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Bioremediation Performance of Marine Sponge Symbiont Bacteria against Nickel and Mercury Heavy Metal Pollutants

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Abstract*—Heavy metals, mercury, and nickel are toxic contaminants, forming positive ions when concentrated and dissolved, and can accumulate in a specific object, including water. The activity and performance of bacterial bioremediation against toxic heavy metals vary due to bacterial characteristics and internal contaminant factors. This research aims to analyze the activity, performance, and efficiency of bioremediation of nickel and mercury pollutants using marine sponge symbiont bacteria. The bioremediation analysis procedure, suspension of bacteria *Alcaligenes faecalis* strain Cu4-1 (AF), and *Acinetobacter calcoaceticus* strain PHCDB14 (AC) interacted with heavy metal pollutants as contaminants for 15 days. Bioremediation performance and efficiency were measured using AAS. The analysis parameters consisted of the performance, efficiency, and mechanism of bacterial bioremediation against nickel and mercury pollutants. The research results show that the bioremediation performance of AF and AC bacteria can carry out the bioremediation function against Ni^{+2} and Hg^{+2} contaminants. The bioremediation performance of AF bacteria against Ni^{+2} pollutant is, on average 167.64±0.9 mg/L, equivalent to 66.85% bioremediation efficiency, and against Hg^{+2} an average 171.55±0.7 equivalent to the efficiency of 65.47%. The performance of bioremediation of AC bacteria on Ni^{+2} pollutant was 168.92±0.7 or efficiency reached 66.97%, and 145.87±0.8 for $Hg^{+2,}$ equivalent to 58.35% efficiency. The bioremediation performance of AF < AC bacteria against Ni^{+2} pollutants, but against Hg^{+2} pollutants, the bioremediation performance of AF > AC bacteria. The symbiotic bacteria of marine sponges are thought to have bioremediation performance against toxic metal pollutants are bacteria isolated from sponges whose body surface is covered with mucus substances.

***Keywords*—Bioremediation; bacterial; marine sponges; pollutants; nickel, mercury.**

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I. INTRODUCTION

Pollution of heavy metal and hydrocarbon components continues to occur along with industrial activities, especially oil exploration and the dynamics of society in carrying out their lives. Industrial activities and community dynamics in meeting their daily needs generally occur in inland areas, followed by water areas [1]. However, these pollutants eventually end up in lower places because they follow gravity. Thus, as giant containers, marine water areas are most vulnerable to contamination. Heavy metal pollutants include hydrocarbon components, microplastics, pesticide residues, and waste media [1], [2].

Mineral and petroleum mining activities always leave toxic and difficult-to-decompose pollutants. The two components are hydrocarbon compounds, hefty metals, and several inorganic components. Many activities in the ocean occur continuously, especially oil processing and mineral mining activities, transportation activities for oil tankers, cargo ships, passenger ships and fishing vessels, including the cultivation of several types of marine life and the distribution of petroleum through underwater pipelines [3], [4], [5]. Activities in these marine areas have the potential to contribute to the contribution of pollutant materials in the sea, especially in the event of an accident due to these activities, such as leakage of petroleum distribution pipelines, ship transportation accidents, so that the ocean is an area that is very vulnerable to exposure to toxic materials, such as heavy metal pollutants [6], [7].

The above conditions cause the marine area to be a potential source and transmission of various types of diseases, especially marine biota and indirectly threaten human health, as many of today's human needs depend on marine resources.

The biogeochemical cycle that occurs in the marine environment with the diversity of existing ecosystems causes the ocean to have a natural ability to maintain its balance, including detoxifying the toxic nature of toxic components that enter marine waters [8], [9]. Thus, the quality of marine biological wealth can be massively maintained and will continue to support the sustainability of human life [10].

The human population on earth is increasing, including various industrial products, which are increasing massively, allegedly contributing to the increasing penetration of marine ecosystem life [11], [12]. The long-term impact that may occur if this condition occurs continuously is that the ability of the sea to produce materials for primary and secondary human needs is weakening. The quality of marine biological resources is also decreasing, and the threat to human health is increasing due to consuming many products from the sea that are contaminated with toxic materials [2], [13].

Observing the conditions described above requires us to take preventive measures and activities that can help restore the quality of the environment and marine ecosystems by applying certain technologies and methods to create environmental sustainability. Many research results show that there are marine biological resources that can restore and improve the quality of marine ecosystems. One of them is the sea sponge as one of the marine biota known to have the ability and function of biodegradation, bioremediation, and destruction of toxic hydrocarbon components, especially polycyclic aromatic hydrocarbons, and the function of bio-adsorption and reduction of heavy metal pollutants [14], [15].

The activity of bio-adsorption and the reduction of toxic properties of heavy metal pollutants by sponges and several other types of marine biota is one of the natural forms of the sea in maintaining the balance of the ecosystem of the living environment to maintain its natural condition, so that it remains in the equilibrium area [16], [17].

Marine waters are exposed to various types of toxic heavy metal pollutants, where heavy metal pollutants are included in the category of hazardous and toxic materials. If it continues without any prevention and recovery efforts, it is feared that it will impact the quality of the marine environment growth and development of various types of existing biota. It is also inhibited [18], [19].

This condition is certain to impact the quality of other marine biotas, especially fish caught by fishers, giving a chain effect on human health threats. One of the efforts to prevent exposure to heavy metals can be made by exploring the natural ability of the sea to maintain the balance of its environment [20]. The activity is tracing and analyzing the ability of sponges and their bacterial symbionts, which are thought to have bio-adsorption or bioremediation functions and activities against the toxicity of various heavy metal contaminants. Nickel and Mercury ions were chosen as the pollutant for the bioremediation performance test of sponge symbiont bacteria based on the assumption that both pollutants are pollutants with relatively high toxicity levels [21], [22].

Efforts that can be made to maintain the quality of the marine environment are to maintain the growth and population of sponges, including other marine biotas that have bio-adsorption and biodegradation functions [3], [23]. The targets described in this research include analyzing types of sponge symbiont bacteria that have a bio-adsorption function against heavy metal pollutants, analyzing bioremediation performance, and determining the level of bio-adsorption efficiency of marine life sponge symbiont bacteria against heavy metal pollutants [24], [25].

The expected target of this research is the existence of engineering, method innovation, and application of technology in increasing the ability and performance of bioremediation against toxic metal pollutants. It is also hoped that this method can be applied continuously to label realistically and cluster sponge symbiont bacteria with bio-adsorption activity and performance against heavy metal contaminants [26], [27]. This study aims to analyze the ability and performance of marine sponge symbiotic bacteria in remediating or reducing the pollutant properties of nickel and mercury based on decreasing concentrations.

II. Materials and Method

A. Materials and Equipment

Two types of bacteria were used as bio-adsorbents, namely: *Alcaligenes faecalis* strain Cu4-1 (AF), isolated from the marine sponge *Coelocarteria singaporensis* and the bacterium *Acinetobacter calcoaceticus* strain PHCDB14 (AC), isolated from the marine sponge *Callyspongia aerizusa* [28]. These two types of sponges were obtained around Samalona Island, the administrative area of Makassar City, where Samalona Island is part of the Spermonde Archipelago Cluster, which is currently a marine tourism area of Makassar City [6], [29], [30].

The previously selected AF and AC bacteria have been characterized by phenotypic and genotypic isolates, including morphological analysis of the two types of sponges that source bacteria; $Ni(NO_3)_2.6H_2O$ pa., $HgCl_2$ pa., NaCl 0,9 % physiological 0.9% NaCl, sterile seawater, Nutrient Agar (NA), Marine Agar (MA), glycerol 25%, HCl pa, 4% formalin, KCl pa, aquabides, paraffin; and 96% alcohol. Equipment: Spectronic: Spectronic-$20D^+$ merk Shimadzu brand, AAS type AA240FS Variance, Shaker incubator, microscope, universal pH indicator paper, digital pH meter, glass set, incubator, micropipette, Ose round, Laminary AirFlow (LAF), oven, tweezers, pipette, test tubes, analytical balance, handskun, Erlenmeyer spirit lamp, colony counter, and Whatman filter paper no. 41 [31], [32].

B. Sample Preparation

The following procedure prepared samples: Bacterial isolates of sponge symbionts were selected according to the criteria (AF) and (AC). The number of cells of these two isolates was doubled using the culture method on NA media by adding 2 mL of physiological 0.9% NaCl solution, shaking, the AF bacterial suspension was inserted into Erlenmeyer, the volume was made up to 250 mL using physiological 0.9% NaCl. A total of 5 mL of AF and AC isolate suspension were put into a vial as a bioremediation reactor, then the AF and AC bacterial suspension was adapted for 1 x 24 hours in the vial. 5 mL of 250 mg/L (Ni^{+2}) solution was added to each reactor. Samples were agitated using a Shaker incubator. The same was done using a solution of Hg^{+2}, both assumed to be pollutants [4], [33].

The interaction period was 15 days. Every three days, intervals were observed, and measured several

bioremediation parameters, such as pH, optical density (OD) of the media, observations of gas bubbles, and the smell of fermentation. After the interaction period is reached, the test sample is filtered. The filtrate was acidified with HCl (pH 3-4), then concentrated. Ni^{+2} uptake was measured using AAS at $\lambda_{max.}$ 228,8 nm. The same treatment for the Hg^{+2} pollutant at $\lambda_{max.}$ 357,3 nm. The determination of the absorption of each test pollutant must fall within the range of the calibration curve that has been made for each test metal pollutant to see the bioremediation performance of the two types of sponge symbiont bacteria as bio-adsorbents against Mercury and Nickel ion pollutants [34], [35].

Experimental bioremediation of AF and AC bacteria is briefly presented in the schematic.

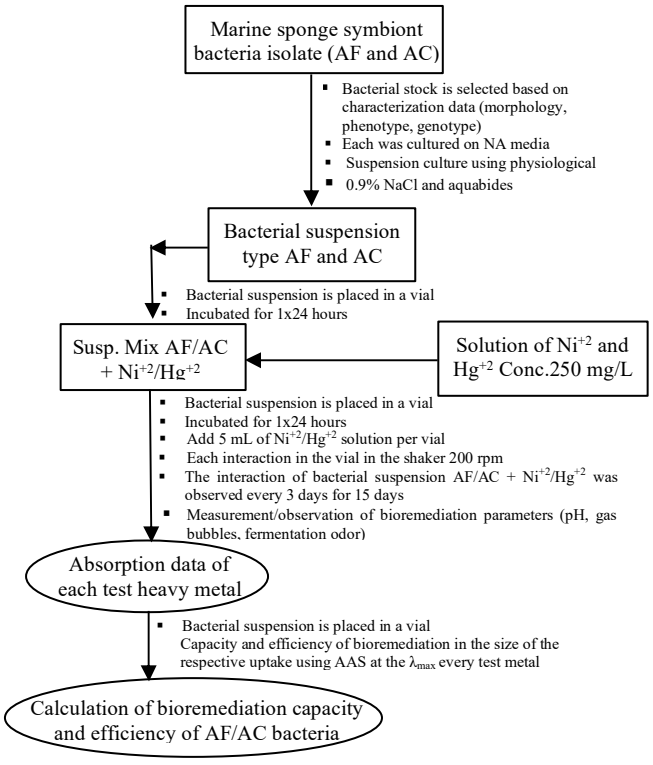

Fig. 1 Experimental flow of AF and AC bacterial bioremediation against Ni^{+2} and Hg^{+2} pollutants

C. Data and Analysis

The analysis of the bioremediation performance of the two types of marine sponge symbiotic bacteria (AF and AC) was based on activity, capacity, and efficiency in reducing or adsorption of heavy metals Ni^{+2} and Hg^{+2}, tests, carried out at the appropriate uptake every three days of interaction using the instrument AAS [2], [36]. The performance and efficiency of bioremediation are determined using the following two equations:

$$Q = \frac{C1 - C2}{m} \, x \, V, \quad (1)$$

$$E\,\% = \frac{C1 - C2}{C1} \, x \, 100\,\%, \quad (2)$$

Note: Q = bio-adsorption capacity; 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), % E = bio-sorption efficiency (%), C1 = concentration before contact (mg/ L); C2 = concentration after contact (mg/L) interaction interval every 3 days.

III. Results and Discussion

The morphological characteristics of the sponges sourced from the isolates of AF and AC bacteria showed that both types of sponges with dark body appearance, the body surface of the sponge *Coelocarteria singaporensis* looked slimy. In contrast, *Callyspongia aerizusa* did not look clear with mucus, but the body surface was smooth. Other characteristics such as growth patterns, oscula, and texture before and after the addition of Et-OH, each in more detail can be seen in the morphology of each marine sponge (Figure 1) [37], [38], [39], [40].

A. Morphology of Marine Sponges Source of Bacterial

The activity and performance of bioremediation of bacterial isolates of AF and AC sponge symbionts against the tested heavy metal pollutants were determined based on several factors thought to affect the achievement of the performance of the heavy metal bioremediation process. The activity of sponge symbiont isolates was analyzed in the bio-adsorption method by grouping the test heavy metals [41], [42]. The details of the morphology of the bacterial source marine sponges are presented in Figure 2.

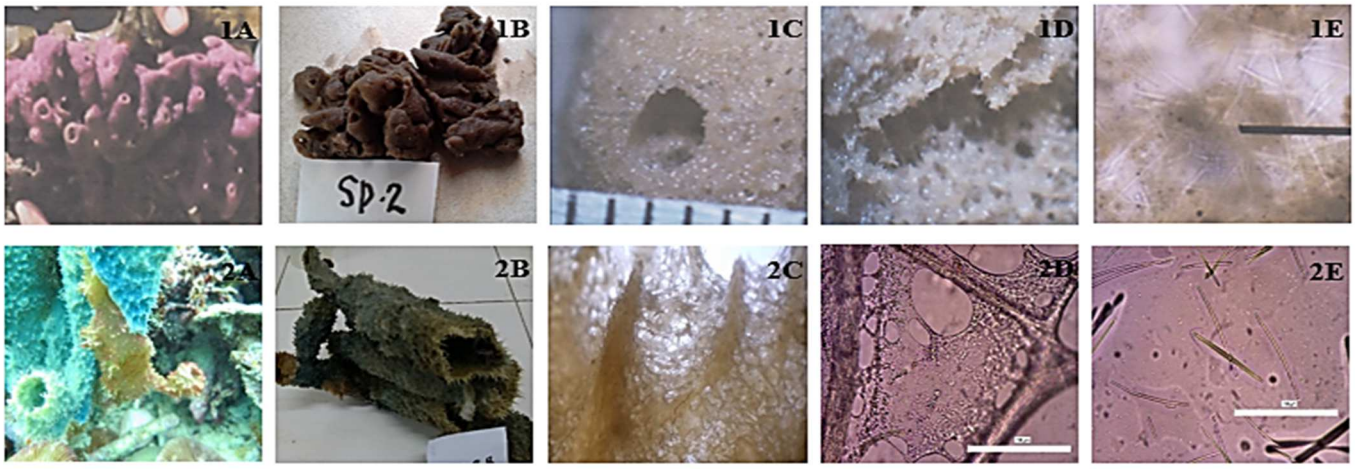

Fig. 2 Sponge morphology *Coelocarteria singaporensis* Family: Chalinidae; Inventor: Carter, 1883A). (1A) Growth forms a massiva, (1B) Oscula scattered along the surface of the sponge body; (1C) staining results with Et-OH; (1D) compressed texture; (1E) surface structure after zooming in 40x [5], [43], [44], and Morphology of sponge *Callyspongia aerizusa* (*Desqueyroux-Faúndez,* 1984) – *Callyspongiidae*. (2A) growth form cylindrical shape sponge, yellow coloured sponge, (2B) colour in Et-OH the colour fades after being prepared with ethanol, (2C) Skeleton primary and secondary fibre, interconnected tract, (2D) fiber high density of fiber, (2E) spicule slender oxea (magnification 40x), no microsclera [45], [46], [47]

B. Bacterial Bioremediation Performance against Test Pollutants

Based on the bioremediation parameters in the bio-adsorption method of AF and AC bacteria on the test pollutant, the two types of bacteria are thought to have bioremediation performance against the test pollutant. Several visible bio-adsorption indicators indicate this ability; (1) there is a change in pH that tends towards a more acidic medium during the interaction period. The decrease in the pH value of the media was seen after the interaction took place in the range of 6 – 12 days, (2) The optical density value increased after three days of interaction, (3) Gas bubbles were detected during the interaction medium after the third day and relatively increased until the twelfth day periods of interaction, (4) Smell of fermentation on the sixth day of interaction. The four indicators are characteristic of the fermentation reaction played by enzymatic-behaving substances [48], [49]. This enzyme substance is thought to be produced by bacteria in response to the test metal pollutants so that the bacterial cells can continue to reproduce by defending themselves. In other words, these two types of bio-adsorbent bacteria can adapt to environments contaminated with Ni^{+2} and Hg^{+2} pollutants [50], [51].

The bioremediation performance of the two types of marine sponge symbiotic bacteria against the test pollutants Ni^{+2} and Hg^{+2} can be seen in the absorption value of each interaction period in 3-day intervals (Table 1). Table 1 shows that the bioremediation performance of AF bacteria against Ni^{+2} pollutants reach an average of 167.64 mg/L and 171.55 mg/L against Hg^{+2}. Both were achieved during an interaction period of 15 days with measurement intervals every three days of interaction [52].

TABLE I
PERFORMANCE OF BIOREMEDIATION OF BACTERIA AF SYMBIONTS ON BIO-ADSORPTION OF HEAVY METAL POLLUTANTS

Type of bacterial test sample	Contact time (days)	Conc. metal ions in media (mg/L)	Bioremediation level (mg/L)	Bioremediation efficiency (% B/V)
Alcaligenes faecalis strain Cu4-1 (AF)	**metal Ni^{+2}**			
	0	250,00	-	-
	3	96,70	153,30	61,32
	6	80,43	169,58	67,83
	9	79,68	170,33	68,12
	12	77,85	172,15	68,84
	15	77,15	172,85	68,14
	average		167,64	66,85
	metal Hg^{+2}			
	0	250,00	-	-
	3	101,08	160,93	59,57
	6	91,08	171,93	63,57
	9	83,30	172,70	66,68
	12	79,68	176,33	68,13
	15	76,45	175,85	69,42
	average		171,55	65,47

The bioremediation efficiency of the two types of bacteria (AF and AC) against the test metal pollutants Ni^{+2} and Hg^{+2} is relatively the same, but in detail, by comparing all the parameters and indicators of bioremediation, it appears that the bioremediation performance of AF-type bacteria isolated from the marine sponge *Coelocarteria singaporensis* is relatively more dominant than AC bacteria, isolates of marine sponge *Callyspongia aerizusa* to Hg^{+2} pollutant and vice versa, AC bacteria bioremediation performance was more dominant than AF bacteria to Ni^{+2} pollutant [55], [56]

TABLE II
PERFORMANCE OF BIOREMEDIATION OF BACTERIA AC SYMBIONTS ON BIO-ADSORPTION OF HEAVY METAL POLLUTANTS

Type of bacterial test sample	Contact Time (days)	Conc. metal ions in media(mg/L)	Bioremediation level (mg/L)	Remediation efficiency (% B/V)
Acinetobact er calcoa-ceticus strain PHCDB14 (AC)	**metal Ni^{+2}**			
	0	250,00	-	-
	3	99,70	150,30	60,12
	6	80,55	169,45	67,78
	9	77,15	172,85	69,14
	12	74,20	175,80	70,32
	15	73,83	176,18	70,47
	average		168,92	66,97
	metal Hg^{+2}			
	0	250,00	-	-
	3	113,88	136,13	54,45
	6	104,43	145,58	58,23
	9	102,15	147,85	59,14
	12	100,33	149,68	59,87
	15	99,88	150,13	60,05
	average		145,87	58,35

The bioremediation efficiency of the two types of bacteria (AF and AC) against the test metal pollutants Ni^{+2} and Hg^{+2} is relatively the same. However, in detail, by comparing all the parameters and indicators of bioremediation. The bioremediation performance of AF-type bacteria isolated from the marine sponge Coelocarteria singaporensis seems relatively more dominant than AC bacteria. Isolates of marine sponge *Callyspongia aerizusa* to Hg^{+2} pollutant and vice versa, AC bacteria bioremediation performance was more dominant than AF bacteria to Ni^{+2} pollutant [55], [56].

Based on the absorption data (Figure 3) strengthens, the assumption that the bioremediation performance of AF bacteria against the test metal pollutant Hg^{+2} is relatively more potent than that of the Ni^{+2} pollutant. This result is thought to be caused by the characteristics and properties of metal ions. In the periodic table of elements, Nickel ions in the periodic system are in group VIIIB period IV, while Mercury ions are in group IIB period VI. According to the position of the elements in the periodic system, the properties and characters (electron affinity, electronegativity, ionization of energy and ion radius) of these elements are different [54], [55].

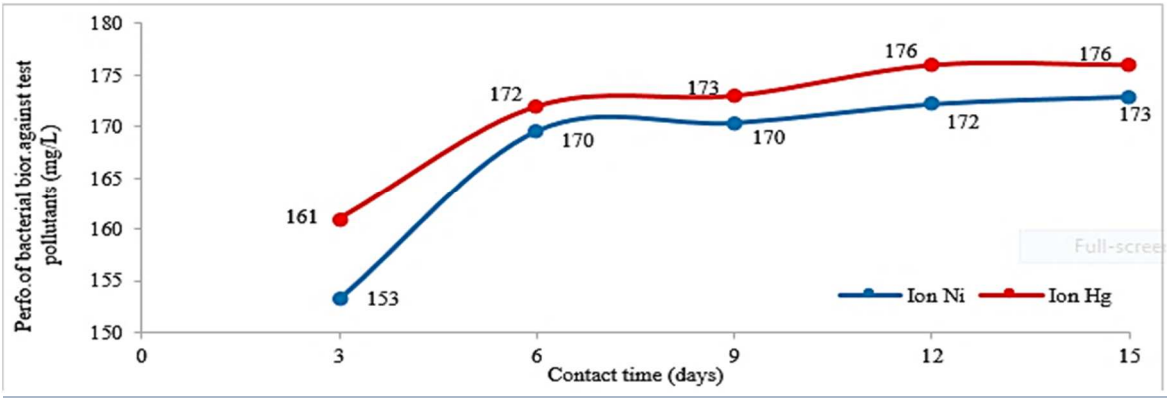

Fig. 3 Bioremediation performance of AF bacteria against test pollutants Ni^{+2} and Hg^{+2}

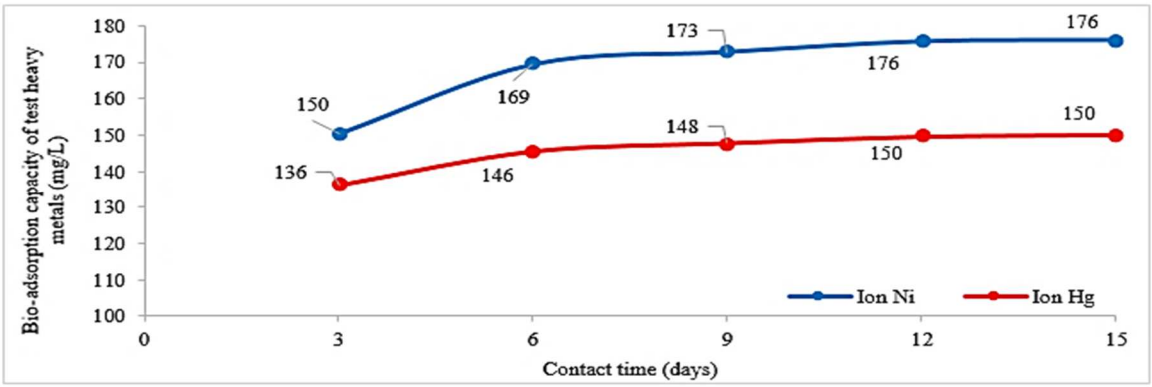

Fig. 4 Performance of AC bacteria bioremediation against test pollutants Ni^{+2} and Hg^{+2}

Bioremediation performance of AF bacteria against test pollutants Ni^{+2} and Hg^{+2}. The bioremediation performance of AC bacteria against the test metal pollutant showed that Ni^{+2} pollutant was easier to remediate than Hg^{+2} metal pollutant (Figure 4). The combination of the two data (Fig. 3 and 4) and (Table 1) shows the bioremediation performance of AF > AC bacteria against Hg^{+2} pollutants, but the contrary to Ni^{+2} pollutants, it appears that the bioremediation performance of AC > AF bacteria [57], [58]. This difference is thought to be due to differences in electronegativity and ionic radii of the two different types of pollutants, so the response of bacteria for adaptation and remediation to the toxic properties of the two types of pollutants is relatively different [59]. However, these differences are only seen in the strength or performance of bioremediation and the efficiency of reducing the toxic properties of the two test pollutant types. This performance shows that the response of bacteria to certain types of pollutants is different [60].

Analysis of the activity and capacity as well as the bio-adsorption pattern shown by the two types of bacteria (AF and AC) of sponge symbionts, as shown in Figures 2 and 3 above, showed that the value of the bio-adsorption capacity of AF bacteria was higher than the performance of AC in heavy metal pollutant bioremediation test [61]. The data (Figures 3 and 3) also confirm that the bioremediation performance of certain bacteria in the bio-adsorption method of heavy metal pollutants is determined by how strong the bacteria can withstand extreme environments contaminated with heavy metal pollutants with high toxic properties. Bacteria in fulfilling bioremediation performance or bioremediation ability against heavy metal contaminants, bacteria as bio-adsorbent material for toxic metal pollutants must have the ability to adapt to environments exposed to heavy metal pollutants, characterized by bacterial cells growing, multiplying rapidly by dividing cells, so that the bacterial population increases. Nutrient availability and oxygen circulation or agitation must be met for bacterial cells to divide in media contaminated with toxic materials [62].

C. Bacterial bioremediation efficiency against test pollutants

The ability of bacteria in bioremediation of toxic heavy metal pollutants can also be seen in the remediation efficiency shown by a type of bacteria as a bio-adsorbent to heavy metal pollutants (Figure. 5). These data show the efficiency of bacterial bioremediation of AF > AC types against Hg^{+2} pollutants. On the contrary, AC > AF bacterial remediation efficiency against Ni^{+2} pollutants. These results strengthen the conclusions taken based on reviewing aspects of the bioremediation performance of the two types of bacteria against Ni^{+2} and Hg^{+2} pollutants [63], [64].

Table 3 shows that the bioremediation performance of AF and AC bacteria against nickel and mercury ion test pollutants, based on adsorption data, shows that the bioremediation of AF bacteria against Ni^{+2} pollutants on average (167.64±0.9 mg/L) is equivalent to an average remediation efficiency of 66.85%, and an average Hg^{+2} pollutant of 163.69±0.7 or equivalent to an efficiency of 65.47%. The average bioremediation performance of AC bacteria against Ni^{+2} pollutants reached 168.92±0.7 or equivalent to an average efficiency of 66.97%, while the average bioremediation performance against Hg^{+2} pollutants

was 145.87±0.8, equivalent to the percentage of bioremediation efficiency reaching 58.35% [60],[65].

Absorption regression equation analysis as the bioremediation performance of the two types of bacteria AF and AC, including the correlation (R^2) (Table 3) between the bioremediation performance of bacteria and heavy metal pollutants, indicates that other external influences are acting on bioremediation, in addition to the internal factors of bacteria and the characteristics of heavy metal pollutants. This influence can come from external factors of bacteria as bio-adsorbents, for example, the availability and adequacy of nutrients in the form of NPK, the fulfilment of oxygen needs, whether the interaction time is right, including the ideal pH by the pH of the bacteria that can work to perform the bioremediation function against pollutants to the fullest [66], [67].

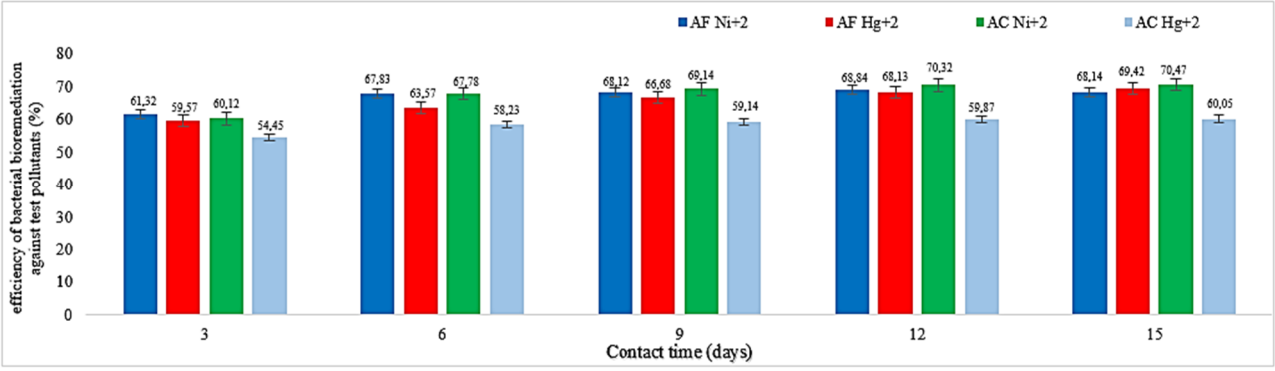

Fig. 5 The relationship between bioremediation efficiency measured in the form of bio-adsorption of AF and AC bacteria on heavy metal Pollutants tested Ni^{+2} and Hg^{+2} based on contact time (days)

The pattern, capacity and level of bioremediation or bio-adsorption of AF and AC bacteria on Ni^{+2} and Hg^{+2} pollutants were seen as the performance of bacterial bioremediation, identified as fast at the initial contact period (day-3 interaction), then decreased significantly in line with the increase in interaction time, indicating that the bioremediation that took place had an ionic reaction mechanism. The ionic reaction mechanism that takes place is based on the balance of negative ions provided by bacteria to the number of positive ions contributed by toxic metal pollutants. The mechanism of this bioremediation is almost identical to that of the EDTA chelating reaction against the central metal ion in complex compound systems [68], [69].

TABLE III
PERFORMANCE RESUME OF BIOREMEDIATION OF AF AND AC BACTERIA, MARINE SPONGE SYMBIONTS AGAINST TOXIC HEAVY METAL POLLUTANTS

Type of bacterial test sample	**Rated parameters**	**Performance of Bio-Adsorption Bacteria against heavy metals Test**	
		Ni^{+2}	**Hg^{+2}**
Alcaligenes faecalis strain Cu4-1 (AF)	Bio-adsorption rate (mg/L)	167,64±0,9	171,55±0,7
	Heavy metal regr. equation test	y = 2,0333x + 145,50	y = 1,3892x + 155,14
	Correlation value (R^2)	0,9425	0,6577
Acinetobacter calcoaceticus strain PHCDB14 (AC)	Bio-adsorption rate (mg/L)	168,92±0,7	145,87±0,8
	Heavy metal regr. equation test	y = 1,9367x + 151,49	y = 1,07x + 136,24
	Correlation value (R^2)	0,73	0,7833

The addition of interaction time at the interval of the second 3 days or the sixth day of interaction did not show an increase in the bioremediation performance of the two types of bacteria against both types of nickel and mercury ion pollutants [11]. This performance indicates that during the interaction period, the bacterial cells were assumed to be saturated and almost there were no bacterial cells to defend themselves because the bacterial cells had been contaminated, even dead [3], [70]. The process of reducing the toxic properties of metal pollutants Ni^{+2} and Hg^{+2} by marine sponge symbiont bacteria resembles the chelation reaction between the ligand and the central atom in the complex formation reaction, wherein this bioremediation of the pollutant Ni^{+2} or Hg^{+2} acts as the central atom and undergoes oxidation while the bacterial cells as ligands [71].

IV. CONCLUSION

Combination and interpretation of bacterial bioremediation performance data against toxic metal pollutants showed that: (a) *Alcaligenes faecalis* strain Cu4-1 (AF), marine sponge symbiont *Coelocarteria singaporensis* and bacteria *Acinetobacter calcoaceticus* strain PHCDB14 (AC), marine sponge symbiont *Callyspongia aerizusa* both can carry out the function of bioremediation against contaminants Ni^{+2} and Hg^{+2}, (b) The bioremediation performance of AF bacteria against pollutants Ni^{+2} is on average 167.64±0.9 mg/L, equivalent to a remediation efficiency of 66.85%, and concerning Hg^{+2}, an average of 171.55±0.7, equivalent to an efficiency of 65.47%, while the performance of AC bacteria bioremediation against Ni^{+2} pollutants averaged 168.92±0.7

or efficiency reached 66, 97%, and concerning Hg^{+2} in the range of 145.87±0.8, equivalent to the percentage efficiency of 58.35%, (c) Bioremediation performance of bacteria AF > AC against Hg^{+2} pollutants, but against Ni^{+2} pollutants, the bioremediation performance of bacteria AC > AF, (d) Selection of the type of bacteria suspected to have performance In bioremediation of toxic metal pollutants, bacteria isolated from sponges whose body surface is covered with mucus substance should be selected.

NOMENCLATURE

Ni^{+2}	the Nickel ion pollutant that is oxidized in the form of $(NO_3)_2.6H_2O$
Hg^{+2}	the Mercury ion pollutant that is oxidized in the form of $HgCl_2$.
Phenotype	analysis carried out using a standard 16-parameter biochemical test to determine the potential and ability of bioremediation.
Genotype	analyses the genetic character of isolates to see DNA pairs and determine the bacterial species of sponge isolates used in pollutant bioremediation.
$y = ax \pm b$	the regression equation for the bioremediation performance of symbiont bacteria against pollutants
Cu4-1	the specific code that shows the characteristics of *Alcaligenes faecalis* (AF) bacteria that are genetically inherited.
PHCDB14	the specific code that shows the characteristics of *Acinetobacter calcoaceticus* (AC) genetically inherited bacteria.

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