Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(3), 953-964 | ISSN:2251-6727

Analysis of Bio-adsorption Activity of Marine Sponge Symbiont Bacteria against Lead and Arsenic Pollutants

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(Received: 16 March 2025 Revised: 20 April 2025 Accepted: 01 May2025)

KEYWORDS

Bio-adsorption activity; Bacteria symbiont Sponge; Lead; Arsenic; Pollutants

ABSTRACT:

Introduction: Arsenic, lead and other heavy metals are toxic pollutants, forming positive ions when concentrated and dissolved in an object. Heavy metal pollutants form accumulations in water areas and are non-biodegradable contaminants.

Objectives: Bacterial bio-adsorption activity against toxic heavy metal pollutants varies due to the influence of bacterial characteristics and internal characteristic factors of metal ion pollutants. This research aims to analyzed the activity, capacity, pattern efficiency and bio-adsorption of As^{+3} and Pb^{+2} pollutants by symbiont bacteria of marine sponges *Bacillus licheniformis* strain ATCC 9789 (BL) and *Pseudomonas stutzeri* strain SLG510A3-8 (PS).

Methods: The analytical method uses a suspension of two types of marine sponge symbiont bacteria interacted with two types of dissolved heavy metal pollutants as contaminants for 20 days. Bio-adsorption capacity was measured using Atomic Absorption Spectrophotometer.

Results: Performance and bio-adsorption capacity of BL bacteria are relatively more dominant than PS bacteria to heavy metal pollutants As^{+3} and Pb^{+2}. Bio-adsorption capacity and efficiency of BL bacteria on $As^{+3} > Pb^{+2}$ pollutants, similarly, the bio-adsorption activity of PS bacteria on $As^{+3} > Pb^{+2}$. The average efficiency of BL bacteria bio-adsorption to As^{+3} (93.68%) and Pb^{+2} (64.49%), while the average efficiency of PS bacteria to As^{+3} pollutants (76.13%) and to Pb^{+2} (59.50 %).

Conclusions: Bacteria Bacillus licheniformis strain ATCC 9789 (BL) sponge symbiont Auletta sp. and Pseudomonas stutzeri strain SLG510A3-8 (PS) sponge symbiont Hyrtios erectus, had bio-adsorption activity against heavy metal pollutants As^{+3} test and Pb^{+2}. The bio-adsorption capacity and performance of BL bacteria is more dominant than PS bacteria, both against pollutants As^{+3} and Pb^{+2}. The bio-adsorption pattern of BL and PS bacteria against heavy metal pollutants follows the growth phase of bacteria.

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JCHR (2025) 15(3), 953-964 | ISSN:2251-6727

1. Introduction

Sea areas, lakes and rivers are the most vulnerable water areas to be polluted by petroleum sludge waste. The main components of crude oil are hydrocarbons and several types of inorganic particles, especially toxic heavy metals (Gawad, 2018; Monem et al., 2013). Almost every effort to exploit and explore petroleum produces waste. The hydrological and gravitational processes are the pathways for this petroleum sludge waste which empties into the sea (Marzuki et al., 2020a; Rahman et al., 2018). Activities and interactions between components in the ocean are very religious, such as ship transportation, marine biota catching activities, including the petroleum processing industry and several other mining businesses, so that the potential for accumulation of petroleum waste and toxic heavy metals is very high (Marzuki et al., 2021a). This condition is slightly offset by the ability of the marine biological environment to naturally detoxify these toxic properties to maintain its balance, however, the balance of marine ecosystems tends to decrease due to increased volume of contaminants accumulated in the sea, especially toxic heavy metal components (Mani et al., 2024). The activity of biodegradation of hydrocarbon components and bio-adsorption of heavy metals by sponges and several types of marine life is one form of maintaining the balance of the marine biological environment in its natural condition, so that it remains in the area of equilibrium consistency (Marzuki et al., 2021b; Ahmad et al., 2019). Marine waters that are contaminated with various types of heavy metals with high toxicity, including in the category of hazardous and toxic materials, are feared to have an impact on reducing on the quality of the marine environment and its growth and development of various types of biota in it, ultimately affecting the quality of fish caught by fishermen and other foodstuffs sourced from sea, has a chain effect on health threats to humans due to consumption of marine products (Marzuki et al., 2021c; Beazley et al., 2013). Observing this situation, concrete efforts are needed to help minimize the massive contamination and accumulation of toxic heavy metal contaminants into the oceans. One of the efforts to prevent exposure to heavy metals can be done through exploration of the natural ability of the sea to maintain environmental balance. The form of activity is in the form of tracing and analyzing the ability of sponges and their bacterial symbionts which are thought to have the function and activity of bio-adsorption and reduction of the toxicity of various types of heavy metals (Marzuki et al., 2021d; Busch et al., 2020).

The population of sponges in the ocean area is very large, with all the uniqueness possessed by these sponges, such as the way of life and obtaining food by filter feeders, symbiosis with several types of microorganisms, especially bacteria, the ability to consume hydrocarbon components as a source of energy to sustain life, so it is very possible to have function and biodegradability inherent in sponges and their micro-symbionts (Jokinen et al., 2020; Huguenin et al., 2018). Heavy metal bio-adsorption activity and function can also be played by micro symbionts and sponge biomass. The role of reduction and possible destruction of chemical pollutant components shown by several types of sponges and sponge symbiont bacteria in heavy metal bio-adsorption activity is interesting for further analysis. (Rusli et al., 2021).

2. Objectives

This research is focused on analyzing the activity pattern of sponge symbiont bacteria on the bio-adsorption of toxic heavy metals. Several previous studies have concluded that not all types of sponges and bacterial symbionts that exist have the ability to bio-adsorb heavy metals (Karimpour et al., 2018). Various literatures related to the function of bio-adsorption of heavy metals by microorganisms, especially sponge symbiont bacteria, explain that there are criteria and requirements that must be considered in selecting the type of sponge and bacterial symbionts that are thought to have a role in bio-adsorption of heavy metals, including: Potential sponge morphology in the bio-adsorption function, if the sponge in its living habitat is suspected of being exposed to petroleum waste, then the sponge surface is generally slimy or dark in color (Khabouchi et al., 2020; Knobloch et al., 2018). Threats to sponges that live in areas contaminated with petroleum and heavy metals, always try to independently maintain their lives, thus stimulating themselves to have symbiosis with various types of microorganisms. The symbiotic bacteria are in a comfort zone and protected from the ferocity of ocean currents (Khan et al., 2021; Marzuki et al., 2020b).

These conditions are used by bacteria to adapt, grow, develop and many of these symbiotic bacteria produce various substances with enzyme characteristics. This enzyme character substance is used by sponges to

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JCHR (2025) 15(3), 953-964 | ISSN:2251-6727

neutralize various predators around them as biological weapons (Maldonado et al., 2021). Substances of this enzyme character, in the process of sponge metabolism are mobilized to the surface of the sponge body, so that most of the surface of the sponge body is covered by this mucus substance. The phenotypic characteristics of the bacteria isolated from the body of the sponge by having symbiotic bacteria of the Gram positive group and at least being able to react with several reagents in the isolate biochemical test, including reacting positively with MR-VP reagent, catalase, lactose and citrate reagents. Isolation of sponge symbionts met the genotype criteria, i.e. at least the length of the nitrogen wet composition (DNA) from the sequenced results reached a minimum of 900 pairs. (Marzuki et al., 2021e; Pita et al., 2018).

The specifics described in this research include analyzing the types of sponge symbiont bacteria that have a bio-adsorption function against heavy metals, the visible bio-adsorption pattern, the reaction mechanism for the formation of complexes or chelating, the bio-adsorption performance of bacteria against heavy metal pollutants is affected by the position of the metal in the periodic arrangement. Periodic system periodical arrangement of elements and physic and chemical observations that appear in the interaction medium during the bio-adsorption process (Sobrinho et al., 2020; Li et al., 2020). This research is one part of several series of researches that are carried out continuously as an effort to realize the desire to compile labeling and clustering of sponge symbiont bacteria that have bio-adsorption activity against heavy metals. The bacterial clusters obtained were combined into one group as a consortium of bacteria and given the term metalloclastic bacteria, which is a group of bacteria with high ability as toxic adsorbents of heavy metals (Liu et al., 2017).

3. Methods

Materials and Equipment

The material used consisted of two types of symbiotic marine sponge bacteria, i.e. *Bacillus licheniformis* strain ATCC 9789 (BL), isolated from the sponge *Auletta* sp; *Pseudomonas stutzeri* strain SLG510A3-8 (PS), isolate the sponge *Hyrtios erectus*. The two types of sponge isolates are stock owned by researchers as in previous publications; As_2O_3 pa.; $Pb(NO_3)_2.6H_2O)$ pa.; NaCl 0,9 % fisiologis; sterile seawater; Agar Nutrients; Marine Agar (MA); 25% glycerol; formalin 4%; aqubides; HCl pa.; KCl pa.; standard biochemical test reagent, paraffin; alcohol 96% and whatman filter paper 41. Equipment: Spectronic-20D$^+$ Shimadzu; Atomic Adsorption Spectrophotometer (AAS) type AA240FS Variance; PCR, Shaker incubator; incubators; Microscope; universal pH indicator paper; digital pH meter; glass sets; laminar air flow (LAF); micropipette; ose round; oven; tweezers; pipette; test tube; analytical balance; Erlenmeyer; hand scoons; spirit lamp and colony counter (Mamun et al., 2019).

Experimental

Cell propagation in sponge symbiont isolates was selected according to the criteria, namely BL and PS bacteria. The doubling of the cells of these two isolates used the culture method on NA media by adding 2 mL of physiological 0.9% NaCl, then shaking, each BL and PS bacterial suspension was put into Erlenmeyer, then the volume was made up to 250 mL using physiological 0.9% NaCl. A total of 5 mL of BL and PS isolate suspensions were put in a vial as a bio-adsorption reactor, then the BL and PS bacterial suspensions were adapted for 1 x 24 hours. In each vial, 5 mL of solution (As^{+3}) and (Pb^{+2}) are assumed to be pollutants with a concentration of 250 mg/L each. Sample in Sheaker incubator. The interaction period was 15 days, every 5 days intervals were observed measuring the pH and optical density of the media. After the interaction period is reached, the test sample is filtered. The filtrate was acidified with HCl (pH 3-4), then concentrated as a pollutant with a concentration of 250 mg/L each. Pb^{+2} uptake was measured using AAS at λ max 224.6 nm and λ max 193.7 nm for As^{+3}. The determination of the absorption of each type of heavy metal test must fall within the range of the calibration curve that has been made for each test metal (Marzuki et al., 2022; Liu et al., 2019).

Analysis

The activity, capacity, percentage and performance as well as the bio-adsorption efficiency of marine sponge BL and PS symbiotic bacteria against heavy metals As^{+3} and Pb^{+2} were determined using the following equation:

$$Y = a \pm b X, \qquad (1)$$

$$Q = (C1\text{-}C2)/m \times V, \qquad (2)$$

$$E\,\% = (C1\text{-}C2)/C1 \times 100\,\%, \qquad (3)$$

Note: Y = Instrument response (absorption), X = metal analysis concentration in solution (mg/L), a = intercept value, b = slope

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value, Q = bio-sorption capacity; m = bacterial cell count (assuming 1 mL of bacterial suspension is equivalent to 1 g bio-sorbent (mg)), C1 = concentration before contact (mg/L); C2 = concentration after contact (mg/L), interaction interval every 5 days; V = cell of volume of solution (L) and % E = bio-sorption efficiency (%).

4. Results

The activity, work pattern, mechanism and performance of BL and PS sponge symbiont isolates against heavy metals were determined based on the consideration of several factors thought to have an effect on the achievement of the performance of the heavy metal bio-adsorption process. The activity analysis of sponge symbiont isolates in the bio-adsorption method was carried out by grouping the heavy metals in the test. Characteristics of bacterial source marine sponges as bio-adsorbents, phenotypic and genotypic characteristics of bacteria, are presented in Table 1.

Table 1. Characteristics of marine sponges, Gram group, bacterial phenotype and Genotype Bio-adsorbent of toxic heavy metal pollutants

Characteristics	Observed parameters	Isolate Type	Isolate Type
Bacterial source sponge morphology	Species	*Auletta* sp Slimy dark blackish	*Hyrtios erectus* Squishy Dark brown
	Surface shape		
	Color in habitat		
Gram stain	Form	basil	basil
	Spores	There are spore	no spores
	Group	Gram positiv (+)	Gram negative (-)
Phenotypic Characteristics (Reaction to some Biochemical reagents)	Tryptone Broth	+	-
	Nitrate Broth	+	+
	Lactose Broth	-	-
	Agar S. Citrate	+	+
	Methyl Red (R-VP Broth)	+	+
	Voges P.(R-VP Broth)	+	+
Genotypic Characteristics	Bacterial sequencing	18 – 917	16 – 906
	Bank gene sequences	10010 – 10953	2142843 - 2143781
	Identity (%)	934/957 (98 %)	913/944 (97 %)
	Species	*Bacillus licheniformis* strain ATCC 9789 (BL)	*Pseudomonas stutzeri* strain SLG510A3-8 (PS)

Based on Table 1 above, it shows that the morphological aspect of the sponge source of bio-sorbent bacteria is a sponge whose body surface is covered by a layer of mucus. This mucus layer is thought to be a substance with enzyme characteristics that is intentionally produced by sponge symbiont bacteria to protect themselves from predators of toxic heavy metal pollutants (Bashir et al., 2019). In Table 1, it also shows that the potential bacteria in the bio-adsorption of heavy metal pollutants are Gram positive and negative groups, with the ability to form positive reactions with several biochemical reagents. The two bacterial isolates were bacillus bacteria for the BL type and Pseudomonas for the PS type. Characteristics that appear based on the genotypic aspect indicate that the two types of marine sponge symbiont bacteria (BL and PS) have a sequence range of 890 – 899 (Tang et al., 2017; Ma et al., 2014) Observing the physic-chemical parameters in the bio-adsorption media in Table 2, it appears that the sponge symbiont bacteria showed the bio-adsorption performance of heavy pollutants during the interaction period of 5 – 10 days, based on the observation of parameters of changes in pH values, tend to be acidic, air bubbles and odors. The bio-adsorption activity of the bacterial isolates was still visible until the interaction period of 15 days, and began to show a significant decrease in the interaction period after the 15-20 days. The target to be achieved in the genotypic analysis using PCR is to find out the type of the two types of isolates isolated from marine sponges, as shown in Table 2.

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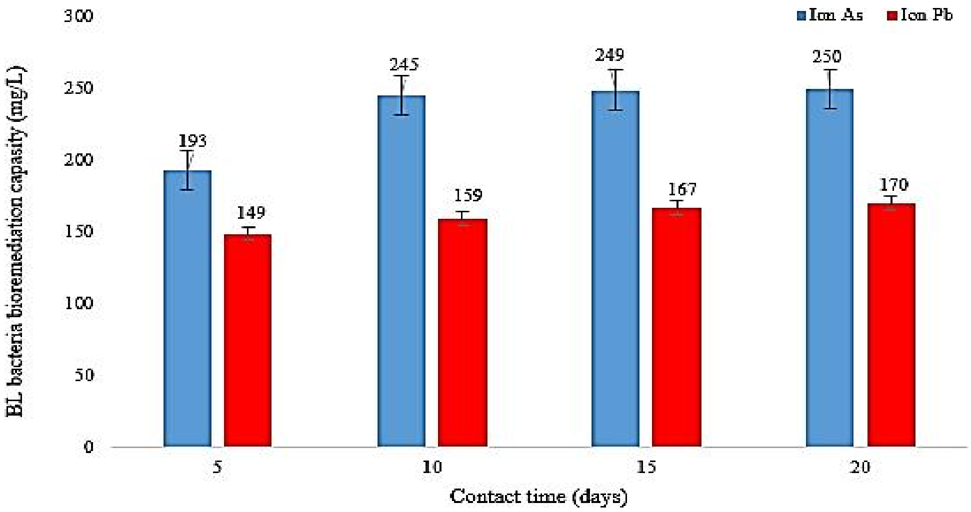
Table 2. Bio-adsorption parameters observed during the interaction between bacterial suspensions of BL and PS sponge symbionts against lead and arsenic pollutants

Type of bacterial test sample	Contact time (days)	Physic-chemical parameters of bio-adsorption media					
		As^{+3}			Pb^{+2}		
		pH	AB	FR	pH	AB	FR
Bacillus licheniformis strain ATCC 9789 (BL)	0	7,2	-	-	7,2	-	-
	5	6,7	O	Δ	7,1	O	Δ
	10	6,2	O	Δ	6,6	O	Δ
	15	6,5	O	Δ	6,7	O	-
	20	6,6	O	Δ	7,0	-	-
Rata-rata	5	6,65			6,93		
Pseudomonas stutzeri strain SLG510A3-8 (PS)	0	7,2	-	-	7,2	-	-
	5	7,0	O	Δ	7,1	O	Δ
	10	6,5	O	Δ	6,7	O	Δ
	15	6,4	O	Δ	6,6	-	-
	20	6,6	O	-	7,0	-	-
Average	5	6,75			6,95		

Note: AB = Air bubble; FR = Fermentation reaction character; O = Air bubbles formed; Δ = Smell the character of a fermentation reaction.

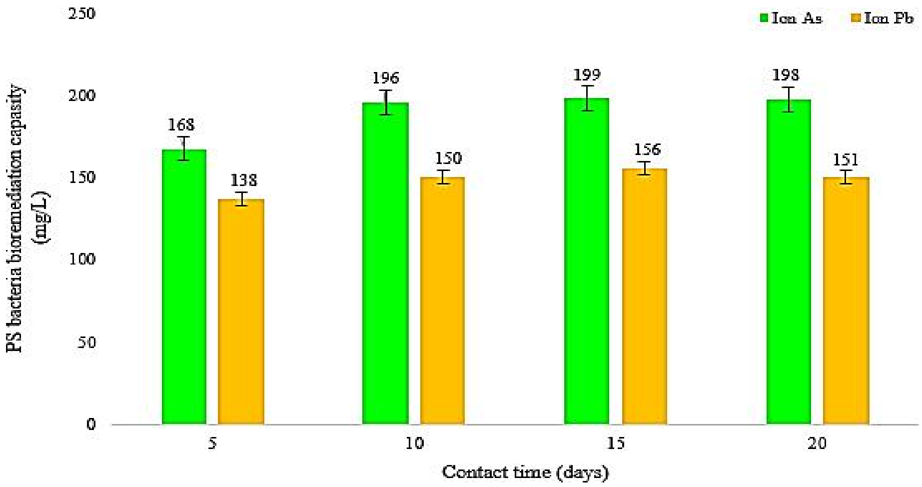
This condition is observed because of the influence of internal factors such as heavy metal pollutant. Other influences, such as the nature and strength of the reducing agent, electron affinity, and the size of the pollutant metal ion, are in accordance with previous studies (Sharma et al., 2021; Tenea et al., 2020). Table 2 above is in accordance with the results of the calculation of the capacity and percentage of bio-adsorption efficiency of sponge symbiont bacteria on heavy metal pollutants, observing the influence of internal factors of heavy metal pollutant (Mariwy et al., 2021).

Figure-1. The relationship between the bio-adsorption of performance of BL bacteria on the tested Heavy metal pollutants (As^{+3} and Pb^{+2}) based on contact time (days).



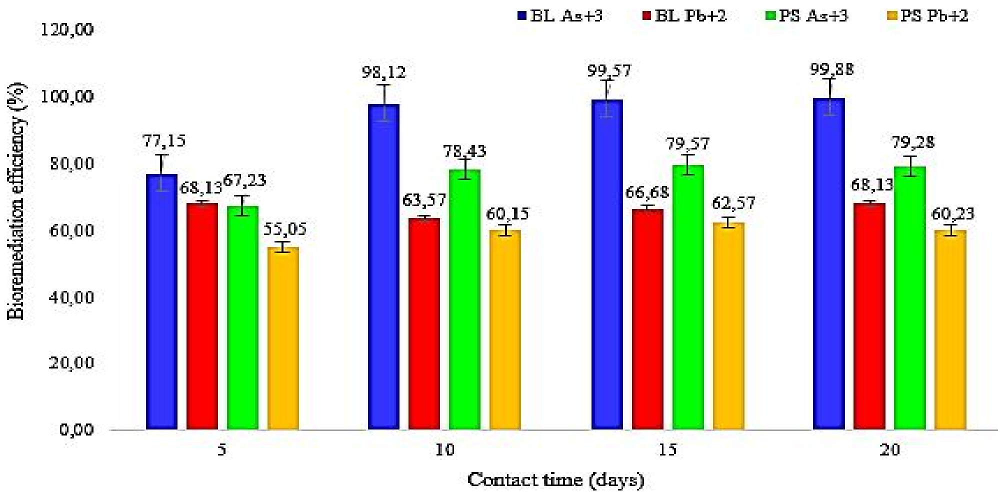
Analysis of the bio-adsorption activity of BL bacteria Figure 1, based on elemental groups in the periodic system, showed that the activity and bio-adsorption performance of PS bacteria were more dominant against metal pollutants As^{+3} than Pb^{+2}. These results are also thought to be due to the characteristics of the two types of heavy metal pollutants as shown by PS bacteria in Figure 2.

Figure-2. The relationship between the bio-adsorption of performance of PS bacteria on the tested Heavy metal pollutants (As^{+3} and Pb^{+2}) based on contact time (days).



Comparing the activity of the two types of bacteria (BL and PS) on the bio-adsorption of lead and arsenic heavy metal pollutants, it was shown that the activity and bio-adsorption performance of BL bacteria were more dominant than PS bacteria against these two types of pollutants in Figures 1, 2 and 3.

Figure-3. The relationship between bio-adsorption efficiency of BL and PS bacteria with Heavy metal pollutant As^{+3} and Pb^{+2} based on contact time (days)



The data shown in Table 3, shows that the activity and bio-adsorption capacity of BL sponge symbiont bacteria against heavy metal pollutants As^{+3} and Pb^{+2} showed differences. The average bio-adsorption performance of BL bacteria on metal As^{+3} (234.20 ± 0.2 mg/L) was equivalent to the bio-adsorption efficiency of ± 93.68%, higher than the bio-adsorption performance of PS bacteria on pollutants. The same (As^{+3}) with an average value of bio-adsorption (190.21 ± 0.5 mg/L) equivalent to 76.13%. The same thing also shows that the bio-adsorption performance of BL bacteria on metal pollutants Pb^{+2} with a value of 161.22 ± 0.7, equivalent to the bio-adsorption efficiency of 64.49%, higher than the bio-adsorption performance of PS bacteria on the test pollutant (Pb^{+2}). The test pollutant (Pb^{+2}) with an average bio-adsorption value of 149.17 ± 0.3, or equivalent to a bio-adsorption efficiency of 59.50% (Kamaruddin et al., 2021; Marzuki et al., 2021b).

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Table 3. Summary of activity, performance and bio-adsorption efficiency of marine sponge BL and PS symbiont bacteria on heavy metal pollutant assays

Type of bacterial test sample	Rated parameters	Bio-adsorption capacity and average efficiency of bacterial isolates against test heavy metal pollutants	
		As^{+3}	Pb^{+2}
Bacillus licheniformis strain ATCC 9789 (BL)	Bio-adsorption average capacity (mg/L)	234,20±0,2	161,22±0,7
	Average efficiency of bio-adsorption (%)	93,68	64,49
	Heavy metal regression equation test	y = 17,41x + 190,68	y = 7,1975x + 143,23
	Correlation value (R^2)	0,6624	0,9616
Pseudomonas stutzeri strain SLG510A3-8 (PS)	Bio-adsorption average capacity (mg/L)	190,21±0,5	149,17±0,3
	Average efficiency of bio-adsorption (%)	76,13	59,50
	Heavy metal regression equation test	y = 9,3225x + 167,01	y = 4,49x + 137,53
	Correlation value (R^2)	0,6543	0,5343

The analysis of the results of the calculation of the regression equation (y = a ± b X) according to equation (1), and the correlation value (R^2), shows that there are several other factors that are thought to have an effect on the activity and performance of the bio-adsorption of sponge symbiont bacteria on heavy metal pollutants. The influence is thought to originate from internal factors of the test metal ion and internal factors of bacteria as biomaterials for adsorption of heavy metal pollutants. The relationship between the performance bacterial bio-adsorption on heavy metal pollutants can be seen in the respective R^2 values.

5. Discussion

The results of the AAS measurements were in the form of absorption and calculation of the capacity and efficiency of the bio-adsorption of sponge symbionts against several test metal pollutants using equations (1), (2) and (3), the maximum occurred at the contact period of days 5 – 10. Performance of bio-adsorption bacteria were still visible until the 15th day of contact, but decreased significantly and continued to slope until the 20th day of the interaction period. The data in Table 2, illustrates that the bio-adsorption activity of sponge symbiont bacteria on the heavy metal pollutants in the test followed the pattern and phase of bacterial growth (Melawaty et al., 2014). Further analysis showed that the pattern and mechanism of action of sponge symbiont bacteria in the bio-adsorption method against heavy metal pollutants resembled the EDTA chelate formation reaction against heavy metals, so it was understood that the maximum bio-adsorption activity took place during the initial interaction period of 5-15 days, then decreased. Significantly following the stationary phase and mass death of bacterial cells (Mostafidi et al., 2021; Wilk et al., 2021). The bacterial activity that still occurs during the 15 - 20 day contact period is influenced by several factors, including the ability of bacterial cells to survive in a toxic environment contaminated with heavy metal pollutants, types of bacteria, and internal factors from heavy metals. The figure below in Figures 1 and 2 provides an indication of how the performance and bio-adsorption pattern of sponge symbiont bacteria against heavy metal pollutants tested. (Marzuki et al., 2021f).

Figure 1, shows that the bio-adsorption activity of BL bacteria against metal pollutant As^{+3} is higher than that of metal Pb^{+2}. This condition is thought to be influenced by many factors, both internal factors of the tested metal pollutants and the type of bacteria used. It is known that the electron affinity and electronegativity of As^{+3} tend to be larger than that of Pb^{+2}, whereas the radius of Pb^{+2} is relatively larger than that of As^{+3}. The characteristics of the two types of test pollutants are thought to contribute to the ability of bacteria to form complex reactions or formation of chelating (Aljahdali & Alhassan, 2023; Selvin et al., 2009). The effect of the oxidation number of the two types of test metal pollutants is also seen as one of the factors that contribute to the activity and

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performance of the bio-adsorption of BL and PS bacteria. (Aljahdali & Alhassan, 2023; Marzuki et al., 2015). It is known that the oxidation number of the As ion is +3, while the Pb ion is +2, even though the effect of this oxidation number, theoretically will have an impact on Pb^{+2} being more easily adsorbed by bacteria compared to As^{+3}, but this aspect is an exception, because deviate from the regular pattern of activity based on the analysis of the influence of internal factors of metal ion pollutant (ionization energy, electron affinity, electronegativity and ion radius) (Muszyńska et al., 2019). This can be understood with the assumption that the process of formation of the oxidation reaction does not occur all at once, but takes place in stages to form the oxidation number +1 to +2 and finally to +3, if the required oxidation reaction conditions can be met.

It is also known that the mechanism of bacterial bio-adsorption of heavy metal ion pollutants resembles the reaction of chelation formation, namely the positive charge of metal ions is neutralized by the negative charge of the chelate, so that the pattern and performance of bacterial bio-adsorption as metal ion chelating must be able to adjust to the ionization energy, electron affinity and electronegativity, as well as the ionic radius of each test metal pollutant. Ensuring the effect of the properties of the test metal ions on the activity, pattern and bio-adsorption capacity of bacteria, requires valid data support and a comprehensive study as material for further studies of this research. (Mostafidi et al., 2021; Muszyńska et al., 2019).

Figure 3, also shows that the bio-adsorption efficiency of BL bacteria is more dominant than PS bacteria, where the bio-adsorption efficiency is directly proportional to the activity and performance of bio-adsorption. These results strengthen the assumption that the bio-adsorption performance of heavy metal pollutants shown by bacteria depends on the characteristics and types possessed by the tested heavy metal pollutants. The performance of bio-adsorption between one type of bacteria and another as a biomaterial or bio-adsorbent for heavy metal pollutants is largely determined by the internal properties inherent in the heavy metal. (Armus et al., 2021; Naghipour & Davoud, 2018).

The R^2 value of BL bacteria on the bio-adsorption performance of As^{+3} (0.66) and Pb^{+2} (0.96), while R^2 for the bio-adsorption performance of PS bacteria against heavy metal pollutants As^{+3} (0.65) and to Pb^{+2} (0.53). This R^2 value, which is relatively much lower than the value of 1.00, indicates that there are other factors that influence the bio-adsorption performance of BL and PS bacteria against heavy metal pollutants As^{+3} and Pb^{+2}. These factors can be analyzed which is part of the continuation of this research (Mustafa et al., 2022; Armus et al., 2021).

The bio-adsorption capacity of bacteria against heavy metal pollutants can be increased by carrying out several engineering and modifications, especially for the types of bacteria used as bio-adsorbents. To fulfill this, several abilities that must be possessed by bacteria include, bacteria are able to adapt to the environment exposed to heavy metal pollutants, bacterial cells must continue to grow and develop rapidly in environmental conditions exposed to pollutants, the number of cells used with a larger population, including agitation or oxygen injection in interaction media, provision of nitrogen, phosphorus and potassium nutrients and also the use of a consortium of bacteria that have bio-adsorption capabilities against heavy metal pollutants (Nedoroda et al., 2021; Orani et al., 2018).

Comparison of several data on the bio-adsorption activity of BL and PS bacteria of marine sponge symbionts, as shown in tables 1, 2, 3, and figures 1, 2, 3, and associated with several assumptions, it can be said that both types of bacteria BL and PS, have activity bio-adsorption of heavy metal pollutants As^{+3} and Pb^{+2}. The order of the bio-adsorption capacity of bacteria against heavy metals is dominantly determined by the internal characteristics of heavy metal pollutants (Page et al., 2021). The pattern of bacterial bio-adsorption of BL and PS against arsenic and lead ion pollutants occurs very quickly and occurs at the beginning of the contact period, then decreases significantly in direct proportion to the increase in interaction time, indicating that bacterial bio-adsorption of heavy metal pollutants takes an ionic reaction mechanism, namely the number of negative poles (Selvam et al., 2021; Rua et al., 2018). The negative charge provided by bacteria is proportional to the amount of ionic charge contributed by the test metal pollutant, until an ionic equilibrium is formed as the end point of the bio-adsorption process or in other scientific terms, namely, the pattern of bacterial bio-adsorption, against heavy metal pollutants similar to the reaction mechanism for the formation of EDTA chelating against ions, where

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the metal ion acts as the central atom in a complex system of compounds.

6. Conclusions

Based on the data and discussion of the findings of this study, it can be concluded as follows: *Bacillus licheniformis* strain ATCC 9789 (BL) symbionts of the sponge *Auletta* sp. and *Pseudomonas stutzeri* strain SLG510A3-8 (PS) symbionts of the sponge *Hyrtios erectus*, have significant bio-adsorption activity against heavy metal pollutants tested As+3 and Pb^{+2}. The bio-adsorption performance of BL bacteria is more dominant than PS bacteria, both against As+3 and Pb^{+2} pollutants. The bio-adsorption pattern of BL and PS bacteria against heavy metal pollutants follows the bacterial growth phase. The physical and chemical characteristics seen in the bio-adsorption of heavy metal pollutants tested by bacteria include changes in the pH of the interaction media, the formation of air bubbles and the smell of fermentation. There are internal factors of the heavy metal pollutant test on the bio-adsorption performance of BL and PS.

Acknowledgements

The author would like to thank the DRTPM of the Ministry of Higher Education, Science and Technology (Kemendiktisainstek) of the Republic of Indonesia, as the funder of the Fundamental research scheme for the implementation of the 2024 year. The author would also like to thank all parties who have helped carry out this research, especially the Manager of the Maros Fisheries Microbiology Laboratory and the Manager of the Instrument Laboratory of the Faculty of Science and Technology, UIN Alauddin Makassar.

Funding

The source of funding for this research is based on the 2024 DRTPM Grant Receipt, number: 0667/E5/AL. 04/2024, May 30, 2024. Research assignment by DRTPM based on contract number: 626/LL9/PK.00.PG/2024, June 20, 2024 and derivative contract number: 1/AI/LPPM-UNIFA/VI/2024, June 21, 2024.

Conflict of Interests

The authors declare no conflict of interest.

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Journal of Chemical Health Risks
www.jchr.org
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