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The Bio-adsorption Pattern Bacteria Symbiont Sponge Marine Against Contaminants Chromium and Manganese In The Waste Modification of Laboratory Scale

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Abstract. The use of sponge symbionts bacteria as marine biomaterials in the heavy metal bio-adsorption method is an effort to save the marine environment from contamination of heavy metal contaminants. The ocean is a giant container, most vulnerable to contamination of pollutants. The target of the research is to determine the potential, capacity and pattern of bio-adsorption of sponge symbionts bacteria against various pollutants so that the toxic properties of heavy metal contaminants can be minimize. The method used is to interact with the bacterial suspension on the test metal concentrations that have been determined. The parameters measured were optical density, pH and concentration of heavy metals after the interaction lasted several days and the calculation of capacity, efficiency and bio-adsorption patterns of bacterial isolates from sponges. Results: The pattern and bio-adsorption power of AC bacteria to Cr and Mn ions were higher than BS bacteria, the adaptability of AC and BS bacteria was stronger in Cr (III) contaminated media compared to Cr (VI) toxic media, causing bacterial cell population BS and AC in Cr (III) and Mn (II) media are more abundant than in Cr (VI) and Mn (VII) media, capacity and bio-adsorption efficiency of BS and AC bacteria agains Cr (III) > Cr (VI) ions and Mn (II) > Mn (VII), It is suspected that there is an influence of reactivity and toxic properties of the metal ion test on the performance of the sponge symbionts in bio-adsorption.

Keywords:
bacterial sponge symbionts;
bio-adsorption capacity;
heavy metals

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Introduction

Heavy metal pollution comes from natural sources by nature and human activities, especially industrial and domestic activities. Heavy metal contaminants are generally in the form of particulates that contaminate the air and water air environment. Exposure to heavy metals in water is usually in the form of ions or oxidized molecules, where it is known that most heavy metals have more than one oxidation number (Sharma et al., 2020). The toxic nature of heavy metal contaminants when accumulated in water areas can cause serious problems for the life of marine life. On the other hand, the presence of heavy metals is difficult to avoid because heavy metals generally much needed in various types of industries.

Chromium metal is widely used in the electroplating industry, corrosion resistant steel, paint fillers, inks, ceramics and other uses, while manganese is widely used in the battery, pigment, precursor and other utilization industries (Alaboudi, Ahmed, & Brodie, 2018).

Accumulation of heavy metal chromium (Cr) when exposed to humans through both acute and chronic processed, has the potential to cause health problems such as dermatitis and skin irritation. Cr (VI) is one of the very toxic, carcinogenic, even mutagenic metal ions, so it is classified as one of the hazardous and toxic substances (B3), although at trace levels Cr (III) is an essential ion for the body's metabolic processes (Ziarati et al., 2015; Marzuki et al., 2019a). Manganese microorganisms (Mn) are also needed by the body in the metabolism system, but in most ceses this metal exposure is rarely found in trace amounts, because it is not decomposed and in the body forms

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an accumulation system, so that in long-term exposure it is certain to exceed the threshold value tolerated by the body (Marzuki, 2016).

Natural dynamics that occur in nature under normal conditions are processes leading to equilibrium, but these natural dynamics are seen as unable to compensate for the negative impacts due to heavy metal toxicity, due to the massive contribution that occurs in industrial activities and the contribution of domestic contaminants in uncontrolled volumes, so the equilibrium process natural that takes place continuously decreases and is not able to fight heavy metal pollution. This situation requires the contribution and active role of related parties to produce new knowledge, methods, and appropriate technology in an effort to overcome and control the volume of contamination of the impact of heavy metal contaminants on the environment, including the expected health effects on humans (Naghipour & Davoud, 2018; Pawar, 2017).

Many natural materials both from animals and plants known to have the ability to adsorb several types of heavy metals this process known as the bio-adsorption method. Some marine biota is known to have bio-adsorption ability, one of which is sponge (Marzuki et al., 2019b). Previous research by Alimardan et al. (2016) and Riyaz et al. (2020), explained that some types of plants as bio-adsorbents of heavy metals and are natural materials in the environment of the chemical group far away. Even by Melawaty et al. (2014) and Orani et al. (2018) states that several types of marine sponges are bioindicator and biomonitoring some types of heavy metals. Several types of heavy metals found in the sea both in sediments, seawater and in some marine biota bodies, indicate that heavy metal exposure to the marine environment occurs very massively (Bibi et al., 2016; Yang et al., 2019). Several studies that have raised the theme of heavy metal adsorption using materials from plants and animals in remediation and phytoremediation methods with fairly good results (Marzuki et al., 2019c; Alaboudi, Ahmed, & Brodie, 2018), but no one has provided information about the kinetics of bio-adsorption of heavy metals by microorganisms or other natural materials.

Conditions and some facts related to bio-adsorption of heavy metals and the level of pollution to the marine environment compared to research findings (Wibowo et al., 2019), guide research on the kinetics of bacterial bio-adsorption on chromium and manganese heavy metals based on contact time to provide information about the types of marine sponge symbionts bacteria that have the ability to bio-adsorption of heavy metals and how the kinetics occur and the mechanism of adsorption. The findings of this research are expected to be useful in preparing the formulation of a heavy metal adsorption consortium which is termed metalloclastic bacteria.

Experimental

Material and Methods

Bacteria Bacillus sp. AB353f parsial (BS) isolates from Neopetrosia sp. and Acinetobacter calcoaceticus strain PHCDB14 (AC), sponge isolates Callyspongia aerizusa, $K_2Cr_2O_7$ pa, $Cr(NO_3)_3$ pa, $MnCl_2.4H_2O$ pa, $KMnO_4$ pa, Nutrient agar (NA), Sea Agar (MA), NaCl 0.9% physiological solution, KCl solution pa, HCl pa, KOH pa, 25% glycerol, 4% formalin, 96% alcohol, aquades, Atomic Absorption Spectropho-tometry (AAS) type AA240FS variance, Spectronic 20D series D* Shimadzu, incubators, test tubes, autoclaves, analytic balances, digital pH meters, Universal pH indicators, LAF, eppendorf micropipette, Ose round series , counter colony, buchner funnel, aluminum foil, paper disk, petri dish, shaker incubator, wattle, a set of glassware.

Sample Preparation

Samples of Bacillus sp. AB353f partial AB353f (BS) and Acinetobacter calcoaceticus strain PHCDB14 (AC) used as bio-adsorbents were obtained from the Biochemistry Laboratory of the Department of Chemistry at Hasanuddin University, a stock of bacteria belonging to researchers isolated from a sponge a year before. Both types of sponge symbionic bacteria were chosen because they met the required requirements based on the results of morphological, phenotypic and genotypic analysis in accordance with the characterization carried out in previous studies. Culture of the two selected bacterial samples was carried out to multiply cells. Cultured cells were suspended in 250 mL of 0.9% NaCl solution physiologically, shaken until homogeneous. Also made a solution of Cr (III), Cr (VI), Mn (II) and Mn (VII), each with a volume of 250 mL and a concentration of 250 mg/L (Marzuki et al,. 2016; De Rosa et al., 2003).

Experimental Design

Prepared 2x3x6 wattle labeled (2 types of bacterial suspensions, 3 repetitions and 5 contact times + 1 pial for negative control), each pial filled with 3 mL BS bacterial suspension, incubated 1 x 24 hours, so that bacterial cells can adapt to the new environment, then each wattle input 5 mL of modified Cr (III) contaminated waste concentration of 250 mg/L. Furthermore, the BC suspension that has interacted with Cr (III) in wattle was measured initial pH and optical density (λ max. 600 nm), then agitated using a Shaker incubator at 100 rpm. Measurement of optical density, pH and concentration of Cr (III) is done every 3 days of interaction 5 times as many measurements, (3, 6, 9, 12 and 15 contact days) (Agarwal & Bagla, 2013). Subsequently the sample was filtered and the filtrate obtained was acidified until it reached pH 4. Determination of the metal content in the sample was measured using AAS at the appropriate max λ. Maximum uptake for metal Cr λ max. : 357, 9 nm and Mn λ max.: 279,5 nm. The same procedure was carried out using AC bacteria and modified Cr (VI), Mn (II) and Mn (VII) waste.

Data and Analysis

The measurement and observation points in this study are: (1) Optical Density (OD) interaction media; (2) media of pH; and (3) maximum absorption of heavy metal bio-adsorption by BC and AC type test bacteria. The capacity and efficiency of the bio-adsorption of the heavy metal ions of the test [Cr (III), Cr (VI), Mn (II), and Mn (VII)] were determined using the equation:

$$Q = \frac{C1-C2}{C1} x\ V \qquad [1]$$

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

Note:
Q = bio-adsorption capacity (mg/L);
C1 = concentration before contact (mg/L);
C2 = concentration after contact (mg/L);
m = Mass Absorbance (mg);
V = volume of solution (L) and
% E = bio-adsorption efficiency (Riyaz et al., 2020).

Result and Discussion

Optical Density

Measurement of optical density (OD) of the interaction media carried out to ascertain whether the bacterial cells of the symbiont sponge are suspended, able to adapt and experience growth. Bacterial cell activity begins with the adaptation of bacterial cells to a new environment (log phase), usually occurring 1-3 days incubation period. The next phase is the stage of growth and development of bacterial cells (exponential phase). This stage, the size and number of cells increases so that the suspension appears physically more turbid, determined by measuring the OD value of the interaction media. If there is an increase in the OD value from the initial value before the interaction, it indicates an increase in the number of bacterial cells, as shown in Figure 1-4, follows:

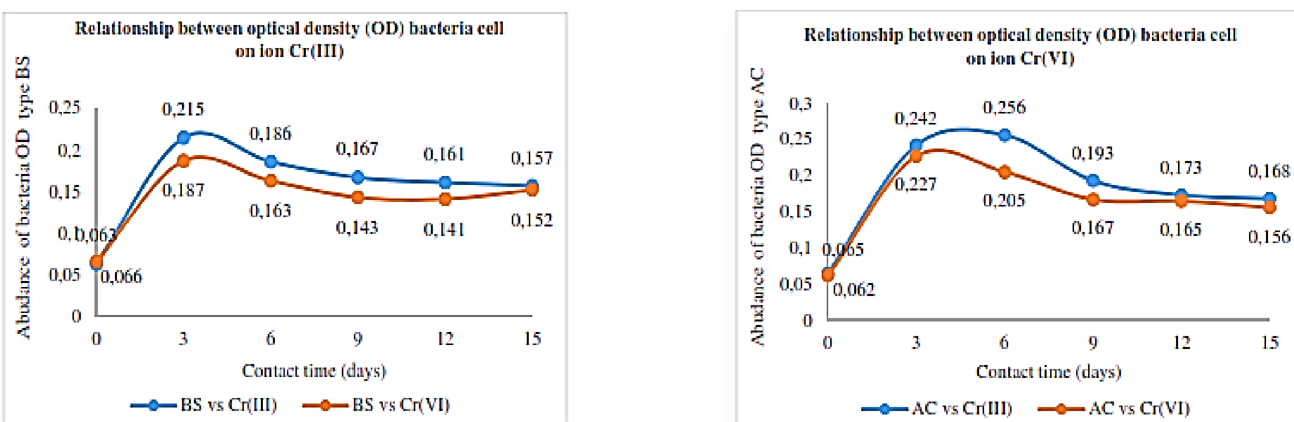
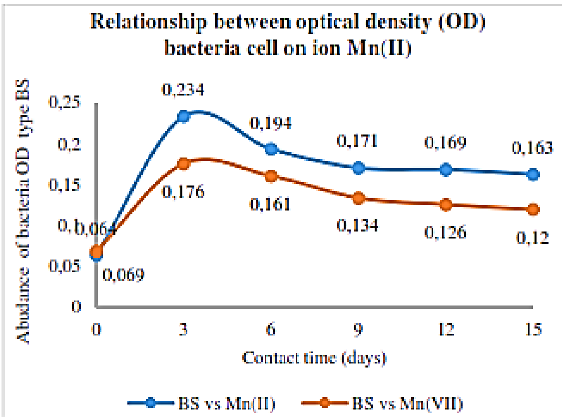


Figure. (1) Changes in OD values of BC type bacterial cells in the interaction medium to Cr (III) and Cr (VI) concentrations based on contact time (days); **(2)** Changes in OD values of AC type bacterial cells in the interaction media to Cr (III) and Cr (VI) concentrations based on contact time (days).

Figure 1 shows the OD value of BC cells in Cr (III) contaminated interaction media compared to in Cr (VI) contaminated media. This indicates that the population of BC cells in Cr (III) contaminated media is greater than the population in Cr (VI) contaminated media. The relatively similar situation shown in Figure 2, it can said, that BS and AC bacterial cells are better able to adapt in media exposed to Cr (III) contaminants than in Cr (VI) media. When compared to the population of cells of the two types of bacteria, it shows that AC bacterial cells are more dominant than BS cells in the same interaction media.

The pattern of changes in OD values of BS and AC bacterial cells in Mn (II) contaminated media (Figures 3 and 4) is relatively not much different from the patterns of OD values of BS and AC in chromium contaminated media (Figures 1 and 2). Comparing the population of BS and AC bacteria in the media exposed to Mn (II) appears to be more dominant when compared to the population of the two types of bacteria in the medical Mn (VII). This shows that bacterial cells are more able to adapt in Mn (II) media than in Mn (VII) media, so it can be predicted that the ability of BS and AC bio-adsorption against Cr (III) and Mn (II) is higher than that of Cr (III) VI) and Mn (VII). Ensuring the ability of bio-adsorption of these two types of bacteria can see in the pH value, capacity and bio-adsorption efficiency of each of these bacteria against the heavy metals test.



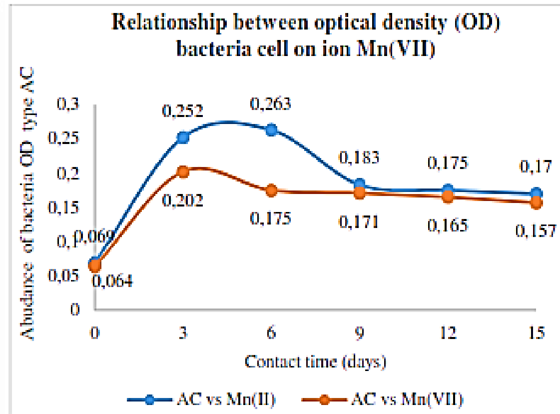


Figure . (3) Changes in OD values of BS bacterial cells in the interaction medium to the concentration of Mn (II) and Mn (VII) based on contact time (days); (4) Changes in OD values of AC bacterial cells in the interaction medium to the concentration of Mn (II) and Mn (VII) based on contact time (days).

The Degree of Acidity (pH) of The Interaction Media

The change in the acidity (pH) of the media is directly proportional to the number of bacterial cell populations, the more the bacterial cell population grows. The pH value of the suspension tends to be lower, meaning that the acidic nature of the media is getting stronger. Table 1. the following shows the pH value of the interaction media between BS and AC bacteria on toxic heavy metal chromium and manganese contaminants.

Table 1. The pH values of interaction media between BS and AC bacterial cells against heavy metal chromium and manganese contaminants.

Bacteria type	Contact time (days)	The degree of acidity (pH) in the Interaction media			
		Cr(III)	Cr(VI)	Mn(II)	Mn(VII)
BS	0	7,0	7,0	7,0	7,1
	3	6,7	6,7	6,6	6,7
	6	6,6	6,7	6,6	6,7
	9	6,7	6,8	6,7	6,8
	12	6,9	6,9	6,8	6,9
	15	6,9	6,9	6,9	7,0
AC	0	7,1	7,0	7,0	7,1
	3	6,6	6,6	6,6	6,7
	6	6,6	6,6	6,6	6,7
	9	6,7	6,8	6,7	6,8
	12	6,8	6,9	6,8	6,9
	15	6,9	7,0	6,9	7,0

Based on Table 1 it shows that the pH value of the interaction media contains a combination of suspension BS and Cr (III) as well as a combination of AC and Cr (III) is relatively smaller than the interaction media combination of BS and Cr (VI) and AC and Cr (VI), with thus it can be said that the population of BS and AC bacterial cells in Cr (III) contaminated media is more abundant than in Cr (VI) contaminated media. The same thing is also shown in the combination of interaction media with a combination of suspension BS and Mn (II) and AC and Mn (II) pH values tend to be lower than in a combination of BS and Mn (VII) and AC and Mn (VII). These results are consistent with the analysis of OD change value data (Figure 1-4), both pH values and OD values both indicate that the population of BS and AC bacterial cells in Cr (III) contaminated media is relatively more than in Cr (VI) contaminated media and BS and AC bacterial cell populations in Mn (II) contaminated media also tend to be larger than in Mn (VII) contaminated media. Lebij further said by Zahirnejad, Ziarati, & Asgarpanah, (2017) that an increase in bacterial cell population is usually accompanied by a tendency to decrease in pH.

The Bacteria of Bio-adsorption capacity and efficiency against test metals

The activity of bacterial bio-adsorption on heavy metals can in the ability of bacterial cells to continue to grow and develop in media exposed to heavy metal waste. The capacity and efficiency of bacterial bio-adsorption of waste containing heavy metal contami-nants shows the power or strength of bacterial bio-adsorption as bio-adsorbent against heavy metal adsorption. The capacity of bacterial bio-adsorption of heavy metals is directly proportional to the value of turbidity of the media. If the OD value is high, the capacity of bacterial bio-adsorption also increases and vice versa. Figure 5-8 shows the value and capacity of bio-adsorption of BS and AC bacteria on chromium metal ions.

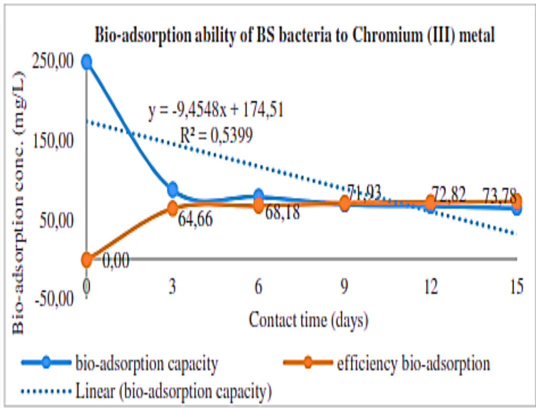


Figure 5. Relationship between capacity and efficiency of BS bio-adsorption on Cr (III) heavy metals.

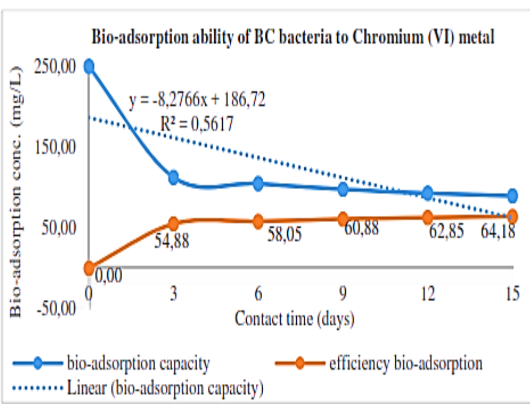


Figure 6. Relationship between capacity and efficiency of BS bacteria agains bio-adsorption on Cr (VI) heavy metals.

The bio-adsorption capacity of BS bacteria against Cr (III) contaminants is lower when compared to the bio-adsorption capacity of BS against Cr (VI) contaminants, meaning that the bio-adsorption capacity of BS bacteria in Cr (III) exposure media is stronger than in exposed media Cr (VI) thought to relate to the toxicity of both.

Comparing the capacity and efficiency of the bio-adsorption of BS bacteria symbiont sponge against chromium heavy metals, (Figures 5 and 6), shows the value of the bio-adsorption capacity of BS against Cr (VI) higher compared to Cr (III), or the efficiency of bacterial bio-adsorption BS against Cr (III) is higher than against Cr (VI). This condition shows that the strength of bio-adsorption of BS bacteria against Cr (III) metal is more dominant than that of Cr (VI). This corroborated by the results of regression analysis and the value of R2 with their respective values in the range of 0.5399 and 0.5617 shows that the bio-adsorption work of bacteria on the heavy metals of the test in an interrelated relationship, meaning that the bacteria as bio-adsorbent is an independent variable to heavy metal and vice versa or in other words there is a reciprocal relationship between the two according to the statement (Sharma et al., 2020; Marzuki et al., 2019b).

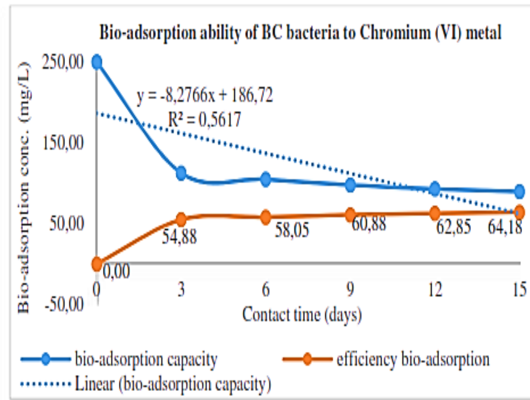


Figure 7. Relationship between capacity and efficiency of AC bio-adsorption on heavy metal Cr (III).

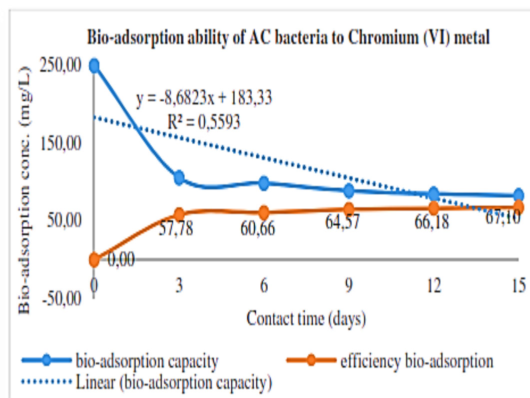


Figure 8. Relationship between capacity and efficiency of AC bio-adsorption on heavy metal Cr (VI).

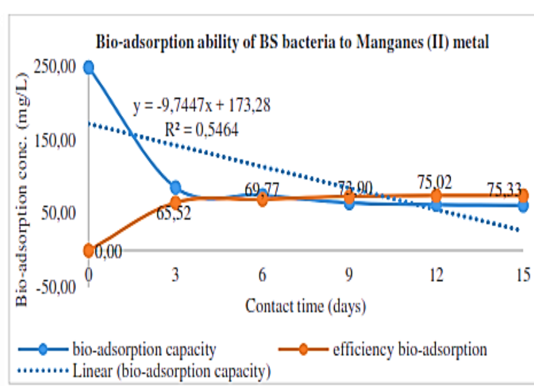


Figure 9. Relationship between capacity and efficiency of BS bio-adsorption on heavy metal Mn (II)

The apparent difference between AC bacteria and BS, on the value of capacity and efficiency of bio-adsorption The value of AC bacterial capacity is relatively smaller than that of BS bacteria or the efficiency of bio-adsorption of AC is higher compared to BS against Cr (III) and Cr (VI), so it can be said that the strength of AC bio-adsorption against Chromium metal is higher compared to BS bacteria.

These results were estimated initially by looking at the AC bacterial suspension OD value higher than the BS bacterial OD value in the same media.

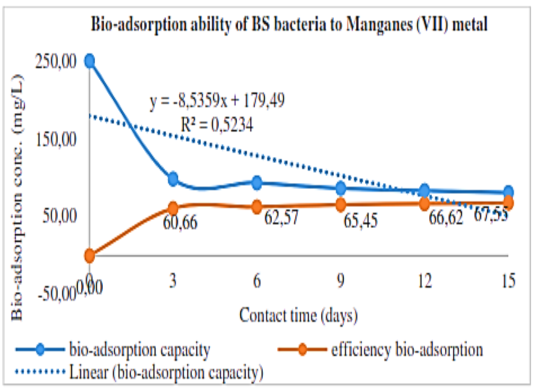


Figure 10. Relationship between capacity and efficiency of BS bio-adsorption on heavy metal Mn (VII).

The pattern of bio-adsorption capacity and efficiency of BS bacteria towards Manganese adsorption is relatively no different compared to Chromium adsorption, as shown in Figures 9 and 10. The difference only appears in the value of bio-adsorption capacity and efficiency, meaning that the bio-adsorption capacity of BS bacteria against Mn (II) is relatively lower compared to Mn (VII) or the bio-adsorption efficiency of BS is higher compared to Mn (II) compared to Mn (VII), but when compared to the efficiency of BS between Chromium and Manganese metals, it appears that the efficiency of BS to Mn (II) higher than Cr (III) as well as things between Cr (VI) and Mn (VII). If we see the results of regression analysis and the value of R2, respectively in the range of 0.5464 and 0.5234, it shows that the bio-adsorption action of bacteria on the heavy metals of the test in an interrelated relationship means that there is a reciprocal relationship between the two, so in this case also applies to the equilibrium equation: bacterial reactivity heavy metal activity. This situation illustrates that there are 2 velocities of bio-adsorption, each k1 to the right and k2 to the left, until a balance is reached between the two where the speed of bio-adsorption goes to zero or the bio-adsorption activity of bacteria against the test metal reaches the point saturated (Riyaz et al., 2020), however, for the investigation and analysis of the bacterial bio-adsorption kinetics of heavy metals, a deep study and experiment are needed.

The pattern of changes in bio-adsorption capacity and efficiency based on the contact time by AC bacteria to the heavy metal Mn (Figures 11 and 12) is also relatively similar to that seen in Cr metal (Figures 7 and 8).

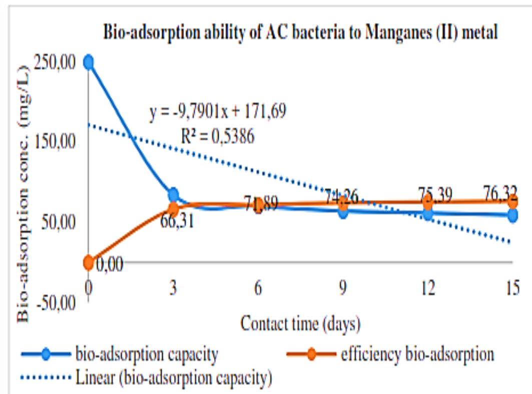


Figure 11. Relationship between capacity and efficiency of AC bio-adsorption on heavy metal Mn (II)

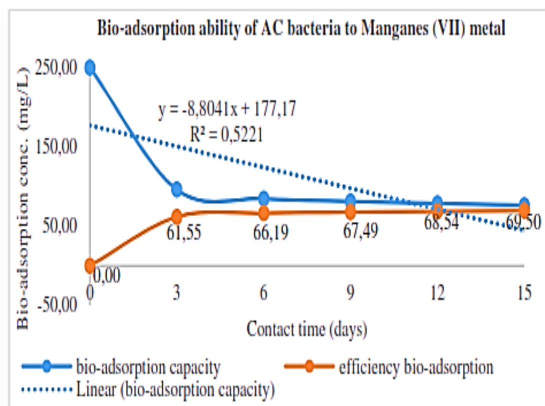


Figure 12. Relationship between capacity and efficiency of AC bio-adsorption on heavy metal Mn (VII)

The difference is only in the capacity value and the efficiency value of bio-adsorption which can be interpreted that the AC bio-adsorption strength of the Mn metal is higher than that of the Cr metal (Orani et al., 2018). The capacity and efficiency bio-adsorption of BS and AC bacteria on heavy metals Cr and Mn ions strengthened by the results of the calculation of average capacity and optimum bio-adsorption capacity, as shown in Table 2, below:

Table 2. Average strengths and optimum values of bio-adsorption of BS and AC bacteria on Chromium and Manganese metals.

Bacteria type	Power Bio-adsorption	Heavy metal ion concentration test				Contact time (days)
		Cr (III)	Cr (VI)	Mn (II)	Mn (VII)	
BS	optimum (mg/L)	161,66±09	137,21±06	163,79±08	151,66±11	3
	average (mg/L)	175,68±12	150,42±14	179,77±07	161,43±07	15
AC	optimum (mg/L)	162,77±16	144,44±08	165,77±15	153,88±14	3
	average (mg/L)	177,24±11	158,14±16	182,08±13	166,64±08	15

Based on Table 2, in general it can be said that the average bio-adsorption power of BS and AC bacteria to Mn is higher than that of Cr, as well as the optimum value of bio-adsorption BS and AC to Mn relative to the metal Cr. The optimum value of bio-adsorption achieved at 3 days of interaction. In Table 2, it also shows the AC bio-adsorption power of both Cr and Mn metals, appears to be higher than that of BS bacteria, where when compared to BS and AC activity on the same metal contaminants, it appears that the AC and BS bio-adsorption power is superior in Cr (III) interaction media were compared in Cr (VI) contaminants. This also occurs in Mn contaminants, where the bio-adsorption power of BS and AC is more dominant in the Mn (II) contamination media than in Mn (VII) contaminants. Comparison of the bio-adsorption power of BS and AC bacteria against Cr and Mn metals in detail shown in Figures 13 and 14, below.

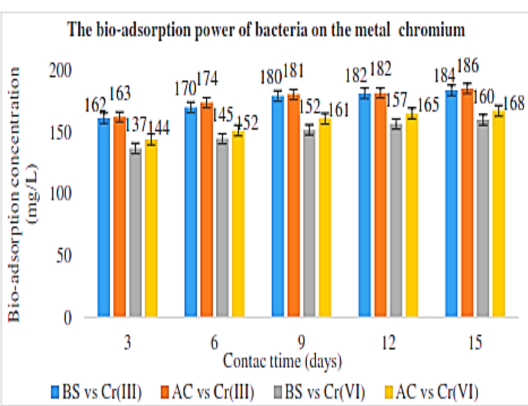


Figure 13. Relationship of bio-adsorption power of sponge symbiont bacteria to Chromium ions.

Figure 13 shows that the bio-adsorption power of AC bacteria against Cr (III) is relatively higher compared to BS bacteria, as well as the bio-adsorption power of AC bacteria to Cr (VI) metal also relatively higher compared to the use of BS bacteria.

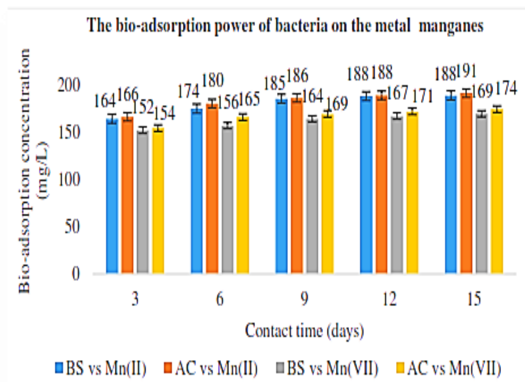


Figure 14. Relationship of bio-adsorption power of sponge symbionts bacteria to Manganese ions

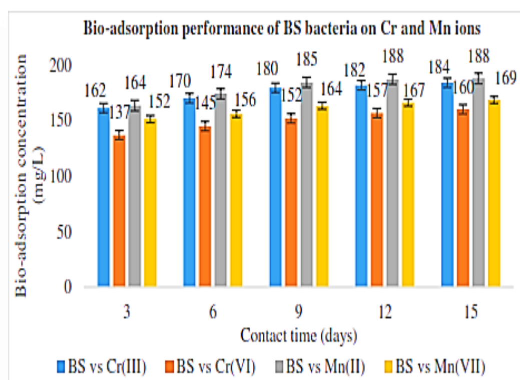


Figure 15. Bio-adsorption performance of BS bacteria on chromium and manganese heavy metals

The data in Figure 14 is also not much different from Figure 13. The AC bio-adsorption power of Mn (II) relatively higher compared to BS bacteria and the bio-adsorption strength of AC bacteria to metal Mn (VII) tends to higher compared to bacterial bio-adsorption power BS.

Comparison of bacterial bio-adsorption performance against the same test metal based on differences in oxidation numbers, shown in Figures 15 and 16, follows:

Bio-adsorption performance between BS bacteria against Cr (III) is higher than that of Cr (VI), likewise the performance of bio-adsorption of BS against Mn (II) is more dominant when compared to Mn (VII). This shown in Figure 15. This situation reflects that there is an effect of reactivity of heavy metal ion tests on the performance of bacterial bio-adsorption. It known that Cr (VI) ions are more toxic and more reactive than Cr (III) ions and Mn (VII) ions are relatively more toxic than Mn (II) ions, so the bio-adsorption activity of bacteria tends to be weaker against Cr (VI) metal ions compared to Cr (III), this result is relatively similar to the statement Naghipour & Davoud, (2018).

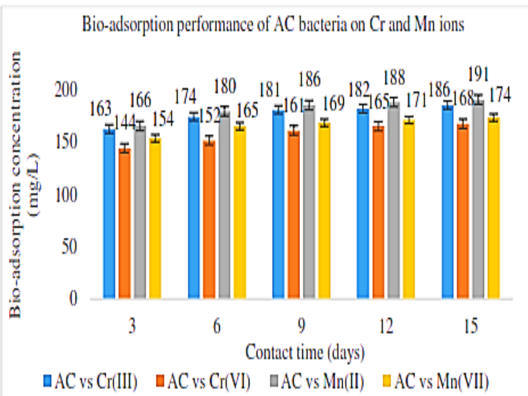


Figure 16. Performance of AC bacterial adsorption on chromium and manganese heavy metals

Looking at Figure 16, it also shows that the bio-adsorption performance of AC bacteria on Cr (III) ions much higher compared to Cr (VI) ions. Likewise, the bio-adsorption ratio of AC bacteria to Mn (II) ions compared to Mn (VII) ions. This reinforces the suspicion that the toxic nature and reactivity of Cr (VI) and Mn (VII) ions influence the performance of bacterial bio-adsorption. This assumption related to the value of affinity, electronegativity, ionization energy and ionic radius, in the periodic arrangement of elements. Cr metal in period 4 position is group 6B, whereas Mn metal is period 4 group 7B, meaning that affinity, ionization energy and electronegativity of Mn are relatively greater than that of Cr metal. This based on the bio-adsorption pattern shown by the two types of sponge symbionts on Cr (III) and Cr (VI) metal ions as well as on Mn (II) and Mn (VII) metal ions. This result is in line with previous research (Karimpour & Davoud, 2018; Konkolewskaet al., 2020). This means that the bio-adsorption pattern of BS and AC bacteria to Cr (VI) ions is always lower when compared to Cr (III) also the bio-adsorption of BS and AC to Mn (VII) is weak compared to Mn (II) in all parameters bio-adsorption observed.

Conclusion

Bio-adsorption power of AC bacteria against Cr and Mn ions is more dominant than AC bacteria. The capacity and efficiency of bio-adsorption of BS and AC bacteria against Cr (III) > Cr (VI) and Mn (II) > Mn (VII) ions. The adaptability of AC and BS bacteria is stronger in Cr (III) contaminated media than in toxic (Cr) VI media, as well as AC and BS adaptation in Mn (II) exposed media compared to Mn (VII) reactive media. The population of BS and AC bacterial cells in Cr (III) and Mn (II) media is greater than in Cr (VI) and Mn (VII) media. It suspected that there is an effect of reactivity and high toxic properties of the test metal ions on the performance of bacterial bio-adsorption.

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