the environment, is still high and does not guarantee safety for living creatures around it [4,98].

Bioremediation technology continues to develop today. One of the developments and advances in bioremediation technology is the innovation of using a consortium of bacteria to remediate toxic and carcinogenic PAHs [22,105,124]. The study of the performance and efficiency of biodegradation in the application of bacterial consortia or groups of tolerant bacteria to the toxicity of PAHs (hydrocarbonoclastic) [68,113] is presented in Figure 2.

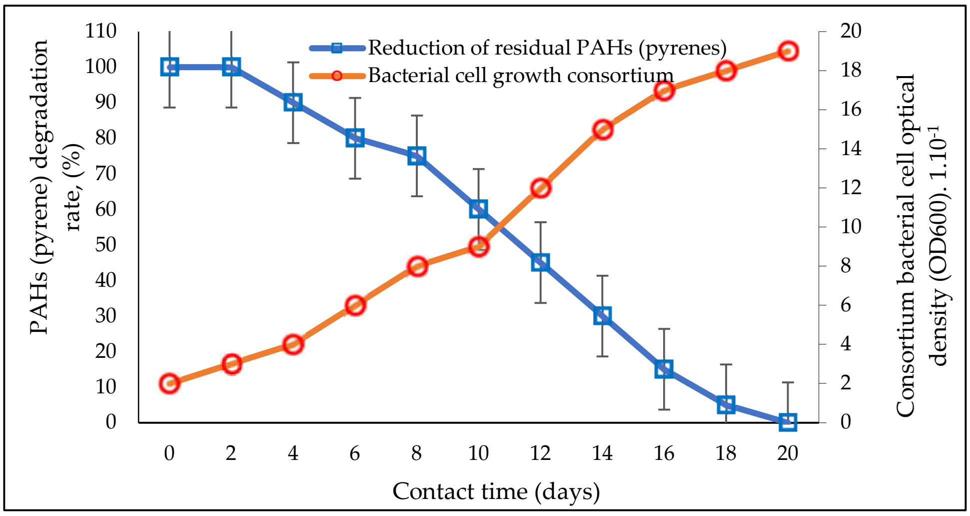

Figure 2. Comparison of the percentage of PAHs (Pyrene) bioremediation with the bacterial cell growth rate of the consortium based on interaction time.

Studies on using hydrocarbonoclastic biodegradators in the bioremediation of PAHs can give better results than using single species bacteria [81,106]. The application of consortium bacteria in the bioremediation of PAHs is considered to have higher performance and substrate degradation power and is more efficient because it is estimated that the bioremediation performance of PAHs increases to 100% (Fig. 2) [27,60,126]. The time needed by the consortium bacteria to degrade potential PAHs is less than 20 days, which can be seen from the consortium bacterial cells that still show growth [51,92]. This condition indicates that bacterial cells can still carry out cell division and degradation of hydrocarbon components as an energy source [5,31].

Table 1. Results of a recent study on the application, performance and efficiency of bacteria in the remediation of polycyclic aromatic hydrocarbons (PAHs) and heavy metal pollutants.

Type of contaminant	Bioremediation method	Test System	interaction duration	Removal Efficiency	Conclusion	References
Pyrene (±10 mg/kg)	Biosurfactants (Biodegradation)	soil microorganism	10 days	±60%	The biodegradation process can occur due to the ability of rhamnolipids to convert carbon into energy sources	[111]
Phenanthrene (±1.0 mg/L)	Biodegradation	using soil adsorption reactor	±90%	more than 50 days	No significant effect of the observed biodegradation efficiency of surfactants	[54]
PAHs (±574 mg/kg)	Biodegradation	Soil microorganism	84 days	72-77%	The formation of surfactants marks the ongoing biodegradation process	[74]
Pyrene (±100 mg/L)	Biodegradation	*Sphingobacterium* sp. strain 21	30 days	±38,29%	The biodegradation performance of pyrene increases at the contact period of 6-20 days	[71]
Pyrene, phenanthrene and others (±6 mg/kg)	Biosurfactants (Biodegradation)	Soil microorganism	±35 days	58-72%	Biosurfactants *(rhamnolipids)* can only carry out biodegradation until the 7th day, then the PAHs biodegradation process does not appear until the 35th day	[85]
Phenanthrene (±1.0 mg/L)	Biodegradation	flake model	14 days	60%	*Rhamnolipids* as surfactants can increase the efficiency of biodegradation at a concentration of 100 mg/L	[127]
PAHs (±1.5 mg/g)	Biostimulation	Soil microorganism	56 days	±99%	Biostimulant effect can increase biodegradation kinetics	[94]
Pyrene (100 mg/L)	Biodegradation using vial reactor	*Alcaligenes faecalis* strain Cu4-1 *Bacillus Cereus* strain MER-8	25 days	97.65% 93, 15%	The products of pyrene biodegradation by the two types of bacteria are relatively different, indicating that there are different metabolic pathways that are influenced by these types of bacteria	[64]
Petroleum refinery waste (±144 g/kg)	Combined biostimulation and bioaugmentation	*Microorganisms in vial*	120 days	57-75%	modification of the method by applying a combination of biostimulation and bioaugmentation to increase remediation efficiency	[128]
Alkanes (initial concentration not determined)	bioaugmentation	*activated microcosm consortium*	85 days	35-66%	The use of the adapted microcosm consortium is able to degrade the hydrocarbon component as a substrate to produce biosurfactants	[95]
Pb(II) and Cd(II)	bioadsorption	*Burkholderia fungorum*	7 days	50 mg/L and	*B. fungorum* strain FM-2 is tolerant to PB(II) and Cd(II) or can	[129]

		FM-2		400 mg/L	carry out the function of bioadsorption of heavy metals	
Pollutant Cd and Hg	Bioadsorption	*Pseudomonas* sp., *Salinobacter* sp., *Streptomyces* sp., *Roseobacter* sp., *Vibrio* sp., *Saccharomonospora* sp. *and others isolated from marine sponge Fasciospongia cavernosa*	7 days	preliminary test	This sponge symbiotic bacteria is able to survive in habitats contaminated with heavy metals mercury and cadmium	[130]
Pb(II) and Cd(II)	remediation in oxidation method	*Natural adsorbent available in aquatic habitat*	2 days	9.03 mg/g and 8,85 mg/g	Natural adsorbent found in the aquatic environment in remediating Pb(II) and Cd(II) pollutants. The isotherm data was processed using the Langmuir approach, showing that lead remediation is endothermic and cadmium is exothermic	[28]
Ions Co, Pb, Cu, Zn	Bio-adsorption	*Rumex crispus L*	7 days	83.5-91.0%	The findings revealed that the heavy metal absorption mechanism occurs on the surface of the bio-sorbent to form a metal-biosorbent complex	[60]

Studies conducted regarding the bioremediation of hydrocarbon components using microorganisms applying different methods, such as biodegradation, biostimulation, bioaugmentation or a combination of the two methods (Table 1), show that none of the experiments has succeeded in degrading hydrocarbon pollutants with efficiency reaching 100% [94,128]. The study of heavy metal bioremediation by microorganisms also showed that no single type of bacteria could absorb heavy metal contaminants in waste with 100% efficiency [52,116,130].

The results of this search indicate the need to develop bioremediation technology for hydrocarbon pollutants (PAHs) and heavy metals. One of the innovations and remediation engineering is the use of several types of bacteria that can bioremediate PAHs and heavy metals to achieve 100% remediation efficiency [37,60,66].

Table 2. Various studies on the biodegradation performance of sponge symbiont bacteria on hydrocarbon components.

Types of hydrocarbon contaminants	Sponge symbiont bacterial species	Type of sea sponge	interaction duration	Removal Efficiency	Conclusion	References
Pyrene (± 100 mg/L)	*Bacillus licheniformis* strain ATCC 9789 (Bl)	*Auletta* sp.	30 days	±39,00	Performance and biodegradation kinetics increased during the contact period of 10-25 days, then slowed down to day 30	[71]
PAHs (Anthracene and pyrene)	*Bacillus pumilus* strain GLB197	*Niphates* sp.	25 days	Antracene (21.89%) Pyrene (7.71 %)	The consortium of three types of bacteria isolated from sea sponges can carry out the function of biodegradation of pyrene and anthracene components, but the performance is less significant, presumably due to competition for carbon as an energy source	[113]
	Pseudomonas stutzeri strain SLG510A3-8	*Hyrtios erectus*				
	Acinetobacter calcoaceticus strain SLCDA 976	*Clathria (Thalysias) reinwardtii*				
PAHs	*Pseudomonas* sp. strain Hi1	*Auleeta sp*	Preliminary test on PAHs contaminated media	observation (qualitative)	All types of sponge symbiont bacteria showed activity on media exposed to PAHs	[78]
	Bacillus subtilis strain BAB-1684	*Clathria reinwardti*				
	Pseudomonas stutzeri strain RCH2	*Callyspongia sp*				
	Bacillus flexus strain PHCD-20	*Hyrtios erectus*				
Naphthalene	*Bacillus* sp.	*Neopetrosia* sp	25 days	±51.37%	Both types of spongy symbiont bacteria can degrade naphthalene, characterized by several parameters, namely increased acidity of the interaction medium, increased optical density (OD600), smells of fermentation and gas bubbles are formed	[58]
	Acinetobacter Calcoaceticus	*Callyspongia Aerizusa*		±37.26%		
Pyrene	SpAB1 and SpAB2	*Hyrtios erectus* (SpA)	Preliminary test on pyrene contaminated media	The activity of the two isolates is weak	The activity of isolates against pyrene generally came from sponges whose body surface was covered with mucus. This mucus is thought to have an enzyme character	[76]
	SpBB1 and SpBB2	*Clathria (Thalysias) reinwardti* (SpB)		Both isolates did not show activity		
	SpCB1 and SpCB2	*Niphates* sp. (SpC)		Both isolates showed strong activity		

PAHs Naphthalene and Anthracene	SpDB1 and SpDB2	*Callyspongia* sp. (SpD)	20 days	Both isolates showed moderate activity	The biodegradation activity of Sp6.B2 isolates against naphthalene and anthracene appeared to be more dominant than Sp8.B1 isolates.	[124]
	Isolate Sp6.B2	*Auletta* sp.		There is biodegradation activity		
	Isolate Sp8.B1	*Callyspongia Aerizusa*				
Aliphatic Components	*Bacillus cohnii* strain DSM 6307 *Bacillus pumilus* strain GLB197	*Niphates* sp.	25 days	Average 48.11%	GC-MS and FTIR detect new organic compounds of alcohol, aldehyde and carboxylic acid groups	[31]
petroleum sludge	*BacillusFlexus* strain PHCDB20.	*Callyspongia* sp.	35 days	Identified 18 types of aliphatic comp. and 2 aromatic comps.	All hydrocarbon components in the degraded sludge are characterized by a decrease in abundance	[13]

Sea sponges generally have a mutualistic symbiosis with microorganisms, especially bacteria [31,113]. Research on bioremediation of waste containing hydrocarbon components (aliphatic, aromatic) using several types of marine sponge symbiont bacteria shows the ability of these bacteria to degrade hydrocarbon components (Table 2) [12,100]. The search results above show that there are 3 (three) groups of symbiotic sponge bacteria (Bacillus, Pseudomonas and Acinetobacter) [38,52,127]. These bacteria showed biodegradation performance against hydrocarbon components [116]. Research findings related to tracking the remediation performance of sponge symbiont bacteria against pollutants containing hydrocarbon components are that these bacteria are generally isolated from sponges whose body surface is covered with mucus or dark-coloured sponges [73,130]. It has to do with the dynamics experienced by sponges suspected of being exposed to pollutants in their habitat, thus stimulating themselves to survive in that environment by producing mucus substances [13,20,131].

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Table 3. Various studies on the bio-adsorption performance of sponge symbiont bacteria against heavy metal contaminants.

Types of hydrocarbon contaminants	Sponge symbiont bacterial species	Type of sea sponge	interaction duration	Removal Efficiency	Conclusion	References
Chromium (VI) Manganese (VII)	*Acinetobacter calcoaceticus* strain PHCDB14	*Callyspongia aerizusa*	15 days	±63.21% ±66.80%	Both types of pollutants are absorbed maximum at a contact period of 3 days	[65]
Cr, Zn, Cu, Fe, Co, Mn, Ag and Cd	*Bacillus cohnii* strains DSM 6307	*Niphates* sp.	16 days	Heavy metal pollutant removal efficiency varies	All types of heavy metals tested can be absorbed by the symbiont bacteria isolate Niphates sp. and Clathria (Tha-lysias) rein-wardtii with varying biosorption performance. Optimum biosorption occurs at a contact period of 4 days	[77]
	Pseudomonas stutzeri RCH2	*Clathria (Thalysias) reinwardti*				
Cd²⁺ and As³⁺	Isolate Sp6.B2	*Auletta* sp.	20 days	83.19%, and 82.24%	Optimum biosorption occurred at a contact duration of 5 days, then weakened until the 20th day of the contact period	[124]
	Isolate Sp8.B1	*Callyspongia Aerizusa*		99.89%, and 99.89%		
Cr(VI) and Cd(II)	*Bacillus pumilus* strain GLB197	*Niphates* sp.	15 days	56.30% and 61.23%	The optimum bioadsorption of these two types of sponge symbiont bacteria against the two types of heavy metal pollutants tested occurred in the range of 3-6 days of contact	[32]
	Pseudomonas stutzeri strain SLG510A3-8	*Hyrtios erectus*		52.74% and 57.80%.		
As^{3+} and Hg^{2+}	*Bacillus licheniformis* strain ATCC 9789	*Auletta* sp.	16 days	99.95%, and 88.49%,	Bio-sorption takes place optimally at a contact duration of 3-6 days. Another supporting indicator is the increase in optical density (OD600), gas bubbles detected in the interaction medium	[131]

Similar research has also been carried out to analyze the ability and performance of remediation of sponge symbiont bacteria against heavy metal pollutants (Table 3). The search results provide information that several types of bacteria isolated from sea sponges whose body surface is coated with mucus can also carry out the adsorption function of several kinds of heavy metal pollutants [32,58,77,132]. The bioremediation ability varies, but in general, the sponge symbiont bacteria are efficient and have high performance against heavy metal adsorption [6,25,133].

The adsorption pattern of sponge symbionts on several types of heavy metal pollutants is different from the biodegradation of PAHs, which tends to be directly proportional to the duration of the interaction [6,28,109]. The adsorption of heavy metal pollutants by bacteria occurs extracellularly through the binding of heavy metal ions by extracellular polymers produced by bacterial cells, which act as negatively charged biosorbents

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on the cell surface to bind and form complexes with positively charged heavy metal ions [59,127].

Analysis of the bioremediation pattern of marine sponge symbiont bacteria against several kinds of heavy metals showed that the optimal adsorption of heavy metal pollutants by bacteria generally occurred at a contact duration of 2-6 days or when the bacteria had passed the adaptation phase in a new environment exposed to heavy metal toxins [20,130]. Bacterial remediation activity decreased until it reached a contact time of 20 days. This biosorption pattern is illustrated in Figure 3.

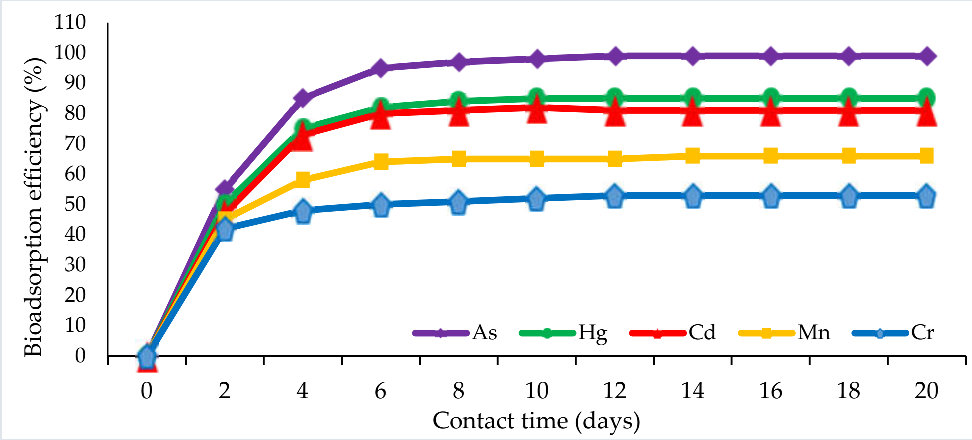

Figure 3. The pattern of adsorption of sponge symbiont bacteria on some heavy metal pollutants

This phenomenon provides information that the adsorption model is an extracellular ionic bond between the positive pole of heavy metals and the negative pole of the bacteria on the surface so that adsorption can occur very quickly when the negative surface of the bacteria is active [60,65,132,134]. The contact duration of 6 days is considered the general period required for the fishery to reach the saturation stage, where most of the negative poles of the bacterial surface have formed ionic bonds with heavy metal ions [35,124]. Under these conditions, the bacterial cells can no longer continue their adsorption activity, and the process towards the division phase and cell growth is declared to have stopped [28,107].

4. Process and mechanism of pollutant bioremediation by marine sponge symbiont bacteria

The degradation process of PAH components by bacteria differs from the heavy metal adsorption process [65,131]. The same thing also differs in the degradation mechanism of PAHs with the mechanism of heavy metal adsorption by bacteria as a bioremediator, although using the same bacterial species. Different types of pollutants (PAHs and heavy metals) as a material to remove [16,24,25]. The search and analysis of several types of research on bioremediation using bacteria raise several assumptions that can be scientifically justified [17,42,43].

4.1. Processes and mechanisms of biodegradation of PAHs

The Pyrene biodegradation process using Bacillus pumilus strain GLB197 isolated from marine sponge Niphates sp. (Table 2) [124] is illustrated in Figure 4. The illustration illustrates that the degradation performance in using one type of bacteria cannot provide significant degradation results, or the degradation takes place incompletely [9,11,31,135].

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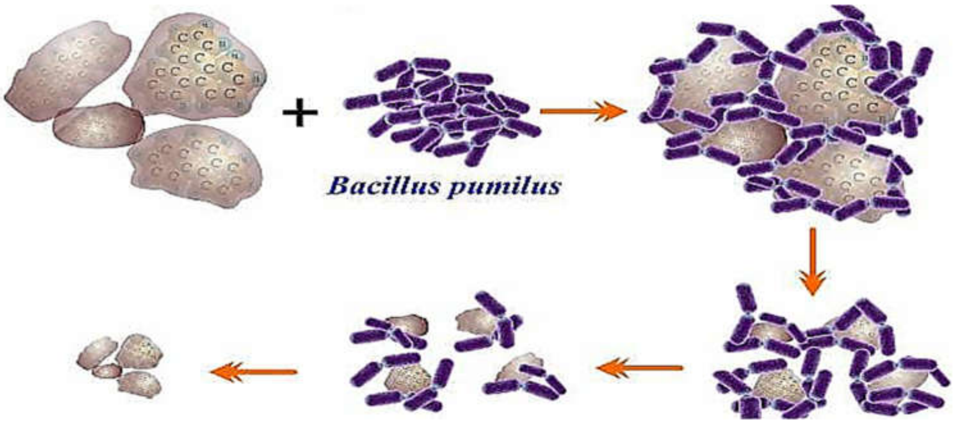

Figure 4. Description of the biodegradation process of PAHs (pyrene) contaminants using one type of bacteria, Bacillus type

The process of pyrene degradation by bacillus begins with adaptation in the medium, which usually lasts 1-3 days [129]. Then the bacteria enter the enlargement phase, cell division to form colonies, crowd around the pyrene pieces until the cells enter the pyrene body and carry out the activity of cutting the pyrene component until it decomposes into small particles (Fig. 4) [46,136]. Suppose the bacteria are still able to carry out the remediation activity. In that case, each of these bacterial communities takes pieces to continue the degradation process until the bacterial cell activity stops, perhaps because it has died by changing the medium to become more acidic [15,34,137].

The process of pyrene degradation by bacillus is similar to the bioremediation mechanism that occurs, and the difference is that the bioremediation mechanism takes place at the molecular level or metabolic substances (micromolecules). In contrast, the remediation process occurs at the component or macromolecular level [11,34,118]. The degradation mechanism of PAHs (pyrene) by bacteria is known as a cycle. Namely, one cycle shows a series of changes in the molecular structure of PAHs from pyrene (4 aromatic benzene rings) to phenanthrene (3 aromatic rings). The following cycle changes phenanthrene to naphthalene (2 aromatic rings), see imaginary line (Fig. 5), and so on until the conversion of benzene (1 aromatic ring) into simple non-aromatic organic compounds that are environmentally friendly is achieved [83,117].

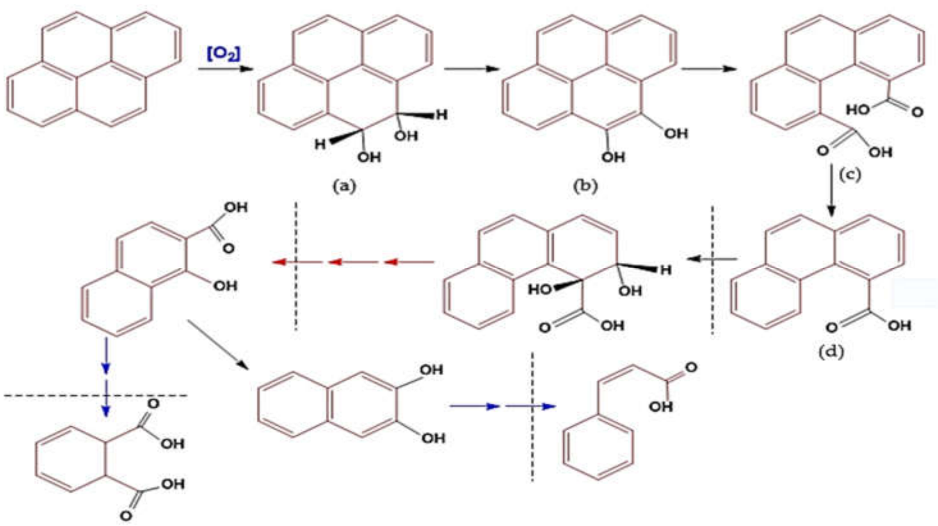

Figure 5. Illustration of the mechanism of biodegradation of pyrene components using *Bacillus* bacteria.

The mechanism of pyrene degradation by *Bacillus pumilus* strain GLB197 [113] is similar to the degradation pathway of PAHs by *Cycloclasticus* sp. [110,112,116] and adopted the mechanism of pyrene degradation by Mycobacterium sp. [91,99,132], combined with genomic analysis and experimental analysis (Fig. 5). The mechanism of pyrene degradation is described through an oxidation reaction in four stages of change or one cycle, i.e., starting with the change to produce the cis/trans transition product 4,5-dihydrodiol- pyrene (Fig. 5(a)), then converted to 4,5-dihydroxy pyrene (Fig. 5(b)), then to 4,5-dicarboxylic acid-phenanthrene (Fig. 5(c)) and finally to the product phenanthrene-4-carboxylate transition (Fig. 5(d)) [17,46,64]. The fourth stage of the first cycle is the first vulnerable point for bacteria because, at that stage, a carboxylic acid transition product is formed, which causes the acidity of the media to increase [110,112,113]. In this condition, the bacterial cells are susceptible, so the potential cell activity decreases drastically and can even experience mass death [91,138]. The mechanism of conversion of pyrene to the transition product of phenanthrene can be called destruction, namely the destruction of the pyrene molecular structure or an open aromatic ring [117,136].

4.2. Process and mechanism of heavy metal bioabsorption

The process of heavy metal adsorption using bacteria is similar to the process of forming a heavy metal complex (X) with ethylene diamine tetra acetate (EDTA) molecules forming an X-EDTA complex [55,136 The mechanism of heavy metal adsorption is forming extracellular ionic bonds on the surface of bacterial cells that are negatively charged with positively charged heavy metals [16,118]. The process and mechanism of adsorption of heavy metal ions last for a shorter duration than the degradation of PAHs [25,128]. The adsorption process continues until the saturation point is reached [65'96]. The mechanism and changes in heavy metal adsorption by bacterial cells can be observed using analytical instruments such as SEM, EDS and XRD while determining the adsorption efficiency can use AAS or ICP [125,139,140].

5. Parameters of Pollutant Bioremediation

Several changes can be observed either directly by observation or by using analytical instruments as the performance of microorganisms (bacteria) in the bioremediation process of PAHs and heavy metal pollutants [141]. These changes are indicators and parameters that can be used as the basis for the occurrence of pollutant bioremediation activities by bacteria [27,95].

5.1. Biodegradation of PAHs

The biodegradation parameters of PAHs, which indicate the presence of degradation activity as a performance of bacteria, include: (1) The growth of bacterial cells can be seen from the increase in the optical density of the interaction medium (OD600) [5,53]. The increase in OD600 medium as an indicator of bacterial cells has passed the adaptation phase and heading to the phase of enlargement and division cells [16,33]. At this stage, the degradation activity of PAHs has taken place. (2) The increase in the acidic properties of the media is a manifestation of the work of bacterial cell degradation that has succeeded in destroying the molecular structure of PAHs, forming several new components, one of which is the formation of carboxylic compounds, which results in increased media acidity [110,112 At this stage, it is called a vulnerable period for bacteria which can result in cells not being able to enlarge and divide, and maybe even the bacterial cells are threatened with mass death [103,134]. (3) The increase in the temperature of the interaction media due to the formation of new compounds resulting from degradation. This parameter has only increased by a few points, generally in the 0.4 – 1.2 ^{0}C [23,37]. (4) The emergence of gas bubbles is suspected that this bacterium is an aerobic group that requires oxygen in carrying out PAH remediation. The oxygen demand of the interaction media is carried out by aeration using a shaker or can be injected directly (5). The smell of fermentation is a characteristic feature of the enzymatic reaction that occurs at the stage of destruction of the molecular structure of PAHs [17,46,68]. The existing enzymes are produced by bacteria as a response of cells to defend themselves in extreme environments exposed to PAHs

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[66,67] and (6) New peaks were identified on the GC-MS chromatogram [120,121]. The peaks recorded with varying abundance are authentic evidence for all the previously described narratives (points 1-5) [8,126]. (7he detection of functional groups of organic compounds from the FTIR chromatogram strengthens the GC-MS data that the degradation products produce organic compounds, one of which is a carboxylic component containing carbonyl and hydroxyl groups [122,142,143].

5.2. Heavy Metal Bioadsorption

Parameters of heavy metal adsorption by bacteria that can be observed and measured include increased media turbidity, a limited change in the pH range of 0.2 - 0.4, temperature increase in a narrow range of 0.3-0.8 ^{0}C, gas bubbles appearing, and the smell of fermentation is very weak [3,50]. Changes during the adsorption process are not strong enough to be described in detail as in the biodegradation of PAHs [87,106]. It is because the remediation occurs in the form of adsorption with the extracellular ionic bonding model of the negative part of the bacterial surface to the positive charge of heavy metals [7,37,61]. Therefore, at a certain contact duration, a saturation point can occur in the media, where this saturation point cannot be observed directly. The saturation point is known if the adsorption efficiency is determined by running every two days of contact using the AAS instrument [20,60]. In this case, the saturation point is assumed to occur at the contact period of 6 - 20 days (Fig. 3).

6. Development and Formulation of Remediator Bacteria Consortium

The performance and efficiency of PAHs pollutant biodegradation can be improved by making experimental modifications, especially using consortium bacteria. Similarly, the capacity and level of bacterial adsorption to heavy metal contaminants with the modification of a consortium of bacterial biodegradators [35,92,130].

6.1. Hydrocarbonoclastic bacteria

The application of hydrocarbonoclastic bacteria (R-1) in the biodegradation of PAHs is one of the modifications that is considered to improve the performance and efficiency of bioremediation [34,95]. One of the efforts to improve the performance and efficiency of biodegradation is made by using several types of bacteria, for example, Bacillus pumilus, Pseudomonas stutzeri and Acinetobacter calcoaceticus which are known [9,127], all of which can biodegrade PAHs (hydrocarbonoclastic bacteria) [99,144]. These bacteria were formulated as a consortium bacterial suspension and interacted with pollutant PAHs, e.g. pyrene (Fig.6) [112,145].

This modification is believed to increase the degradability of PAHs because each type of bacteria can take on the role of degradation in parallel so that it can complete one cycle of conversion of pyrene to phenanthrene in a possibly shorter duration of time [71,90]. The study of the potential of the R-1 consortium bacteria was intended to remediate waste containing hydrocarbon components, especially PAHs, with high remediation performance and efficiency [38,145].

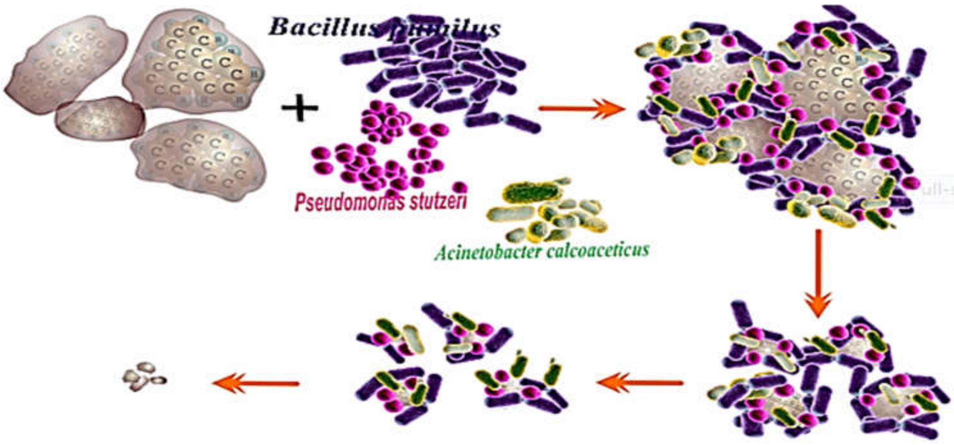

Figure 6. The process of biodegradation of PAH contaminants uses a consortium of hydrocarbonoclastic bacteria. An illustration

The degradability of bacteria was significantly increased, resulting in a high-efficiency degradation. Thus, all pyrene could be degraded to produce the final product in the form of simple non-aromatic organic compounds that are environmentally friendly (Fig. 6) [44,117]. The problem of limiting factors in the PAHs degradation process when it comes to the formation of transition products of carboxylic acid compounds is also assumed to be minimized so that bacterial cells can work continuously to convert carbon elements into energy [82,110].

6.2. Metalloclastic bacteria

Performance, capacity and efficiency of heavy metal adsorption by using several types of bacteria formulated in the form of a consortium of bacteria adsorb heavy metals or bacteria of the metalloclastic category (R-2) [9,131]. The increase in the capacity and efficiency of heavy metal pollutant adsorption occurs due to the abundance of negative poles from the bacterial cell surface, allowing the formation of ionic bonds that occur very much at the surface at almost the same time [28,77].

The difference in adsorption efficiency due to differences in the reactivity and affinity of each heavy metal, as well as the presence of several types of bacterial cells in the consortium formulation, allows the barriers that suppress the abundance of ionic bond formation to be overcome because each type of bacteria has a specific adsorption model [6,20]. The positive charge of each heavy metal also affects the bonds formed. The adaptability factor of bacterial cells to the environment exposed to heavy metals greatly determines the adsorption process. In contrast, the external influence of adsorption, such as the provision of nutrients, and the presence of aeration, does not positively impact the number of ionic bonds formed [35,113]. The consortium of bacteria-coded R-2 has high adsorption power and efficiency, which is intended to be applied in the remediation of waste exposed to heavy metals [77,131].

6.3. Metallo-hydrocarbonoclastic bacteria

In addition to containing aliphatic and aromatic hydrocarbon components, Sludge waste originates from petroleum processing and contains several types of heavy metals [65,96]. This condition requires using bacteria that have a biodegradation function and an adsorption function [60]. Several studies have shown that several types of sponge symbiont bacteria can degrade PAH components and adsorb heavy metals, although these ability tests were carried out separately [6,45,146]. Groups of bacteria with multiple abilities are called Metallo-hydrocarbnoclastic (R-3) bacteria.

Several types of research have been conducted; it was found that several sponge symbiont bacteria can be included in the group of bacteria coded R-3, namely a collection of bacteria that can biodegrade PAHs and also have the ability to adsorb heavy metals [34,109].

This study concludes the findings in the form of recommendations and suggestions for developing bioremediation technology for GTP, especially PAHs and heavy metals are important to produce and improve remediation efficiency [19,110]. The bacterial consortium of marine sponge symbionts, both hydrocarbonoclastic (R-1), metalloclastic (R-2) or Metallo-hydrocarbonoclastic (R-3) bacteria, have the potential to be applied to increase remediation efficiency in various types of waste [51,81,92]. Screening is important to find and categorize bacteria (R-1; R-2; R-3) with different abilities in GTP remediation [1,7,19]. Bacterial formulation codes R-1, R-2, and R-3 can also be developed in the future into crystalline preparations so that these bacteria are easily mobilized for rapid culture at sites exposed to GTP [2,7,147]. The search for marine sponge symbiont bacteria for bioremediation with high performance and efficiency can be traced only to marine sponges whose body surface is covered with mucus or dark in colour [59,88].

Supplementary Materials: Suppplementary material are available online.

Author Contributions: Conceptualization, I.M., R.R., A.M., E.K., E.S. and B.B.; methodology, I.M., R.R., A.M., S.S., I.S., Early.S.; software, R.R., K.K., E.H., I.S.; validation, I.M., R.R., A.M., A.A. and T.M.; formal analysis, I.M., A.M., T.M., S.H.; investigation, I.M., R.R., A.S., A.M., R.A.; resources, R.R., S.S., M.M., E.S.; data curation, I.M., R.R., E.R., A.M. and K.K.; writing—original draft preparation, I.M., A.M., B.I., B.B., K.N.; writing—review and editing, I.M., R.R., A.M., Early.S., A.A., R.A., K.N.; visualization, E.H., A.S., B.I.; supervision, I.M., S.G., E.K., R.R.; project administration, A.A., E.R., M.M., S.H. S.G.; funding acquisition, I.M., R.R., A.M., R.A. All authors have read and agreed to the published version of the manuscript.

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