学　位　論　文　内　容　の　要　旨

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学　位　論　文　題　名

Development of bioremediation methods for soil and water contaminated with heavy metals in Kabwe mine

(Kabwe 鉱山において重金属により汚染された土壌および水に対するバイオレメディエーション法の開発)

Heavy metal contamination of both soil and water is known the world over especially from abandoned mining activities. The abandoned Kabwe Mine is known for chronic lead (Pb) poisoning in humans and polluting soil, water, and sediments in and the surrounding areas. For example, the current blood levels of Pb in children exceeds 0.45 mg/L, much higher than the recommended level of 0.05 mg/L by the World Health Organization. The high Pb level in blood is attributed to the airborne Pb metallic dust emanating from the mine waste dump. Additionally, a high level of Zinc (Zn) in water have been reported. Therefore, to address this challenge, developed bioremediation methods for alleviating heavy metal contamination for both soil and water using novel bioremediation techniques. Bioremediation plays a significant role in the restoration of a contaminated site, as it is less disruptive and sustainable compared to other methods. In this context, the objectives of this study were focused on developing novel bioremediation techniques for alleviating both soil and water contamination caused by the abandoned Kabwe Mine site, Zambia. In order to bioremediate soil, microbially induced calcium carbonate ($CaCO_3$) precipitation (MICP) was adopted using three species of bacteria: *Pararhodobacter* sp. isolated from Okinawa, Japan and two locally isolated bacteria from the mine. Furthermore, Pb and Zn removal from the water was investigated using another indigenous bacteria species isolated from Kabwe as well as a novel cellulose-based biosorbent formulated by the fusion protein.

In Chapter 1, the research background, the literature review, the research objectives, scope and the originality of the thesis are presented.

In Chapter 2, the stabilization of mine waste was undertaken using *Pararhodobacter* sp. The bacteria strain was applied and evaluated for the purpose of bioremediation using MICP technique. In this chapter, we demonstrated that Pb^{2+} can be removed by bioprecipitation using *Pararhodobacter* sp. The bacteria was capable of complete removal of 1036 mg/L Pb^{2+}. Furthermore, sand solidification test by the bacteria revealed that optimal unconfined compressive strength (UCS) was obtained when four bacterial injections were used. Biocemented mine wastes (kiln slag (KS) and leach plant residue (LPR)) from Kabwe mine had leachate Pb^{2+} concentrations below the detection limit of <0.001 mg/L, resisted slaking, and had maximum UCS of 8 MPa for KS and 4 MPa for KS/LPR mixture. Furthermore, biocemented mine waste exhibited lower water absorption coefficient values, which could potentially reduce the water transportation of Pb. The results of this chapter show that MICP can reduce Pb 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.

In Chapter 3, we introduced two new strains of ureolytic bacteria for the MICP process: *Oceanobacillus profundus* KBZ 1-3 and KBZ 2-5 isolated from Kabwe mine site. Both strains of bacteria were Pb tolerant and capable of mediating the formation of $CaCO_3$ bioprecipitates, which was confirmed to be calcite by XRD analysis. The biocemented sand achieved maximum UCS values of 4.0 MPa and 5.7 MPa, which could be useful enough to prevent Pb dust particles from being blown away by prevailing winds and to prevent water erosion. Combined with a reduced hydraulic conductivity of 9.6 x 10^{-8}m/s and 8.9 x 10^{-8}m/s mediated by *O. profundus* KBZ 1-3 and KBZ 2-5, respectively, the process is expected to retard heavy metal leaching due to the lack of oxygen and water resulting from reduced infiltration. The results of immobilization by locally isolated bacteria indicated that they can be utilized at the abandoned mine. Isolation and identification of effective microorganisms for biotechnological applications, represents a green and sustainable approach to remediation, eliminating the current environmental problems without significantly changing the local ecological integrity.

In Chapter 4, Pb-tolerant *Oceanobacillus profundus* KBZ 3-2 isolated from the site was investigated for the removal of heavy metal from the water. The results indicated biosorption conditions were favorable at pH 6.5, the temperature of 30°C, and a contact time of 120 minutes. The bacteria were found to accumulate Pb and Zn in different cellular parts such as cell proteins, cytoplasm, and cell surface as well as extracellular polymeric substance (EPS). The results indicated that of all the cellular parts, the EPS excreted by the bacteria is largely responsible for heavy metal sequestration by complexation. *Oceanobacillus profundus* KBZ 3-2 is highly efficient as a biosorbent and has great potential in upscaling its biosorption purpose for bioremediation at the Kabwe Mine site.

In Chapter 5, a novel cellulose-based biosorbent was developed. The fusion protein composed of metallothionein (MT) from *Synechococcus elongatus* PCC 7942 and cellulose binding module (CBM) from *Clostridium thermocellum* was successfully constructed. The fusion protein was immobilized on cellulose to form cellulose-MT-CBM biosorbent. The biosorption condition was favorable at a pH 6.5 at room temperature with 20 minutes contact time. This study provides insight into the application of naturally occurring cellulose and MTs as biosorbents to remove toxic trace elements from water which can consequently enable its commercial application. The regeneration and reusability make the biosorbent a sustainable product formulated from totally bio-based renewable materials.

Finally, Chapter 6 summarized all the results obtained in each chapter and provides some prospects for future works in the above areas.