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Bioremediation of Trivalent Chromium in Soil Using Bacteria

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Abstract

Trivalent chromium classified as one dangerous contaminant that require further treatment. Trivalent chromium levels that exist in surface water and soil increased as many industries utilize chromium as a raw material. Bioremediation is a technique that is widely used to process pollution caused by heavy metals. Bacteria is one of the agent that has been used as trivalent chromium remediator. Bacteria can defend themselves from trivalent chromium exposure. *Azotobacter S8, Bacillus subtilis* and *Pseudomonas putida* have been studied and capable to remove trivalent chromium in soil. The highest chromium removal percentage in preliminary test shown by *Azotobacter S8* that was equal to 10,53%, followed by *Bacillus subtilis* by 5.68%. *Azotobacter S8* chosen as chromium removal agent in chromium contaminated soil. Chromium concentration of 50mg/L (19mg/Kg) and 100mg/L chromium trichloride (38mg/Kg) used as variable in bioremediation of chromium contaminated soil. Result showed that *Azotobacter S8* was capable to remove up to 22.82% for 19mg/Kg chromium contaminated soil and 11.08% for 38mg/Kg chromium contaminated soil.

Keywords: *Azotobacter S8, Bacillus subtilis*, Bioremediation, *Pseudomonas putida*, Trivalent Chromium

INTRODUCTION

The application of chromium in various industry such as electroplating, leather tanning, stainless steel coating, metal processing, and textile has caused a widespread environmental contamination (Shukla et al., 2007). Chromium generally exists in two forms, Cr(III) and Cr(VI) (Kaur and Kumar, 2014). Cr(III) is less toxic and less mobile than Cr(IV) (Cheng and Li, 2009). However, Cr(III) is more stable than Cr(VI), when Cr(VI) reacts with other particles in the air, the form will be changed into Cr(III) (Evelyne and Ravinskar, 2014).

Chromium pollution can be treated using psycho-chemical or biological treatment. Biological treatment is relatively more environmental friendly because its waste-less than psycho-chemical treatment. Bioremediation is one of technology that used for treating soil and wastewater containing chromium (Evelyne and Ravinskar, 2014). It uses living organisms to reduce the amount of contaminants (Vidali, 2001). The principle of bioremediation is utilizing metabolic reaction of living organisms to degrade the contaminants in environment (Mahimairaja et al., 2011).

Bacteria is one of living organism that usually use for treating heavy metal contaminant. Bacteria have an ability to resist and reduce heavy metals at certain concentration (Kaur and Kumar, 2014). This ability consist of several processes such as adsorption, absorption, accumulation and transformation inside its cell (Zahoor and Rehman, 2009). Biosorption usually used as the most significant bacteria's ability to reduce the ammount of pollutant (Oves et al., 2013). Biosoption mechanism consist of adsorbtion, absorption, accumulation and transformation of pollutant inside bacteria's cell. Because of this ability, bacteria usually used for bioremediation of heavy metal pollution in environment (Deepali, 2011).

In this study, bacteria used were *Azotobacter S8*, *Bacillus subtilis,* and *Pseudomonas putida* which resistance and able to reduce heavy metal contaminant. *Azotobacter S8* has an ability to resist Chromium(VI) up to 300 mg/L (Pavel et al., 2012) when *Pseudomonas* is able to resist Chromium(III) up to 400 µg/mL (Bahig et al., 2012). *Bacillus subtilis,* Gram-positive bacteria, was known to survive in medium containing Chromium(III) up to 400 µg/mL (Bahig et al., 2012). The objective of this study were to measure the trivalent chromium removal percentage by bacteria for remediating chromium contaminated soil.

MATERIALS AND METHODS

Bacteria Screening Test

The aim of screening test was to obtain the best bacteria for chromium contaminated soil remediation. Bacteria screening test was carried by inoculating bacteria in 50 mg/L chromium trichloride (Merck, Germany) solution. Inoculum were bacteria that has been shaken for half an exponential time to obtain OD600 = 0.5 (Purwanti et al., 2015). Inoculum was added to chromium solution as much as 10% of the total volume of the reactor (Deepali, 2011). Bacteria shaken on a shaker (Innova 2000, Germany) for exponential time (4hours) by 150 rpm (Purwanti et al., 2015). Total chromium in the beginning and the end of test period were measured using Atomic Absorption Spectrophotometer (Rayleigh WFX 210, Beijing).

Trivalent Chromium Removal in Contaminated Soil Test

Trivalent chromium removal in contaminated soil was done by inoculating the best bacteria culture from the screening test. The inoculated bacteria already been shaken for half an exponential time to obtain OD600 = 0.5 (Purwanti et al., 2015). Number of bacteria added to reactor was equal to 15% volume per volume (v/v) (Deepali, 2011) and the chromium concentration used were 19 and 38mg/Kg using sand medium. The total sand medium used for each reactor was 425mg in 350ml glass reactor.

The experiment was carried out in Environmental Remediation Laboratory, Department of Environmental Engineering. Total 4 reactors were used for this experiment. The concentration of chromium trichloride spiked into the sand were 19mg/Kg (representing 50mg/L) and 38mg/Kg (representing 100mg/L). After spraying into the sand, the sand containing chromium was stirred manually to homogeneous and left for 14 days prior the biomag. Four reactors used in this stage coded C1B1(1), C2B1(1), C1B1(2) and C2B1(2). C1 used for 19mg/Kg chromium contaminated medium and C2 used for 38mg/Kg chromium contaminated medium while B1 representing the addition of 15% v/v *Azotobacter S8*. Each concentration was done duplo by coding (1) and (2). Spiked soil was made by mixing 1000mg/L chromium trichloride stock solution to sand medium by its bulk density as the formula below:

$$X\frac{mg}{Kg} = \frac{1000(\frac{mg}{L})\ x\ Volume\ based\ on\ bulk\ density\ (L)}{Weight\ of\ medium\ (Kg)}$$

The bacteria used in this stage was 24hours bacteria that already been shaken for half an exponential time to obtain OD600 = 0.5. Bacteria then centrifuged 4000rpm for 15minutes before washed with 0.85% sodium chloride solution (Merck, Germany). Washed bacteria then diluted by 0.85% sodium chloride solution to certain volume to obtain OD600 = 0.5 (Purwanti et al., 2015). 85% of sand bulk density filled with chromium solution as calculated before and other 15% filled with bacteria OD600 = 0.5.

Reactors then incubated for 14days in room temperature. Reactor aeration was done manually by completely stirring medium using inert stirrer once a day. Parameters tested within incubation period were pH, temperature, number of bacterial colonies and total chromium. Temperature and pH parameters were tested once every two days when the number of bacterial colonies and total chromium parameter were tested twice within incubation period (at the beginning and the end of the incubation period) (Pranowo and Titah, 2016). The result of chromium removal in reactor contain bacteria will be compared with chromium removal in reactor contain only contaminated medium.

RESULT AND DISCUSSION

Bacteria Screening Test

Percentage of chromium removal after 4hours test is depicted in Figure 1. The highest removal of chromium (10.53%) achieved by *Azotobacter S8*. Erni (2011) suggested that *Azotobacter S8* has exopolysaccharide (EPS) which can adsorb metals and forming ligands, together with EPS. Figure 1 indicated that *Pseudomonas putida* didn't show any removal percentage because of its low growth checked by visual turbidity. Meanwhile this case also happened due to the lack of test period. The 4hours test period was not significant enough for all bacteria to give their best metabolism to remove trivalent chromium. (Erni, 2011). In other side, Pranowo and Titah (2016) suggested that measurement in exponential phase could be used as preliminary screening test. Based on the result, *Azotobacter S8* will be used on trivalent chromium removal in contaminated soil test.

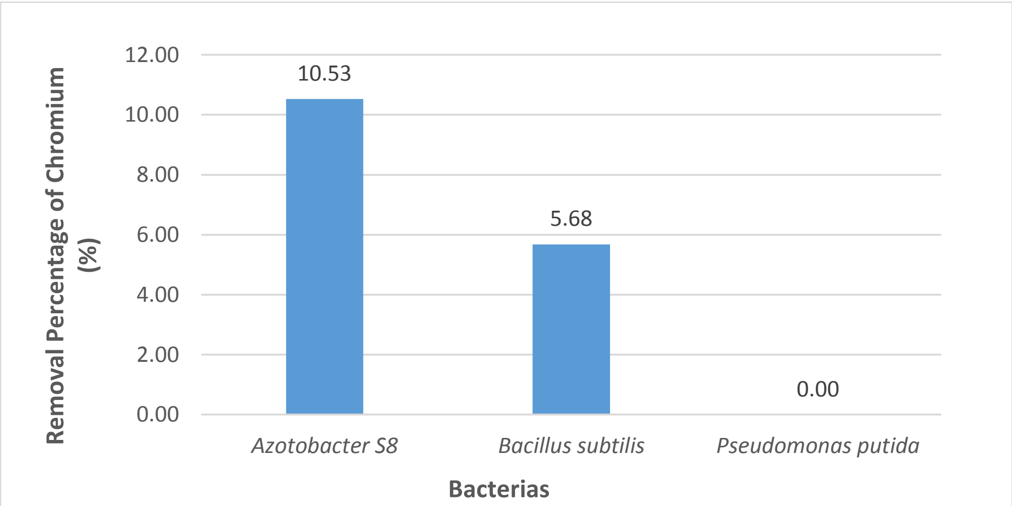

Figure 1: Trivalent Chromium Removal in Wastewater by Bacteria

Trivalent Chromium Removal in Contaminated Soil

The time that used as the test period for chromium removal in contaminated soil was 14 days (Purwanti et al., 2015). At this phase, main parameters that will be tested were temperature, pH, total chromium and number of bacterial colonies. Average temperature (Figure 2), average pH (Figure 3), the total chromium parameter (Figure 4) and number of bacterial colonies (Figure 5) were measured as parameters in this stage.

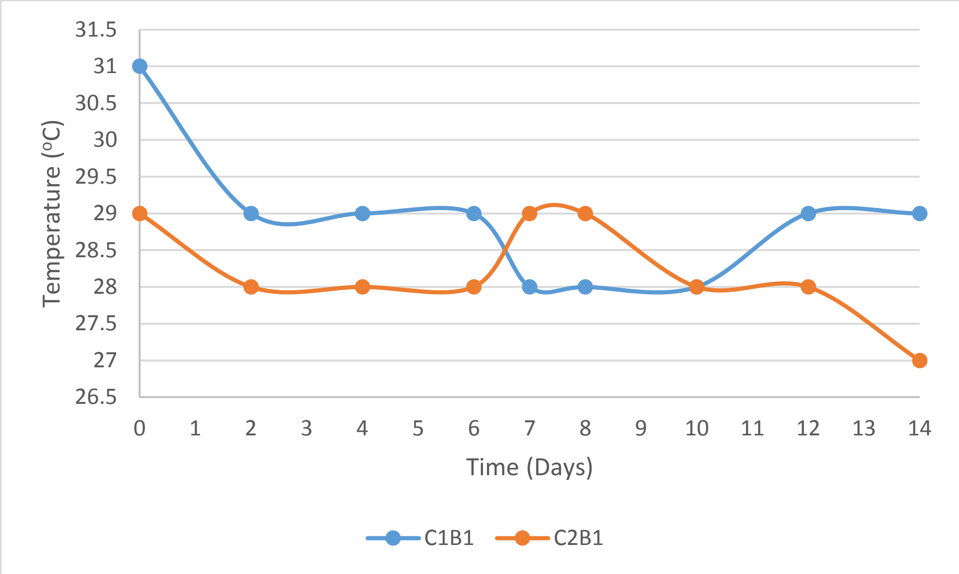

Figure 2: Temperature Measurement on Chromium Removal in Contaminated Soil

Based on Figure 2, temperature measured on all reactor were fluctuating and ranged from 27 to 31.5ºC. Reference (Wedhastri, 2002) states that Azotobacter's growth is very sensitive to temperature above 35ºC. Soil temperature tested in all reactor were still below 35ºC and above 20ºC and it still included in Azotobacter's optimum growth condition as mesophilic bacteria.

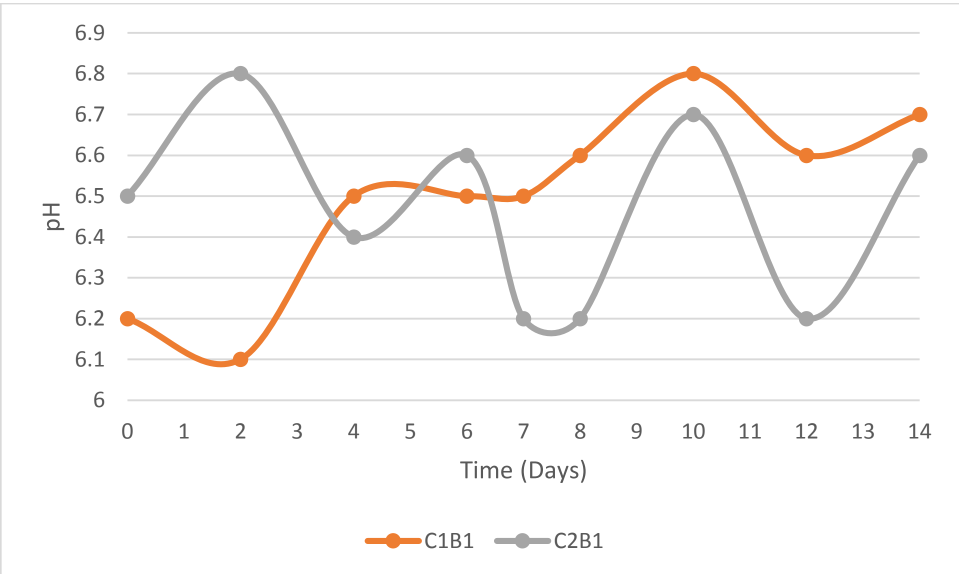

Figure 3: pH Measurement on Chromium Removal in Contaminated Soil

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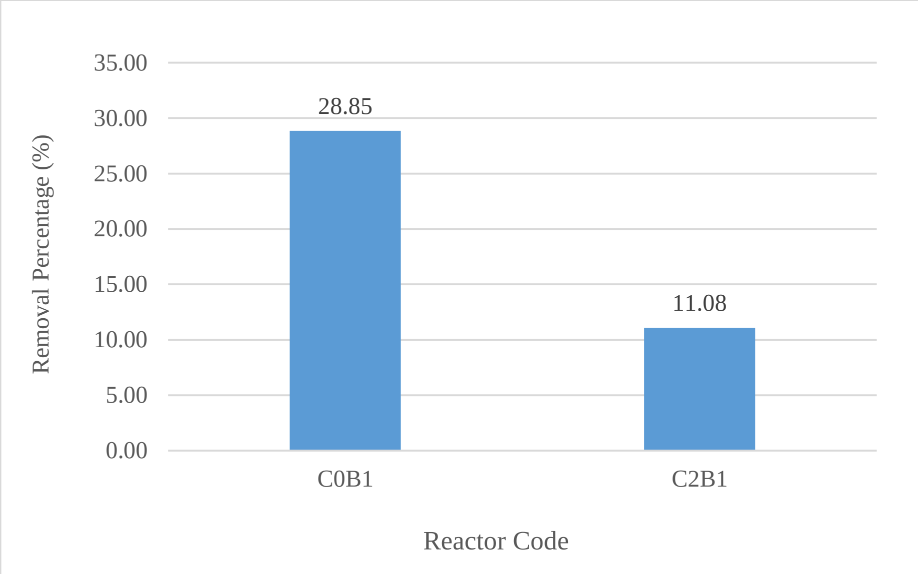

Figure 4: Trivalent Chromium Removal in Contaminated Soil

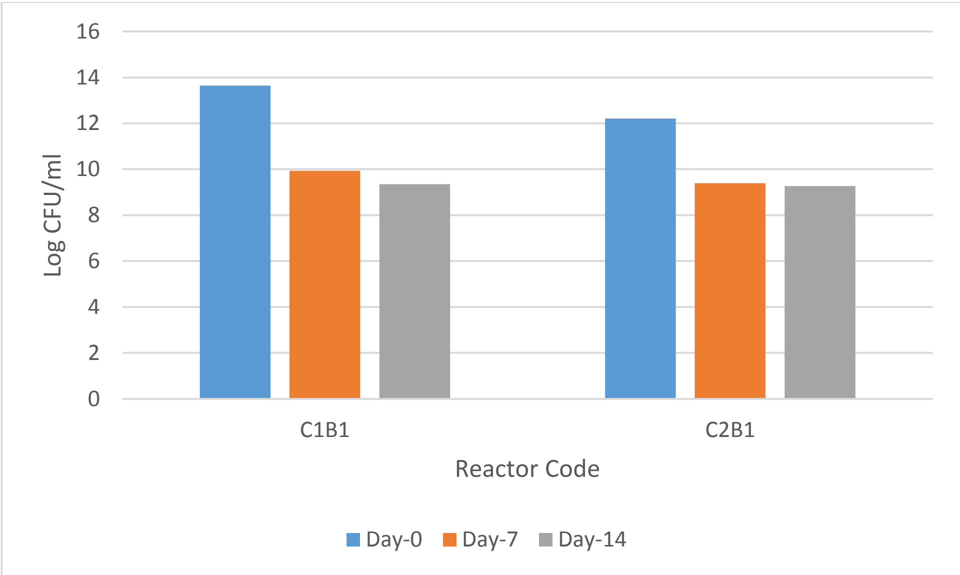

Figure 5: Number of Bacterial Colonies on Chromium Removal in Contaminated Soil

Figure 3 showed that pH tested in all reactor were ranged from 5 to 6.9. Abat (2006) states that Azotobacter can live in medium with pH ranged from 4.5 to 8.5. Wedhastri (2002) also states that Azotobacter was so sensitive to acidic condition. Based on Wedhastri (2002) and Abat (2006), all pH tested were still included in Azotobacter optimum growth condition.

There were correlations between Figure 4 and 5, the higher concentration of chromium added to the medium, the lower number of bacteria living in reactor. It also could be seen that the number of bacteria living in all reactor tend to be decreased by the time along test period. In addition of 15% *Azotobacter S8* to reactor contain 19 and 38mg/kg chromium, removal percentage of trivalent chromium decreased as the chromium concentration in medium increased. This showed that the existence of chromium in medium inhibit the bacterial metabolism, bacteria metabolism to reduce chromium in medium also decreased when the chromium concentration increased.

CONCLUSION

Based on results and discussion above, the conclusions can be determined from this research were:

1. Azotobacter S8 showed the best chromium removal percentage in screening test by 10.53% during 4hours test period.

2. The addition of Azotobacter S8 in C1B1 reactor was capable to remove up to 22.82% in 19mg/kg trivalent chromium contaminated soil.

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