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Forming a cemented crust in sloped sand with enzyme induced carbonate precipitation

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ABSTRACT

Enzyme Induced Carbonate Precipitation (EICP) is a novel biogeotechnical ground improvement technique in which calcium carbonate is precipitated to form a weakly cemented soil. The technique uses the hydrolysis of urea catalyzed by plant-derived free urease enzyme. An EICP application in development is the mitigation of rainfall-induced erosion in sloped sandy soil by creating a cemented crust. In bench-scale tests and small field trials in native sand, significant runoff of the treatment solution happened before it could percolate. The outcome was a thinner, less cemented crust than intended and waste of treatment materials because the solution did not percolate completely. The paper discusses the experimental observations and lessons for developing this EICP application, including the thickness of the cemented crust, calcium carbonate content, and strength of the cemented crust in sloped sand specimens.

INTRODUCTION

Soil erosion is a natural or human-induced process that can impact the performance of new and existing civil infrastructure as well as the quality of soil and surface water or groundwater. Erosion involves the displacement of soil particles by wind or water (Fay et al., 2012). Several factors affect soil erosion, such as the ground slope angle (Collin et al., 2008; Fay et al., 2012; Fang et al., 2015). Stormwater runoff on unprotected ground surfaces dislodges and mobilizes soil particles. Erosion may result in structural instability and increased infrastructure maintenance needs.

Enzyme-Induced Carbonate Precipitation (EICP) is a biogeotechnical ground improvement technique that is in development for various geotechnical engineering applications, including mitigation of rainfall-induced soil erosion (Ossai, 2021; Rivera and Bandini, 2024). The EICP reaction is abiotic and involves the hydrolysis of urea catalyzed by a plant-derived free urease enzyme to induce the precipitation of calcium carbonate within the soil. The EICP treatment can form a weakly cemented soil crust with sufficient thickness to reduce soil erosion (Ossai, 2021;

Rivera and Bandini, 2024). EICP has shown promising results in treating level soil for mitigation of fugitive dust (Hamdan and Kavazanjian, 2016). However, achieving formation of a uniform, thicker crust on sloped soil surfaces is still under development and has presented some challenges (Shen et al., 2023; Rivera and Bandini, 2024). Understanding what factors lead to treatment issues on sloped ground is essential to develop viable treatment application methods.

Few studies have addressed the biocementation treatment of soil on sloped ground and the issues of applying the treatment solution on sloped surfaces. Research found that part of the treatment solution sprayed onto the soil surface on sloped specimens or field plots experienced runoff during treatment (Xiao et al., 2022; Gowthaman et al., 2023; Shen et al., 2023; Rivera and Bandini, 2024). Cementation in the initial treatment cycles impacted the solution percolation in subsequent treatment cycles. Additional treatment solution running down the soil surface impacted cementation thickness, reducing the overall effectiveness of the treatment. Effectiveness of biocementation (i.e., soil cementation) has been evaluated by crust thickness and strength, and calcium carbonate content (Xiao et al., 2022; Gowthaman et al., 2023; Shen et al., 2023; Rivera and Bandini, 2024). Assessing soil cementation has some limitations, such as the difficulty in corroborating cementation uniformity across the sloped ground. Evaluating cementation uniformity is particularly important given the problems related to solution runoff during treatment.

This study aimed at assessing the effectiveness of an EICP solution application method and two solution concentrations for sand specimens with sloped surfaces. The number of treatment cycles and volume of solution applied in each cycle were considered. Laboratory tests were conducted at two scales. Small block specimens with two relative densities were treated with two solution concentrations, and the findings of these preliminary tests informed a larger-scale experiment to assess solution percolation and crust thickness. The effectiveness of the treatment conditions was evaluated based on the thickness of the cemented crust, calcium carbonate content precipitated during EICP treatment, and strength of the cemented crust in the sloped specimens.

METHODS AND MATERIALS

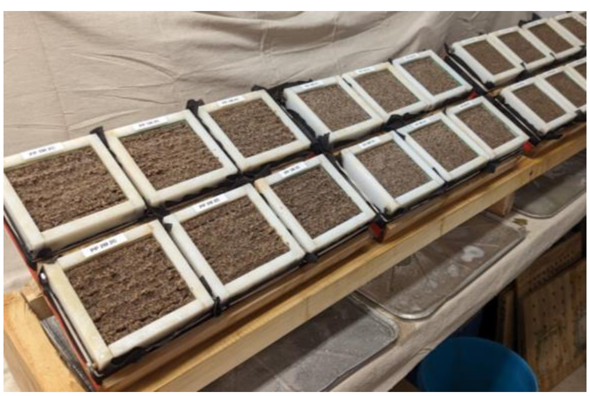
The test plan consisted of EICP treatment of block specimens and box specimens. Block specimens were used to confirm that the sand was amenable to EICP cementation and assess effectiveness of solution concentration and volume. Box specimens were used to study thickness and relative strength of the cemented crust in sloped sand. Calcium carbonate content, microscopy (Hitachi FE-SEM SU7000), and needle penetrometer were used to study the cementation characteristics.

Soil Characteristics. The specimens were prepared with sand from a quarry located in Chaparral, New Mexico, referred to as Chaparral #2 sand. The sand contained 0.9% of fines (i.e., material smaller than 0.075 mm, passing sieve No. 200) and was classified as poorly graded sand (SP) according to the Unified Soil Classification System (ASTM International, 2017). The coefficients of uniformity and curvature were 2.81 and 1.25, respectively. The maximum and minimum void ratios were 0.71 and 0.49, respectively, and the specific gravity was 2.64. The maximum dry unit weight and optimum water content were 16.2 kN/m^3 and 5.6%, respectively.

EICP Solution. EICP solutions with 1 and 2 molar (M) concentrations were used. 1 M solution was composed of 1 M urea, 0.67 M calcium chloride dihydrate ($CaCl_2$-$2H_2O$, ACS grade), 20 ml/l crude extract urease enzyme solution, and 4.0 g/l nonfat powder milk. These components were doubled for 2 M solution. The solutions were prepared with tap water. Nonfat milk was used because it may help delay urease denaturation (Nemati and Voordouw, 2003) and increase the strength of the cemented sand (Almajed et al., 2019). The crude extract of urease was prepared by breaking down jack beans using a mallet until all pieces were 50 mm or smaller. Tap water was added before breaking the beans further in a kitchen blender on high-speed setting for four minutes or until the mixture had a uniform consistency. The liquid from this slurry was extracted with a juicer, and the crude extract was strained through cheesecloth. Approximately 50 ml of crude extract urease was obtained from 63 g of jack beans blended in 200 ml of water.

Block Specimen Preparation. The internal length, width, and height of the molds were 102 mm (4 in), 102 mm (4 in), and 51 mm (2 in), respectively. The top and bottom of the molds were open. The specimen target height was 45 mm (1.75 in), leaving 6 mm (0.25 in) from the soil surface to the top of the mold to catch any solution runoff during treatment. The base of the mold was wrapped in landscape fabric secured with rubber bands to allow solution drainage during treatment. The sand was placed by dry pluviation in two layers using a funnel, maintaining a drop height of approximately 50 mm (1.97 in). Each layer was compacted with a dolly tamper to a target relative density (D_r). The specimens were placed on easels inclined 30° that had holes to allow drainage of excess solution (Figure 1a). Average results of duplicate specimens were reported.

Box Specimen Preparation. Six box specimens (Figure 1b) were prepared in wooden boxes with internal length, width, and height of 914 mm (36 in), 280 mm (11 in), and 300 mm (12 in), respectively. The boxes were open at the top, had wooden baseboards with holes for solution drainage, and were lined with landscape fabric. The soil was placed in three layers to the target D_r. The boxes were prepared and treated outdoors and were inclined 27° during treatment and curing.



(a) **(b)**

Figure 1. (a) Block specimens. (b) Box specimens.

Solution Application Methods. Tables 1 and 2 provide the test conditions for the block specimens and box specimens, respectively. The results of the block specimens informed the test conditions for the box specimens. Applying the cementing solution multiple times helps increase carbonate precipitation. Thus, the cementing solution was applied to the soil either two or three times in 24-hour intervals. Each solution application is referred herein as a treatment cycle. The volume of cementing solution used in a cycle (V_{sol}) was expressed as a percentage of the estimated pore (or void) volume of the dry sand specimen (V_v). The cementing solution was applied with a method called two-step percolation (or PP), first proposed by Ossai (2021), consisting of separating the treatment into two solutions, applying one right after the other within a treatment cycle (Ossai, 2021; Rivera and Bandini, 2024). One solution contained crude extract urease and nonfat powder milk. The second solution contained calcium chloride and urea. These two solution parts were applied on the specimen top beginning with the urease and milk solution, followed by the calcium chloride and urea solution. The solutions were slowly poured on the block specimens using a needless syringe and on the box specimens using a handheld sprayer with the spout fully open.

Table 1. Test conditions of block specimens.

Series label	Treatment condition label	Solution concentration (M)	Treatment cycles	Solution volume, V_{sol} (%)			D_r (%)
				Cycle 1	Cycle 2	Cycle 3	
BLa1	BL-1M-45-C2a	1	2	100	50	-	45
BLa2	BL-1M-70-C2a	1	2	100	50	-	70
BLa3	BL-2M-70-C2a	2	2	100	50	-	70
BLb1	BL-1M-45-C2b	1	2	100	75	-	45
BLb2	BL-1M-70-C2b	1	2	100	75	-	70
BLc1	BL-1M-45-C2c	1	2	100	100	-	45
BLc2	BL-1M-70-C2c	1	2	100	100	-	70
BLd1	BL-1M-45-C3d	1	3	100	40	30	45
BLd2	BL-1M-70-C3d	1	3	100	40	30	70
BLd3	BL-2M-70-C3d	2	3	100	40	30	70

Table 2. Test conditions of box specimens.

Series label	Treatment condition label	Solution concentration (M)	Treatment cycles	Solution volume, V_{sol} (%)			D_r (%)
				Cycle 1	Cycle 2	Cycles 3-5	
BX-A1	BX-1M-55-C5	1	5	100	40	30	55
BX-A2	BX-2M-55-C5	2	5	100	40	30	55
BX-A3	BX-1M-55-C3	1	3	100	40	30	55
BX-B1	BX-1M-80-C5	1	5	100	40	30	80
BX-B2	BX-2M-80-C5	2	5	100	40	30	80
BX-B3	BX-1M-80-C3	1	3	100	40	30	80

Evaluation of Cementation of Block Specimens (Percent Mass Loss). After the last treatment cycle, block specimens were cured at room temperature for seven days. After curing, the specimens were removed from the molds, and soaked in tap water for at least 30 min and rinsed to remove byproducts of the EICP reactions and determine the percent of sand that did not cement, referred herein as percent mass loss (PML), calculated as the ratio (in %) of the dry mass of the uncemented sand particles (after soaking) and the initial dry mass of sand used to prepare the specimen.

Needle Penetrometer Test on Box Specimens. Relative cementation strength among treatment conditions and throughout the crust of a given box specimen was assessed with a needle penetrometer (Mecmesin AFG-1000N). For each box specimen, eleven measurements in a quincunx pattern were recorded after curing. The needle penetrometer does not provide adequate sensitivity for strength characterization of EICP treated sands (Khodadadi Tirkolaei et al., 2018); however, the measurements allowed comparison of cementation among the various conditions.

Calcium Carbonate Content. The calcium carbonate content (CCC) was determined by acid digestion (acid washing) on 18 samples from EICP-treated box specimens (after rinsing), as described by Madrigal et al. (2025). Acid digestion may dissolve some minerals in the soil that are not EICP precipitate and may remove fines particles in the silt- and clay-size ranges. Thus, acid digestion on triplicate samples of untreated sand was used to determine a baseline value. Net CCC values are reported, calculated as CCC from acid digestion minus baseline CCC of untreated sand.

RESULTS AND DISCUSSION

Block Specimens

Average PML values for the block specimens are shown in Figure 2. PML varied within a wide range (18-75%) depending on the test conditions. Series BLd1 and BLd2, which were prepared with three treatment cycles and 1 M solution, had the smallest PML values (i.e., more cementation), showing the positive effect of multiple treatment cycles. Series BLd1 and BLd2 had the lowest PML values among all the series despite having used the smallest V_{sol} in the second cycle (see Table 1). On the other hand, the largest PML values (i.e., less cementation) were for series BLa3 and BLd3, which were the only specimens prepared with 2 M solution.

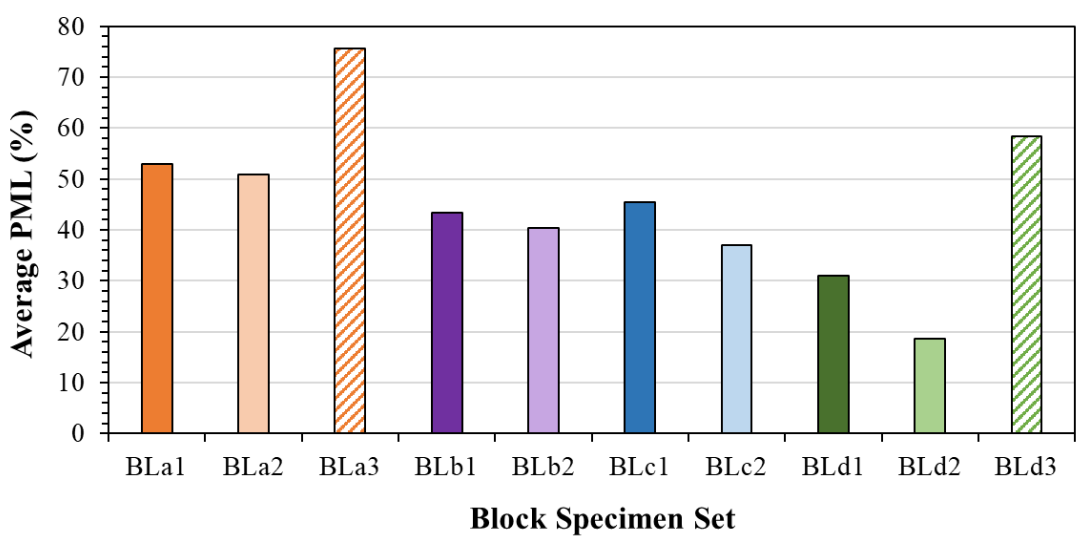
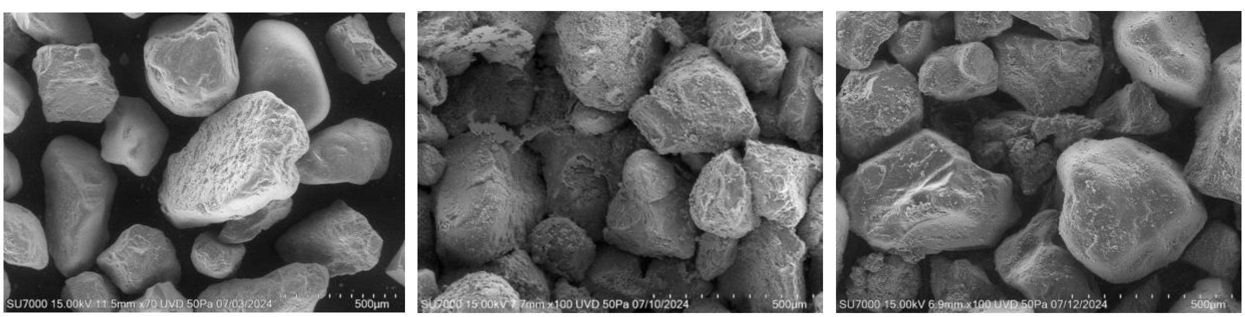


Figure 2. Percent mass loss (PML) results for block specimens.

For block specimens treated with 1 M solution, the specimens formed at D_r = 45% had on average greater PML than the corresponding specimens with D_r = 70%. The effect of D_r was more significant when comparing series BLd1 (loose sand) and BLd2 (dense sand) (see Figure 2), which were the two series with greatest cementation. When only considering specimens treated with 1 M solution and two cycles, the PML order from greatest (i.e., least cemented) to lowest (i.e., most cemented) was for series BLa, BLb, and BLc, which seemed to indicate that using larger volume of solution in the second cycle (V_{sol} = 50, 75, and 100%, respectively) led to more cementation. However, adding a third treatment cycle (series BLd) was significantly more effective in cementing the sand even though the combined solution volume of cycles 2 and 3 (V_{sol} = 40% + 30% = 70%) was smaller than the solution volume applied in the second cycle in series BLb (V_{sol} = 75%) and BLc (V_{sol} = 100%).

Box Specimens

Microscopy. Scanning Electron Microscope (SEM) images of crust samples from box specimens were used to assess the cementation. Figure 3 shows SEM images of untreated sand and crust samples from two box specimens with D_r = 80%, one treated with 1 M solution (BX-B1) and the other treated with 2 M solution (BX-B2). In sample BX-B1, sand grains were heavily coated by relatively small calcite crystals and had numerous sites of particle cementation (see Figure 3b). In contrast, sample BX-B2 had significantly less calcite particle coating and fewer cementation sites (see Figure 3c). This observation is consistent with the block specimen results and confirmed that treating Chaparral #2 sand with 2 M solution resulted in poor or negligible carbonate precipitation and cementation. In all the cemented samples, it was evident that the carbonate precipitation did not clog the pore space (see Figure 3b for example), which is consistent with the relatively small effect of EICP treatment on the hydraulic conductivity of sand (Madrigal et al., 2025).



(a) (b) (c)

Figure 3. SEM images of (a) untreated sand and crust samples from box specimens (b) BX-B1 and (c) BX-B2.

Penetration Resistance. Penetration resistance (PR) values varied widely among the box specimens (16-233 N) depending on the test conditions. The PR variability throughout a given box specimen in terms of standard deviation was also considerable. The average PR for BX-A1, BX-A2, BX-A3, BX-B1, BX-B2, and BX-B3 was 154, 26, 66, 173, 34, and 108 N, respectively (Standard deviation, SD = 45, 9, 29, 33, 7, and 37 N, respectively). Specimens BX-A1 and

BX-B1, which were treated with five cycles of 1 M solution, had the highest average PR. Five treatment cycles led to higher average PR compared with three treatment cycles (specimens BX-A3 and BX-B3) because each cycle provides additional carbonate precipitation increasing particle cementation. Penetration resistance of specimens BX-A2 and BX-B2, which were treated with five cycles of 2 M solution, was negligible. This result is consistent with SEM observations (compare Figures 3b and 3c) and prior studies (Ossai 2021). In specimens treated with 2 M solution, the precipitate may have included calcium carbonate species different from calcite, creating weaker bonds that resulted in very low penetration resistance. When comparing average PR for the same test condition, denser specimens had higher penetration resistance.

The relationship between location of PR measurements and penetration resistance for box specimens is shown in Figure 4. Based on the slopes of the best-fit lines through the data in Figure 4, the lower half of the sloped box specimens had greater PR except for specimen BX-B1, whose trendline was nearly horizontal (see Figure 4b). There was a poor correlation between PR and location for specimens BX-A1, BX-A3, BX-B1, and BX-B3, indicating heterogeneity of the cementation level along the specimen slope. However, variability of the crust cementation is likely to be scale-dependent (e.g., block specimen, box specimen, test plot, field plot). Specimens BX-A2 and BX-B2 had strong positive correlation between PR and location (Figure 4) because of their lesser carbonate precipitation and poor cementation.

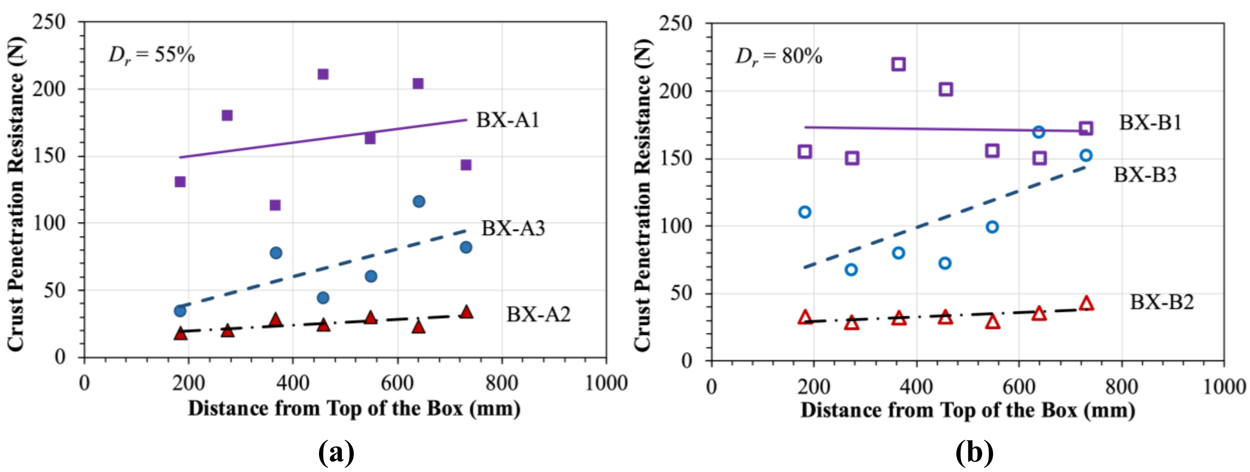


Figure 4. Average crust penetration resistance of box specimens: (a) D_r = 55%, (b) D_r = 80%.

Crust Thickness. Crust thickness measurements were taken at four locations along the specimen length, revealing variability and a broad range of thickness among specimens, from 8-48 mm depending on location and treatment conditions. The averages of crust thickness for BX-A1, BX-A2, BX-A3, BX-B1, BX-B2, and BX-B3 were 39, 9, 27, 40, 18, and 37 mm, respectively (SD = 5.0, 0.6, 9.9, 7.0, 4.5, and 11.2 mm, respectively). The relationship between crust thickness and location is shown in Figure 5. On average, specimens BX-A1 and BX-B1 had the thickest crusts, followed by specimens BX-A3 and BX-B3. Specimens BX-A2 and BX-B2 had the thinnest crusts because of their limited cementation. These observations correlate well with the PR measurements.

Calcium Carbonate Content. Net CCC values for crust samples ranged from 0.30-1.78% (Figure 6). Average net CCC for crust sample of specimens BX-A1, B-A2, BX-A3, BX-B1, BX-B2, and BX-B3 was 1.37, 0.59, 1.37, 1.41, 0.61, and 1.15%, respectively (SD = 0.22, 0.25, 0.34, 0.37, 0.41, and 0.24%, respectively). Specimens with thicker crust (and greater penetration resistance) had greater net calcium carbonate content (all values greater than 1.0%), whereas the weak and thin crust of specimens treated with 2 M solution (BX-A2 and BX-B2) had very low net calcium carbonate content (mostly less than 1.0%), as shown in Figure 6.

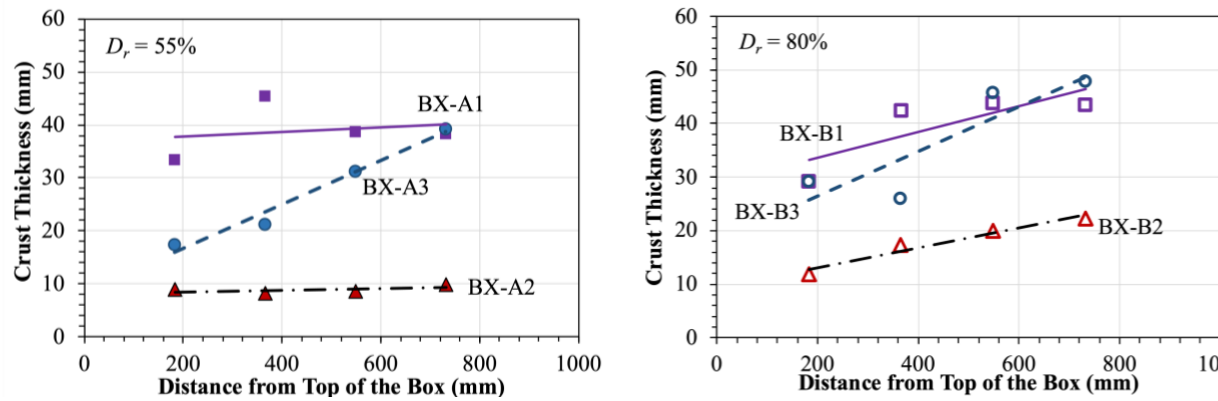


Figure 5. Crust thickness of box specimens: (a) D_r = 55%, (b) D_r = 80%.

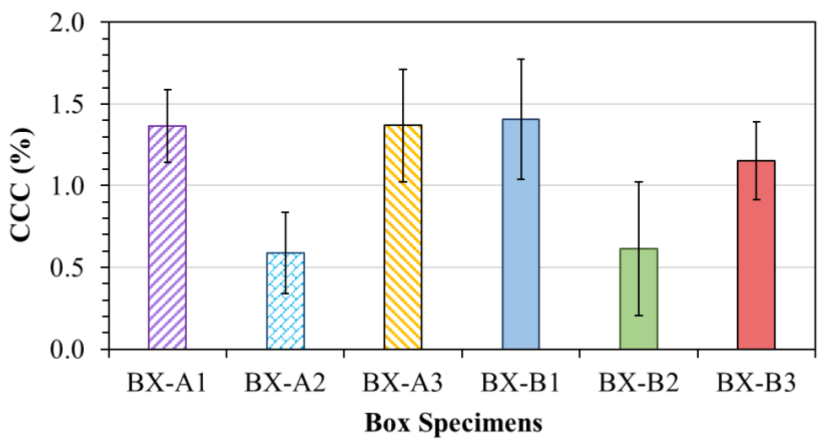


Figure 6. Average net calcium carbonate content (CCC) and error bars for box specimens.

CONCLUSIONS

The results from this study contributed to the limited data available concerning the effect of EICP treatment on soil properties and treatment of sloped surfaces. Some unique aspects of this study were the cementing solution application method and the treatment of sloped sand. The following conclusions were drawn from the results:

- Increasing the number of treatment cycles from three to five led to more cementation (i.e., thicker, stronger crust, more calcium carbonate precipitated). Density also had a positive (but modest) effect on cementation, likely because a denser, tighter particle arrangement has more particle contacts where calcite can precipitate to form bonds.

- Treating sloped sand at the bench scale resulted in issues with crust uniformity in terms of relative strength and thickness. Results from the box specimen showed a tendency for more precipitation and particle cementation near the specimen toe. As the treatment solution was applied, gravity directed the solution toward the toe although pooling of the cementing solution on the surface during treatment was not observed. To achieve greater crust uniformity at the bench scale and field scale, adjustments could be made to the solution volume applied in each treatment cycle and solution application method. Problems associated with treatment solution runoff on sloped soil surfaces are expected to decrease as the size of the treated area increases.
- Increasing the concentration of all components in the EICP solution (2 M solution) resulted in a notable reduction in calcium carbonate precipitation and poor or negligible particle cementation. Various factors, including urea and calcium chloride concentrations, influence the precipitation of calcium carbonate (Krajewska, 2018). Although elevated urea and calcium chloride levels can facilitate carbonate precipitation, the literature suggests a threshold beyond which further increases in concentration may hinder precipitation (Krajewska, 2018). Lack of precipitation with 2 M solution may be attributed to the concentration exceeding this threshold.
- The EICP treatment application method impacts the soil cementation. The two-step percolation method has shown promising results at the bench scale (Ossai, 2021) and in small field plots (Rivera and Bandini, 2024), but further research is needed to increase effectiveness in treating sloped soil surfaces.

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REFERENCES

ASTM International (2017). *Standard Practice for Classification of Soils for Engineering Purposes (Unified Soil Classification System).* ASTM D2487-17. West Conshohocken, PA. DOI: 10.1520/D2487-17

Almajed, A., Tirkolaei, H. K., Kavazanjian, E., and Hamdan, N. (2019). "Enzyme induced biocementated sand with high strength at low carbonate content." *Scientific Reports*, *9*, 1135-1135. https://doi.org/10.1038/s41598-018-38361-1

Collin, J. G., Loehr, J. E., and Hung, C. J. (2008). *Highway Slope Maintenance and Slide Restoration: Reference Manual*. FHWA NHI-08-098, National Highway Institute. https://rosap.ntl.bts.gov/view/dot/50373

Fang, H., Sun, L., and Tang, Z. (2015). "Effects of rainfall and slope on runoff, soil erosion and rill development: An experimental study using two loess soils." *Hydrological Processes*, 29(11), 2649-2658. https://doi.org/10.1002/hyp.10392

Fay, L., Akin, M., and Shi, X. (2012). *Cost-Effective and Sustainable Road Slope Stabilization and Erosion Control*. National Cooperative Highway Research Program (NCHRP) Synthesis 430, The National Academies Press. https://doi.org/10.17226/22776

Gowthaman, S., Koizumi, H., Nakashima, K., and Kawasaki, S. (2023). "Field experimentation of bio-cementation using low-cost cementation media for preservation of slope surface." *Case Studies in Construction Materials*, 18, e02086. https://doi.org/10.1016/j.cscm.2023.e02086

Hamdan, N., and Kavazanjian, E. (2016). "Enzyme-induced carbonate mineral precipitation for fugitive dust control." *Géotechnique*, 66(7), 546-555. https://doi.org/10.1680/jgeot.15.P.168

Krajewska, B. (2018). "Urease-aided calcium carbonate mineralization for engineering applications: A review." *Journal of Advanced Research*, 13, 59-67. https://doi.org/10.1016/j.jare.2017.10.009

Martin, K., Khodadadi, H., Krishnan, V., Adam, R., Ramirez, J., Shurley, D., and Kavazanjian, E. (2018). "*Bench-scale bio-grouted column formation using enzyme-induced carbonate precipitation*." In *Proc. Biomediated and Bioinspired Geotechnical Conference*, Atlanta.

Madrigal, N., Marmolejo, E., and Bandini, P. (2025). "Hydraulic conductivity of a sand cemented with enzyme induced carbonate precipitation." *Proc. GeoFrontiers 2025*, Louisville, Kentucky, March 2-5, 2025 (In print).

Nemati, M., and Voordouw, G. (2003). "Modification of porous media permeability, using calcium carbonate produced enzymatically in situ." *Enzyme and Microbial Technology*, 33(5), 635-642. https://doi.org/10.1016/S0141-0229(03)00191-1

Ossai, R. (2021). *Development of Enzyme-Induced Carbonate Precipitation (EICP) Treatment Application Methods for Erosion Control of Sand in Sloping Ground*. Ph.D. Dissertation, New Mexico State University.

Rivera, L. S., and Bandini, P. (2024). "Erosion testing on sloped ground treated with Enzyme-induced Carbonate Precipitation." In *Geotechnical Engineering Challenges to Meet Current and Emerging Needs of Society*. *Proc. XVIII European Conference in Soil Mechanics and Geotechnical Engineering.* Eds. N. Guerra et al., pp 2589-2592. London: CRC Press. https://doi.org/10.1201/9781003431749

Shen, D., Liu, Z., Song, Z., and Wu, C. (2023). "Reinforcement mechanism and erosion resistance of loess slope using enzyme induced calcite precipitation technique." *Sustainability*, 15(2), 1044. https://doi.org/10.3390/su15021044

Xiao, Y., Zhou, W., Shi, J., Lu, H., and Zhang, Z. (2022). "Erosion of biotreated field-scale slopes under rainfalls." *Journal of Performance of Constructed Facilities*, 36(3). https://doi.org/10.1061/(ASCE)CF.1943-5509.0001732

Zomorodian, S. M. A., Nikbakht, S., Ghaffari, H., and O'Kelly, B. C. (2023). "Enzymatic-induced calcite precipitation (EICP) method for improving hydraulic erosion resistance of surface sand layer: A laboratory investigation." *Sustainability*, 15(6), 5567. https://doi.org/10.3390/su15065567