Efficacy of biocementation of lead mine waste from the Kabwe Mine site evaluated using *Pararhodobacter* sp.

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Acknowledgments

Abstract

Biocementation of hazardous waste is used in reducing the mobility of contaminants but studies on evaluating its efficacy have not been well documented. Therefore, to evaluate the efficacy of this method, physicochemical factors affecting stabilized hazardous products of in-situ microbially induced calcium carbonate precipitation (MICP) were determined. The strength and leach resistance were investigated using the bacterium *Pararhodobacter* sp. Pb-contaminated kiln slag (KS) and leach plant residue (LPR) collected from Kabwe, Zambia were investigated. Biocemented KS and KS/LPR had leachate Pb concentrations below the detection limit of <0.001 mg/L, resisted slaking, and had maximum unconfined compressive strengths of 8 MPa for KS and 4 MPa for KS/LPR. Furthermore, biocemented KS and KS/LPR exhibited lower water absorption coefficient values, which could potentially reduce the water transportation of Pb^{2+}. The results of this study show that MICP can reduce Pb^{2+} mobility in mine wastes. The improved physicochemical properties of the biocemented materials therefore, indicates that this technique is an effective tool in stabilizing hazardous mine wastes and consequently, preventing water and soil contamination.

Keywords: *Abandoned mine, solidification; leaching; strength; efficacy*

1 Introduction

Mining and smelting operations generate large quantities of mine wastes that result in contamination of both soil and water resources. The wastes pose a significant risk for biota since they do not undergo biodegradation (Jaishankar et al. 2014). Stabilization or solidification (S/S) is a method commonly used for immobilizing hazardous wastes (Al-Kindi 2019). Recent studies have accomplished S/S using Portland cement (Al-Kindi 2019), fly ash (Tsang et al. 2014), geopolymers (Li et al. 2018), phosphates (Huang et al. 2016) and organic materials (Ashrafi et al. 2015). However, most of these methods are costly and have sub-optimum performance (Jena and Dey 2016). One of the promising techniques for heavy metal remediation is immobilization by microbially induced calcium carbonate precipitation (MICP) using ureolytic bacteria (Ivanov and Stabnikov 2016; Zhang et al. 2016). Although Achal et al., 2013; Nam et al., 2016; Zhu et al., 2016; Chen et al., 2017; and Kim and Lee, 2018 recommended the use of in-situ MICP to immobilize hazardous mine waste, they did not evaluate the physicochemical stability of the method. Understanding the physicochemical properties of stabilized mine wastes is essential in evaluating the long-term potential of the technique to prevent water and soil pollution thus extending the existing knowledge. The effectiveness of stabilized mine wastes is defined by strength and leach resistance (Li et al. 2018; Al-Kindi 2019; Pan et al. 2019). Strength of stabilized mine wastes depend on mechanical strength, slaking and water absorption whereas the leaching concentration of an element of concern depends on pH, its mineralogy and hydraulic conductivity (Fig 1). In this study, MICP was investigated as a method for stabilizing Pb-contaminated kiln slag (KS) and leach plant residue (LPR) collected from Kabwe, Zambia. Several researchers have reported Pb contamination general in Kabwe (Blacksmith 2013) and particularly in soils and both surface and groundwater (Kříbek et al. 2019) and in humans (Yabe et al. 2018) and livestock (Yabe et al. 2011). Therefore, stabilization of hazardous materials in Kabwe is critical in both mitigating and controlling pollution in Kabwe. The technique can be extended to other regions where the legacies of closed mines have raised background levels of heavy metals in ecosystems. Biocementation can also be used to mitigate and rehabilitate environments impacted by anthropogenic activities during and after the closure of mining companies.

The objective of this study was to characterize the physical and chemical stability of stabilized mine wastes and therefore, evaluate the effectiveness of in-situ MICP in mitigation and controlling heavy metal pollution. The authors found no previous studies that evaluated the efficacy ofin-situ MICP for

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stabilizing mine wastes. Such investigations would be valuable in facilitating the long-term design of bioremediation systems using MICP.

2   Materials and methods

2.1   Mine waste samples

The mine wastes - KS and LPR - used in this study were collected from the abandoned Kabwe Mine of Central Province, Zambia. Fig. 2 shows the location of the study area and sampling points for KS (Site 1) and LPR (Site 2). The mine waste was permitted under approval No. SEP/055/17. KS is granular round ill-sorted pebbles with mean particle diameter size of 1000 μm, density of 2.88 g/cm^3 with bioavailable Pb concentration of 5.40 mg/L whereas LPR is very fine to fine sand with a mean particle diameter size of 9 μm, density of 2.39 g/cm^3 and bioavailable Pb concentration of 7.87 mg/L.

2.2   Culturing of bacteria

Pararhodobacter sp., the biosafety level 1 bacterium used in this study, was cultured in ZoBell 2216 medium. The ZoBell 2216 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 using Aquamarin (Yashima Pure Chemical Co. Ltd.) and the chemical composition is listed in Table 1. The final solution was adjusted to a pH of 7.6–7.8 using 1 M NaOH (Wako Pure Chemical Industries, Ltd, Tokyo, Japan). *Pararhodobacter* sp. was precultured for 24 h in 5 mL of ZoBell 2216 medium. Then 1 mL of preculture was introduced into 100 mL of the main culture at 30 °C for 48 h with continuous aeration at 160 rpm. This culture was used for subsequent solidification experiments. All supplies and media were sterilized in an autoclave (LSX-500, Tomy Seiko Co., Ltd., Tokyo, Japan) at 121 °C for 20 min.

2.3   Biocementation of mine wastes by bacteria

To minimize interferences from antecedent microbes, the LPR and KS samples were oven dried at 110 °C for 48 h. Each dried sample was hand packed into a 35-mL sterile syringe (mean diameter, $D_{50} = 2.5$ cm and height, h = 7 cm). A volume of 16 mL of grown bacteria suspension ($OD_{600} = 4.0$) was initially added. Bacteria fixation was added on days 1, 3, 5, and 7. After 2 h a flush of 20 mL of cementation solution (0.5 M urea, 0.5 M calcium chloride, 0.02 M sodium hydrogen carbonate, 0.2 M ammonium

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chloride, and 3 g/L nutrient broth) were added sequentially to the syringe and drained at a constant rate of 2 mL/min for a 14-day period at 30 °C. Two sets of experiments were conducted with procedures formulated to mimic possible conditions for in-situ injection of treatment solutions into contaminated ground. The two procedures are illustrated in Fig. 3. In the first set of experiments, 2 mL of cementation solution was left above the surface of the waste to mimic saturated conditions, so this procedure was called the immersed method (Fig. 3a). The second set of experiments was conducted such that solution was added sequentially with all solution drained, so this procedure was called the flow through method (Fig. 3b). Additionally, KS/LPR was an amendment formulated to mimic the mixing of 50% KS and 50% LPR by weight to represent the mix of materials in waste piles at the mine site. In the control distilled water was used instead of bacterial suspension.

2.4   Physical testing procedures

2.4.1 Needle penetration test

Unconfined compressive strength (UCS) was measured using a needle penetration device (SH-70, Maruto Testing Machine Company, Tokyo, Japan) according to ISRM (International Society for Rock Mechanics 2015). To determine the strength of a sample, the needle penetration inclination (Np) value of each sample were measured using needle penetration device and the UCS was estimated from Np value. The strength of the sample (Np value) was calculated using Equation 1

$$N_P = F/D \tag{1}$$

Where, F is the penetration load (N) and D is the depth of penetration (mm). The unit of Np is N/mm. From the chart of UCS-Np correlation, estimated UCS value was determined.

2.4.2 Capillary water absorption tests

The water absorption tests were conducted in accordance with ASTM C1585-13 (ASTM International 2013). The surface subjected to water absorption was immersed in water (2 ± 1 mm depth) in a shallow tray and covered to prevent air circulation and contact with atmospheric water vapor. The water absorption coefficient [S, $kg/(m^2 \cdot h^{0.5})$] was determined by the slope of the curve of the water absorbed per unit area (kg/m^2) and the square root of the sinking time ($sec^{0.5}$). The tests were conducted on untreated and treated KS and KS/LPR that was prepared by both immersed and flow through methods.

2.4.3 Jar slake test

The jar slake tests were conducted according to ASTM D4644-16 (ASTM International 2016). In brief, samples were oven-dried at 105 °C, cooled and placed into empty plastic antistatic weighing dishes. Distilled water was poured into the dishes to submerge each sample for 24 h, and the samples were observed and described after 30 min and after 24 hours. The samples were classified as 1-6 with corresponding descriptions as follows: (1) degrades to a pile of flakes or mud; (2) breaks rapidly and/or forms many chips; (3) breaks slowly and/or forms few chips; (4) breaks rapidly and/or develops several fractures; (5) breaks slowly and/or develops few fractures; and (6) no change. The tests were conducted on untreated and treated KS and KS/LPR that were prepared by both immersed and flow through methods.

2.4.4 Hydraulic conductivity

A hydraulic conductivity was conducted by the falling head method using a DIK 4000 system (Daiki Rika Kogyo Co., Ltd., Saitama, Japan) according to ASTM D5084-03 (ASTM International 2003). The molds for DIK 4000 were used for solidification of samples which has outside dimension of width 530 mm x diameter 260 mm x height of 380 mm (Fig. 4). To calculate the hydraulic conductivity, Equation 2 was used.

$$K = \frac{2.3a.L}{A.t} Log^{10} \frac{h_1}{h_2} \tag{2}$$

Where K (m/sec) is the hydraulic conductivity; a = 0.503 cm^2 cross-sectional area of the scale tube, L = 5.1 cm which is thickness of the sample; A = 19.6 cm^2 which is the sectional area of sample; h_1 = 17 cm; h_2 = 7 cm; and t is the time (sec) taken for water level to drop from top to bottom of the scale tube.

The methodology described in section 2.3 was used for biocementation, but the bacteria and cementation solutions were scaled up according to the mass of the mine waste in the mold. The samples were saturated, then placed in a desiccator for 48 h before hydraulic conductivity was determined.

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2.5 Chemical and mineralogical characterization methods

2.5.1 XRD analysis

X-ray diffraction (XRD) analysis (MiniFlex™, Rigaku Co., Ltd., Tokyo, Japan) was conducted using Ni-filtered Cu 1.5406 Å radiation to determine the mineral phases of both the untreated and treated KS and KS/LPR. Scans were recorded from 5 to 80° 2θ at a rate of 20°/min.

2.5.2 SEM and SEM-EDS

The microstructure of fractions of the samples was examined by scanning electron microscopy (SEM) (Miniscope TM3000, Hitachi, Tokyo, Japan) and by energy-dispersive X-ray spectroscopy (EDS) (JSM-IT200, Joel Ltd., Tokyo, Japan). Micrographs and element compositions were acquired under the microscope using samples mounted on carbon tape.

2.5.3 Leaching test of biocemented KS and KS/LPR

USA EPA Method 1315 for mass transfer rates of constituents in monolithic materials using a semi-dynamic tank leaching procedure was used to determine the cumulative release of Pb from the biocemented KS and KS/LPR, as shown in Fig. 5 (U.S. EPA 2017). To determine the rate of release, biocemented KS and KS/LPR samples treated by the flow through method, were immersed in deionized water with the liquid–surface area ratio (L/A) maintained at 9 ± 1 mL/cm^2. Leaching was allowed to continue for 231 days with leachate collected in a sealed high-density polyethylene (HDPE) bucket at defined intervals. Leachate was collected after 8 hours, 1 day, 2 days, every 7 days from day 7 through day 49, after 63 days, and every 14 days from day 63 through day 231. Pb^{2+} concentration, pH, and electrical conductivity were measured for the collected leachate at the end of each interval. The leachate was filtered through a 0.45 µm nylon filter into a polypropylene centrifuge tube, acidified, and stored in a refrigerator at 4 °C for analysis by ICP-AES.

2.6 Statistical analysis

The data was analyzed by comparing means by independent two-sample t-test using R Statistical Package Version 3.5.2 (Team R. Core 2018).

3 Results and discussion

3.1 Physical characterization of the biocemented waste

3.1.1 Needle penetration test

Fig. 6 shows untreated and biocemented KS and KS/LPR prepared by the immersed and flow through methods whereas Table 2 shows the corresponding estimated UCS results. KS prepared by the immersed method, had a maximum estimated UCS of 8.0 MPa, compared to 5.0 MPa when prepared by the flow through method. KS/LPR prepared by the immersed method, had a maximum UCS of 4.0 MPa, compared to only 2.8 MPa when prepared by the flow through method. Statistical analysis revealed that there was non-significant difference found between the control and KS treated by immersed method ($p = 0.10$). This result could be due to accumulation of reactants and bacteria at the injection point hence only top of the specimen was solidified whereas comparison between the means of the control and flow through method yielded a significant difference ($p = 0.02$). This further agrees with the results of a previous study that found that the flow through method models environmental conditions and is more conducive for effective formation of calcium carbonate ($CaCO_3$) crystals at various degrees of saturation (Cheng et al. 2013). For KS/LPR, statistical analysis revealed that there was no significant difference between the control and both immersed ($p = 0.933$) and flow through ($p = 0.06$) treatment methods. The fine nature of the LPR that retards the flow of bacteria down. Therefore, flow through was the most optimal of the two methods. The top of the column closest to the injection point is probably exposed to significantly more reactants than the bottom as described by a previous studies that observed clogging due to accumulation of bacteria and reactants near the injection point (Cheng and Cord-Ruwisch 2014; Eryürük et al. 2015; Dhami et al. 2016). Additionally, it was observed the stabilized products of in-situ MICP were not blown by wind. A handheld blower was used to blow on unstabilized and stabilized mine waste at different angles. No dust was observed for biocemented KS and KS/LPR prepared by either the immersed or the flow through methods, while the unstabilized waste was completely dispersed. Similar results were reported in a previous study by Doostmohammadi et al., (2017). The increased particles size resulting from MICP makes the aggregated material less susceptible to being blown by wind, eliminating a current exposure pathway to humans and animals in and around the mine site.

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3.1.2 Capillary water absorption

Table 3 shows the water absorption results of untreated KS and KS/LPR, as well as biocemented KS and KS/LPR prepared by the immersed and flow through methods. The water absorption coefficient of unstabilized KS was 3.56 kg m^{-2} h$^{-0.5}$ and that of KS/LPR was 6.56 kg m^{-2} h$^{-0.5}$. The water absorption coefficient for biocemented KS was 1.31 kg m^{-2} h$^{-0.5}$ for the immersed method and 1.96 kg m^{-2} h$^{-0.5}$ for the flow through method, whereas the water absorption coefficient for KS/LPR was 2.42 kg m^{-2} h$^{-0.5}$ for the immersed method and 5.92 kg m^{-2} h$^{-0.5}$ for the flow through method. It was observed that all the treatment methods were statistically significant ($p < 0.05$). The results in this study are similar to those observed in previous studies for materials such as mca20/80 and handmade bricks (Karagiannis et al. 2016), and concrete (Demirci and Sahin 2014). The low water absorption ability of the biocemented mine waste can be attributed to the in-situ precipitation of $CaCO_3$ in the pore spaces of the material. This deposition increased the compressive strength and decreased capillary absorption rate. Possible sources of capillary water include rainfall, runoff from slopes of the mine waste, and capillary rise of groundwater. These results suggest that MICP can bind loose waste material to reduce water absorption, thereby reducing infiltration and increasing runoff, which could prevent water transport of Pb-laden mine wastes into the surrounding water bodies. The findings are in agreement with results reported by Achal et al. (2013) when they stabilized Chromium contaminated slag from China.

3.1.3 Slaking behavior

Slaking tests were conducted to determine the stability of the immobilized mine wastes and to qualitatively predict resistance to erosion. Fig. 7 shows the slaking behavior of untreated and treated KS, and KS/LPR at 0 min, 30 min, and 24 hours. Untreated KS and KS/LPR easily degraded into a pile of mud after the addition of water and were given a classification of 1. These results indicate that untreated KS and KS/LPR cannot resist disruptive forces such as rain or wind; and therefore, will continue to leach Pb^{2+} freely and disperse easily to the surrounding flora and fauna. However, the biocemented KS and KS/LPR, prepared by both immersed and flow through methods, resisted slaking. They were given a classification of 6 since no reaction occurred after immersion in water for 24 h. This observation was further confirmed after five cycles of wetting and drying of the same sample. These results suggest that the biocemented KS and KS/LPR is stable and resist the disruptive forces of wind and rain.

3.1.4 Hydraulic conductivity

It was observed that the unstabilized KS had the hydraulic conductivity of 1.2×10^{-3} m/s while that of unstabilized K/LPR was 8.8×10^{-4} m/s. Results further revealed that treatment by immersed method resulted in a KS stabilized material with a hydraulic conductivity value of 3.9×10^{-5} m/s and also stabilized KS/LPR material with a hydraulic conductivity value of 5.8×10^{-5} m/s. Whereas the flow through method showed that KS and KS/LPR had hydraulic conductivity values of 3.2×10^{-4} m/s and 2.1×10^{-6} m/s, a reduction of one and two orders of magnitude respectively. KS had a lower hydraulic conductivity compared to KS/LPR probably due to pore size distribution (Ren and Santamarina 2017). For stabilized hazardous materials, reduced hydraulic conductivity is desired because it reduces the ability of water to contact contaminants, and, therefore, reduces contaminant leaching rates.

3.2   Chemical testing procedures

3.2.1 XRD

Results obtained from XRD analysis of the untreated waste revealed that the mineral composition of the stabilized mine waste material KS included quartz, hematite, goethite, magnetite, cerussite, and anglesite (Fig. 8). Similar findings were reported by Gutiérrez et al. (2016) and Liu et al. (2018) who noted that the minerals observed in this current study are typical of waste from sites that have the footprints of mining activities. The quartz, hematite, goethite and magnetite mineral components were due to the ore host rock which is characteristic of the geology in Kabwe. The Pb-based minerals identified - cerussite, and anglesite - are associated with sulfide-bearing mine wastes, which are the main source of contamination in and around the Kabwe mine site. The XRD patterns of the biocemented waste were similar to those of the original waste, with calcite as the most abundant mineral components. The precipitated $CaCO_3$ in the voids of the wastes, acted as a binder to the wastes and rendering them less soluble and less likely to cause toxicity to humans, animals and the environment in Kabwe and similar sites.

3.2.2 SEM and EDS

The results of SEM analysis of untreated and biocemented KS are shown in Fig. 9. Fig. 9a and 9b shows that the untreated KS is rough and separated, with no particle bonding. However, the biocemented KS (Fig. 9c and 9d) had spherical $CaCO_3$ deposited on the surface and between the sand grains. The

deposited, spherical CaCO$_3$ caused bridging, which further roughened the surface and decreased the pore space size by filling the voids between particles and causing particle binding. The decrease in pore space size has been reported in a number of previous studies (Ng et al. 2012; Rowshanbakht et al. 2016; Mujah et al. 2017).

The EDS analysis revealed that untreated KS consisted mainly of C, O, S, Mg, Ca, Fe, Si, Zn, and Pb, whereas the biocemented KS and KS/LPR prepared by flow through and immersed methods consisted of C, O, S, Mg, Ca, Fe, Zn, Pb, Si, P, Na, Cl and Al. This composition is due to the heterogeneous nature of the orebody and subsequent MICP process. The element composition is consistent with the minerals identified by XRD - quartz, hematite, goethite, magnetite, cerussite, and anglesite. Figure 10 shows typical, representative EDS elemental mapping of Pb and Ca in untreated and biocemented KS and KS/LPR prepared by both flow through and immersed methods. The presence of Ca and Pb in treated wastes is evident, which indicates that Pb, after treatment, was immobilized, resulting in the prevention of Pb migration out of the solid phase.

3.2.3 Leaching test results

The results of the leaching stability test results are shown in Fig. 11. After continuous leaching for 231 days, the concentrations of leached Pb^{2+} were below 0.001 mg/L for biocemented KS and KS/LPR prepared by the flow through method. This means that leachability, which is the material's ability to release a contaminant from a solid phase into a contacting liquid, was prevented. Therefore, results of the current study indicate that Pb from the biocemented monolith was negligible. Furthermore, it means that the stabilized mine waste was not influenced by contact with an eluent, binders and by the solubility of the contaminant (Bates and Hills 2015). These results indicate that Pb release from the biocemented monolith was negligible. Compared with stabilized materials which had a leachability of below 0.001 mg/L, the leachable concentrations of KS of 5.40 mg/L and that of KS/LPR of 7.87 mg/L Pb^{2+} was observed. This further reveal that biocementation can be used to prevent pollution of ecosystems whereby pollutants like heavy metals cannot be leached into ecosystems to raise background levels of these pollutants. The leachability test results confirm that the Pb^{2+} was effectively immobilized preventing toxic, water-soluble Pb from leaching out of the wastes. The water-soluble fraction of Pb is considered the most toxic fraction due to its potential to contaminate the food chain, surface water, and groundwater (Akbar et al. 2016; Tang et al. 2016; Yutong et al. 2016). The pH values of the leachate

from the biocemented KS and KS/LPR are shown in Fig. 11a. The biocemented KS and KS/LPR changed the initial pH of deionized water from pH 6.5 to a maximum pH of 9.5. The increased pH of the leachate, compared to the initial deionized water, could be due to the buffering capacity of the $CaCO_3$ in the biocemented material (Zhang et al. 2016b). The pH of the leachate was weakly alkaline and was within the bounds of the Zambia Environmental Management Agency (ZEMA) guidelines of 6.0 and 9.5 for effluent and wastewater discharge into the environment, indicated by the purple and blue lines in Fig. 11a (ZEMA 2013). The electrical conductivity of the leachate from the KS and KS/LPR is shown in Fig. 11b, ranging from 130 to 550 µS/cm. Schedule III 7(2) of the ZEMA (2013) guidelines for wastewater discharge into the environment is electrical conductivity of less than 4,300 µS/cm.

3.3 Strategy for immobilization of mine waste in Kabwe

Strength and leach resistance were used to assess the effectiveness of MICP in making stabilized products critical in controlling pollution (Pan et al. 2019). It was observed that that biocemented KS had increased strength, with maximum UCS of 8 MPa and KS/LPR had 4 MPa (Table 2). The increased strength is attributed to the hydrolysis of urea and the precipitation of $CaCO_3$, which significantly reduced hydraulic conductivity. Results revealed that leachability of Pb^{2+} was below 0.001 mg/L for both the biocemented KS and KS/LPR. The result was obtained after leaching for 231 days. Furthermore, reduced slaking (Fig. 7), and reduced water absorption capacity (Table 3) of the biocemented samples was noted. This minimizes the mobility of pollutants into lower horizons and consequently, prevent the pollution of aquifers. It was noted that biocemented wastes were not dispersed by a handheld fan. This means that airborne transportation of Pb contaminated dust particles can be eliminated. Consequently, MICP has potential to reduce the risk of exposure to contaminated particulate matter to living organisms, humans inclusive. The findings of the current study is in agreement with the results of Wang et al. (2018) who reported that MICP can alleviate cracking and wind erosion and can control the diffusion of dust from desert sand. The overall conceptual model for biocementing the Kabwe mine wastes in situ, based on the results of this study, is shown in Fig. 12.

4 Conclusion

This research promotes the utilization of biocementation as an alternative technique for effectively biocementing mine wastes. The maximum strength value of biocemented KS was observed to be 8 MPa

while that of KS/LPR was 4 MPa. The slaking test results revealed that biocemented KS and KS/LPR did not disintegrate and therefore, are stable and resist erosion by wind and rain. The water absorption coefficient reduced compared to the unstabilized sample in the treated wastes, which would also improve resistance to water and wind erosion. Finally, leachable Pb^{2+} was below the detection limit after 231 days of continuous leaching. This technique is potentially eco-friendly, easy to manage, and cost-effective because it uses onsite materials. Very few low-cost, in situ heavy metal treatment processes for bioremediation of Pb are available worldwide. Solidification of waste by MICP has the potential for application as a low cost and eco-friendly method for heavy metal remediation not only at the Kabwe mine but also at other heavy metal contaminated sites.

Declarations of interest: none

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Figure List

Fig. 1 Flowchart showing the research methodology adopted in this study to evaluate both the leachability and strength of immobilized mine waste.

Fig. 2 Location of the study area and sampling sites for KS (Site 1) and LPR (Site 2).

Fig. 3 Experimental setups for (a) immersed and (b) flow through methods for immobilization of mine wastes

Fig. 4 (a) DIK 4000 system for measuring hydraulic conductivity where 1 = scale tube; 2 =weight for supporting scale tube; 3 = silicon rubber ring; 4 = O ring; 5 = cylindrical sample holder; 6 = casing for sample holder; 7 = water tank (b) DIK 4000 system used in this study with a stopwatch

Fig. 5 USA EPA Method 1315 setup of the 3D leaching configuration of (a) biocemented KS and (b) biocemented KS/LPR

Fig. 6 Comparative view of untreated and treated KS and KS/LPR prepared by the immersed and flow through methods

Fig. 7 Slaking test images of untreated KS and KS/LPR and biocemented KS and KS/LPR for dry specimen, 30 minutes after immersion in water, and 24 hours after immersion: Left: KS/LPR Right: KS
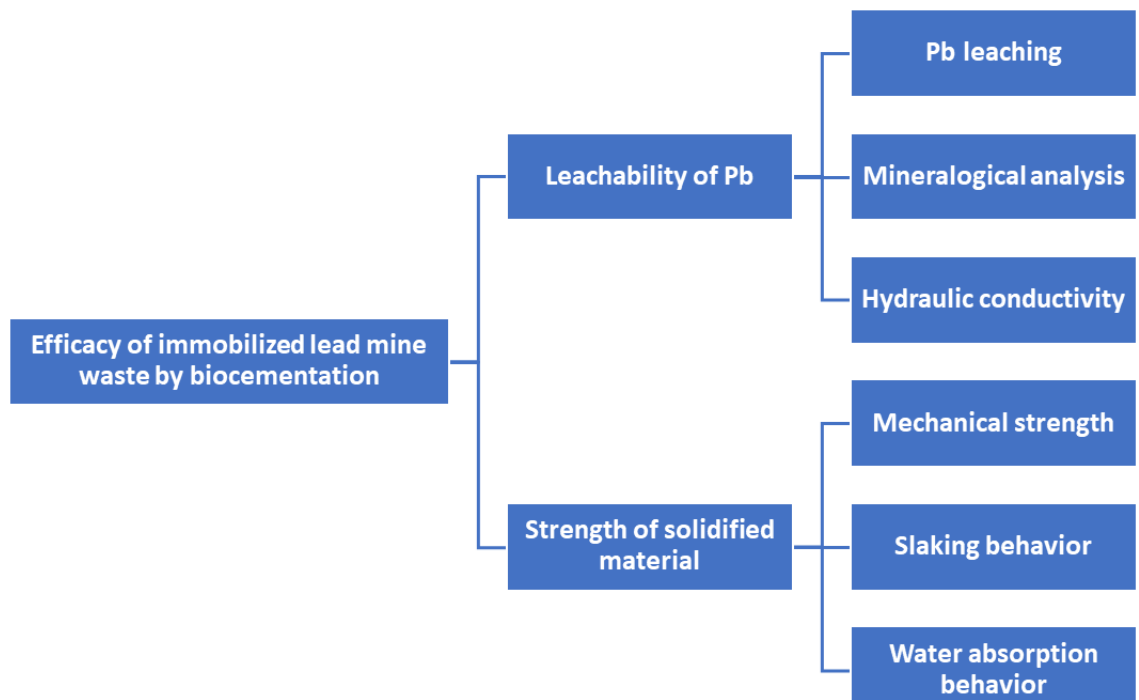
Fig. 8 X-ray diffraction (XRD) patterns of untreated and biocemented KS and KS/LPR

Fig. 9 Scanning electron microscope (SEM) images of (a) untreated KS at x100 magnification, (b) untreated KS at x300 magnification, (c) biocemented KS prepared by immersed method at x100 magnification, and (d) biocemented KS prepared by immersed method at x300 magnification

Fig. 10 Scanning electron microscopy (SEM) and energy-dispersive x-ray spectroscopy (EDS) images of untreated and biocemented KS and KS/LPR

Fig. 11 Results of leaching test for biocemented KS and KS/LPR prepared by flow through method: (a) pH and (b) hydraulic conductivity

Fig. 12 Conceptual model for immobilization of Pb-contaminated mine wastes at Kabwe Mine site using MICP by *Pararhodobacter* sp.
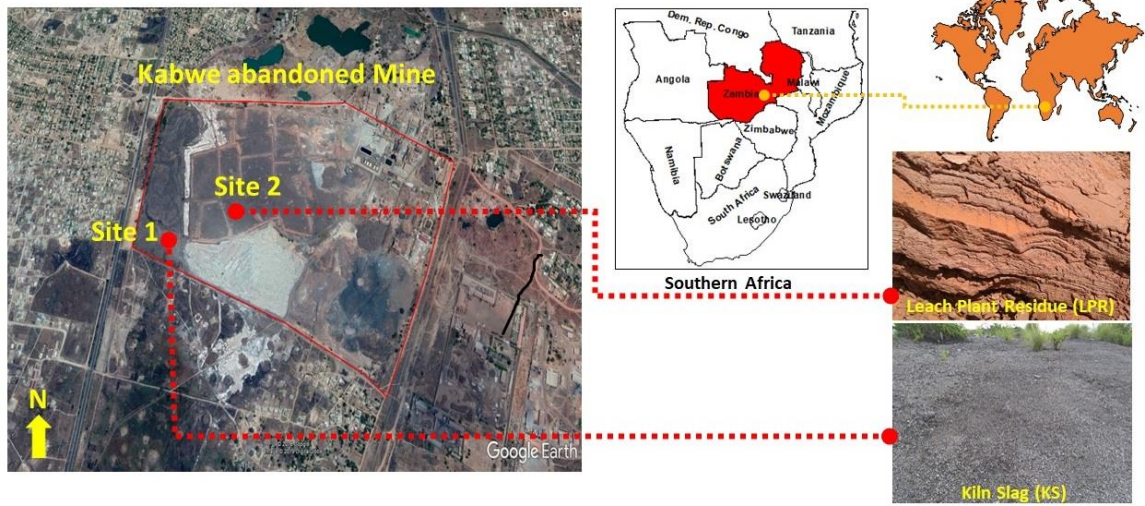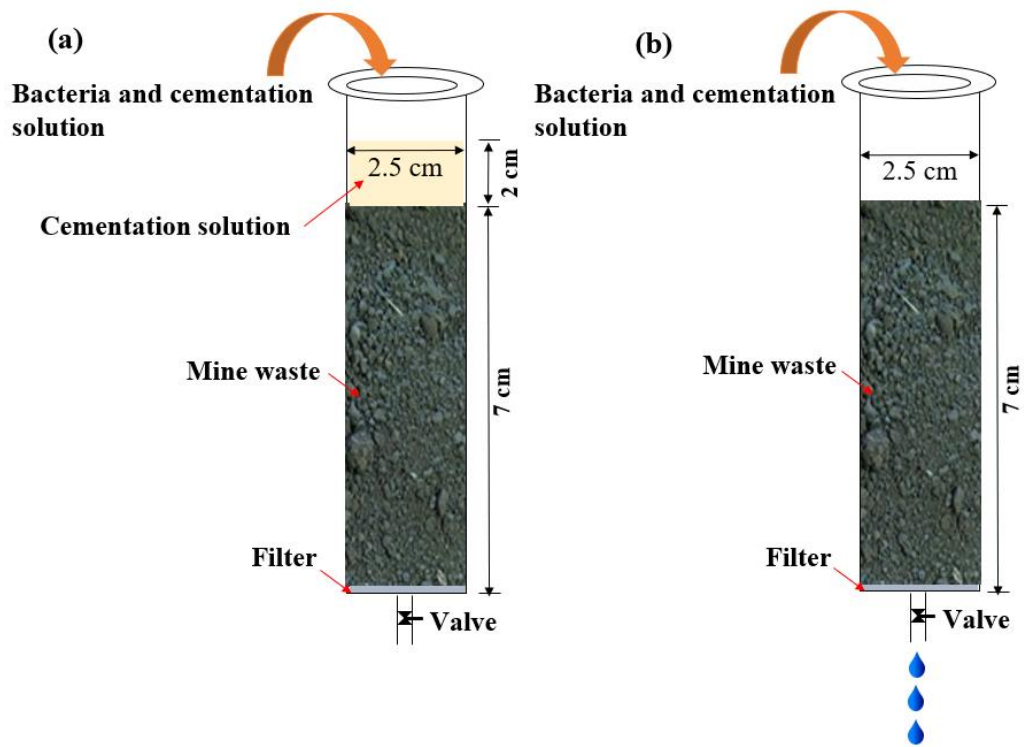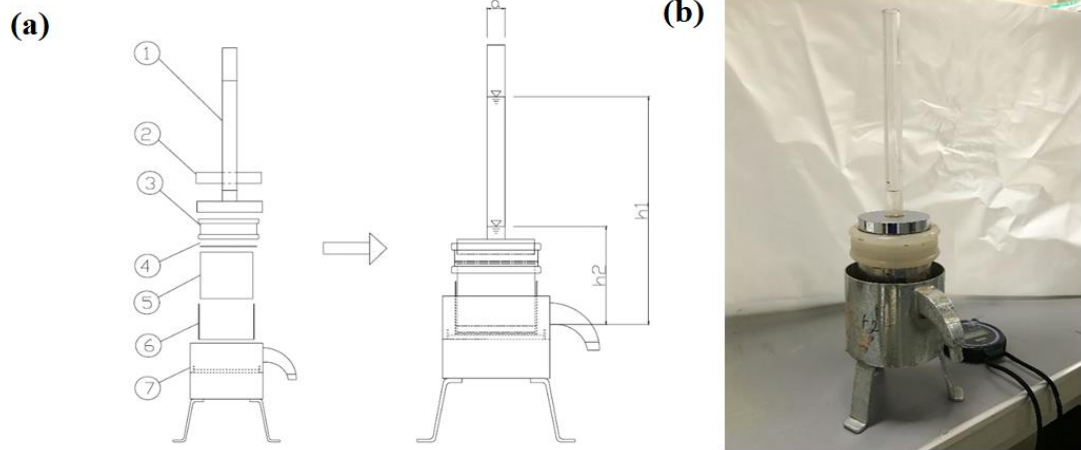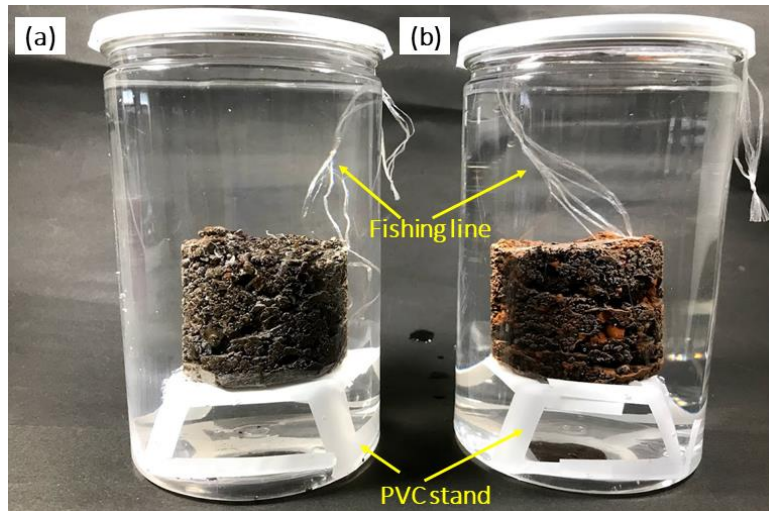
Fig. 13

Fig. 14

(a)

Bacteria and cementation solution

2.5 cm

2 cm

Cementation solution

Mine waste

7 cm

Filter

Valve

(b)

Bacteria and cementation solution

2.5 cm

Mine waste

7 cm

Filter

Valve

Fig. 15

Fig. 16

Fig. 17

Fig. 18

| | dry specimen | after 30 min in water | after 24 h in water |

Untreated

Immersed

Flow through

Fig. 19

Untreated KS

Biocemented KS by flow through

Untreated KS/LPR

Biocemented KS/LPR by flow through

Calcite
Quartz
Cerussite
Anglesite
Hematite
Magnetite
Geothite

2θ

Fig. 20

27

Fig. 21

	SEM	Pb	Ca
Untreated KS			
Flow through KS			
Immersed KS/LPR			

Fig. 22

29

Fig. 23

Rain

No dust particles
from mine waste

Wind Direction

Increased runoff

Biocemented KS

Biocemented KS/LPR

LPR

Clay

Storm water drain

Fig. 24

Table 1:. Chemical composition of artificial seawater (g/L)

NaCl	MgCl$_2 \cdot$6H$_2$O	Na$_2$SO$_4$	CaCl$_2 \cdot$2H$_2$O	KCl
24.53	11.11	4.094	1.535	0.695
KBr	SrCl$_2$	H$_3$BO$_3$	NaF	NaHCO$_3$
0.101	0.043	0.027	0.003	0.201

Table 2: Estimated Unconfined Compressive Strength (UCS) of untreated and biocemented KS and KS/LPR by the immersed and flow through method.

Type of treatment	Estimated UCS of KS (MPa)			Estimated UCS of KS/LPR (MPa)		
	Top	**Middle**	**Bottom**	**Top**	**Middle**	**Bottom**
Untreated	0	0	0	0	0	0
Biocementation by immersed method	8	1.8	1.7	4	0	0
Biocementation by flow through method	5	4.6	2.5	2.8	2.1	0.5

Table 3: Water absorption results of untreated and biocemented KS and KS/LPR prepared by immersed and flow through methods

Type of material	Untreated	Immersed condition after biocementation (kgm^{-2}h$^{-0.5}$)	Flow through method after biocementation (kgm^{-2}h$^{-0.5}$)	Water absorption of conventional materials (kgm^{-2}h$^{-0.5}$)	Source of Results
KS	3.56	1.31	1.96	-	This study
KS/LPR	6.56	2.42	5.92	-	This study
mca20/80 brick	-	-	-	3.2	Karagiannis et al. 2016
Handmade brick	-	-	-	4.5	Karagiannis et al. 2016
Concrete	-	-	-	1.00	Demirci and Sahin, 2014