Bioremediation of Lead-Contaminated Mine Waste Using Microbially Induced Carbonate Precipitation

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Abstract

The aim of this study was to use microbially induced calcium carbonate precipitation (MICP) technique to bioremediate lead using bacterium *Pararhodobacter* sp. Laboratory scale experiments conducted, achieved complete removal of lead. This result was further confirmed by SEM and XRD analysis that indicated coprecipitation of calcium carbonate ($CaCO_3$) and cerussite ($PbCO_3$). Furthermore, syringe test demonstrated that MICP based sequestration of heavy metals via coprecipitation with calcium carbonate may be useful for lead bioremediation. Very few low-cost in situ heavy metal treatment processes for lead bioremediation are available; therefore, bioimmobilization of lead by MICP has the potential for application as a low-cost and eco-friendly method for heavy metal remediation.

Keywords: Biominerals, Bioimmobilisation, Hazardous mining waste

1. Introduction

Lead makes up only about 0.0013% of the earth's crust and has been used for centuries because of its malleability, ductility, and a poor conductor of electricity. However, despite its usefulness, lead has caused both air, water and soil pollution. According to the WHO lead causes brain and central nervous system to cause coma, convulsions and even death [1]. The major challenge of conventional physico-chemical lead remediation technologies involves huge cost for removal, treatment or containing to prevent contaminant from migrating to surrounding areas. In this study, we explore the possibility of bioremediation by Bioimmobilisation process based on microbially induced calcium carbonate precipitation (MICP) by *Pararhodobacter* sp. [2]. MICP involves the hydrolysis of urea into ammonium and carbamate by urease catalysis which results in $CaCO_3$ formation in the presence of Ca^{2+} ions (Eq. (1) - (3))[3].

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{Urease}} H_2NCOO^- + NH_4^+ \quad \ldots\ldots(1)$$

$$H_2NCOO^- + H_2O \longrightarrow HCO_3^- + NH_3 \quad \ldots\ldots(2)$$

$$Ca^{2+} + HCO_3^- + NH_3 \longrightarrow CaCO_3 + NH_4 \quad \ldots\ldots(3)$$

Pararhodobacter sp. was studied because it has shown high urease activity and can maintained its activity for a long time [4]. This characteristic was utilized in this study as it is useful for remediating soils in-situ. Previous researchers conducted solidification using *Pararhodobacter* sp. for ground improvement purposes only [2][5], however, in this study, *Pararhodobacter* sp. was investigated for possible heavy

metal contaminated mine waste stabilization and immobilization. Therefore, the specific objectives of this study were to: investigate the effect of lead on microbial growth; determine the effectiveness of *Pararhodobacter* in lead removal; and determine the effect of varying the injection interval of the bacteria on unconfined compression strength (UCS) for fine and coarse grained sand for possible application in bioremediation activities.

2. Methodology

2.1 Effect of lead on microbial growth

Pararhodobacter sp., an ureolytic bacterium isolated by our laboratory from the soil near beachrock in Okinawa, Japan [6] was used in this study. *Pararhodobacter* cells were precultured in 5 mL medium at 30°C with shaking at 160 rpm for 24 hour, and then a 1 mL of the preculture was inoculated into 100 mL. Different concentrations of Pb^{2+} were prepared and added to the 100 mL medium and incubated for 48hrs and the number of colony forming units (CFU/mL) in solution determined. To determine the colony forming units per mL of sample (CFU/mL), Miles And Misra method was adopted and equation (4) was used[7].

$$\frac{CFU}{mL} = \frac{\text{(Number of colonies} \times \text{Dilution factor)}}{\text{Volume of liquid culture sample plated}} \quad \text{…. (4)}$$

2.2 Bioprecipitation Experiments

Bioprecipitation experiment was carried out by culturing *Pararhodobacter* sp. at 30 °C after addition of urea and calcium chloride in the presence of lead and precipitate formed, separated from supernatant by centrifugation. Residual lead in the supernatant was determined by inductively coupled plasma atomic emission spectroscopy whereas the precipitate was analysed by X-ray diffraction (XRD) and scanning electron microscope (SEM).

2.3 Sand Solidification

Two types of sand samples were used for this study: fine sand (mean diameter, D_{50} = 170 μm) and coarse sand (mean diameter, D_{50} = 1.2 mm). Initially, sand was packed into the syringe, then bacteria and solidification solution were added according to previous study by Danjo and Kawasaki [2]. After the curing period of 14days, the estimated unconfined compression strength (UCS) of the specimen was determined using a penetrometer.

3 Results and Discussion

3.1 Effect of lead on microbial growth

Lead containing liquid media showed a decrease in number of cells following lead treatment compared to the control (Figure 1). These results imply that the bacterium can be used for bioremediation, as the effect of lead on the bacteria is negligible for the concentration evaluated.

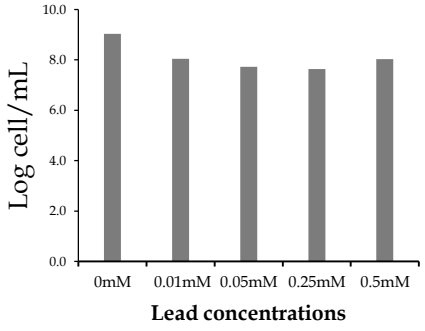

Figure 1 - Effect of Pb^{2+} concentrations on the growth of *Pararhodobacter* as measured by CFU/mL (n=3)

3.2 Lead Bioprecipitation

Figure 2 shows that *Pararhodobacter* was effective in complete removal of lead. This result agrees with results for microbial growth in that *Pararhodobacter* can be used for bioremediation of lead. It is postulated that calcium carbonate incorporated Pb^{2+} in/onto their surfaces through the replacement of the Ca^{2+} in the calcium carbonate matrix and form cerussite ($PbCO_3$) which is a stable and non-toxic form of lead.

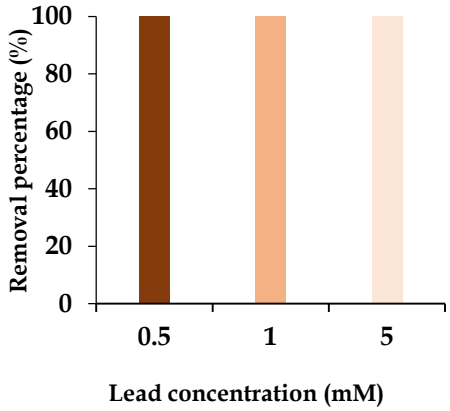

Figure 2 - Removal percentage of lead during bioprecipitation experiment using *Pararhodobacter*. Triplicates of experiment were conducted

3.3 Syringe solidification experiments

Pararhodobacter increased the pH to maximum value of 9.5. A high pH is very important for calcium carbonate precipitation. SEM (Figure 3a) and XRD (Figure 3b) results shows formation of calcium carbonate in the form of calcite and vaterite. This study has clearly confirmed calcium carbonate formation and free metal ion incorporated in/onto the surfaces of calcium carbonate. This could have been due to the replacement of the Ca^{2+} in the calcite/vaterite matrix and formation of cerussite ($PbCO_3$) which is chemically stable and non-toxic form of lead[8][9].

Figure 4 shows that increase in the number of injection interval of bacteria increases the UCS value of the solidified sand. Therefore, addition of bacteria several times would be more advantageous as it allows more nucleation sites than adding once. Therefore, MICP technique not only can it immobilized the lead but also induce high resistance of the contaminated materials to erosion [10]. Therefore, the data obtained using the syringe test demonstrated that MICP is a viable option for use in coarse and fine grained sand.

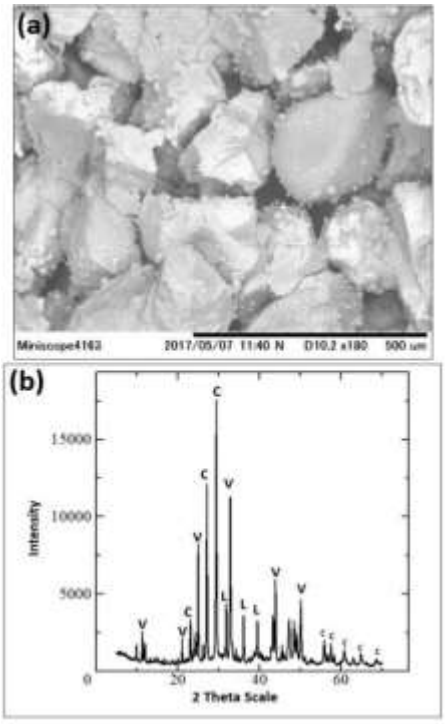

Figure 3 - (a) SEM images and (b) XRD analysis of precipitate formed by *Pararhodobacter* sp. in the presence of Pb^{2+} (b). (C = Calcite ($CaCO_3$); V = Vaterite ($CaCO_3$); L = Lead Oxide (PbO))

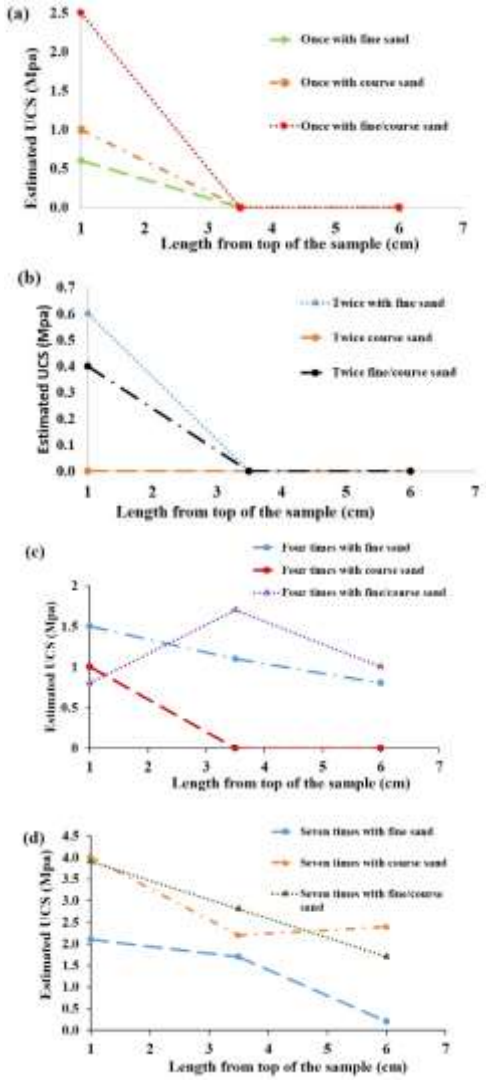

Figure 4 - UCS comparison of the results from varying the bacteria injection interval (a) once (b) twice (c) four times (d) seven times

4. Conclusion

Pararhodobacter sp. was effective in complete removal of lead by elevating the pH to alkaline condition. SEM and XRD further confirmed transformation of toxic free Pb^{2+} ions to a more stable form of lead which were bioprecipitated by calcite or vaterite which were predominant in the pattern. Furthermore, the syringe experiments revealed that UCS increased with increasing injection interval of bacteria. These results are useful and can be applied for the bioremediation of lead in both fine and coarse materials as an eco-friendly and sustainable method of heavy metal remediation.

Acknowledgment

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References

[1] V. Achal, X. Pan, D. J. Lee, D. Kumari, and D. Zhang, (2013). Remediation of Cr(VI) from chromium slag by biocementation," *Chemosphere*, vol. 93, no. 7, pp. 1352–1358.

[2] T. Danjo and S. Kawasaki, (2016). Microbially Induced Sand Cementation Method Using *Pararhodobacter* sp. Strain SO1, Inspired by Beachrock Formation Mechanism," *Mater. Trans.*, vol. 57, no. 3, pp. 428–437.

[3] V. S. Whiffin, L. A. van Paassen, and M. P. Harkes, (2007). Microbial Carbonate Precipitation as a Soil Improvement Technique, *Geomicrobiol. J.*, vol. 24, pp. 417–423.

[4] M. Fujita, K. Nakashima, V. Achal, and S. Kawasaki, (2017). Whole-cell evaluation of urease activity of Pararhodobacter sp. isolated from peripheral beachrock, *Biochem. Eng. J.*, vol. 124, pp. 1–5.

[5] M. N. H. Khan, G. G. N. N. Amarakoon, S. Shimazaki, and S. Kawasaki, (2015). Coral Sand Solidification Test Based on Microbially Induced Carbonate Precipitation Using Ureolytic Bacteria, *Mater. Trans.*, vol. 56, no. 10, pp. 1725–1732,.

[6] T. Danjo and S. Kawasaki, (2013). A Study of the Formation Mechanism of Beachrock in Okinawa , Japan : Toward Making Artificial Rock, vol. 5, no. 1, pp. 634–639.

[7] A. Miles, S. Misra, and J. Irwin, (1938). The estimation of the bactericidal power of the blood," *J. Hyg. (Lond),* pp. 732–749.

[8] A. J. Mugwar and M. J. Harbottle, (2016). Toxicity effects on metal sequestration by microbially-induced carbonate precipitation, *J. Hazard. Mater.*, vol. 314, pp. 237–248.

[9] C. H. Kang, S. H. Han, Y. Shin, S. J. Oh, and J. S. So, (2014). Bioremediation of Cd by microbially induced calcite precipitation," *Appl. Biochem. Biotechnol.*, vol. 172, no. 6, pp. 2907–2915.

[10] E. Salifu, E. MacLachlan, K. R. Iyer, C. W. Knapp, and A. Tarantino, (2016). Application of microbially induced calcite precipitation in erosion mitigation and stabilisation of sandy soil foreshore slopes: A preliminary investigation," Eng. Geol., vol. 201, pp. 96–105.