Proceedings of the ICBBG2025
2025 International conference on Bio-mediated and Bio-inspired Geotechnics
18-20 May, 2025 | Tempe, Arizona, USA
https://doi.org/10.53243/ICBBG2025-76

ICBBG
18-20 May 2025

The Challenge of Stabilizing Copper Tailings Using EICP: The Role of Inhibitors

Bemah Ibrahim[1], Md Mizanur Rahman[1], Md Rajibul Karim[1], Khoi Nguyen[1] and Edward Kavazanjian Jr. [2]

[1]UniSA STEM, University of South Australia, Mawson Lakes, Adelaide, Australia, E-mail: bemah.ibrahim@mymail.unisa.edu.au
[2]Center for Bio-Mediated and Bio-Inspired Geotechnics, School of Sustainable Engineering and the Built Environment, Arizona State Univ., Tempe, AZ 85281

ABSTRACT

Enzyme-induced carbonate precipitation (EICP) is being studied as a promising method for stabilizing mine tailings against liquefaction, building on recent studies of its applications in natural soil stabilization. However, tailing composition (unlike natural soils), including heavy metals and flotation reagents, creates a harsh environment that can inactivate the urease enzyme essential to successful EICP. This study investigates the effects of tailing compositions, specifically the concentration of copper (Cu) and flotation collectors (i.e., potassium amyl xanthate (PAX)) on EICP efficiency. A series of test tube experiments were conducted to assess these influences. The results indicate that increasing Cu concentrations can completely inhibit EICP. A Cu concentration of 5.22 mg/L can reduce the maximum precipitation by as much as 50%. PAX reduced enzyme activity by 43.25% at a concentration of 100 mM. Therefore, the standard EICP treatment process, while highly successful for Adelaide industrial sand, is likely to be unsuccessful for copper tailings. The study proposes the use of L-cysteine to protect the urease enzyme from inactivation. The results indicate that L-cysteine is effective at preventing enzyme inactivation in tailing stabilization.

INTRODUCTION

The increasing production of mineral commodities, driven by a rising global demand, has resulted in a significant surge in tailings generation (Kossoff, Dubbin et al. 2014). It is concerning that 10% of all tailing storage facilities (TSF) have reported stability issues, with instability becoming more prevalent as tailing height, stored volume, and seismic hazards increase (Franks, Stringer et al. 2021, Karim, Rahman et al. 2023). Enzyme induced carbonate precipitation (EICP) has gained increasing attention as a sustainable alternative to traditional tailing stabilization techniques, owing to its environmental benefits and ability to be applied with minimal disturbance (Han, Li et al. 2022). While EICP offers promise for tailings stabilization, the complex composition of tailings presents significant hurdles for EICP application. In particular, the constituents of tailings, as influenced by the geochemistry of the ore and the extraction processes used to liberate ore from gangue minerals, can inhibit the application of EICP.

Ore deposits, enriched by hydrothermal fluids, are polymetallic and contain metals such as copper, gold, nickel, and cobalt. These metals can inhibit enzyme activity by binding to amino acid residues containing sulfur (cysteine), nitrogen (histidine), and oxygen (aspartic and glutamic acids) (Krajewska 2009, Buikema, Zwissler et al. 2017). This can inactivate the urease enzymes essential to EICP by interacting with thiol cysteine residues, forming mercaptides that hinder the enzyme's mobility and block access to the active site, leading to decreased catalytic activity (Olech, Zaborska et al. 2014, Mazzei, Cianci et al. 2018) and ineffective biotreatment.

In addition to inhibition by metals, flotation reagents used to liberate valuable minerals from gangue may present challenges to EICP application. Xanthates, such as potassium amyl xanthate (PAX) are widely used in flotation circuits (especially for sulphidic ores) to render the metal mineral hydrophobic (Langa, Adeleke et al. 2014). Typically, 10–300 grams of xanthates are required per ton of ore during the flotation process (Bulatovic 2007). However, only about half of these chemicals are consumed during the process, with the remainder being discharged with the tailings. Previous studies have shown that xanthates can inhibit microbial activity in soil (Bararunyeretse, Yao et al. 2017). As ores become increasingly complex, additional chemical reagents such as frothers, depressants, activators, and pH modifiers (such as lime) are often required to optimize the flotation system. These combined factors contribute to the complexity of tailings, creating a particularly hostile environment for urease enzymes. Given the urgent need to prevent tailings dam failures due to liquefaction and the growing interest in EICP, it is critical to understand how tailing composition impacts biotreatment processes.

In this study, a baseline investigation was conducted to evaluate the impact of heavy metals and flotation reagents (PAX) on the efficiency of EICP through control test tube experiments. In addition, urease enzyme incubation in leachate from tailing and Adelaide Industrial sand (AI Sand) prior to EICP was investigated test tube. The results from test tube experiments were implemented to treat the tailing and AI sand. The protection of the urease enzyme from inactivation was also explored.

MATERIALS AND METHODS

Material. Powdered crude paddy melon enzyme with activity of 2.0 KU/g was used (Ahenkorah, Rahman et al. 2023, Ahenkorah, Rahman et al. 2024, Rahman, Ahenkorah et al. 2024, Tran, Rahman et al. 2024). L-cysteine, $\mathrm{CuNO_2 \cdot 3H_2O}$, and urea were purchased from Sigma-Aldrich. $\mathrm{CaCl_2 \cdot 2H_2O}$ and skim milk powder were purchased from Scharlau and Woolworth, respectively.

Tailings were collected from one of the largest open-pit copper mines. The principal economic minerals in ores from which these tailings were produced are chalcopyrite, bornite and molybdenite, which predominantly occur in veins and fractures. The copper tailing is produced from the mine site via grinding lines, with three lines consisting of semi-autogenous mills and ball mills and two lines consisting of autogenous mills and ball mills. The grinding stage reduces the ore to the consistency of sand to liberate copper. To recover copper minerals, the ground ore is processed through a bulk sulphide flotation circuit containing a series of reagents such as fuel oil, potassium amyl xanthate and potassium isopropyl xanthate. To comprehensively ascertain the sole effect of tailing composition, the particle size distribution (PSD) of the tailing was modified to match that of AI sand, which is used in this study as a control material. It was prepared by performing a sieve analysis to match the particle size distribution of AI sand. After sieving the AI sand, the percentage of material retained on each sieve was used to create the modified tailing by sieving the original tailing and retaining the same mass as the AI sand to obtain 500 g of modified tailing. The resultant PSD is shown in Figure 1. The elemental composition (Table 1) was determined using Inductively coupled plasma atomic emission spectroscopy (ICP-OES).

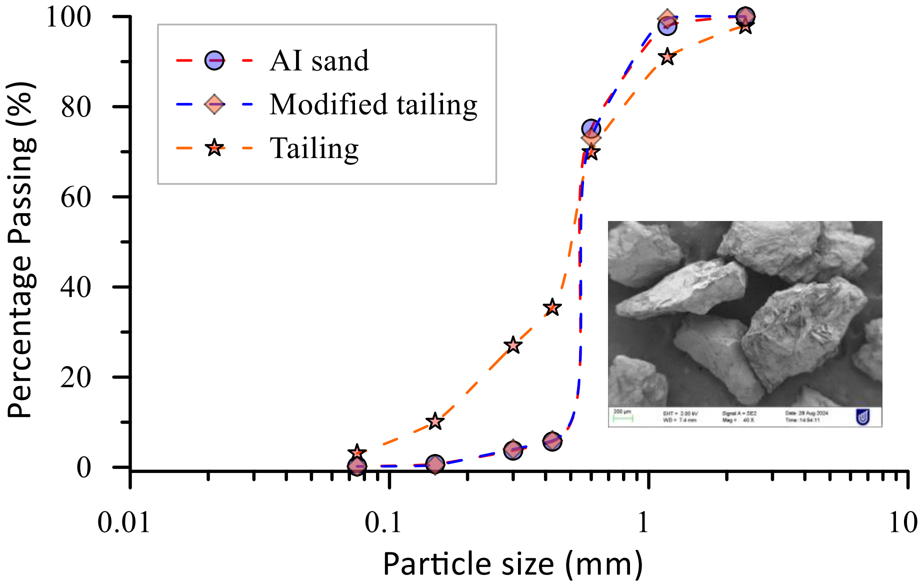

Figure 1. Particle-size distribution for the tailing, modified tailing and AI sand with inset SEM image of grain size and shape for modified tailing

Table 1. ICP-OES analysis results of modified tailing and AI sand

Material	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)	Cr (mg/kg)	Co (mg/kg)	Fe(mg/kg)
Tailing	1687.17	<LOD	22.42	32.89	<LOD	<LOD	6981.64
A.I Sand	14.03	19.04	<LOD	<LOD	67.36	<LOD	22683.61

Test Tube Precipitation. Three sets of test tube precipitation experiments were conducted using 50ml test tubes to investigate: (1) The influence of reaction time on precipitation ratio (PR = mass of precipitate $CaCO_3$/theoretical mass of $CaCO_3$) for uninhibited EICP (2) Cu inhibited EICP and (3) the effect of increasing Cu concentration on PR.

In the first set, 16 individual test tubes (without replication) were prepared, each filled with 20ml solution of equimolar EICP solution consisting of 0.5M urea and $CaCl_2$, and 5g/L of crude paddy melon enzyme (Tran, Rahman et al. 2024). The test tubes were sacrificed and analyzed at different time intervals. In the second set, another 16 test tubes were prepared with the same EICP solution composition plus 3.69 mg/L Cu, and were similarly sacrificed and analyzed at the same time intervals. In the third set, 40 test tubes (including random duplicates) were prepared with 20ml of EICP solution consisting of urea, $CaCl_2$, urease enzyme (Rahman, Ahenkorah et al. 2024), and varying concentrations of Cu. The concentration of urea and $CaCl_2$ was maintained at 0.5 M. The test tubes were incubated in a temperature-controlled incubator at 30°C for 3 days (Almajed, Khodadadi Tirkolaei et al. 2018, Ahenkorah, Rahman et al. 2021, Muhammed, Kassim et al. 2021).

After incubation, the amount of $CaCO_3$ precipitated was then evaluated by means of the following procedure:

a) the solution was filtered through the 2.5 μm filter paper.
b) the test tube and the filter paper were dried, and the precipitate was weighed.
c) the total mass of $CaCO_3$ was calculated as the sum of the material deposited on the filter paper and on the bottom of the test tube.

Leachate inhibition. Six test tube experiments were conducted using 50 ml test tube to evaluate the effect of tailing leachate on EICP. 50 g of each soil sample was suspended in 50 mL deionized water for two days. The suspension was shaken at 200 rpm for 1 h using an overhead shaker, centrifuged at 4500 rpm for 30 min. The leachate was filtered using a Whatmanfilter paper with a 2.5 μm pore size. A 10 mL aliquot of the leachate was then used to prepare an enzyme solution at a concentration of 5 g/L. Control samples were prepared using deionized water. The enzyme solution was incubated in an oven at 30°C for 15 minutes, after which it was mixed with 10 mL of substrate solution containing 0.5M urea and $CaCl_2$ to initiate EICP. The experiments were left to stand for 3 days after which , the PR was determined using the procedure described in previous section.

Determining the effect of xanthate collectors on paddy melon enzyme activity. PAX was collected from the Mineral Processing Laboratory of the University of South Australia. The enzymatic reaction was performed in 20 ml of solution containing 0.5 M urea and an increasing dose of PAX (0-100 mM). The reaction was initiated by adding 5g/L solution of urease to the reaction mixture, and the electrical conductivity (EC) was monitored for 15min. The rate of change of EC was used as the measure of enzyme activity.

Tailing Treatment. In treating tailing and AI sand, a PVC split mold (50 mm diameter, 100 mm height) was used, with the specimens constructed in three layers. The substrates (urea (1.2-2.0M), $CaCl_2$ (1.2-2.0M), and 5g/l milk powder) and enzyme solution were premixed and allowed to percolate into the tailings via gravity. A constant ratio of enzyme to substrate concentration was maintained at 11g/L/M. Tailings and AI sand were treated with the same EICP solution. However, in the protected treatment of tailing, the enzyme was incubated with L-cysteine for 10 minutes before being mixed with the substrates. A cysteine to enzyme ratio of 0.01 M/g/L was maintained for all protected treatment. Skim milk was used in all treatment to provide nucleation sites (Almajed, Tirkolaei et al. 2019).

RESULTS AND DISCUSSION

Figure 2 shows the PR in the test tubes with and without 3.69 mg/L Cu as an inhibitor. It shows that PR increases with reaction time, irrespective of inhibition. However, the maximum PR of 100 % was achieved in 15 hours for uninhibited EICP, whereas a maximum PR of 87% was realized in 24 hours for Cu-inhibited EICP. Interestingly, in the first hour, uninhibited and inhibited EICP achieved PRs of 35% and 34%, respectively. This suggests that the enzyme remained almost unaffected during the initial stage. However, beyond this point, the PR consistently increased at a higher rate in the uninhibited reaction compared to the inhibited reaction. This is a characteristic of slow-binding inhibitors as they do not act on enzymes instantly but display a slow onset of the inhibition (Zaborska, Krajewska et al. 2004, Morrison and Walsh 2006).

Figure 3 shows the effect of Cu concentration, with increasing concentration significantly reducing PR due to enzyme inactivation. The sigmoidal shape of the precipitation curves aligns with previous studies on ureolysis (Krajewska 2008) and can be represented using the Hill equation as:

$$PR\ (\%) = PR_{max} + (PR_{min} - PR_{max}) \times \left(\frac{[\mathrm{Cu}]^n}{k^n + [\mathrm{Cu}]^n}\right)$$

where, k and n are fitting parameters.

As shown in Figure 3, the concentration of Cu at which precipitation was reduced by 50% (HPR_{50}) was determined as 5.22 mg/L. HPR_{50} can be used to determine the relative inhibition strength of various heavy metals on EICP efficiency and be used in developing mathematical equations for complex inhibited EICP reactions.

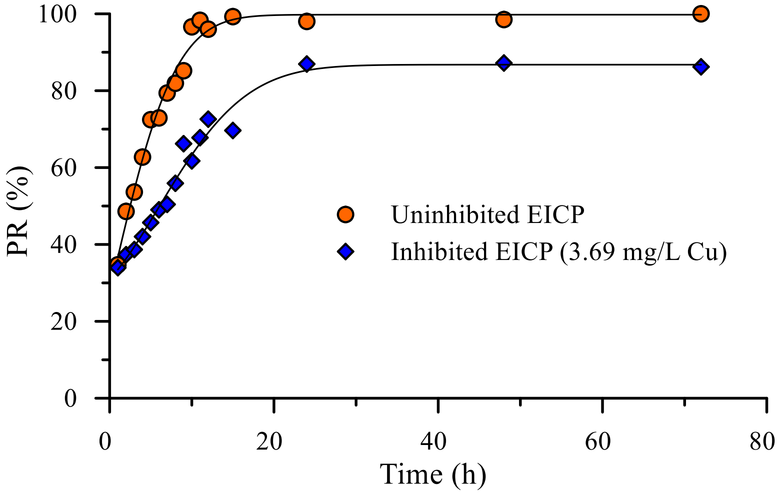

Figure 2. Precipitation ratio vs time plot for uninhibited EICP and inhibited EICP with 3.69 mg/L Copper

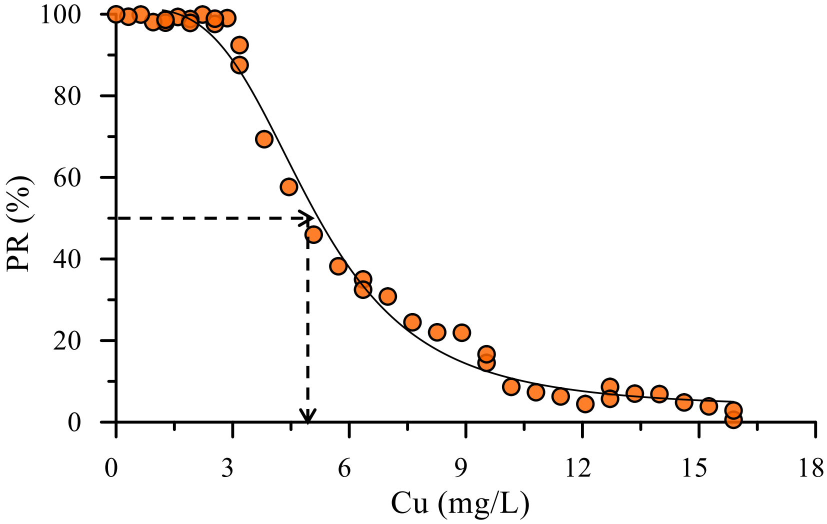

Figure 3. Plot of: (a) Precipitation ratio vs copper concentration

A summary of the initial rate of change EC as plotted against PAX concentrations is shown in Figure 4. The results demonstrate a negative correlation between urease activity and increasing PAX concentrations, consistent with previous findings on the inhibitory effects of sodium isopropyl xanthate and sodium isobutyl xanthate on soil microbial and enzyme activities (Bararunyeretse, Yao et al. 2017). 100mM PAX reduced enzyme activity by 43.25%.

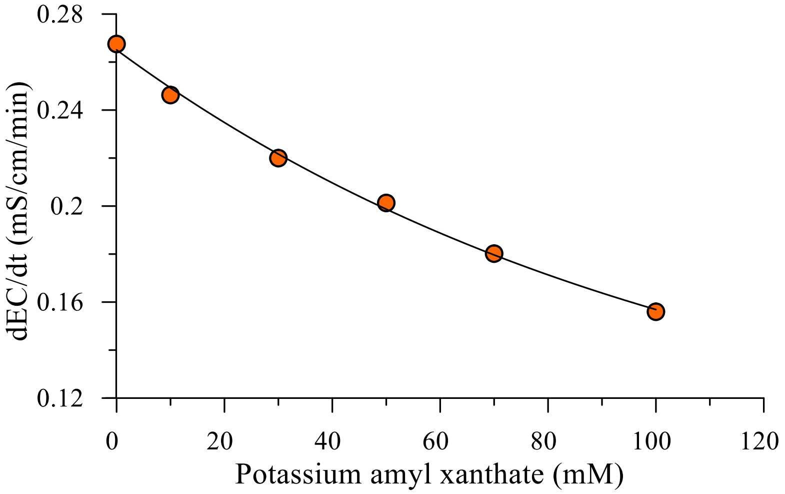

Figure 4. Rate of change in enzyme activity vs concentration of PAX

Figure 5 presents the average PR obtained after incubating enzyme in leachate from tailing and AI sand prior to EICP. The PR was lowest in tailing leachate at 86%, while the leachate from AI sand and the control (deionized water) achieved PR of 96% and 97%, respectively. The reduced PR observed in tailing leachate suggests that the composition of the tailings inhibited urease enzyme activity during incubation.

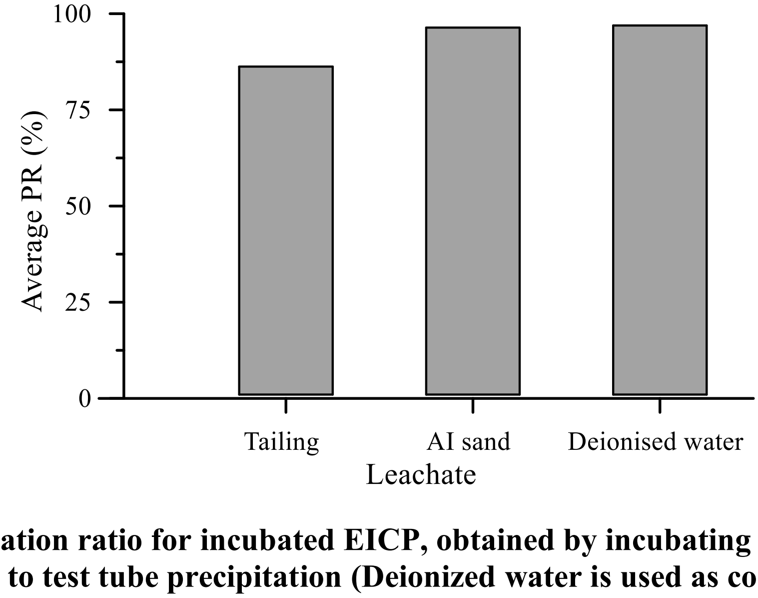

Figure 5. Precipitation ratio for incubated EICP, obtained by incubating urease in leachate prior to test tube precipitation (Deionized water is used as control)

As shown in Figure 6a, EICP treatment of tailing was ineffective, while treatments of AI sand were successful (Figure 6b). The paddy melon urease enzymes, with inherently low substrate affinity, exhibit slow urea hydrolysis (data not shown). Flotation reagents like xanthates present in the tailings compete with urea for the enzyme's active site, significantly slowing hydrolysis. Although reduced urease activity can promote uniform precipitation homogeneity (Sun et al., 2024; H. Wang, L. Miao, et al., 2023; H. Wang, X. Sun, et al., 2023), prolonged exposure to heavy metals in the tailings leads to the formation of mercaptides at the enzyme's mobile flap, resulting in enzyme inactivation. As ureolysis is crucial for $\mathrm{CaCO_3}$ precipitation in EICP, the reduced activity, and metal-induced inactivation render the process ineffective. However, pre-incubating the urease enzyme in L-cysteine made EICP effective (Figure 6c). Literature evidence explains this protective mechanism: L-cysteine in the pre-incubation mixture covalently bind to urease thiol groups, thereby protecting them from interactions with the heavy metal (Riddles, Andrews et al. 1983, Zaborska, Krajewska et al. 2004, Krajewska and Ciurli 2005, Krajewska and Brindell 2011). Once, the incubation mixture is introduced, the unbounded L-cysteine binds with the heavy metal through disulphide interchange between the thiol of L-cysteine and the heavy metals, leading to the formation of cysteine-heavy metal complexes (Krajewska, Zaborska et al. 2004, Krajewska 2008).

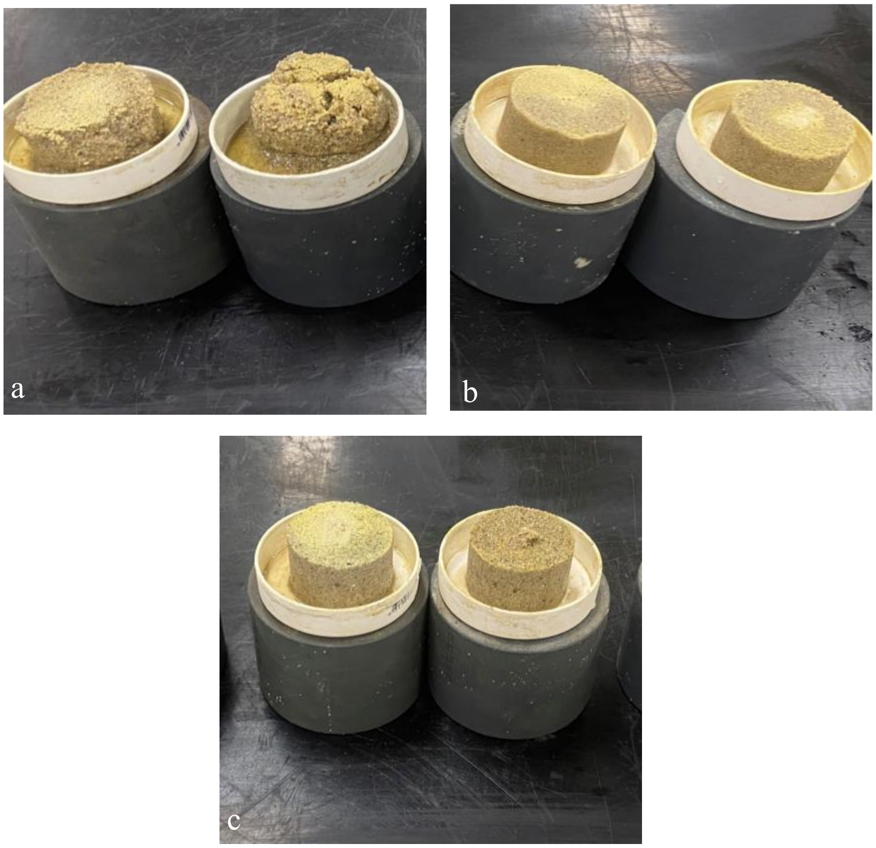

Figure 6. Images of freshly removed samples of: (a) tailings (b) AI sand and (c) tailings treated with EICP + L-cysteine

CONCLUSIONS

In this study, a baseline investigation was conducted to understand the effect of tailing composition on the EICP process with a focus on heavy metals (Cu) and flotation collectors (PAX). Test tube precipitation tests as well as laboratory scale EICP treatment of modified copper tailings and Adelaide industrial sand, were performed. The main findings of this study are summarized below.

i. Increasing heavy metal concentrations can completely inhibit EICP. The concentrations of Cu that lead to a 50% reduction in maximum calcium precipitation (HPR_{50}) was determined to be 5.22 mg/L. Cu also delays precipitation.
ii. Increasing concentration of PAX leads to reduction in enzyme activity. Activity reduced by 43.25% for PAX concentration of 100 mM.
iii. Standard EICP was ineffective at stabilizing copper tailings due to harsh tailing environment.
iv. The use of L-cysteine was effective at protecting urease enzyme from inactivation leading to effective treatment of copper tailing.

ACKNOWLEDGMENTS

The tailing material was collected as part of TAILLIQ (Tailings Liquefaction), which is an Australian Research Council (ARC) Linkage Project supported by financial and in-kind contributions from Anglo American, BHP, Freeport-McMoRan, Newmont, Rio Tinto, and Teck. The TAILLIQ project is being carried out at The University of New South Wales (UNSW), The University of South Australia, The University of Western Australia (lead university), and The University of Wollongong. The authors acknowledge the support and contributions of project personnel at each of the supporting organizations. The authors specifically thank the mine site personnel who assisted in the sampling works that provided the material used for this study. The authors acknowledge the technical assistance of Dr Patrick Sherman and Susie Ritch of the University of South Australia.

REFERENCES

Ahenkorah, I., M. M. Rahman, M. R. Karim and S. Beecham (2021). "Optimisation of chemical constituents on enzyme-induced carbonate precipitation in test-tube and soil." *Geotechnical Research* 8(3): 66-84.

Ahenkorah, I., M. M. Rahman, M. R. Karim and S. Beecham (2023). "Unconfined compressive strength of MICP and EICP treated sands subjected to cycles of wetting-drying, freezing-thawing and elevated temperature: Experimental and EPR modelling." *Journal of Rock Mechanics and Geotechnical Engineering* 15(5): 1226-1247.

Ahenkorah, I., M. M. Rahman, M. R. Karim and S. Beecham (2024). "Cyclic liquefaction resistance of MICP- and EICP-treated sand in simple shear conditions: a benchmarking with the critical state of untreated sand." *Acta Geotechnica*.

Almajed, A., H. Khodadadi Tirkolaei and E. Kavazanjian Jr (2018). "Baseline investigation on enzyme-induced calcium carbonate precipitation." *Journal of Geotechnical and Geoenvironmental Engineering* 144(11): 04018081.

Almajed, A., H. K. Tirkolaei, E. Kavazanjian and N. Hamdan (2019). "Enzyme Induced Biocementated Sand with High Strength at Low Carbonate Content." *Scientific Reports* 9(1).

Bararunyeretse, P., J. Yao, Y. Dai, S. Bigawa, Z. Guo and M. Zhu (2017). "Toxic effect of two kinds of mineral collectors on soil microbial richness and activity: analysis by microcalorimetry, microbial count, and enzyme activity assay." *Environmental Science and Pollution Research* 24(2): 1565-1577.

Buikema, N. D., B. E. Zwissler, E. A. Seagren, T. Oommen and S. Vitton (2017). "Stabilisation of iron mine tailings through biocalcification." *Environmental Geotechnics* 5(2): 94-106.

Bulatovic, S. M. (2007). *Handbook of flotation reagents: chemistry, theory and practice: Volume 1: flotation of sulfide ores*, Elsevier.

Franks, D. M., M. Stringer, L. A. Torres-Cruz, E. Baker, R. Valenta, K. Thygesen, A. Matthews, J. Howchin and S. Barrie (2021). "Tailings facility disclosures reveal stability risks." *Scientific Reports* 11(1).

Han, L.-J., J.-S. Li, Q. Xue, M.-Z. Guo, P. Wang and C. S. Poon (2022). "Enzymatically induced phosphate precipitation (EIPP) for stabilization/solidification (S/S) treatment of heavy metal tailings." *Construction and Building Materials* 314: 125577.

Karim, M. E., M. M. Rahman, M. R. Karim, A. B. Fourie and D. Reid (2023). "Characteristics of copper tailings in direct simple shearing: A critical state approach." *Journal of Geotechnical and Geoenvironmental Engineering* 149(5): 04023018.

Kossoff, D., W. E. Dubbin, M. Alfredsson, S. J. Edwards, M. G. Macklin and K. A. Hudson-Edwards (2014). "Mine tailings dams: Characteristics, failure, environmental impacts, and remediation." *Applied Geochemistry* 51: 229-245.

Krajewska, B. (2008). "Mono- (Ag, Hg) and di- (Cu, Hg) valent metal ions effects on the activity of jack bean urease. Probing the modes of metal binding to the enzyme." *Journal of Enzyme Inhibition and Medicinal Chemistry* 23(4): 535-542.

Krajewska, B. (2008). "Mono-(Ag, Hg) and di-(Cu, Hg) valent metal ions effects on the activity of jack bean urease. Probing the modes of metal binding to the enzyme." *Journal of enzyme inhibition and medicinal chemistry* 23(4): 535-542.

Krajewska, B. (2009). "Ureases I. Functional, catalytic and kinetic properties: A review." *Journal of molecular catalysis B: Enzymatic* 59(1-3): 9-21.

Krajewska, B. and M. Brindell (2011). "Urease activity and<scp>l</scp>-ascorbic acid." *Journal of Enzyme Inhibition and Medicinal Chemistry* 26(3): 309-318.

Krajewska, B. and S. Ciurli (2005). "Jack bean (Canavalia ensiformis) urease. Probing acid–base groups of the active site by pH variation." *Plant Physiology and Biochemistry* 43(7): 651-658.

Krajewska, B., W. Zaborska and M. Chudy (2004). "Multi-step analysis of Hg2+ ion inhibition of jack bean urease." *Journal of Inorganic Biochemistry* 98(6): 1160-1168.

Langa, N. T. N., A. A. Adeleke, P. Mendonidis and C. K. Thubakgale (2014). "Evaluation of sodium isobutyl xanthate as a collector in the froth flotation of a carbonatitic copper ore." *International Journal of Industrial Chemistry* 5(3-4): 107-110.

Mazzei, L., M. Cianci, A. Gonzalez Vara and S. Ciurli (2018). "The structure of urease inactivated by Ag(<scp>i</scp>): a new paradigm for enzyme inhibition by heavy metals." *Dalton Transactions* 47(25): 8240-8247.

Morrison, J. F. and C. T. Walsh (2006). The Behavior and Significance of Slow-Binding Enzyme Inhibitors, John Wiley & Sons, Inc.: 201-301.

Muhammed, A. S., K. A. Kassim, K. Ahmad, M. U. Zango, C. S. Chong and J. Makinda (2021). "Influence of multiple treatment cycles on the strength and microstructure of biocemented sandy soil." *International Journal of Environmental Science and Technology* 18(11): 3427-3440.

Olech, Z., W. Zaborska and M. Kot (2014). "Jack bean urease inhibition by crude juices of Allium and Brassica plants. Determination of thiosulfinates." *Food chemistry* 145: 154-160.

Rahman, M. M., I. Ahenkorah, M. R. Karim and S. Beecham (2024). "In vitro screening of crude urease extract from native Australian plants and weeds for bio-cementation of sands." *Géotechnique*.

Riddles, P. W., R. K. Andrews, R. L. Blakeley and B. Zerner (1983). "Jack bean urease VI. Determination of thiol and disulfide content: reversible inactivation of the enzyme by the blocking of the unique cysteine residue." *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology* 743(1): 115-120.

Tran, M. V., M. M. Rahman, M. R. Karim and I. Ahenkorah (2024). Powdered Enzyme from Australian Weed for Bio-stabilisation. *Lecture Notes in Civil Engineering*. Singapore, Springer Nature. 442: 1191-1198.

Tran, M. V., M. M. Rahman, M. R. Karim and I. Ahenkorah (2024). Powdered Enzyme from Australian Weed for Bio-stabilisation, Springer Nature Singapore: 1191-1198.

Zaborska, W., B. Krajewska and Z. Olech (2004). "Heavy metal ions inhibition of jack bean urease: potential for rapid contaminant probing." *Journal of Enzyme Inhibition and Medicinal Chemistry* 19(1): 65-69.