BIODIVERSITAS
Volume 22, Number 12, December 2021
Pages: 5536-5543

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d221237

Performance of cultured marine sponges-symbiotic bacteria as a heavy metal bio-adsorption

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Manuscript received: 24 September 2021. Revision accepted: 26 November 2021.

Abstract. *Marzuki I, Ahmad R, Kamaruddin M, Asaf R, Armus R, Siswanty I. 2021. Performance of cultured marine sponges-symbiotic bacteria as a heavy metal bio-adsorption. Biodiversitas 22: 5536-5543.* One source of heavy metal waste could be coming from industrial disposal. Contamination and accumulation of hazardous heavy metal waste are most likely to occur in water areas, It has become a global issue that urgently requires appropriate technology to address. Two marine sponge-symbiotic bacteria from the Spermonde islands, Indonesia, i.e., *Bacillus cohnii* strain DSM 6307 (BS) and *Pseudomonas stutzeri* RCH2 (PS), were used in this study. The purpose of this study is to investigate the mechanism of heavy metal bio-adsorption of two sponge symbiotic bacteria by contact technique and are assessed their activity, capacity, and efficiency of bio-adsorption against different heavy metals (Cr, Mn, Fe, Co, Cu, Zn, Ag, and Cd),. The bio-adsorption capacity was evaluated by Atomic Absorption Spectroscopy (AAS) after a predetermined contact time (4, 8, 12, and 16 days). The results demonstrated that two sponge symbiotic bacteria had bio-adsorption activities against eight different heavy metals. BS bacteria exhibited higher bio-adsorption capacity (Cr > Zn > Cu > Fe > Co >Mn and Ag > Cd) than that of PS bacteria (Zn > Co > Fe > Mn > Cu > Cr and Cd > Ag). The difference in the atomic number of heavy metals causes the characteristics of these metals to vary which affects the capacity and efficiency of bio-adsorption of sponge symbiotic bacteria to heavy metals.

Keywords: Bacteria, bio-adsorption, heavy metal, marine sponge-symbiotic

Abbreviations: BS: *Bacillus cohnii* strain DSM 6307; PS: *Pseudomonas stutzeri* RCH2

INTRODUCTION

In today's world, pollution has targeted all living areas with various types of pollutants. Heavy metals are a form of pollutants that is extensively distributed in soil, water, and air. Water areas are very sensitive to heavy metal contamination from various sources, particularly human activities. Petroleum exploration efforts on land and at sea will almost certainly produce sludge containing hazardous chemicals, carcinogenic and mutagenic Polycyclic Aromatic hydrocarbon components, and heavy metal pollutants with high toxicity (Melawaty 2014; Marzuki 2019a, 2020a). Both hazardous chemicals have also been verified to end up in the water, particularly in the ocean, in the hydrological cycle of sewage sludge (Siahaya et al. 2014; Marzuki 2020a). It is associated with the life cycle of living organisms, and it would harm human existence (Marzuki et al. 2019b, 2021a).

To maintain the balance of the biological environment, and to overcome the potential adverse effects caused by heavy metal toxic contaminants, therefore, it is needed to obtain a waste management strategy to prevent and anticipate serious impacts that may occur, particularly on the survival of marine life (Gupta et al. 2013; Marzuki et al. 2019a). It is important to adopt preventive actions to predict the emergence of chronic conditions on poor marine biota life and chronic impact on the long-term existence of living organisms, including humans (Gjorgieva et al. 2011; Alimardan et al. 2016; Marzuki et al. 2020b).

According to some literature and previous research findings, sponges are a type of marine biota that have bioindicator functions of several heavy metal pollution based on the accumulation of heavy metals in the body of sponge (Shama et al. 2010; Siahaya et al. 2014; Pawar et al. 2017). The function of sponges as bioindicators against heavy metals is related to the uniqueness of the sponge, i.e., small body, slow growth, and the specific color of the body surface, and some of them have slime-covered body surfaces, and they are classified as a marine animal that filters heavy metals (Melawaty et al. 2014; Wibowo et al.

2019; Marzuki et al. 2020b). Sponges can suck and spray mud to obtain nutrients, symbioses with various types of microorganisms, especially bacteria, decompose hydrocarbons as an energy source, and form complex compounds with certain heavy metals between sponges and their microsymbionts by chelation mechanism (Pawar et al. 2017; Orania et al. 2018; Lajayer et al. 2019).

The role of sponges as bioindicators of heavy metals is interesting to be studied in more depth (Melawaty et al. 2014; Han et al. 2015) regarding the utilization of sponges and their symbiotic bacteria to overcome heavy metal pollution in the water (Zhou et al. 2011; Marzuki et al. 2019a, 2020c). Another sponge-related scientific finding is the involvement of symbiotic bacteria as heavy metal bioindicators, especially metal biosorption by sponge symbiotic bacteria (Chávez et al. 2017; Ziarati et al. 2017; Marzuki et al. 2021b).

In this paper, the activity and application of sponge symbiont bacteria in the biosorption of heavy metals were described, especially the capacity, efficiency, type of heavy metal biosorption, biosorption pattern and mechanism, and the type of sponge symbiont bacteria in terms of elemental property theory (affinity), electrochemistry and biosorption pattern and mechanism. (Zhang et al. 2012; Gupta et al. 2013; Marzuki et al. 2021c, 2019b).

MATERIALS AND METHODS

Collection of marine sponge-symbiotic bacteria

The sponge-symbiotic bacteria *Bacillus cohnii* strains DSM 6307 (BS) and *Pseudomonas stutzeri* RCH2 (PS) were collected from the previous study (Marzuki et al. 2021b) and have been molecularly identified using a modified technique of (Kamaruddin et al. 2014; Kamaruddin et al. 2020; Minarti et al. 2020). These two bacteria were isolated from the sponge *Niphates* sp., and *Clathria* (*Thalysias*) *reinwardti* from around Kodingareng Keke Island (119°17'19.399" E and 5°6'21.423"S), which is part of the Spermonde Archipelago area in Makassar, South Sulawesi. *Niphates* sp. was obtained from 3.6 meters of sea level; the salinity of 29.3; pH 6.8; a temperature of 28ºC and approx. 64% of the body's surface covered with a slightly dark mucus substance. *Clathria* (*Thalysias*) *reinwardti* was obtained from 3.1 meters of sea level; the salinity of 29.7; pH 7.0; a temperature of 29ºC, and approx. 42% of the body surface was coated with a thin mud-like substance. The sponge samples were observed for their morphological characters (Marzuki et al. 2021). The isolated bacterial symbionts were observed for their phenotypic and genotype characters (Marzuki et al. 2021b).

Experimental design

Bacteria were selected based on spore-producing and Gram-positive bacteria. Selected isolates were cultivated on Nutrient Agar (NA) media (Liu et al. 2017). Cultivated cells were suspended in 250 ml of 0.9% NaCl solution and agitated physiologically in an Erlenmeyer flask. A total of 64 sterile vials were prepared with the following treatments: two species of bio-adsorbent bacteria, four duration of contact (four, eight, twelve, and sixteen days), and eight types of heavy metals. Five mL of bacterial suspension was put in each vial and adapted for 24 hours. After adaptation, 5 mL of heavy metal solution at a concentration of 250 mg/L was added to each vial and then placed in a shaker incubator. The sample was filtered after the contact period was achieved (Marzuki 2020a; Tam and Wong 2008). The filtrate was acidified with HCl (pH 3-4) and concentrated. Physicochemical observations and measurements of the absorption of each metal at the proper wavelength were carried out (Seo et al. 2012; Zhang et al. 2012).

Data and analysis

Several parameters of heavy metal bio-adsorption by bacteria were observed and analyzed for (1) Sponge-symbiotic bacteria activity, (2) capacity, (3) efficiency, and (4) The bio-adsorption performance of BC and PS bacteria on heavy metal ions (Cr, Mn, Fe, Co, Cu, Zn, Ag, and Cd). The average efficiency was calculated using four concentrations of heavy metal ions, carried out every 4 days for 16 days (Orania et al. 2018; Marzuki et al. 2020c; Amaral et al. 2020). The bio-adsorption capacity was calculated using the following equation:

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

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

While:

Q : Bio-adsorption capacity (mg/g);

C1 : Concentration before contact (mg/L);

C2 : Concentration after contact (mg/L);

m : Absorbent Mass (mg), assumption: 1 mL of bacterial suspension bio-sorbent is equivalent to a mass of 1 g absorbent

V : Solution volume (L) and

%E: Bio-adsorption efficiency.

RESULTS AND DISCUSSION

The capacity of bacterial bio-adsorption on different heavy-metals

The bio-adsorption activity of sponge symbiont *B. cohnii* strains DSM 6307 (BS) and *P. stutzeri* RCH2 (PS) on eight different heavy metals was presented in Figure 1.

The test for the bio-adsorption capacity of two sponge bacterial symbionts against different metal ions was carried out under the same contact conditions, such as heavy metal ion concentration, contact duration, and volume of media. The results revealed that BC bacteria generally had a stronger bio-adsorption capacity than PS bacteria (Figure 1A-G), except for Cd metal ion bio-adsorption (Figure 1H). PS bacteria have a better bio-adsorption capacity for Cd ions than BC bacteria.

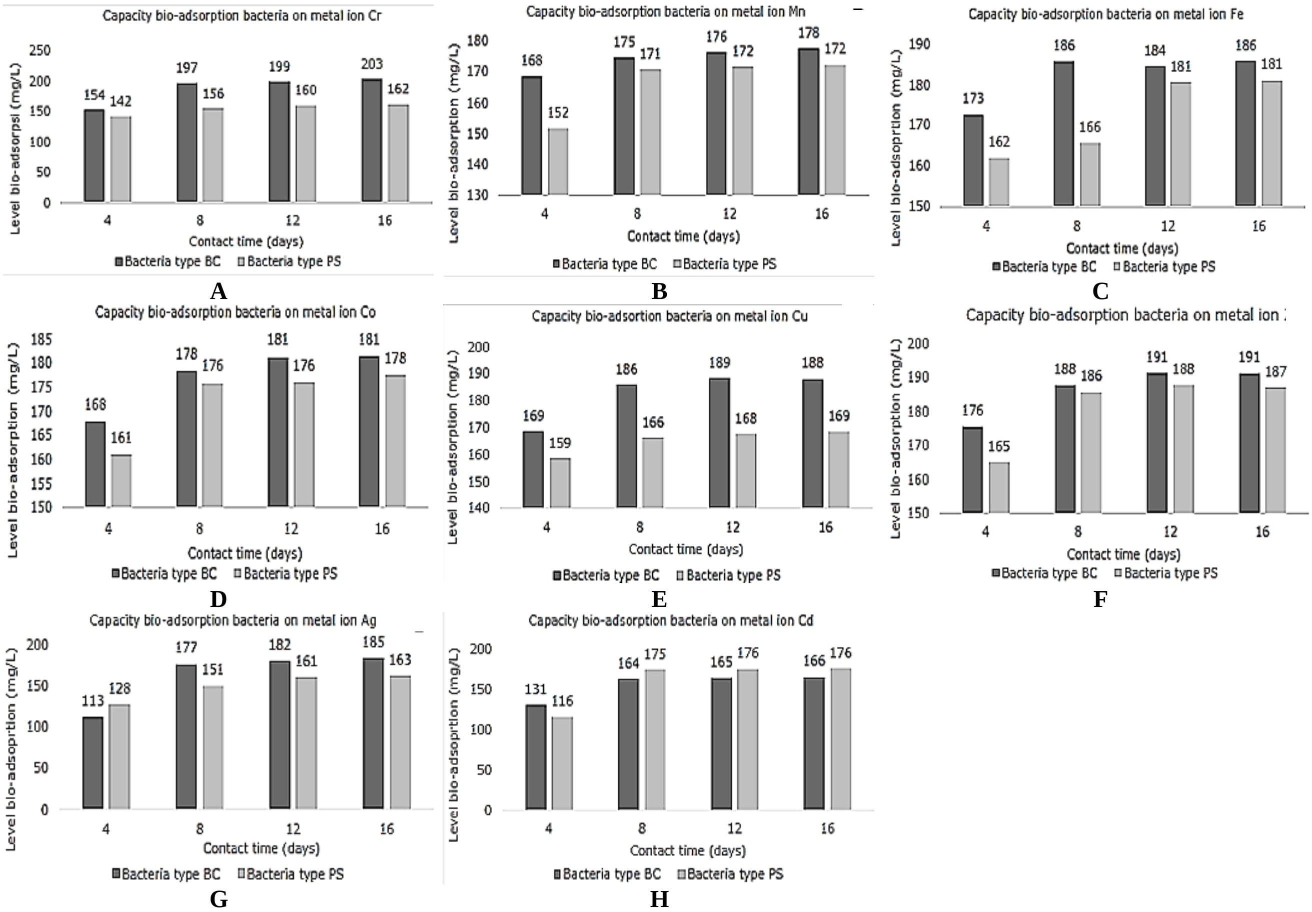


Figure 1.A-H. Bio-adsorption capacity of sponge symbiont BC and PS bacteria against eight different heavy metal ions

Bio-adsorption pattern

The bio-adsorption patterns of BC and PS bacteria to various heavy metal ions are shown in Figures 2 and 3. The pattern and bio-adsorption capacity of BC bacteria against heavy metal ions exhibited a similar tendency to the properties of elements in the periodic table, in order from high to low: Zn > Cu > Co > Fe > Mn > Cr (Figure 2.A and B) and Ag < Cd (Figure 2.C).

The pattern and bio-adsorption capacity of PS bacteria against eight different heavy metal ions, in the following order: Zn > Cu > Co > Fe > Mn > Cr, and Ag > Cd (Figure 3A-C). The characteristics of the heavy metal elements employed in the periodic system are comparable to those of the bio-adsorption sequence. Due to the bio-adsorption capacity of Ag and Cd by PS bacteria being considerably lower than that of the other six elements, it is assumed that these two elements are in period 5 of the periodic system, whereas the other metal elements are in period 4.

Table 1 indicated that 2 species of sponge symbiont bacteria, from left to right, have the higher maximal capability for bio-adsorption of heavy metals: (Cr, Mn, Fe, Co, Cu, Zn), and decrease from top to bottom following the periodic system's elemental sequence (Ag, Cd). All maximum bio-adsorption capacities were obtained after a 4-day contact time. Data on the average and maximum effectiveness of bacterial bio-adsorption of 8 heavy metal ions were shown in Figures 4 and 5. The maximum bio-adsorption capacity of heavy metal contaminants was simply based on the order of heavy metals in the periodic table of elements. Any interaction between bacterial biosorbents against one type of heavy metal was influenced by many factors, Analysis of maximum bio-adsorption capacity of bacterial biosorbents of sponge symbionts against heavy metals was reviewed based on the length of contact time rather than by comparing the heavy metals. The bio-adsorption of heavy metal contaminants by one type of bacteria follows a specific mechanism (Marzuki et al. 2020b, 2021e). This indicates that the maximum performance of BC and PS bacteria bio-adsorption against 8 types of heavy metals was achieved after 4 days of contact, despite an increase in bio-adsorption over the next 4 days of interaction (8, 12, and 16 days). It was, however, not as significant as the bio-adsorption achieved by BC and PS bacteria in the first four days of interaction. Bacterial cells may be unable to divide further due to the toxic nature of heavy metal ions, resulting in a decrease in the performance of bacterial bio-adsorption on the test metal. This circumstance has an impact on the average bio-adsorption capacity of BC and PS bacteria, which is greater than the maximum capacity (Marzuki 2020a; Marzuki et al. 2021f).

The bio-adsorption ability of BC and PS bacteria on the eight heavy metals tested did not increase from left to right according to the periodic table (Table 1). Several factors influenced bacterial bio-adsorption ability, including the type, characteristics, and adaptability to a contaminant. The properties and characteristics of the adsorbed heavy metals are different because each heavy metal tested has a different atomic number, resulting in different ionic radii, affinity, electronegativity, and ionization energy.

Maximum bio-adsorption and average capacity of sponge symbiotic bacteria against 8 different heavy metal ions

The average and maximum bio-adsorption capabilities of heavy metal ions of sponge symbiotic bacteria are presented in Table 1.

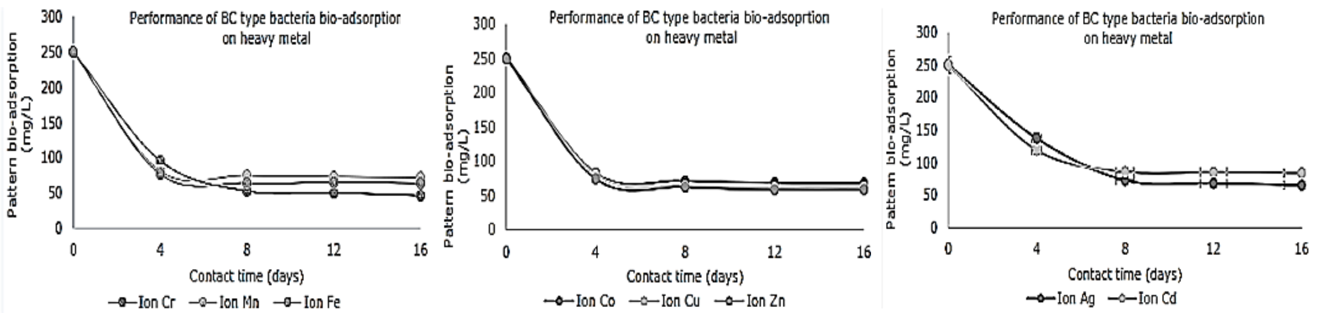
The maximum bio-adsorption efficiency is the difference between the initial and final concentrations of heavy metal, which is obtained based on the interaction time regardless of the amount of bacterial suspension bio-sorbent used (Eq. 2). Maximum bioadsorption is the bacterial bioadsorption that is measured based on interaction time and the amount of bacterial suspension bio-sorbent used (Eq. 1).

Table 1 showed that the concentration of heavy metals in the test as the performance of BC and PS bacteria measured based on contact time, implying that the value represents the concentration of heavy metals in the test that is not adsorbed after several days of contact. Figure 4 illustrates the amount of heavy metal concentration of the test adsorbed by BC and PS bacterial cells over time. Table 1 describes the heavy metal concentrations in the assay as the performance of BC and PS bacteria measured by contact time, implying that these values represent the concentrations of heavy metals in the assay that were not adsorbed after several days of contact. Figure 4 illustrates the amount of the test heavy metal concentration adsorbed by BC and PS bacterial cells from time to time

Table 1. Performance of bio-adsorption and average capacity of sponge symbiotic BC and PS bacteria against heavy metal ions

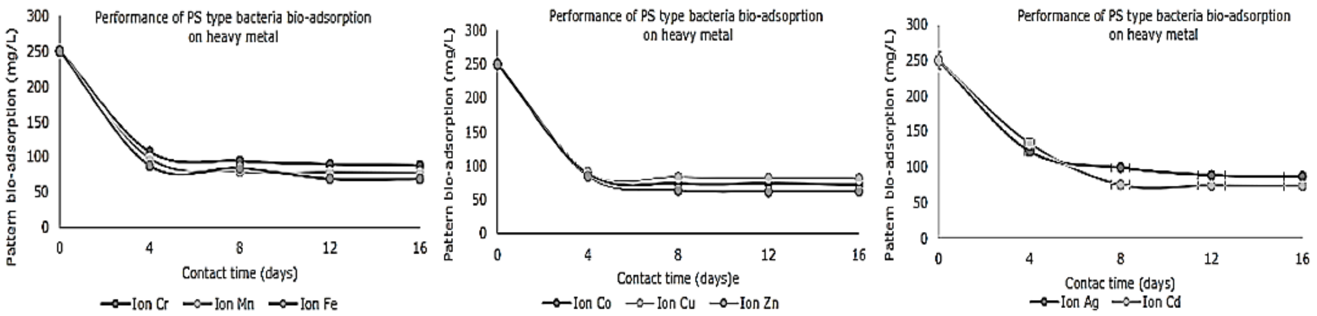
Bacteria	Bio-adsorption capability	Heavy Metal ions								
		Cr	Mn	Fe	Co	Cu	Zn	Ag	Cd	
BC	Maximum (mg/L)	153.72	168.44	172.65	167.87	168.66	175.53	112.55	130.88	Ct 4 days
	Average (mg/L)	188.30	174.22	182.12	177.25	182.87	186.47	163.97	156.28	
PS	Maximum (mg/L)	142.36	151.63	162.02	161.02	158.66	165.06	127.59	116.25	Ct 4 days
	Average (mg/L)	154.96	166.55	172.34	172.62	165.34	181.46	150.63	160.79	

Note: BC: *Bacillus cohnii* strains DSM 6307; PC: *Pseudomonas stutzeri* RCH2; Ct: Contact time



A B C

Figure 2. Bio-adsorption pattern of BC bacteria against eight different heavy metal ions. A. against Cr, Mn, and Fe, B. against Co, Cu, and Zn, C. against Ag and Cd ions



A B C

Figure 3. Bio-adsorption pattern of PS bacteria against eight different heavy metal ions. A: against Cr, Mn, and Fe ions, B: against Co, Cu, and Zn ions, C: against Ag and Cd ions

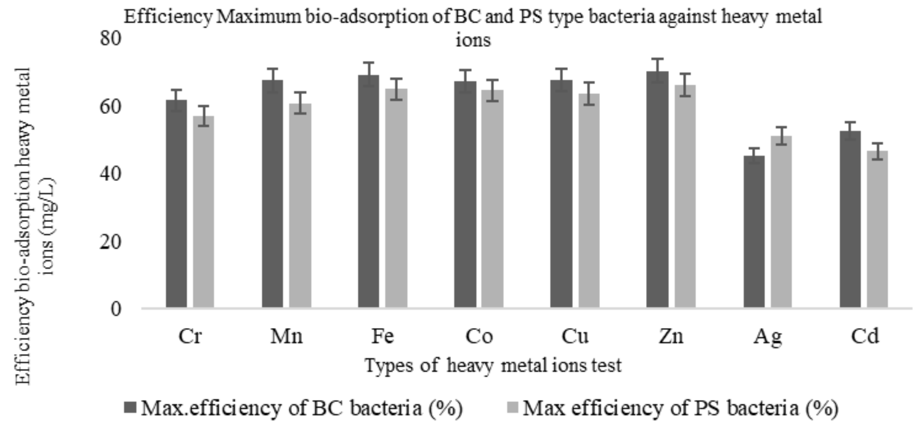


Figure 4. The highest bio-adsorption efficiency of BC and PS bacteria against eight different heavy metal ions

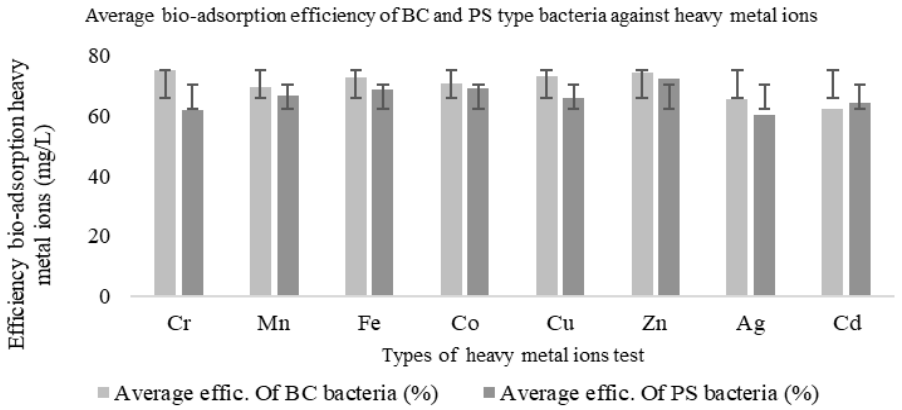


Figure 5. The average efficiency bio-adsorption of BC and PS bacteria against eight different heavy metal ions

The average bioadsorption efficiency (Figure 5) is the average value of bacterial bioadsorption calculated by dividing the total value of several interaction time measurements by the number of measurements, regardless of the amount of biosorbent used. The average capacity showed that the bioadsorption efficiency of BC and PS bacteria isolated from marine sponges was determined by summing the results of the bioadsorption capacity measurement (Equation 1) for the interaction period of 4, 8, 12, and 16 days for each heavy metal test and then dividing by the number of measurements. The average efficiency is relatively identical to the method used to determine the average capacity, but the data used is the result of measuring the efficiency (Equation 2) for each metal.

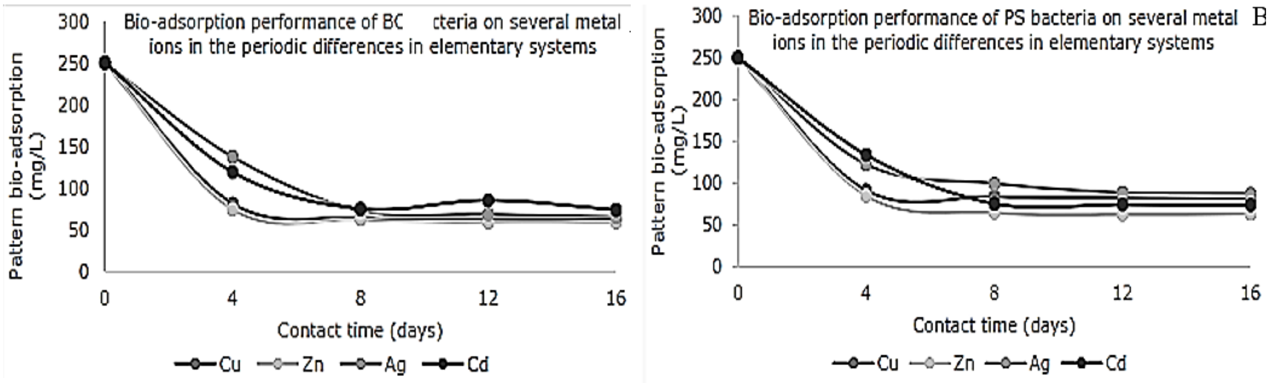
Bio-adsorption pattern

The bio-adsorption pattern of four heavy metals is dependent on the metal's location in the periodic table of elements: Cu and Ag are in group IB and periods 4 and 5. Whereas, Zn and Cd are in group IIB likewise with periods 4 and 5, as demonstrated in Figure 6.

Discussion

The bio-adsorption abilities of *B. cohnii* strains DSM 6307 and *P. stutzeri* RCH2 which are marine sponge-symbiotic bacteria were evaluated against various heavy metals by examining several variables that were considered significant, such as activity, capacity, average and maximum efficiency, and bio-adsorption patterns based on differences in elemental properties in the periodic system of elements. In general, *B. cohnii* had a better bio-adsorption capacity than *P. stutzeri* bacteria (Figure 1A-G), except for Cd metal ion bio-adsorption (Figure 1H.).

After comparing the order of eight types of heavy metal ions in the periodic table of elements, it appears that the bio-adsorption capacity of bacteria tends to follow a pattern based on elemental properties (affinity, ionization energy, electronegativity) from left to right in the elemental periodic system tends to be higher. Meanwhile, the bio-adsorption capacity from top to bottom is decreasing. The theoretical description of the characteristics is consistent with the increase in heavy metal bio-adsorption capacity demonstrated by the two species of bio-adsorbent bacteria (Han et al. 2015; Marzuki et al. 2019a).



A B

Figure 6. Bacterial bio adsorption of the test heavy metals was arranged based on the difference in the periodic arrangement of the elements. A. Bio-adsorption performance of BC type bacteria against Cu, Zn, Ag, and Cd. B. Bio adsorption performance of PS type bacteria against Cu, Zn, Ag, and Cd

This is supported by a comprehensive examination of the bio-adsorption pattern of bacteria, which corresponds to the nature of the elements in the periodic system as shown in Figures 2 and 3. The first six test metals (Cr, Mn, Fe, Co, Cu, Zn,) are in period 4 of the periodic table of elements, from left to right of the sequence with higher affinity, ionization energy, and electronegativity. However, the pattern and bio-adsorption capacity of Ag and Cd elements in period 5of the periodic system showed Ag has a higher bio-adsorption capacity than Cd (Shama et al. 2010; Gebregewergis 2020; Marzuki et al. 2021a, 2021d). This was thought to be connected with sample preparation accuracy i.e. human error and the instrument used that may affect the results; absorbance measurement by AAS; and other treatments relevant to the nature of *B. cohnii* (Figure 3A-C). This may be caused by the bio-adsorption capacity of Ag and Cd by PS bacteria is considerably lower than that of the other six elements. It is assumed that these two elements are in period 5 of the periodic system, whereas the other metal elements are in period 4.

The bio-adsorption pattern of *B. cohnii* and *P. stutzeri* was similar, with a very high capacity in the first 4 days of contact and progressively decreasing with increasing contact time, even tending to reach zero. The phenomenon of biosorption of 8 heavy metals by sponge symbiont bacteria, indicated that the bio-adsorption process leads to a chelation mechanism between anions donated by bio-adsorbent bacteria and cations from heavy metal ions (Alimardan et al. 2016; Ziarati et al. 2017; Marzuki et al. 2020b). The decrease in bio-adsorption capacity was caused by bacterial cell division, where at the initial contact (4-8 hours) the number of anions from bacteria was saturated by cations from heavy metals so that bacterial cells could not survive and the formation of chelate become zero. In this situation, it is assumed that the bacterial cells have been intoxicated by heavy metal, and resulting in bacterial cell death. whereas the enzymatic reaction mechanism is used in the Polycyclic Aromatic Hydrocarbon biodegradation technique by bacteria (Konkolewska et al. 2020; Marzuki et al. 2019b, 2020c).

The maximum bio-adsorption capacity value for heavy metal ions in period 5 elements differed, it did not consistent with the order of the elements in the periodic system (Zhou et al. 2011; Motaghi and Ziarati 2016). Bio-adsorption of heavy metals, particularly those in the period 5 position on the periodic system by bacteria required further studies especially the type of heavy metals and the bacterial species. The consistency of the maximum capacity of bio-adsorption of heavy metals by two species of sponge symbiont bacteria followed the order based on the character and properties of the elements in the periodic system, as presented in data on the average capacity for bio-adsorption with a contact period of 16 days. The order of the components in the periodic system affects adsorption (Muszyńska et al. 2019; Marzuki et al. 2019b, 2021d). The maximum bio-adsorption efficiency of both sponge symbiont bacteria (BC and PS) against heavy metals (Figure 4) increased consistently. In the periodic table of elements, the maximum efficiency value increase from left to right and from top to bottom, notably for BC bacteria, but the maximum efficiency pattern for PS bacteria is slightly different, especially for heavy metal ions period-5 periodic system (Ag and Cd). In general, the maximal effectiveness of heavy metal bio-adsorption by these two species of sponge symbiont bacteria follows the character and characteristics of the elements in the periodic table (affinity, electronegativity, ionization energy) with a standard variation of 5%. The highest effectiveness of bio-adsorption was achieved by both species of bio-adsorbent bacteria after a four-day contact time.

The average efficiency of bio-adsorption of heavy metals by BC and PS bacteria (Figure 5) showed that the performance of BC and PS bacteria against 8 different heavy metal ions did not follow the pattern and consistency of average efficiency according to the character and properties of elements in the periodic table, which means that the average efficiency value of heavy metal bio-adsorption by BC and PS bacteria did not follow the pattern. Several factors may affect the pattern of heavy metal bio-adsorption efficiency by BC and PS bacteria, namely (i) Stages of bacterial growth and development; (ii)

The mechanism of bioadsorption of heavy metals by two bacterial species was based on a chelation reaction in which the activity of the bacteria was saturated at first contact, and resulting in suboptimal development and growth phase was not optimal; (iii) The measurement of bio-adsorption of the heavy metal ion should be carried out every day for 10-days maximum contact for obtaining an accurate bio-adsorption, (iv) Physicochemical measurements (pH, air bubbles) on the bio-adsorption media can determine the pattern of the average effectiveness of bacterial bio-adsorption on the heavy metals studied (Karimpour et al. 2018; Marzuki et al. 2019a).

Figure 6 reveals that the bio-adsorption of bacteria to the tested heavy metals did not follow a consistent pattern based on the positions of the elements in the periodic table. This indicates that bio-adsorption takes place in erratic and varied patterns. These findings indicate that the characteristics of the elements in the periodic table are varied, affecting the pattern, capacity, and efficiency of heavy metal bioadsorption by bacteria. of heavy metal bio-adsorption by bacteria (Zhou et al. 2011; Pawar et al. 2017; Marzuki et al. 2021c) especially sponge symbiont bacteria. The bio-adsorption mechanism is based on the formation of chelating enzymes produced by bacteria against heavy metal cations in an equilibrium system so that the saturation point of chelation formation reaches zero if the number of anions donated by bacteria is equal to the number of heavy metal cations, and thus the bio-adsorption capacity cannot be achieved. The contact time between the bacterial suspension and the dissolved heavy metal ions could be improved. The capacity and effectiveness of bacterial bioadsorption of heavy metals can be increased by increasing the concentration of bacterial cells in the contact medium. Increasing the concentration of bacterial cells in the contact medium can improve the capacity and effectiveness of bacterial bioadsorption on heavy metals. There are two ways to multiply the number of bacterial cells in the contact medium: (i) using a bacterial consortium known as metalloclastic bacteria (a collection of heavy metal bioadsorbing bacteria), and (ii) increasing the adaptation time of the bacterial suspension until the bacteria enter the logarithmic phase to achieve maximum growth and development while being agitated with nutrients.

In conclusion, sponge-symbiont bacteria BC and PS had bio-adsorption activity against eight different heavy metals. The bio-adsorption capability of BC bacteria against eight heavy metal ions was better than that of PS bacteria. The order of bio-adsorption capacity of *B. cohnii* against heavy metals was as follows: Cr > Zn > Cu > Fe > Co > Mn and Ag > Cd, while the order in *P. stutzeri* bacteria was as follows: Zn > Co > Fe > Mn > Cu > Cr and Cd > Ag; The pattern and maximum bio-adsorption efficiency of heavy metals by bacteria were obtained after 4 days of contact. The character of the elements in the periodic table affects the performance of bacterial bio-adsorption on the tested heavy metals. The mechanism of bacterial bio-adsorption of heavy metals was performed through the chelation formation reaction pathway, and the bacterial consortium formula can be used to determine the bio-adsorption capability of heavy metals by bacteria.

ACKNOWLEDGEMENTS

We express our gratitude for the support from the Ministry of Science and Technology / National Agency for Research and Innovation of the Republic of Indonesia (RISTEK/BRIN) and the Ministry of Education and Culture of the Republic of Indonesia, through the Applied Research Grant scheme for the second year of 2020. Thanks are also conveyed to the Makassar Regional Research Center for Industry and Trade as an implementing partner and the Makassar City Fisheries, Marine, and Agriculture Office as a user partner for research results. We also thank Universitas Muhammadiyah Semarang, Indonesia for the kind of collaboration in writing and reviewing.

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