Bioremediation of lead-contaminated mine waste by *Pararhodobacter* sp. based on the microbially induced calcium carbonate precipitation technique and its effects on strength of coarse and fine grained sand

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Abstract

Lead (Pb^{2+}) is a toxic heavy metal that has a severe negative effect on human health and the environment. Physical, chemical and biological remediation techniques have long been used to remediate lead contamination. However, because of the great danger posed by lead contamination, there is increasing interest to apply eco-friendly and sustainable methods to remediate lead. Therefore, this study was conducted to use the microbially induced calcium carbonate precipitation (MICP) technique in conjunction with the bacterium *Pararhodobacter* sp. to bioremediate lead. Laboratory scale experiments were conducted and complete removal of 1036 mg/L of Pb^{2+} was achieved. These results were further confirmed by scanning electron microscope (SEM) and X-ray diffraction (XRD) analysis, which indicated coprecipitation of calcium carbonate ($CaCO_3$) and lead. The unconfined compressive strength increased with an increase in injection interval with maximum unconfined compressive strength of 1.33 MPa for fine sand, 2.87 MPa for coarse sand and 2.80 MPa for mixed sand. For *Pararhodobacter* sp. to efficiently induce lead immobilisation the bacterial interval required is four times with a calcium and urea concentration of 0.5 M and bacterial concentration of 10^9 CFU/mL. 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: *biomineral; bioremediation; microbially induced calcium carbonate precipitation; lead*

1. Introduction

Lead (Pb^{2+}) has been studied extensively worldwide because of its effects on human health and the environment (Yabe et al., 2015; Lin et al., 2009). The main characteristics of lead are that it persists in all parts of the environment, cannot be degraded or destroyed and enters the body through food, drinking water, and air. Lead poisoning results in brain and central nervous system damage, coma, convulsion and even death, mostly in children because they absorb 4-5 times as much lead as adults (Lanphear, 2005). One such area that is heavily contaminated with lead due to mining and smelting is Kabwe Mine, Zambia (Nyambe et al., 2013), which was selected as the study area for this investigation.

The Kabwe mine was a Pb-Zn mine that operated from 1902 to 1994, during which time it caused lead poisoning affecting close to 300,000 people. Despite closure of the mine, its wastes have continued to pollute the soil and water. Plant residue leachate of waste from tailings and thickeners and kiln slag are the major sources of lead, covering an area of approximately 86,600 m^2 and 164,800 m^2, respectively (ZCCM-IH, Zambia Copper and Cobalt Mining-Investment Holding Plc., 2002). In the past, a portion of the coarse kiln slag was used to cover the fine leached plant residue to prevent water erosion and windblown dust.

Several physical, chemical and biological remediation techniques have been used for many years to remediate lead contamination. However, the major challenge of physical-chemical lead remediation technologies involves a huge cost for complete removal or containment of contaminants to prevent them from migrating to surrounding areas. In this study, we seek to investigate the possibility of bioremediation of lead-contaminated mine waste in Kabwe based on microbially induced calcium carbonate precipitation (MICP). This technique involves the hydrolysis of urea into ammonium and carbamate by urease catalysis (Eq. (1)), resulting in $CaCO_3$ formation in the presence of Ca^{2+} ions (Eqs. (2)–(3)) (Whiffin et al., 2007).

$$CO(NH_2)_2 + H_2O \longrightarrow H_2NCOO^- + NH_4^+ \quad (1)$$

$$H_2NCOO^- + H_2O \xrightarrow{\text{Urease}} HCO_3^- + NH_3 \quad (2)$$

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

The role of calcium ion in bioremediation using the MICP technique takes advantage of the incorporation of the Pb^{2+} onto their surfaces through substitution in the calcite lattice (Equation 4), after which Pb^{2+} are changed from soluble form to insoluble forms hence detoxifying the toxic lead ions (Anbu et al., 2016). The calcium carbonate formed can either be immobilize or form undissolved substances, with the free ions being attached onto the surfaces of calcium carbonate to yield a chemically stable and non-toxic form. (Volodymyr and Viktor, 2017).

$$Pb^{2+} + CO_3^- \longrightarrow PbCO_3 \quad (4)$$

Bioremediation using this technique is promising for heavy metals (Cd^{2+}, Ni^{2+}, Pb^{2+} etc.) contaminated soils, sediment and water and has been successfully applied in recent studies (Kang et al., 2014; Zhu et al., 2016; Achal et al., 2012). In this study, we determine for the very first time the use of *Pararhodobacter* sp. for laboratory scale bioremediation of lead. *Pararhodobacter* sp. was selected for investigation because it has shown high urease activity and can maintain the enzyme activity for a long time (Fujita et al., 2017). This characteristic is useful in bioremediation as the bacteria is resilient when injected in the ground and its enzyme activity sustained for a longer period to accomplish biocementation and immobilization of the toxic ions. Previous researchers conducted solidification using *Pararhodobacter* sp. for ground improvement methods for marine (Danjo and Kawasaki, 2016) and land usage purposes (unpublished). However, in this study, *Pararhodobacter* sp. was investigated for both mine waste immobilization and to determine if it exhibits any Pb^{2+} resistance and its applicability for bioremediation. Further investigation on the bacteria would be necessary before possible application of this bacteria in-field.

An improved understanding of this process and mechanism will provide a scientific basis for the development of sound strategies for the provision and sustainable use of ureolytic bacteria for bioremediation of lead contaminated sites such as the Kabwe area in Zambia.

The rationale for choosing this technique for mine waste bioremediation is to prevent wind-blown dust from uncovered waste dump sites, as well as to prevent erosion that leads to the transportation of sediments downstream. To achieve this, we determined the solidification and bioremediation conditions necessary for remediation using in vitro experiments by bioaugmentation, then will scale these up for field application via biostimulation (Figure 1). Studies by Gomez et al., 2017 and Gat et al., 2016 reported that biostimulation offers important economic and environmental benefits through the elimination of expensive nonnative monoclonal bacterial cultivation as well as avoid changes to indigenous microbial population which may be affected to an unknown extent. Thus, the specific objectives of the present study (steps 1 and 2) were to: investigate the effects of lead on urease activity and microbial growth; determine the effectiveness of lead removal by *Pararhodobacter* sp.; determine the effects of varying the injection interval of the bacteria on unconfined compressive strength (UCS) for fine and coarse-grained sand; and evaluate the usefulness of *Pararhodobacter* sp. for lead bioremediation. The results from this study will be used in future studies as outlined in Figure 1.

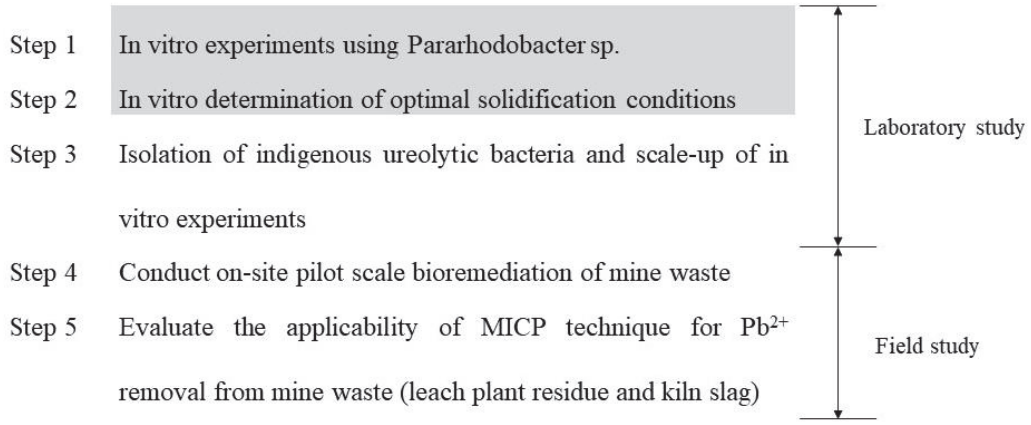

Figure 1: Flowchart of the study. The steps described in this study are highlighted in grey.

2. Materials and methods

2.1 Bacteria, media, and Pb^{2+} stock solution preparation

Pararhodobacter sp., a ureolytic bacterium isolated from the soil near beachrock in Okinawa, Japan, was used in this study (Danjo and Kawasaki, 2013). Cells were cultured in

ZoBell2216 medium, which contained 5.0 g/L hipolypeptone (Nihon Seiyaku Co., Ltd., Tokyo, Japan), 1.0 g/L yeast extract (BD Biosciences Advanced Bioprocessing, Miami, FL, USA), and 0.1 g/L $FePO_4$ (Junsei Chemical Co., Ltd., Tokyo, Japan) prepared with artificial seawater with the final solution pH adjusted to 7.6–7.8 using 1 M NaOH (Fujita et al., 2017). Stock solution of lead was prepared using $PbCl_2$ (Wako Pure Chemical Industries Ltd., Tokyo, Japan), which was dissolved in distilled water, sterilized with a 0.22 μm filter and preserved at 4°C. Different working concentrations of lead were then obtained by serial dilution.

2.2 Effect of lead on microbial growth and urease activity

The tolerance of *Pararhodobacter* sp. to lead was evaluated to elucidate its usefulness in bioremediation. Different concentrations of Pb^{2+} (0 mM, 0.01 mM, and 0.5 mM) were prepared in 100 mL Erlenmeyer flasks containing *Pararhodobacter* sp. and ZoBell2216 culture medium and the time course of cell growth was determined based on the optical density (OD) values at 600 nm by UV-visible spectrophotometry (V-730, Jasco International Co., Ltd., Tokyo, Japan) for 14 days in the presence of Pb^{2+}.

The urease activity of bacterial cells was determined by monitoring ammonium ions generated in urea hydrolysis using the indophenol blue method based on Berthelot's reaction in a water bath maintained at 30°C (Weatherburn, 1967; Natarajan, 1995). After bacterial cultivation for 48 hours, a bacterial suspension (1 mL) (OD_{600} = 1.0) was added to 0.1 M phosphate buffer (100 mL) containing 0.1 M urea. To determine the effects of lead on urease activity, different concentrations of lead (0–0.5 mM) were added. Samples were then collected at intervals of 5 minutes (0, 5, 10 and 15 minutes) and passed through a 0.22 μm filter to remove cells. Next, a 2 mL aliquot of the filtered sample was mixed with phenol nitroprusside reagent (4 mL) (0.25 M phenol in 100 mL of deionized water containing 23 μM of sodium nitroprusside) and hypochlorite reagent (4 mL) (0.05 M sodium hydroxide in 100 mL of deionized water containing 7.5 mL bleach (5% NaOCl)). Each reaction mixture was vortexed and then incubated at 50°C–60°C for 10 minutes. The amount of ammonium

ion released because of urea hydrolysis was determined by referring to a previously prepared standard curve relating the absorbance at 630 nm to ammonium ion concentration (0–10 mg/L). Using this curve, one unit of urease activity (U) was defined as the amount of enzyme that would hydrolyze 1 μmol of urea per minute. The release of 2 μmol of ammonia is equivalent to the hydrolysis of 1 μmol of urea.

2.3 Bioprecipitation of calcium carbonate and lead

To elucidate the coprecipitation mechanism of lead and calcium, Pb^{2+} bioprecipitation experiments were carried out. Specifically, *Pararhodobacter* sp. was precultured for 24 hours in 5 mL ZoBell2216 medium, after which 1 mL of preculture was inoculated into 100 mL of the main culture at 30°C for 48 hours with continuous aeration at 160 rpm. The bacterial suspension (5 mL) was then added to 2 mL calcium chloride (0.5 M) and 2 mL urea (0.5 M), after which different lead final concentrations were added (0 mM, 0.01 mM and 5 mM). The mixture was subsequently incubated for 6 hours at 30°C with shaking (160 rpm) and then further centrifuged (15,000 rpm for 5 minutes) to collect the precipitate. The concentration of lead in the supernatant was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES; ICPE-9000, Shimadzu Corporation, Tokyo, Japan), whereas the precipitate was analyzed by XRD/SEM as described in section 2.5. Precipitation experiments were conducted in triplicate.

2.4 Syringe solidification of coarse- and fine-grained sand

The most practical way of remediating a contaminated site is via in situ treatment of a contaminated material facilitated by the injection of microorganisms and nutrients. To understand the optimal injection interval of bacteria, four different injection intervals were tested in syringe solidification experiments. All syringe solidification experiments were conducted in an incubator at 30°C. A method described by Danjo and Kawasaki was adopted for the syringe solidification experiments (Danjo and Kawasaki, 2016). Briefly, 40 g of fine

sand (mean diameter, D_{50} = 170 µm) and/or coarse sand (mean diameter, D_{50} = 1.2 mm) was oven dried at 105°C for 48 hours and then placed in a 35 mL syringe (mean diameter, D_{50} = 2.5 cm and height, h = 7 cm) (Figure 2). The laboratory experiment was designed to mimic the field conditions in Kabwe mine as closely as possible based on grain size, with the very fine and coarse sand representing the plant residue and kiln slag, respectively. The mixed system was selected to mimic slag used to cover leached plant residue to prevent water and wind erosion on site. Bacterial culture (16 mL) (OD_{600} = 1.0 = 10^9 cfu/mL) and 20 mL of solidification solution (0.5 M urea; 0.5 M calcium chloride; 0.02 M sodium hydrogen carbonate; and 0.2 M ammonium chloride and nutrient broth 3 g/L) were sequentially added to the syringe and drained, leaving about 2 mL of solution above the surface of the sand to maintain wet conditions as conceptualized in Figure 2a. The outlet solution from the syringe was measured for pH and Ca^{2+}. After 14 days of curing, the UCS was estimated using a needle penetration device (SH-70, Maruto Testing Machine Company, Tokyo, Japan).

2.5 XRD and SEM analyses

X-ray diffraction (XRD; MiniFlex™, Rigaku Co., Ltd., Tokyo, Japan) and a scanning electron microscope (SEM; Miniscope TM3000, Hitachi, Tokyo, Japan) were used to qualitatively analyze the MICP precipitate. The sample for XRD was dried at 105°C and ground into powder using a Multi-beads shocker (Yasui Kikai, Tokyo, Japan). X-ray diffraction was conducted under Ni-filtered Cu1.5406A radiation. Scans were recorded from 5 to 80° 2θ, at a rate of 20°/min. The crystalline phases were identified using the International Centre for Diffraction Data (ICDD) database and MATCH 3.4 (Klaus and Putz, 2017). SEM images were taken under the microscope after mounting on a carbon tape.

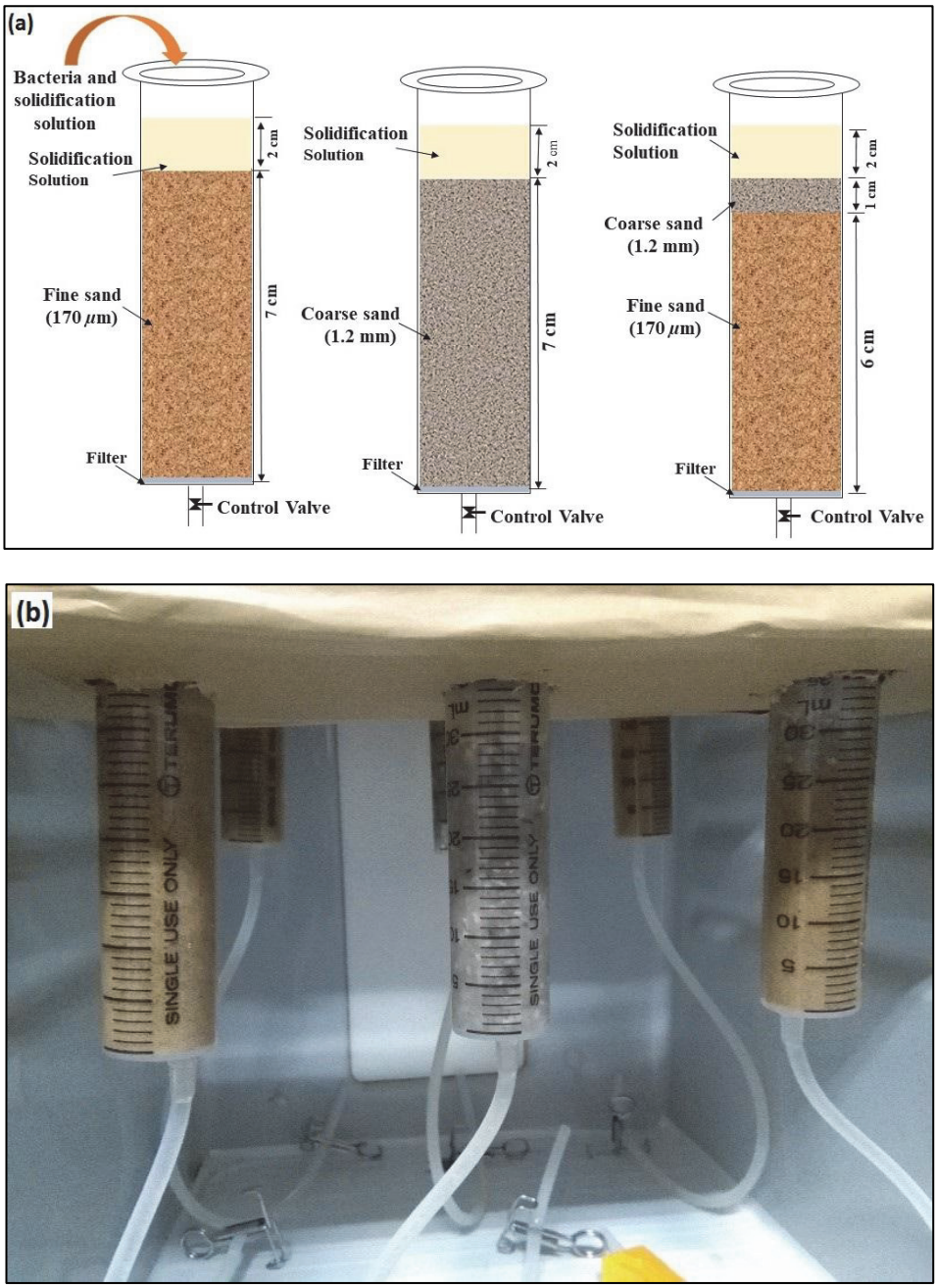

Figure 2: (a) Conceptual setup and (b) images of the syringe solidification of coarse- and fine-grained sand.

3. Results and discussion

3.1 Effect of lead on microbial growth

Pararhodobacter sp. clearly exhibited increased growth following the lag, logarithmic, stationary and retardation phases in lead free media (Figure 3). In the presence of lead, the logarithmic phase was slightly retarded and an overall decrease in growth was observed. The

effects of lead on microbial growth were negligible, whereas no growth was observed for concentrations greater than 1 mM (data not shown). These results indicate that *Pararhodobacter* sp. can be used for bioremediation of lead, even though it was not isolated from a lead contaminated site (Kang et al., 2015). The measured pH of the solution decreased during the logarithmic phase, then increased consistently. It is unclear why a high pH was maintained during incubation; however, this could have occurred because of the release of metabolites in the solution, such as ammonia, by the live cells. The ammonia generation could have originated from amino acids contained in the yeast extract. In many studies, increased pH was only recorded when urea was added to the growth media. These findings are in tandem with those of previous studies, although the effects of lead ions differ for different microorganisms because of different inhibitory concentrations among organisms (Govarthanan et al., 2013; Naik and Dubey, 2013).

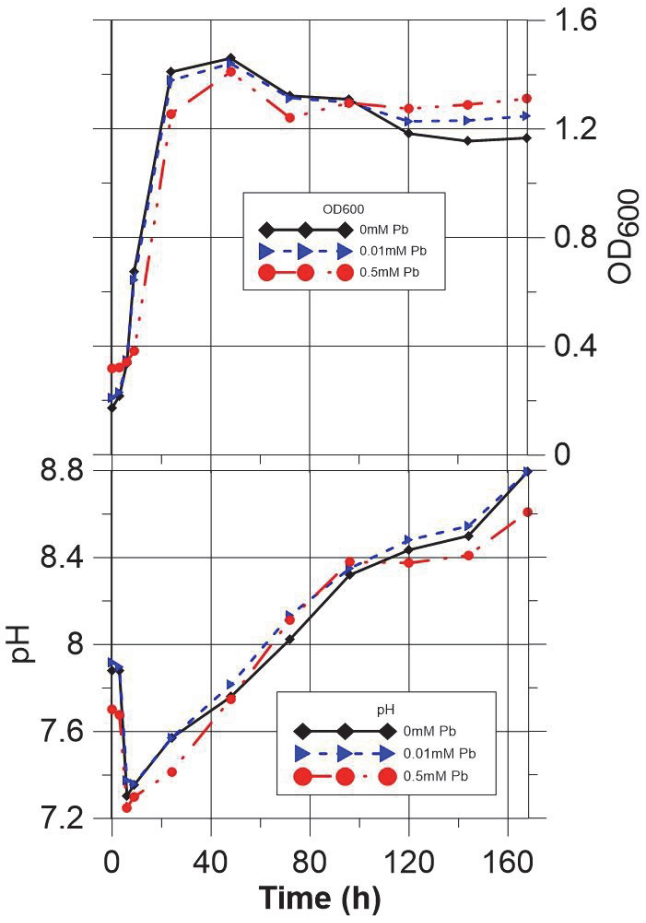

Figure 3: Effect of lead on *Pararhodobacter* sp. growth curve. Values are averages from two independent experiments.

3.2 Effect of lead on urease activity

Urease activity of bacterial cells was studied because it serves as a good bioindicator of enzyme activity in the MICP bioremediation technique and is used to assess environmental health changes occurring in the environment. As shown in Figure 4, the urease activity was not significantly affected by lead. This pattern, which was probably because of the effects of lead on enzyme activity, reinforces the findings reported by Fujita et al. (2017), who found that urease accumulated in/on cells of *Pararhodobacter* sp. However, several authors have reported a significant decrease in enzyme activity and microbial activity because of heavy metals pollution of aquatic and soil ecosystems (Sadler and Trudinger, 1967; Nwuche and Ugoji, 2008; Begum et al., 2009). The present study revealed a negligible decreased in urease and microbial activity. These results were probably because of other factors such as synergy of heavy metals as reported by Nwuche and Ugoji (2008), who observed inhibition of soil microbial activities in response to a combination of Cu and Zn.

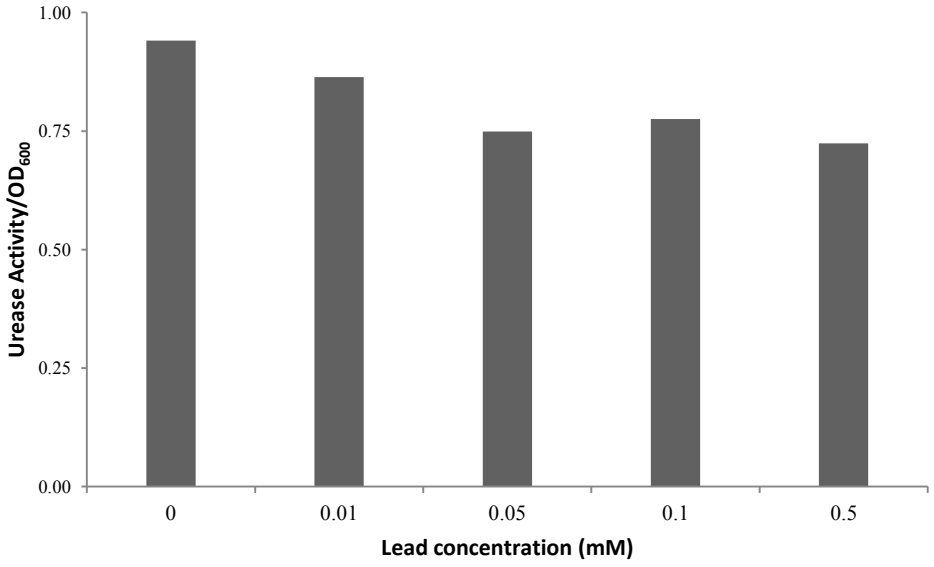

Figure 4: Effect of lead on urease activity based on the OD_{600} of *Pararhodobacter* sp. Values are averages from two independent experiments.

3.3 Lead bioprecipitation

Pararhodobacter sp. was effective at complete removal of Pb^{2+}. Comparison of the removal percentage of lead during bioprecipitation between this study and previous studies

shows comparable figures to other ureolytic bacteria isolated from various sites such as *Rhodobacter spharoides* isolated from an oil field achieved 90.31% (Li et al., 2016); *Enterobacter cloacae* isolated from an abandoned mine achieved 68.1% (Kang et al., 2015); *Sporosarcina pasteurii* achieved 100% (Mugwar and Harbottle, 2016); and *Terrabacter tumescens* achieved 100% (Li et al., 2015). The capability of *Pararhodobacter* to completely remove lead lies in its ability to efficiently hydrolyze urea to generate carbonate ions and elevate the pH to alkaline conditions (8.0–9.1), which promotes precipitation of lead and calcium carbonate.

Figure 5 shows SEM images and XRD patterns of the control (a, c) and bioremediated (b, d) precipitates. Figure 6a shows the spherical particles of calcium carbonate precipitated in the absence of lead and confirmed by XRD analysis in Figure 6c. This finding has been observed from previous studies that reported different polymorphs of calcium carbonate in the form of vaterite and calcite being formed when biomineralization occurs via mediation by bacteria (Park et al., 2010; González-Muñoz et al., 2010). Vaterite does not occur in abundance in the natural environment but is an important precursor in calcite formation of a more stable form of calcium carbonate (Nehrke and Van Cappellen, 2006). Furthermore, Figure 6b shows the SEM image of precipitate in the presence of lead. In the figure, framboidal aggregates were identified as vaterite, whereas spherical and rhombohedral shaped precipitates were identified as calcite. In the MICP process, calcium carbonate can adsorb or incorporate free toxic ions Pb^{2+} through substitution of the divalent calcium ion in the $CaCO_3$ lattice (Figure 6d). Detoxification of the lead to insoluble form occurs when the toxic free ion is transformed into a chemically stable and non-toxic form of lead as elucidated in another study by Li et al., (2013).

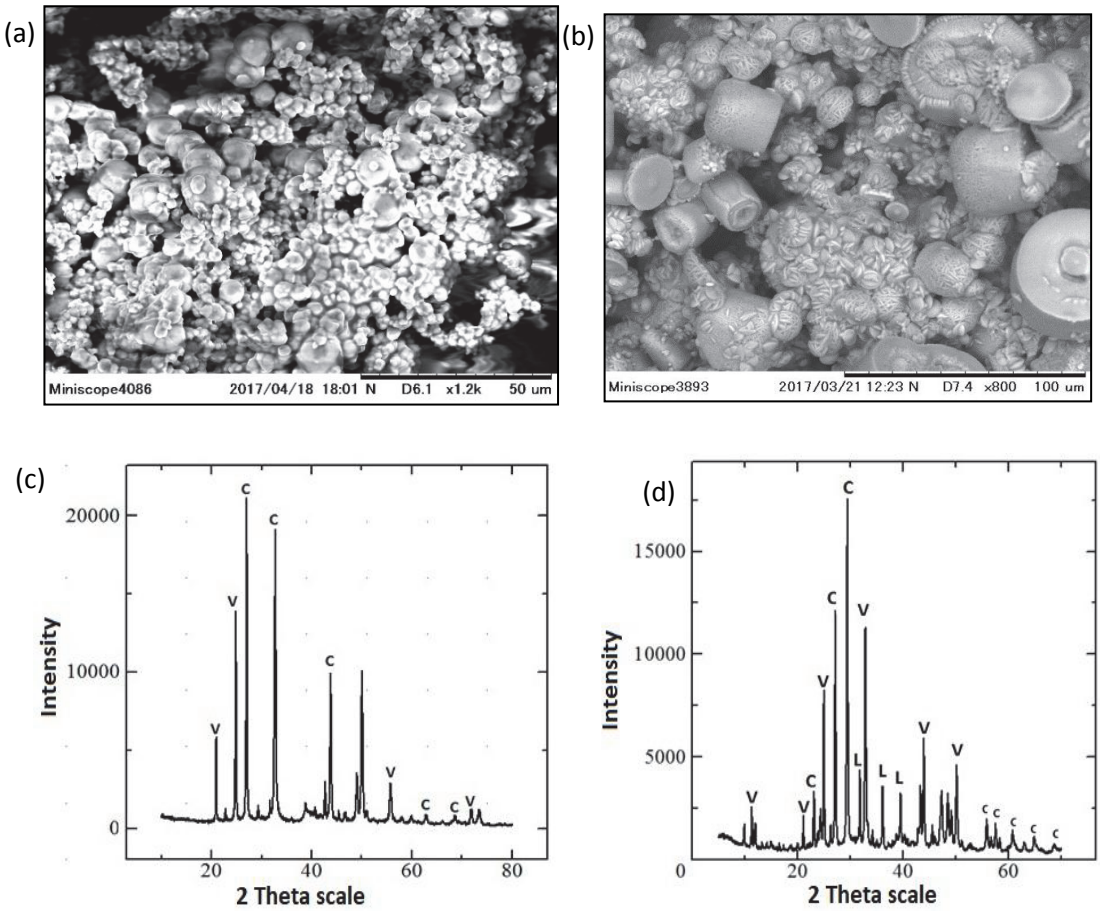

Figure 5: SEM images (a, b) and XRD data (c, d) of precipitates formed by *Pararhodobacter* sp. in the absence (a, c) and presence of 5 mM Pb^{2+} (b, d). (C = Calcite ($CaCO_3$); V = Vaterite ($CaCO_3$); L = Lead Oxide (PbO)).

3.4 Syringe solidification experiment

Pictorial images of sand samples from all syringe solidification experiments after 14 days are shown in Figure 6. The UCS comparison of the results obtained after varying the injection interval (once, twice, four and seven times) is summarized in Table 1 and graphically shown in Figure 7. The classification scheme adopted in this paper was based on that developed by Shafii-Rad and Clough (1982), in which weakly cemented sand was defined as having a UCS of less than 0.3 MPa, moderately cemented sand was defined as having a UCS between 0.4 Mpa and 1 MPa and solidified sand was that with greater than 1 MPa (Shafii and Clough, 1982). Generally, UCS increased with increasing injection interval as well as the top part of the sample was solidified more than the bottom. This increase in

UCS at the top can be attributed to calcite crystals forming cohesive bonds between sand grains mediated by *Pararhodobacter*, which accumulated at the top of the sample.

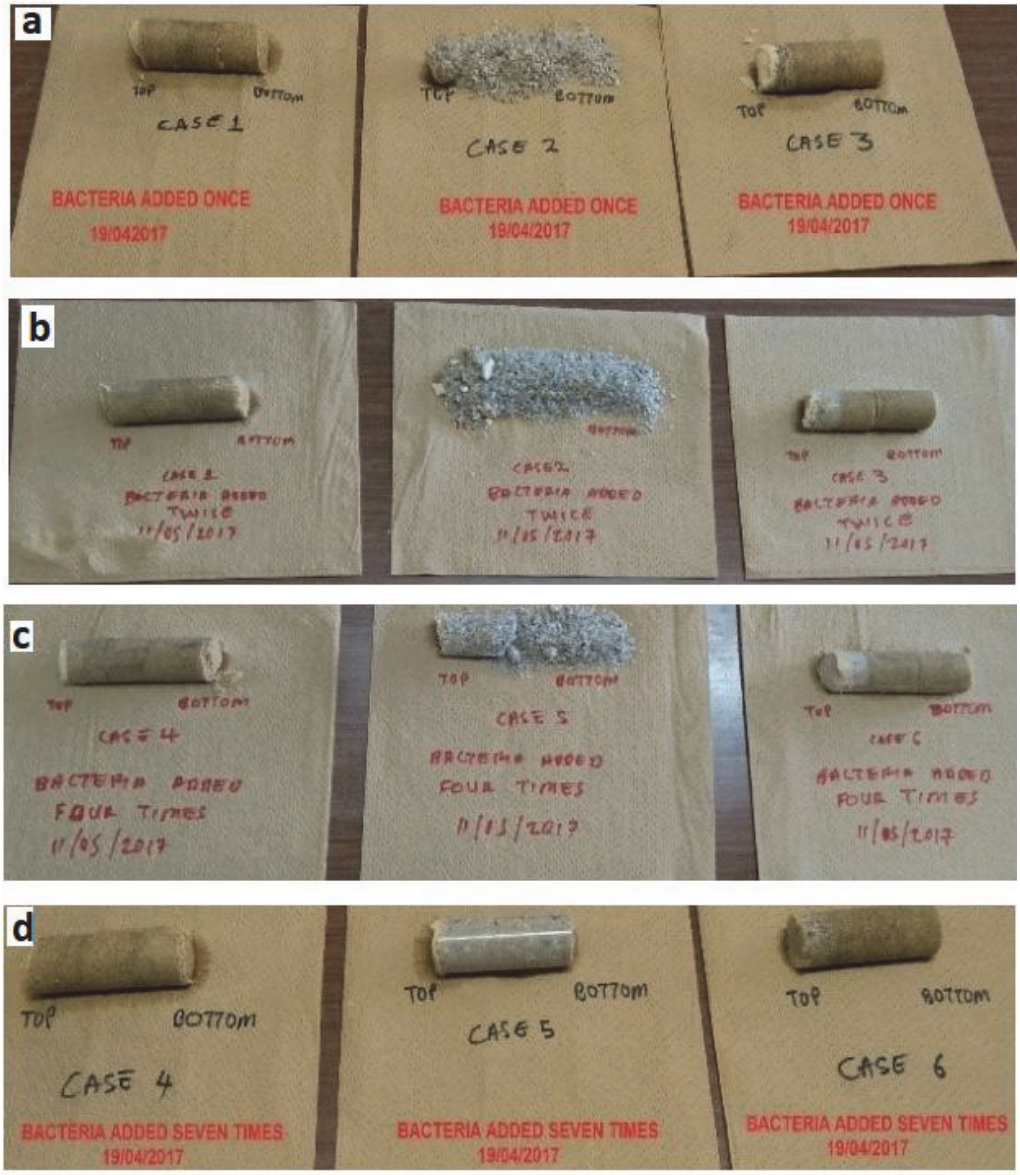

Figure 6: Pictorial images of the results of all syringe tests after 14 days while varying the bacterial injection interval to (a) one (b) two (c) four and (d) seven times. Left, fine sand; center, coarse sand; right, mixture of course and fine sand.

Table 1: Solidification conditions of the injection intervals.

	Sand particle size	Bacterial injection times	Solidification solution injection	Bacterial OD_{600}	Temp. (°C)	Timing (Day)	Condition of solidification		
							Top	Middle	Bottom
Case 1	170 µm	Once	Daily	1.0	30	14	Moderately solidified	Not solidified	Not solidified
Case 2	1.2 mm	Once	Daily	1.00	30	14	Solidified	Not solidified	Not solidified
Case 3	170 µm/1.2 mm	Once	Daily	1.00	30	14	Solidified	Not solidified	Not solidified
Case 4	170 µm	Twice	Daily	1.00	30	14	Moderately solidified	Not solidified	Not solidified
Case 5	1.2 mm	Twice	Daily	1.00	30	14	Not solidified	Not solidified	Not solidified
Case 6	170 µm/1.2 mm	Twice	Daily	1.00	30	14	Weakly solidified	Not solidified	Not solidified
Case 1	170 µm	Four times	Daily	1.00	30	14	Solidified	Solidified	Solidified
Case 2	1.2 mm	Four times	Daily	1.00	30	14	Solidified	Not solidified	Not solidified
Case 3	170 µm/1.2 mm	Four times	Daily	1.00	30	14	Moderately Solidified	Solidified	Solidified
Case 4	170 µm	Seven times	Daily	1.00	30	14	Solidified	Solidified	Solidified
Case 5	1.2 mm	Seven times	Daily	1.00	30	14	Solidified	Solidified	Solidified
Case 6	170 µm/1.2 mm	Seven times	Daily	1.00	30	14	Solidified	Solidified	Solidified

Increasing cohesive bonds result in cementation leading to decreased permeability, as was observed during the investigation. Therefore, MICP can both immobilize the lead and induce high resistance of the contaminated materials to erosion. This phenomenon was also observed when conducting a steep slope experiment using *Sporosarcina pasteurii* (Salifu et al., 2016). Overall, the data obtained using the syringe test demonstrated that MICP is a viable option for use in coarse- and fine-grained sand. In syringe experiments, the pH value ranged from 8.0 to 9.5, which is similar to what has been observed in other studies (Stocks-Fischer et al., 1999). The calcium ion concentration was lower in the first 7 days of the experiment, indicating deposition of calcite in the column and a decrease in deposition in the latter half. Clogging of the porous media was greater in coarse sand than in finely graded sand. This clogging was because of the cell growth and calcium carbonate formation that accompany the MICP process.

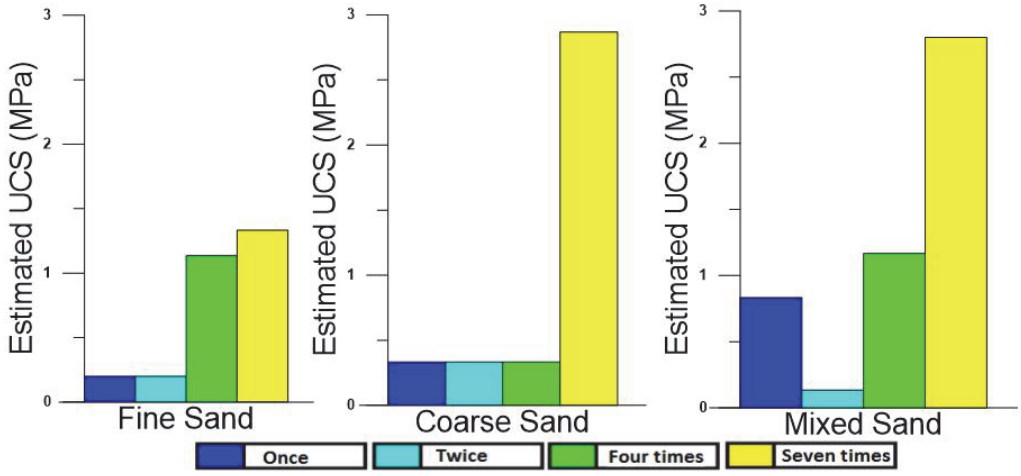

Figure 7: UCS comparison of the results obtained when varying the bacteria injection for fine, coarse and mixed sand.

4. Conclusion

In this study, we demonstrated for the first time that lead (Pb) can be bioremediated by *Pararhodobacter* sp. and that the microorganism was capable of complete removal of 1036 mg/L Pb^{2+} during 6 hours of incubation via elevation of the pH to alkaline conditions (8.0–9.1). SEM and XRD further confirmed transformation of toxic free Pb^{2+} ions to a more stable

form of lead that bioprecipitated together with calcite or vaterite, which were predominant. Furthermore, syringe experiments revealed that UCS was greater when seven injection were performed as opposed to less injection interval of bacteria. The unconfined compressive strength increased with an increase in injection interval with maximum unconfined compressive strength of 1.33 MPa for fine sand, 2.87 MPa for coarse sand and 2.80 MPa for mixed sand. For Pararhodobacter to efficiently induce lead immobilisation the bacterial interval required is four times with a calcium and urea concentration of 0.5 M and bacterial concentration of 10^9 cfu/mL. These results will facilitate the bioremediation of lead in both fine and coarse materials as an eco-friendly and sustainable method of heavy metal remediation.

Acknowledgments

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References

Achal, V., Pan, X., Zhang, D., Fu, Q., 2012. Bioremediation of Pb-contaminated soil based on microbially induced calcite precipitation. J. Microbiol. Biotechnol. 22, 244–247. doi:10.4014/jmb.1108.08033.

Anbu, P., Kang, C.-H., Shin, Y.-J., So, J.-S., 2016. Formations of calcium carbonate minerals by bacteria and its multiple applications. Springerplus 5, 250. doi:10.1186/s40064-016-1869-2.

Begum, A., Harikrishna, S., Khan, I., 2009. Analysis of heavy metals in water, sediments and fish samples of Madivala lakes of Bangalore, Karnataka.pdf. Int. J. ChemTech Res. 1, 245–249.

Danjo, T., Kawasaki, S., 2016. Microbially Induced Sand Cementation Method Using *Pararhodobacter* sp. Strain SO1, Inspired by Beachrock Formation Mechanism. Mater. Trans. 57, 428–437. doi:10.2320/matertrans.M-M2015842.

Danjo, T., Kawasaki, S., 2013. A Study of the Formation Mechanism of Beachrock in Okinawa , Japan : Toward Making Artificial Rock 5, 634–639.

Fujita, M., Nakashima, K., Achal, V., Kawasaki, S., 2017. Whole-cell evaluation of urease activity of Pararhodobacter sp. isolated from peripheral beachrock. Biochem. Eng. J. 124, 1–5. doi:10.1016/j.bej.2017.04.004.

Gat, D., Ronen, Z., Tsesarsky, M., 2016. Soil Bacteria Population Dynamics Following Stimulation for Ureolytic Microbial-Induced CaCO3 Precipitation. Environ. Sci. Technol. 50, 616–624. doi:10.1021/acs.est.5b04033.

Gomez, M.G., Anderson, C.M., Graddy, C.M.R., DeJong, J.T., Nelson, D.C., Ginn, T.R., 2017. Large-Scale Comparison of Bioaugmentation and Biostimulation Approaches for Biocementation of Sands. J. Geotech. Geoenvironmental Eng. 143, 4016124. doi:10.1061/(ASCE)GT.1943-5606.0001640.

González-Muñoz, M.T., Rodriguez-Navarro, C., Martínez-Ruiz, F., Arias, J.M., Merroun, M.L., Rodriguez-Gallego, M., 2010. Bacterial biomineralization: new insights from *Myxococcus* -induced mineral precipitation. Geol. Soc. London, Spec. Publ. 336, 31–50. doi:10.1144/SP336.3.

Govarthanan, M., Lee, K.J., Cho, M., Kim, J.S., Kamala-Kannan, S., Oh, B.T., 2013. Significance of autochthonous Bacillus sp. KK1 on biomineralization of lead in mine tailings. Chemosphere 90, 2267–2272. doi:10.1016/j.chemosphere.2012.10.038.

Kang, C.H., Han, S.H., Shin, Y., Oh, S.J., So, J.S., 2014. Bioremediation of Cd by microbially induced calcite precipitation. Appl. Biochem. Biotechnol. 172, 2907–2915. doi:10.1007/s12010-014-0737-1.

Kang, C.H., Oh, S.J., Shin, Y., Han, S.H., Nam, I.H., So, J.S., 2015. Bioremediation of lead by ureolytic bacteria isolated from soil at abandoned metal mines in South Korea. Ecol. Eng. 74, 402–407. doi:10.1016/j.ecoleng.2014.10.009.

Klaus and Putz, H.B., 2017. Match! - Phase Identification from Powder Diffraction, Crystal Impact.

Lanphear, B.P., 2005. Childhood Lead Poisoning Prevention. J. Am. Med. Assoc. 293, 2274. doi:10.1001/jama.293.18.2274.

Li, M., Cheng, X., Guo, H., 2013. Heavy metal removal by biomineralization of urease producing bacteria isolated from soil. Int. Biodeterior. Biodegradation 76, 81–85. doi:10.1016/j.ibiod.2012.06.016.

Li, M., Cheng, X., Guo, H., Yang, Z., 2015. Biomineralization of Carbonate by Terrabacter Tumescens for Heavy Metal Removal and Biogrouting Applications. J. Environ. Eng. C4015005–C4015005. doi:10.1061/(ASCE)EE.1943-7870.0000970.

Li, X., Peng, W., Jia, Y., Lu, L., Fan, W., 2016. Bioremediation of lead contaminated soil with Rhodobacter sphaeroides. Chemosphere 156, 228–235. doi:10.1016/j.chemosphere.2016.04.098.

Lin, G.Z., Peng, R.F., Chen, Q., Wu, Z.G., Du, L., 2009. Lead in housing paints: An exposure source still not taken seriously for children lead poisoning in China. Environ. Res. 109, 1–5. doi:10.1016/j.envres.2008.09.003.

Mugwar, A.J., Harbottle, M.J., 2016. Toxicity effects on metal sequestration by microbially-induced carbonate precipitation. J. Hazard. Mater. 314, 237–248. doi:10.1016/j.jhazmat.2016.04.039.

Naik, M.M., Dubey, S.K., 2013. Lead resistant bacteria: Lead resistance mechanisms, their applications in lead bioremediation and biomonitoring. Ecotoxicol. Environ. Saf. 98, 1–7. doi:10.1016/j.ecoenv.2013.09.039.

Natarajan, K.R., 1995. Kinetic Study of the Enzyme Urease from Dolichos biflorus. J. Chem. Educ. 72, 556–557. doi:10.1021/ed072p556.

Nehrke, G., Van Cappellen, P., 2006. Framboidal vaterite aggregates and their transformation into calcite: A morphological study. J. Cryst. Growth 287, 528–530. doi:10.1016/j.jcrysgro.2005.11.080.

Nwuche, C.O., Ugoji, E.O., 2008. Effects of heavy metal pollution on the soil microbial activity. Int. J. Environ. Sci. Technol. 5, 409–414. doi:10.1007/BF03326036.

Nyambe, I.A., Chirwa, M., Chaziya, B., 2013. Impacts of past mining activities on the Ecosystem and on human health in Kabwe Town, Zambia, in: Workshop on Environmental and Health Impacts of Mining Activities in the Sub-Saharan African Countries. p. 33.

Park, S.J., Park, Y.M., Chun, W.Y., Kim, W.J., Ghim, S.Y., 2010. Calcite-forming bacteria for compressive strength improvement in mortar. J. Microbiol. Biotechnol. 20, 782–788. doi:10.4014/jmb.0911.11015.

Sadler, W.R., Trudinger, P.A., 1967. The inhibition of microorganisms by heavy metals. Miner. Depos. 2, 158–168. doi:10.1007/BF00201912.

Salifu, E., MacLachlan, E., Iyer, K.R., Knapp, C.W., Tarantino, A., 2016. Application of microbially induced calcite precipitation in erosion mitigation and stabilisation of sandy soil foreshore slopes: A preliminary investigation. Eng. Geol. 201, 96–105. doi:10.1016/j.enggeo.2015.12.027.

Shafii, R.-N., Clough, W., 1982. The influence of cementation on the static and dynamic behavior of sands. Stanford, California, California.

Stocks-Fischer, S., Galinat, J.K., Bang, S.S., 1999. Microbiological precipitation of CaCO3. Soil Biol. Biochem. 31, 1563–1571. doi:10.1016/S0038-0717(99)00082-6.

Volodymyr and Viktor, S.I., 2017. Construction Biotechnology: Biogeochemistry, Microbiology and Biotechnology of Construction Materials and Processes. Springer Nature, Singapore. doi:10.1007/978-981-10-1445-1.

Weatherburn, M.W., 1967. Phenol-hypochlorite reaction for determination of ammonia. Anal. Chem. 39, 971–974. doi:10.1021/ac60252a045.

Whiffin, V.S., van Paassen, L.A., Harkes, M.P., 2007. Microbial Carbonate Precipitation as a Soil Improvement Technique. Geomicrobiol. J. 24, 417–423.

doi:10.1080/01490450701436505.

Yabe, J., Nakayama, S.M.M., Ikenaka, Y., Yohannes, Y.B., Bortey-Sam, N., Oroszlany, B., Muzandu, K., Choongo, K., Kabalo, A.N., Ntapisha, J., Mweene, A., Umemura, T., Ishizuka, M., 2015. Lead poisoning in children from townships in the vicinity of a lead-zinc mine in Kabwe, Zambia. Chemosphere 119, 941–947. doi:10.1016/j.chemosphere.2014.09.028.

ZCCM-IH – Zambia Copper and Cobalt Mining- Investment Holding Plc., 2002. Environmental assessment of the Copperbelt and Kabwe area. Kitwe.

Zhu, X., Li, W., Zhan, L., Huang, M., Zhang, Q., Achal, V., 2016. The large-scale process of microbial carbonate precipitation for nickel remediation from an industrial soil. Environ. Pollut. 219, 149–155. doi:10.1016/j.envpol.2016.10.047.