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Performance Analysis of bio-sorption of Heavy Metal and Biodegradation PAH of Isolates Marine Sponges Symbiont Bacteria

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Abstract. Heavy metal pollutants and polycyclic aromatic hydrocarbon contaminants, also known as PAHs, need attention from related parties, considering that their use is very wide, as well as their relatively high natural releases. Careless and uncontrolled handling has the potential to cause problems for creatures, especially in marine ecosystems. The aim of the study was to determine the performance of marine sponge micro symbiont isolates in heavy metal bio-sorption and PAH biodegradation. Pure isolates were obtained by the scratch method using NA media. The pure isolates from the colonization were converted into isolate suspensions using physiological 0.9% NaCl solution. Suspension adapted 24 hours. The isolate suspension was interacted with heavy metals Cd^{2+} and As^{3+}, each with a concentration of 100 mg/L and naphthalene and anthracene with a concentration of 1000 mg/L. Interaction time of 5, 10, 15, and 20 days. Results of the analysis showed the bio-sorption capacity of the bacterial isolate Sp6.B2 to Cd^{2+} = 83.190 %, while Sp8.B1 = 82.240 %. Bio-sorption performance of Sp6.B2 isolates against As^{3+} = 99.890 %, while Sp8.B1 = 99.894 %. The biodegradation performances of Sp6.B2 isolates had a higher aggressiveness towards naphthalene and anthracene test contaminants than Sp8.B1 isolates. These results indicate that the bio-sorption performance of Sp6.B2 > Sp8.B1 isolates against Cd^{2+} contaminants and vice versa Sp6.B2 < Sp8.B1 against As^{3+} bio-sorption, while the biodegradation performance of Sp6.B2 > Sp8.B1 isolates both against naphthalene and anthracene test contaminants. The development of this research in the future is directed at tracing sponge symbiont bacteria that have two functions at once, namely as a biomaterial for PAH degrading and heavy metal bio-adsorbent.

Keywords:
PAHs;
heavy metals;
bio-sorption;
biodegradation;
bacteria;
sponge micro symbionts

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Introduction

Heavy metal pollution can come from natural sources in nature and human activities, especially industrial and domestic activities (Mostafidi et al., 2019). Heavy metal contaminants are generally in the form of particulates that pollute the air environment, water media and soil (Alimardan, Ziarati, & Moghadam, 2016). Exposure to heavy metals in water is usually in the form of oxidized ions or dissolved molecules (Ziarati et al., 2019). Generally, heavy metals have more than one oxidation number. The toxic nature of heavy metal contaminants that accumulate in waters can cause serious problems for the life of aquatic biota, especially biota that live in the marine environment. On the other hand, the presence of heavy metals is difficult to avoid because generally heavy metals are needed in various types of industries (Wibowo, Nurcahyo, & Gabriel, 2019). Cadmium and chromium metals are widely used in electroplating, corrosion-resistant steel, paint fillers, inks, ceramics and other uses, while manganese is widely used in batteries, pigments, precursors and other industries (Mostafidi et al., 2019).

The presence of heavy metals in the environment freely, is classified as a hazardous and toxic substance mainly to living things that have interactions with these heavy metal contaminants. Exposure to heavy metals in the form of oxidized or dissolved ions to humans has the potential to cause serious health problems (Siahaya et al., 2013). Heavy metal ions, for example: Cobalt (Co), Copper (Cu), Chromium (Cr), Zinc (Zn), Silver (Ag), Manganese (Mn) (Marzuki, 2020) Cadmium (Cd), Mercury (Hg), Lead (Pb)

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Selenium (Se), Arsenic (As), and several other heavy metals (Melawaty et al., 2014).

Based on the potential sources of contamination, exposure and health risks, the three types of heavy metals in the form of Cd^{2+}, Pb^{2+} and As^{3+} ions are unstable free radical metal ions, so they need special and specific handling (Orani et al., 2018). One of the ways to handle this is by reducing the toxic properties possessed by applying certain methods such as bacteria in the bio-sorption method (Marzuki et al., 2019).

Other types of contaminants are hydrocarbon pollutants, both aliphatic and aromatic. Model of handling hydrocarbon contaminants, especially aromatic types (PAHs) through physical, chemical and biological methods (Marzuki et al., 2020). Along with the development of science and demands for high, efficient and simple results, this method has been less used since the beginning of the 21st century. Remediation methods and processes, both with the biodegradation model, are an alternative option in reducing PAHs contaminants (Marzuki et al., 2020a). The bioremediation method uses the activity of microorganisms, such as bacteria, fungi and the like. Potential microorganisms in bioremediation are widely distributed in nature, both in soil media and in water media (Marzuki et al., 2017).

Several types of marine bacteria, both in the form of free colonies in seawater and forming symbiosis with other marine biota, such as sponges, are known to be able to degrade toxic hydrocarbons, reduce and convert into simple components of organic compounds by utilizing carbon compounds as an energy source (Marzuki et al., 2016). Biodegradation of hydrocarbons by microbial communities depends on the composition of the community and the adaptive response of microorganisms to the presence of pollutants such as hydrocarbon materials (Marzuki et al., 2015). The rate and mechanism of biodegradation of aliphatic hydrocarbon components are specific and different from the mechanism and rate of bacterial biodegradation of aromatic compounds, the constituents of aliphatic compounds such as oil, generally have long carbon chains and heavy fractions, while aromatic hydrocarbons are persistently toxic to marine biota and microorganisms. (Maldonado et al., 2021). However, several studies have shown that under certain conditions the degradation of complex aliphatic and aromatic hydrocarbons can proceed at varying speeds and with specific mechanisms. (Pita et al., 2018).

Marine sponge symbiont microorganisms have two important roles in the biological life of the sponge system, first, as a source of nutrients for symbiotic microorganisms or with sponges. (Yang et al., 2019). Second, Microorganisms form a symbiosis with sponges, where the sponges position themselves as hosts or living homes for microorganisms, on the other hand, sponges use symbiotic bacteria as self-defense materials to survive, including in habitats contaminated with various types of pollutants. (de Kluijver et al., 2021). Third, sponges are generally able to adapt and survive in extreme environments (polluted environments). (Fang et al., 2020).

Sponges are one of the marine biota that have many unique features, not only because of their ability to adapt to toxic pollutant-contaminated environments, they also have the ability to produce a substance in the form of mucus that behaves like an enzyme. (Marzuki1 et al., 2015a). The production of spongy symbiont mucus is thought to be related to the responsiveness of the sponge to habitat changes due to the presence of predators or due to contamination with toxic pollutants (Marzuki, Kamaruddin, & Ahmad, 2021). The ability of sponges to isolate themselves due to extreme environmental changes is carried out by spreading the mucus they produce to the surface of their bodies as a form of sponge self-isolation against environmental conditions that are not conducive to their life and development processes. (Marzuki et al., 2020b).

The application and development of the use of marine sponge micro symbionts in reducing PAHs and heavy metal contaminants, through biodegradation and bio-adsorption methods is one of the efforts to create and implement management and conservation of the marine environment, so that damage to marine ecosystems and ecology remains a conducive part for all living things. not conducive to the process of life and development (Marzuki et al., 2020; Tereza et al., 2018). These efforts require collaboration and synergy together which are carried out systematically and structured. The enthusiasm, commitment and hard work of various elements of society, the existence of policies that support the atmosphere towards improvement and partiality are the keys to the achievement and realization of these desires, including the involvement and active role of universities in the realm of professionalism in the scientific field.

This research raises novelties related to the multi-function of sponge symbiont isolates, namely the potential function of biodegradation of PAHs contaminants as well as their benefits as heavy metal bio-adsorbents. Another novelty in the aspect of heavy metal bio-adsorption is using marine sponge isolates, where previously the isolates used in the same method were isolated from heavy metal contaminated areas. Likewise in the aspect of biodegradation of aromatic hydrocarbon components,

which is a new thing with the use of isolates from marine sponges.

Experimental

Materials and method

Materials used in this study consisted of two types of bacterial isolates which were micro symbiont sponges *Auletta* sp (Sp6.B2), *Callyspongia* sp (Sp8.B1), naphthalene 1000 mg/L (Supelco), anthracene 1000 mg/L (Supelco), HCl pa, Arsenic Trioxide (As_2O_3) pa (Sigma), Cadmium Sulfate (Cd_SO_4) pa (Sigma), 0.9% physiological NaCl; dichloromethane Pa, Na_2SO_4 pa, NaOH pa, sterile seawater, Nutrient Agar. The equipment consists of Gas Chromatography-Mass Spectrometer (GC-MS) Agilent Technologies 7890A, Fourier Transform Infrared (FTIR) Shimadzu IR Prestige-21, Atomic Absorption Spectrometer (AAS) AA240FS, cotton, plastic wrap, aluminum foil, alcohol, gauze, tissue, and filter paper, a set of glassware, shaker, incubator, autoclave, digital balance.

Sample Preparation

The isolates obtained by isolation from each colony were purified, namely by scratching 1 ose of the colony in a zig-zag direction on a petri dish containing Nutrient Agar (NA) media. Then incubated at room temperature 30 ^oC for 1 x 24 hours. After incubation, new colonies grew, then rescribbled into media containing sterilized Nutrient Agar slanted media. This process was carried out until a single isolate was obtained.

The isolates were propagated by isolate culture method on Nutrient Agar media. The isolate was converted into a suspension by adding 2 ml of 0.9% physiological NaCl, put in a 150 ml volumetric flask, then the volume was compressed with the same solution and shaken until homogeneous. It was put into an Erlenmeyer, then the volume was made up to 150 ml. Each 5 ml of the isolate suspension was pipetted, put into 6 wattles. Then incubated for 1 x 24 hours. 5 ml of dissolved heavy metal Cd^{2+} and As^{3+} with a concentration of 100 ppm were added to each wattle. Then 5 ml of PAH naphthalene and anthracene were added in each vial. Each vial is placed into the shaker incubator 200 rpm rotation, contact time respectively (5, 10, 15, and 20) days for each vial (Marzuki et al., 2021; Akinde & Iwuozor, 2012).

Heavy Metal and Compound PAHs Testing

The contact time between the sponge symbiont isolates with naphthalene and anthracene contaminants, as well as heavy metals Cd^{2+} and As^{3+} were determined 5, 10, 15 and 20 days, then extracted using a 5 mL dichloromethane extractor in a separating funnel, then allowed to stand for 1 minute so that two layers of polar liquid appeared and non-polar. Separated assuming the remaining heavy metal contaminants are not absorbed, incorporated into polar components, and dissolved PAHs are not degraded into non-polar components. The non-polar solution was added + 0.01 gram of Na_2SO_4 powder into the vial containing PAHs, to remove the water content, the solution was ready to be analyzed using GC-MS and FTIR. The polar solution was further processed by filtering using Whatman 42 paper, added H_2SO_4, the solution was ready to be analyzed using AAS.

Sample analysis

The bio-sorption performance of isolates of marine sponge symbiont bacteria was analyzed by determining the absorbance measured in AAS, then determined the concentrations of Cd^{2+} and As^{3+} that were not absorbed using equation (1), the bio-sorption capacity of each isolate (Sp6.B2 and Sp8.B1) against Cd^{2+} and As^{3+}. Determination of the bio-adsorption capacity of isolates is based on the application of equation 2, where the use of isolates is in units of suspension volume, not based on the number of cells used. using equation (2) and the bio-sorption efficiency of sponge symbiont isolates using equation (3).

$$Y = a \pm bX, \quad [1]$$

$$Q=(C1-C2)/m \times V, \quad [2]$$

$$\% E= (C1-C2)/C1 \times 100\%, \quad [3]$$

Note:
Y = Instrument response (absorption),
X = concentration of metal analyte in solution (mg/L),
a = intercept, b = slope, Q = bio-sorption capacity,
C1 = concentration before contact (mg/L),
C2 = concentration after contact (mg/L).
m = bacterial cell count (assuming 1 mL of bacterial suspension is equivalent to 1 g bio-sorbent) (mg),
V = cell of volume of solution (L) and
% E = bio-sorption efficiency (Riyaz et al., 2020).

The biodegradation performance of isolates of marine sponge symbiont bacteria was determined by analyzing the naphthalene and non-degradable anthracene contaminants using GC-MS, while the biodegradation products in the form of simple organic compounds were determined from the chromatogram of the measurement results using the FTIR instrument. (Marzuki et al., 2015a). Qualitative analysis of GC-MS and FTIR recording data in the form of chromatograms by observing retention time, peak height, peak number, component quality, type and name of biodegraded compounds, and functional groups of components of biodegradation products (Marzuki et al., 2021a).

Result and Discussion

General review, the activity of microorganisms that occur is based on the mechanism of the fermentation reaction, although the mechanism used may vary, due to differences in phenotype and genotype as well as strains of symbionts that carry out the function of biodegradation, while the function of bio-sorption generally occurs by the mechanism of complex formation or ionic bonding similar to the formation of compounds. Complex in the complex reaction of chelate formation (Marzuki et al., 2021a; Marzuki et al., 2020; Liu et al., 2019).

Table 1. Parameters of microorganism fermen-tation reaction test

Isolate code	Parameters biodegradation	Contact time (days)			
		5	10	15	20
Sp6.B2	turbidity	+	++	++	+
	gas bubbles	2	3	3	1
Sp8.B	turbidity	+	++	++	+
	gas bubbles	2	3	2	1

Note: ++ = Very cloudy; + = little cloudy
3 = many; 2 = currently and 1 = not enough

The bio-sorption performance of Cd^{2+} and As^{3+} heavy metals and the biodegradation of naphthalene and anthracene PAH compounds by Sp6.B2 and Sp8.B1 bacteria can be considered through the mechanism of a fermentation process involving enzymes produced by isolates in response to contaminated habitat. Table 1 shows that the fermentation process has started to occur after a contact time of 5 days with the presence of turbidity and gas bubbles formed in the vial. The fermentation process gradually decreased at the contact time of the 10 days, continued to weaken until the contact time of the 20 days. During the contact period above the 20 days, it is suspected that the fermentation reaction will no longer occur because it is suspected that the isolates have died. (Melo, 2020; Liu et al., 2019). The bio-adsorption process of heavy metal contaminants is based on the assumption that the isolate is a bio-adsorbent, so that the determination of the bio-adsorption performance is based on the volume of the isolate suspension with the assumption that 1 mL of suspension is equivalent to 1 g of bio-adsorbent mass (Marzuki, 2020; Liu et al., 2019).

The other than the biodegradation and bio-sorption parameters that can be used as performance indicators of isolates besides turbidity and abundance of gas bubbles are changes in the pH of the interaction medium as shown in Table 2. The value of acidity fluctuates especially in the non-polar layer, while in the polar layer it is relatively more acidic with increasing time interaction. Based on visual observations and measurements of several biodegradation parameters, such as an increase in the turbidity of the interaction media, the presence of gas bubbles that appear, odors that characterize the fermentation process, changes in pH and changes in temperature during the interaction, indicate that the degradation process of the tested PAHs components and heavy metal adsorption are assumed to be The process takes place similar to a fermentation reaction by enzymatic activity n several reactions involving enzymes (Marzuki et al., 2017; Obire, O., Aleruchi & Wemedo, 2020; Lundstedt, 2003).

Table 2. Degree of acidity of polar and non-polar contact media after extraction.

Symbiont code	Extract	pH according to contact time (days)			
		5	10	15	20
Sp6.B2	polar	3	3	2	3
	non-polar	4	5	6	5
Sp8.B1	polar	3	3	3	2
	non-polar	4	6	5	4

Non-polar compounds at the contact time of 5 and 10 days, the degree of acidity increased at pH 5-6, which indicated that the fermentation was maximal, and the pH began to decrease on days 20 which indicated that the fermentation process was weakening (Al-Mutary et al., 2019).

Table 3. Bio-sorption performance of sponge symbiont isolates against Cd^{2+} contaminants

Isolate code	Contact time (days)	Absorp-tion	Contaminant level Cd^{2+} (ppm)	Bio-sorption Performanc e (%)
Sp.6.B2	5	0.0088	18,24	81,76
	10	0.0084	17,28	82,72
	15	0.0083	17,26	82,72
	20	0.0082	16,81	83,19
Sp8.B1	5	0.0115	24,67	75,33
	10	0.0104	22,08	77,92
	15	0.0089	18,48	81,32
	20	0.0086	17,76	82,24

Table 4. Bio-sorption performance of sponge symbiont isolates against As^{3+} contaminants

Isolate code	Contact time (days)	Absorp-tion	Cont. level As^{3+} (mg/l)	Bio-sorption Performance (%)
Sp.6.B2	5	0.00488	0,1162	99.8838
	10	0.00521	0.1154	99.8846
	15	0.00518	0.1147	99.8853
	20	0.00491	0.1092	99.8908
Sp8.B1	5	0.00554	0.1221	99.8779
	10	0.00522	0.1156	99.8844
	15	0.00510	0.1131	99.8869
	20	0.00477	0.1065	99.8935

The decrease in metal concentration occurred after contact of the sponge symbiont suspension, which was interpreted by the weakening of the metal's toxicity as a result of the work of the sponge symbiont isolate. The analyte concentrations of Cd^{2+} and As^{3+} tend to decrease with increasing contact time, as shown in Table 3 and Table 4.

The bio-sorption performance of isolates of marine sponge symbiotic bacteria against Cadmium and Arsenic ions is shown in Tables 3 and 4. These data indicate that the bio-sorption of Sp6.B2 isolates was more progressive than Sp8.B1 isolates. This shows that Sp6.B2 isolates have the ability to adapt to Cd^{2+} contaminants compared to Sp8.B1 isolates. Another assumption is related to the progression of the two types of marine sponge isolates, particularly the bio-sorption performance on Cd^{2+}, which is influenced by the characteristics and properties of the ions in the periodic arrangement. (Marzuki, et al., 2020a) In general, the bio-sorption performance of the two isolates (Sp6.B2, Sp8.B1) against As^{3+} was higher than that of Cd^{2+}. Comparison of the performance of bio-adsorption on Cd^{2+}, it appears that the isolate Sp6.B2 was more dominant than the isolate Sp8.B (Table 3), while against As^{3+}, the two isolates showed that the performance of the isolates was relatively not significantly different (Table 4). This situation provides strong information that the bio-sorption strength of the two types of isolates is more influenced by the character of the isolate itself, not from the effect of differences in ionic properties as shown in the periodic table of elements, where Arsenic is in the 4 period position of group VA, compared to Cadmium. in the 5 period position of the transition group IIB (Asad et al., 2019; Jańczuk, Szymczyk, & Zdziennicka, 2021). The main character of a bacterium compared to other types of bacteria lies in its adaptability to the growth environment, even though both types of bacteria come from the same source, both come from the sponge group biota. The difference in bio-sorption performance of the two types of isolates (Sp6.B2 and Sp8.Bi) has implications for differences in bio-sorption capacity and appearance of bio-sorption efficiency (Figure. 1 and Figure. 2). The mechanism of bio-sorption that occurs by one type of bacterial isolate against heavy metal contaminants is different from other types of metal (Marzuki, 2016)

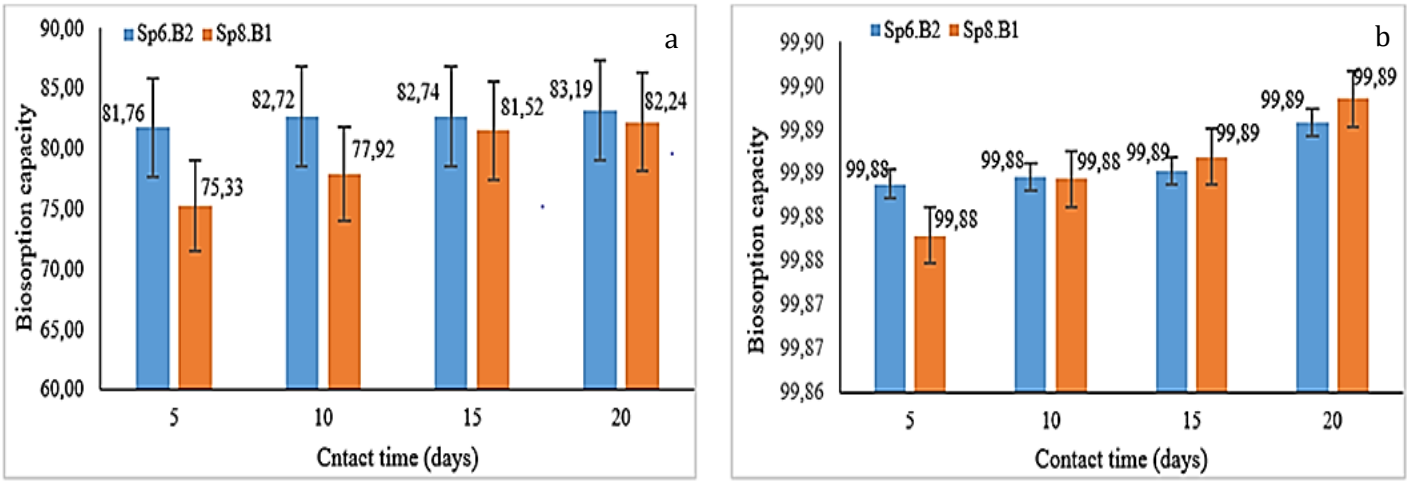

Figure 1. Bio-sorption capacity of bacterial isolates SP6.B2 and SP8.B1 against heavy metal contaminants. (a) Bio-sorption of Metal ion Cadmium (Cd^{2+}) and (b) Bio-sorption of ion Arsenic (As^{3+})

Based on the data in Figure 1, it shows that the bio-sorption performance, especially in the aspect of bio-sorption capacity against Cd^{2+}, isolate Sp6.B2 is relatively stronger than isolate Sp8.B1 (Figure.1a) but against As^{3+}, it is seen that the bio-sorption capacity of isolate Sp8.B1 is relatively higher. Progressive compared to Sp6.B2 isolates, even though it appeared at 5 days contact, the bio-sorption capacity of Sp6.B2 isolates appeared to be greater (Figure. 1b). Based on direct observation on the bioreactor, it was shown that the performance of reducing the concentration of cadmium and arsenic ion contaminants played by the isolates Sp6.B2 and Sp8.B1 by taking a mechanism similar to a fermentation reaction involving enzymes, although it has not been specifically explained in detail the type and mechanism of the enzymatic reaction. This condition is an initial indication for conducting further studies and research related to the mechanism of enzymatic reactions in PAH biodegradation and heavy metal bio-adsorption using sponge symbiont isolates as biomaterial degradators and biosorbents (Liu et al., 2019; Lundstedt, 2003; Marzuki, 2016). Toxicity reduction of Cd^{2+} and As^{3+} which previously had a concentration of 100 mg/L, after a few days of contact the concentration of the two types of contaminants decreased significantly, where the experimental conditions

were isolated from environmental influences, so it was strongly suspected that the decrease in the concentration of heavy metal contaminants was the sole role of the isolate (Karimpour et al., 2018). The performance of bio-sorption and biodegradation of marine sponge symbiont isolates produced products in the form of simple organic components and gases. One of the bio-sorption products is H_2O_2 which is toxic to symbiont isolates, so that cells cannot divide further, and even undergo mass death. Bio-sorption products, one of which is the compound H_2O_2 which is toxic to symbiont isolates, so that the cells cannot divide longer, and can even experience mass death. This situation is often referred to as a stopping factor for the bio-sorption process, when the bio-sorption product has gone through producing peroxide compounds (Iyer , Stepanov & Iken, 2013; Okoro,2010).

The stopping factor for the bio-sorption of heavy metals or the biodegradation of PAHs, when the enzymatic reaction process runs and begins to produce acidic substances or compounds of the carboxylic acid group. This process cannot be avoided because the biodegradation method resembles a fermentation reaction process, where the role of enzymes as biodegradators is to destroy the molecular structure of hydrocarbons, especially aromatic polycyclic types. This process raises the suspicion that a biodegradation process that resembles the involvement of enzymes is produced by isolate cells in response to the toxic nature of PAH contaminants. This process also has implications for the performance of the bio-sorption capacity of the isolates against the heavy metals tested (Figure. 1), also has a contribution to the performance of bio-sorption efficiency against heavy metal contaminants as shown in Figure 2. The performance data of the efficiency aspect of bio-sorption showed that the isolate Sp6B.2, the marine sponge symbiont *Auletta* sp., was more aggressive than the isolate Sp8.B1, the marine sponge symbiont bacterium *Callyspongia* sp., especially against the Cd^{2+} test contaminants (Figure. 2a). However, there was a counter performance of the bio-sorption efficiency against the As^{3+} test contaminants, which showed that the aggressiveness of the Sp8.B1 isolate was more dominant than the Sp6.B2 isolate (Figure. 2b). This situation proves that the bio-sorption mechanism of a type of bacteria against heavy metal contaminants is specific and specific to one type of test metal, it does not necessarily produce the same bio-sorption performance on other test metal contaminants. (Bell et al., 2013; Bendouz et al., 2017).

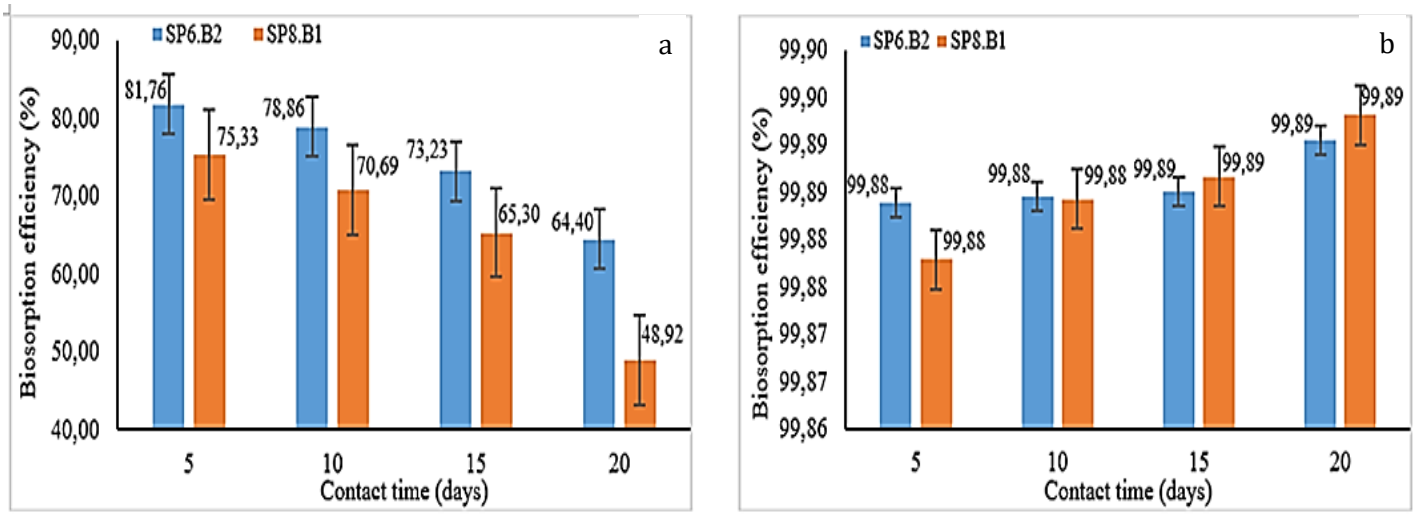

Figure 2. Bio-sorption efficiency of bacterial isolates SP6.B2 and SP8.B1 against heavy metal contaminants. (a) Bio-sorption of ion Cadmium (Cd^{2+}) and (b) Bio-sorption of ion Arsenic (As^{3+}).

Isolates of marine sponge symbiont bacteria have the ability to carry out two reducing roles, namely in addition to being able to carry out the bio-sorption function of heavy metals, they can also carry out the biodegradation function of hydrocarbon components. The biodegradation performance of non-polar compounds in micro symbionts of *Auletta* sp., (Sp6.B2) sponges began to decrease in concentration on days 5 to 20 of contact time, indicated by a decrease in peak height or a decrease in abundance with increasing contact time. This condition can be assumed that the performance of the *Auletta* sp., sponge symbiont Sp6.B2 isolate can carry out the biodegradation function against both types of PAHs tested (naphthalene and anthracene) up to 20 days of contact time. (Table 5).

The biodegradation performance of Sp8.B1 isolates, both against naphthalene and against anthracene, seemed less aggressive than Sp6.B2 isolates. It can be seen that the number of peaks produced is only 6, meaning that there are only 4 types of new peaks that are assumed to be compounds of biodegradation products. Another factor was the peak height of

naphthalene and anthracene in the biodegradation performance of Sp8.B1 isolates which was greater than the peak height of the same component of the biodegradation performance of Sp6.B2 isolates (Table 6), and confirmed on the 20 days of contact time, the abundances of naphthalene and anthracene components after The biodegradation process by the isolate Sp8.B1 was higher than the biodegradation performance of the isolate Sp6.B2, which was analyzed under the same conditions, indicating that the sponge symbiont isolate *Callyspongia* sp., was no longer able to carry out the biodegradation function of the test compounds naphthalene and anthracene. (Arroyo et al., 2021; Baquiran et al., 2020).

Table 5. Biodegradation performance of Sp6.B2 Isolate Sponge symbiont against PAHs contaminants

Peak number	Retention Time (second)	Peak height (10^6)	Quality (%)	Comp. conc. (%)	Compound number
1	6,083	0,50	91	5,66	Cyclotetrasiloxane
2	10,295	6,51	97	63,53	Naphthalene
3	17,482	1,18	59	8,49	3,3,5 Trimethyl bicyclo octan -2,8- dione
4	17,824	0,13	72	1,09	1-Ethyldioxyindol
5	18,571	1,22	95	12,88	Anthracene
6	20,791	0,13	38	0,94	Phosphonic acid
7	21,322	0,34	78	2,86	4,5-Dimethoxyindole
8	21,223	0,45	96	4,56	N,N-Dimethyl-2-phenylcyclopropanet

Table 6. Biodegradation performance of Sp8.B1 Sponge symbiont isolate against PAHs contaminants

Peak number	Retention Time (second)	Peak height (10^6)	Quality (%)	Comp. conc. (%)	Compound namer
1	10,344	18,64	97	75,700	naphthalene
2	17,489	0,47	58	1,03	Thiophene
3	18,592	7,74	95	21,82	Anthracene
4	21,235	0,18	95	0,58	N,N-Dimethyl-2-phenylcyclopropanet
5	24,974	0,14	93	0,39	Phenol
6	26,860	0,15	60	0,48	Tetrephtalic acid

In general, the biodegradation products produced by the two types of sponge symbiont isolates were alcohols, aldehydes, and carboxylic acids. These results can be seen in the FTIR chromatogram analysis (Figure 3). The functional group analysis of the FTIR chromatogram, in particular (Figure. 3a), showed that the PAH naphthalene and anthracene compounds that were in contact with the isolate Sp6.B2, after contact with 20 days, resulted in compounds classified using the Principles of Instrumental Analysis table, including in the region the peak of the range of 4000-2500 cm^{-1} there are types of amine compounds, and alkanes are characterized by the presence of single N-H, C-H, and O-H bonds. In the peak region of the 2000-1500 cm^{-1} range, there are types of aldehydes, alkenes and nitro compounds, characterized by the presence of double bonds such as C=O, C=N, and C=C, while in the peak region the range is 1500-400 cm^{-1} (Table 5) and (Figure. 3a). There are types of aldehydes, alkenes, and alcohol compounds which at this peak are usually known to be the fingerprint region of the infrared spectrum (Fu et al., 2020).

Analysis of the functional groups of the biodegradation performance of Sp8.B1 isolate (Figure.3b), especially the biodegradation products, after being contacted for 20 days, showed that absorption peaks in the absorption peak region of 4000-2500 cm^{-1} contained types of amines, alkanes and alkenes characterized by the presence of N-H, C-H, and single O-H bonds, while the peak region in the range of 2000-1500 cm^{-1} contains types of alkene compounds characterized by the presence of double bonds such as C=O, C=N, and C=C. The peak region of the 1500-400 cm^{-1} range contains types of aldehydes, alkenes, and alcohols (Table 6) and (Figure. 3b) (Nikel et al., 2014; Smułek et al., 2020).

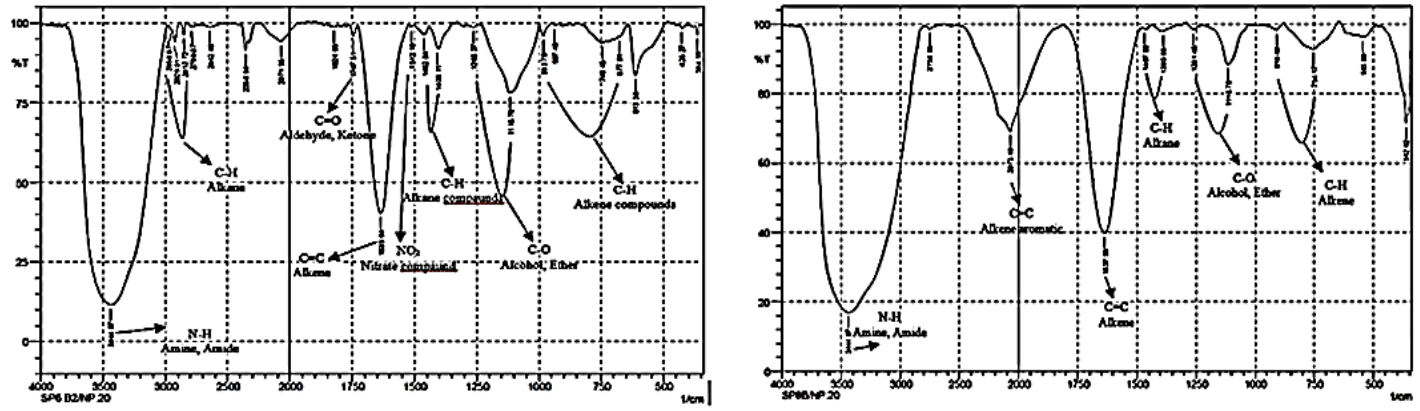

Figure 3. FTIR chromatogram on the performance of the biodegradation of sponge symbiont bacteria isolates against PAHs contaminants, contact time 20 days. (a) Biodegradation performance of isolate Sp6.B2, sponge symbiont *Auletta* sp., and (b) Biodegradation performance of isolate SP8.B1, sponge symbiont *Callyspongia* sp.

Analysis of the functional groups of the FTIR chromatogram which is the biodegradation performance of the Sp6.B2 and Sp8.B1 isolates, it is said that they are relatively not much different, namely the group of organic compounds produced by biodegradation products, so it can be assumed that the aggressiveness of the Sp6.B2 isolate, the symbiotic bacterium of the marine sponge *Auletta* sp., relatively higher in degrading the contaminants of the naphthalene and anthracene tests compared to Sp8.B1 isolates, bacteria isolates of sponge *Callyspongia* sp.

Conclusion

Based on the analysis of the bio-sorption performance of marine sponge symbiont bacteria isolates against heavy metal contaminants and the biodegradation performance of PAHs components, it can be concluded that: (1) Isolates of Sp6.B2 and Sp8.B1 can carry out the function of bio-sorption of Cd^{2+} and As^{3+}, as well as the function of biodegradation of components naphthalene and anthracene aromatic hydrocarbons; (2) Aggressive bio-sorption of Sp6.B2 isolates of Cd^{2+} contaminants compared to Sp8.B1 isolates, and conversely the bio-sorption performance of Sp8.B1 was stronger against As^{3+} contaminants than Sp6.B2 isolates; (3) the biodegradation performance of the Sp6.B2 isolate, both against the naphthalene and anthracene test components, was relatively better than the biodegradation performance of the Sp8.B1 isolate; and (4) biodegradation products of the two types of marine sponge symbiont isolates are simple organic compounds of alcohol, aldehyde and carboxylic acid groups.

Conflict of Interest

The authors declare that there is no conflict of interest.

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