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Effect of Treatment Volume on Bio-cemented Sand Under Cold Temperature

Pukar Joshi, Mohammad Khosravi, Adrienne Phillips, Alfred Cunningham, Sabine Olds, Jessica Rahn
Department of Civil Engineering, Montana State University, United States of America, pukar.joshi@student.montana.edu

Michael Carter
Air Force Research Laboratory, United States of America

ABSTRACT: This study investigates the influence of treatment volume on the strength and carbonate precipitation of Microbially induced carbonate precipitation (MICP)-treated sand under cold conditions (4°C). MICP is a bio-mediated ground improvement technique that increases soil strength through calcium carbonate bonding. Sakrete sand with a mean particle size (d_{50}) of 0.67 mm was used to prepare cylindrical specimens (50 mm diameter × 100 mm height). These were subjected to sequential bacterial and cementation solution injections via a gravity-fed method, with treatment durations of 2, 4, and 6 days, each involving one bacterial and one cementation injection daily. Specimens were incubated at a constant 4°C using a shaker incubator. Post-treatment, samples were oven-dried and analyzed for unconfined compressive strength (UCS), calcium carbonate content through acid digestion, and microstructure via scanning electron microscopy (SEM). The 2-day treated samples showed minimal cementation, making UCS testing infeasible. The 4-day samples exhibited a strength of 260 ± 24 kPa, while the 6-day samples achieved the highest UCS value of 385 ± 35 kPa. Calcium carbonate content increased with treatment duration, supporting strength trends. SEM analysis supported this trend, showing denser and more continuous calcium carbonate precipitation between particles. Overall, the results highlight that prolonged treatment time is essential to achieve appreciable cementation under cold curing conditions, and that limited treatment time or injection volume may constrain MICP performance in such environments.

KEYWORDS: MICP, Cold temperature.

1 INTRODUCTION

Biogeotechnical solutions have emerged as innovative alternatives for enhancing soil properties and mitigating geotechnical issues (DeJong et al., 2006; DeJong et al., 2010). These techniques utilize biological processes to improve soil strength, reduce permeability, and enhance resistance to erosion (Aletayeb et al., 2021; Khosravi et al., 2024; Olds et al., 2024). This process operates through a two-step reaction: first, urea is hydrolyzed into carbonate and ammonium ions in the presence of urease; then, calcium ions in the pore fluid react with carbonate to precipitate calcium carbonate (Phillips et al., 2013). The process, when catalyzed by live bacteria, is known as microbially induced calcium carbonate precipitation (MICP).

MICP has been extensively studied under ambient laboratory conditions, where temperatures between 20 °C and 30 °C support optimal bacterial activity and carbonate precipitation (Kim et al., 2018; Okwadha & Li, 2010). Numerous studies have shown that MICP can significantly increase UCS, with reported values ranging from 0.03 MPa to over 10 MPa, depending on treatment protocol, soil type, and curing conditions (Rahman et al., 2020). Despite this progress, few studies have explored the performance of MICP under cold environmental conditions. Cold climates, where subsurface temperatures may remain near or below 0 °C for extended periods, pose challenges for ground improvement. In North America, seasonally frozen ground dominates much of the northern U.S. and Canada, with typical freezing depths exceeding 2 m in northern zones (Davis, 2001). Soils in these regions are subject to complex thermo-hydro-mechanical processes, including ice lens formation, thaw settlement, and differential heave, which degrade infrastructure performance (Haeberli et al., 2021; Kanevskiy et al., 2011; Sheng et al., 2013).

Applying MICP in cold regions requires an understanding of how low temperatures influence the coupled biochemical and mechanical processes underlying biocementation. Urease activity, the key catalyst for urea hydrolysis, is highly sensitive to temperature (Bachmeier et al., 2002). Enzyme kinetics data show that ureolysis rates decrease below 20 °C. Krajewska et al. (2012) investigated jack bean urease activity between 15°C and 35°C and found that the hydrolysis rate of urea declines with decreasing temperature, while Kim et al. (2018) found highest relative precipitation at 30 °C and decrease in relative precipitation at temperatures above and below 30 °. Moreover, Yasuhara et al. (2012) found that temperature affects both precipitation rate and morphology. Low temperatures can promote formation of less stable polymorphs such as vaterite, leading to weaker interparticle bonding (Cheng et al., 2017; Wang et al., 2023b). More recently, Wang et al. (2023a) demonstrated that ureolysis at 4 °C was markedly slower than at 20 °C and 35 °C, resulting in delayed nucleation and reduced carbonate cluster size. These biochemical limitations directly impact the mechanical behavior of MICP-treated soils.

Despite these temperature-dependent effects, only a limited number of experimental studies have examined MICP performance at cold temperatures such as 4 °C. This presents a significant gap in literature, especially for regions where low temperatures are prevalent during construction or service life. A key factor that may influence the success of MICP under cold conditions is treatment volume, typically defined by the number of bacterial and cementation solution injections. In MICP, repeated injections are often used to increase calcium carbonate content and promote bonding continuity. However, when working in cold environments, each treatment cycle is expected to yield less carbonate due to slower urease kinetics. This raises the question: can increasing the volume of treatment, i.e., the total number of treatments, compensate for the reduction in precipitation caused by cold temperature?

This study focuses on the influence of treatment volume on the effectiveness of MICP under cold curing conditions. Commercially available poorly graded sand (Sakrete®, $d_{50} = 0.67$ mm) was treated using a gravity-fed injection method, with one bacterial and one cementation solution injection administered per day. Treatment durations of 2, 4, and 6 days were selected to represent increasing treatment volumes, while maintaining consistent solution concentration and bacterial density across all specimens. All samples were cured at 4 °C to replicate low-temperature conditions. The objective of this study is to assess whether increased treatment volume can enhance calcium carbonate precipitation and unconfined compressive strength when curing occurs at 4 °C. Strength testing, calcium carbonate quantification, and SEM imaging were conducted to evaluate the effect of treatment duration on cementation patterns and mechanical behavior. This research addresses a critical knowledge gap in cold-climate MICP applications and provides experimental data to support treatment design strategies in temperature-constrained environments.

2 MATERIAL AND METHODS

2.1 *Sample Preparation*

Commercially available Sakrete® sand was used as the base material in this study. The sand has a median particle size (d_{50}) of 0.67 mm and is classified as SP (poorly graded sand) according to the Unified Soil Classification System (USCS). The grain size distribution curve, shown in Figure 1, indicates a narrow particle size range, consistent with the SP classification. The physical properties of the sand, including specific gravity, void ratios, and coefficients of uniformity and curvature, are summarized in Table 1.

Table 1. Soil properties.

Parameter	Symbol	Value
Specific Gravity	G_s	2.66
Median particle size (in mm)	d_{50}	0.67
Minimum void ratio	e_{min}	0.53
Maximum void ratio	e_{max}	0.97
Coefficient of uniformity	C_u	1.65
Coefficient of curvature	C_c	0.91
USCS soil classification	*SP*	SP

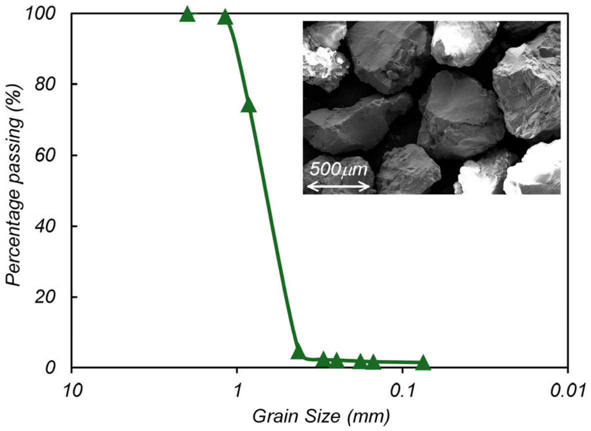

Figure 1. Grain size distribution of Sakrete sand (d_{50} = 0.67 mm), showing its poorly graded nature with a narrow size range.

Cylindrical specimens were prepared using standard plastic concrete molds with internal dimensions of 50 mm in diameter and 100 mm in height. A Ladd's modified wet tamping method (Ladd, 1978) was adopted to ensure uniform and repeatable compaction. Each specimen was prepared at a relative density of 35%, selected to represent a loosely packed condition. To promote uniformity, the specimens were compacted in five equal layers. For each layer, a pre-calculated mass of moist sand was placed and tamped using a standardized rod to minimize layering effects and density variation across the sample height. Moisture conditioning was done prior to placement to ensure consistent distribution and ease of compaction. This approach provided control over specimen uniformity and improved repeatability across triplicate tests.

A total of nine specimens were prepared for treatment, divided into three groups based on treatment duration: 2-day, 4-day, and 6-day treatments. Each group consisted of three identical specimens to allow for triplicate testing and account for experimental variability. The treatment durations were selected to evaluate the progressive influence of cumulative injection volume on bio-cementation. Details of the treatment process, including microbial inoculation and cementation solution application, are presented in the following sections.

2.2 *Microbially induced cementation*

The ureolytic bacterium *Sporosarcina pasteurii* (ATCC 11589) was used to catalyze urea hydrolysis for inducing calcium carbonate precipitation. Bacterial preparation followed a three-step process. First, 1 mL of frozen bacterial stock was inoculated into 99 mL of Brain Heart Infusion (BHI) medium and incubated at 30 °C while shaking at 150 rpm for 24 hours to activate growth. Next, 1 mL of the BHI-grown culture was transferred into 99 mL of yeast extract (YE) medium and incubated for 16 hours under the same conditions. From this enriched culture, 1% (v/v) was then used to inoculate a larger volume of fresh YE medium to generate the desired volume of bacterial solution for daily injection. The recipes for preparing the BHI and YE media, as well as the associated solutions, were adopted from Olds et al. (2024). The final cultures were incubated at 30 °C and 150 rpm for 24 hours reaching an optical density (OD_{600}) between 0.8 and 1.0. To maintain bacterial consistency and effectiveness, this sub-transfer was done only three times as it was observed that OD_{600} decreased during subsequent re-generations (data not shown). This process was repeated as needed to prepare bacterial solutions for subsequent treatment days.

The cementation solution comprised 20 g/L of urea and 49 g/L of calcium chloride dihydrate, yielding a molar concentration of approximately 333 mM for both components. This solution was prepared in deionized water and injected after the retention period using the same gravity-fed setup shown in Figure 2.

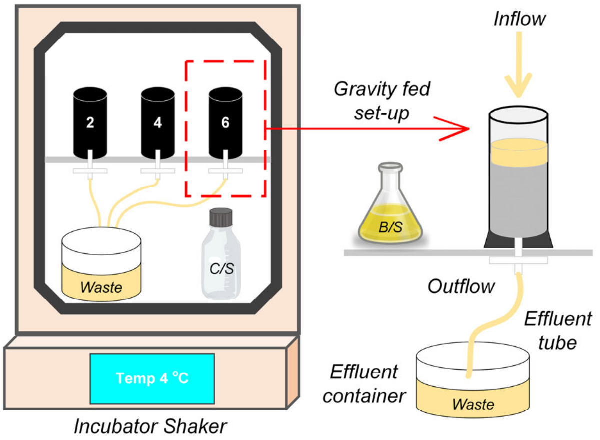

Figure 2. Schematic of the experimental gravity-fed MICP treatment setup. Sand columns were placed inside a shaker incubator maintained at a constant temperature of 4 °C to simulate cold-region conditions. The treatment system involved sequential injections of bacterial solution (B/S) and cementation solution (C/S) into the columns using a gravity-fed approach. Effluent from the bottom of each column was collected in waste containers. Columns underwent 2, 4, and 6 days of treatment, respectively, as indicated.

Each daily treatment cycle consisted of two sequential injections: a bacterial solution (70 mL) followed by a cementation solution (70 mL), corresponding to approximately 1.5 times the initial pore volume (46 mL) of the sand column. After introducing the bacterial solution, columns were incubated at 4 °C for an 18-hour retention period to promote bacterial attachment to the sand grains. This long retention time ensures sufficient interaction between bacteria and the mineral substrate (Wang et al., 2023b). Each treatment involved daily injections of bacterial and cementation solutions, following the injection strategy described above. Throughout the treatment period, all specimens were cured at a constant temperature of 4 °C in a shaker incubator to simulate cold environmental conditions.

2.3 *Unconfined compressive strength testing*

Following MICP treatment, the dried samples underwent UCS testing in accordance with ASTM D2166 (Astm, 2016) to assess their strength. To ensure accurate results, the samples were filed down to eliminate any uneven surfaces. Each sample was then placed individually on the load frame and secured in an upright position with a slotted surcharge weight. The sample was subjected to axial loading at a constant strain rate of 1% per minute until failure. After testing, the samples were retained for further analysis.

2.4 *Acid digestion*

Following UCS testing, acid digestion was performed on soil specimens to determine calcium carbonate content. To assess potential uniformity in cementation, approximately two grams of soil were extracted from the top, middle, and bottom sections of each column. These samples were transferred into pre-weighed centrifuge tubes, and 10 mL of 5% nitric acid was added to each. The tubes were capped and gently shaken daily to facilitate complete dissolution. After digestion, the supernatant was carefully removed, and the remaining material was oven-dried at 50 °C until the mass stabilized. Calcium carbonate content was determined from the difference in mass before and after digestion.

2.5 *Microscopic analysis (SEM)*

Biocemented sand specimens were collected and prepared for SEM to examine carbonate precipitation at the microscale. Prior to imaging, samples were coated with iridium for 60 seconds at a current of 20 mA to minimize surface charging. Imaging was performed using a Zeiss Supra 55VP Ultra Field Emission SEM located at the Imaging and Chemical Analysis Laboratory (ICAL) in Bozeman, Montana. Standard imaging parameters included a working distance of approximately 14 mm, an accelerating voltage of 10 kV, and a 30 µm aperture. These settings provided sufficient resolution to observe spatial distribution of calcium carbonate within the soil matrix.

3 RESULTS AND DISCUSSION

This section presents a comprehensive evaluation of how increasing the number of treatment cycles influences MICP performance at 4 °C, addressing the study's objective of identifying effective protocols for cold-region applications. The discussion begins with visual observations of treated specimens to assess macro-scale cementation and structural integrity. This is followed by unconfined compressive strength testing to quantify mechanical improvements. Calcium carbonate content is then analyzed through acid digestion to examine the relationship between strength gain and precipitation. Vertical uniformity of cementation is evaluated to determine the consistency of treatment distribution, and scanning electron microscopy is used to investigate microstructural changes. The section concludes by synthesizing the influence of low-temperature conditions on MICP effectiveness and identifying key factors for optimizing treatment strategies in cold environments.

3.1 *Effect of treatment cycles on UCS*

Following the MICP treatment, the treated-sand columns were oven-dried and carefully demolded for mechanical testing. Visual conditions of the samples, observed after 2, 4, and 6 days of treatment, are illustrated in Figure 3. Samples treated for 2 days showed limited cementation and lacked structural integrity, resulting in collapse upon demolding and rendering them unsuitable for UCS testing. Meanwhile, the 4-day and 6-day treated specimens maintained structural integrity during demolding. Notably, all samples displayed a prominent cemented crust at the top layer, suggesting that calcium carbonate precipitation was most pronounced near the injection point across all treatment durations.

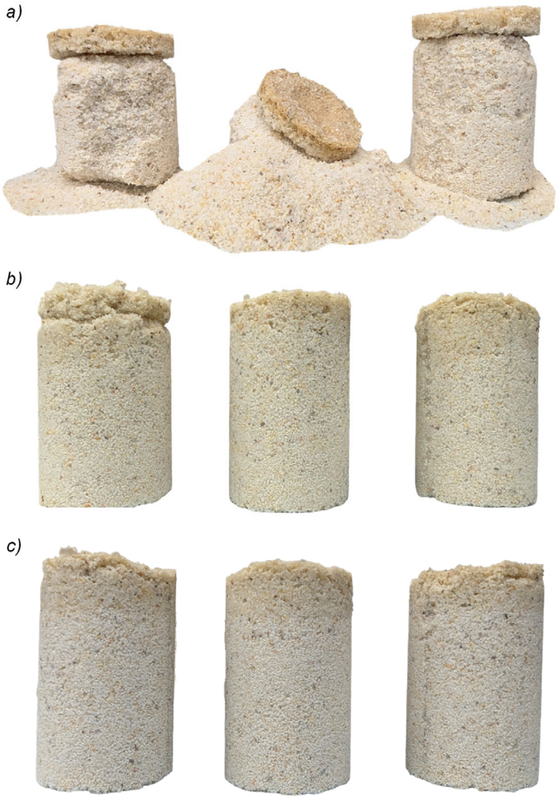

Figure 3. Demolded samples after drying under gravity-fed MICP treatment. (a) 2-day treatment, (b) 4-day treatment, and (c) 6-day treatment. Samples show a visibly stronger cementation layer near the top due to downward infiltration. The 2-day samples lacked sufficient strength for UCS testing, while samples treated for 4 and 6 days developed adequate cementation for UCS testing.

The axial stress–strain behavior of MICP-treated sand specimens is presented in Figure 4, comparing samples subjected to 4 and 6 days of treatment. Each treatment group included triplicate specimens to account for variability. Specimens treated for 4 and 6 days exhibited measurable compressive strength, with UCS values of 260 ± 24 kPa and 385 ± 35 kPa, respectively. The observed increase in UCS with longer treatment duration reflects progressive strengthening resulting from continued calcium carbonate precipitation at interparticle contacts. Given that all specimens were tested under identical unconfined conditions and strain rate, the strength enhancement is primarily attributed to the formation of calcite bridges between sand grains. The repeated introduction of bacteria and reactants over successive treatments likely promoted more extensive precipitation within the pore structure. This progressive cementation process has been well documented in prior studies, where multiple treatment cycles led to improved bonding and enhanced mechanical performance (DeJong et al., 2010; van Paassen et al., 2010).

The observation that 6-day treated samples reached peak strength at lower axial strains compared to 4-day samples suggests that stiffness increased with greater cementation. This behavior is likely attributed to the development of more continuous and denser calcite bridges at particle contacts, which reduces axial deformability and allows the specimen to mobilize peak strength at smaller strains. Similar trends have been observed by Montoya and DeJong (2015), where increasing calcium carbonate content led to greater stiffness and earlier failure in stress–strain response.

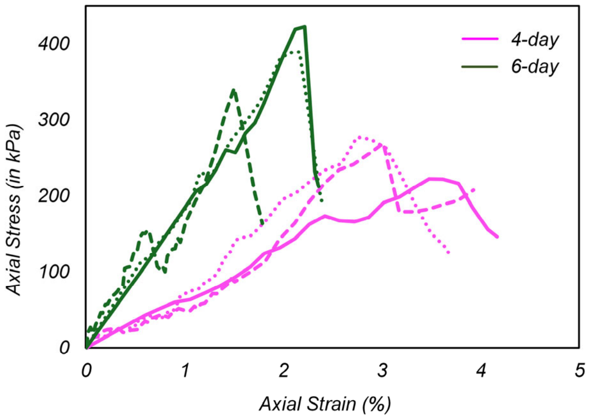

Figure 4. Axial stress–strain response of MICP-treated sand samples. Pink curves represent triplicate specimens treated for 4 days, while green curves represent those treated for 6 days. Samples treated for 2 days lacked sufficient cementation for testing. The 6-day treated specimens exhibited higher peak strength (UCS = 385 ± 35 kPa) and stiffer response, compared to 4-day specimens (average UCS = 260 ± 24 kPa) indicating an increase in strength with longer treatment duration.

3.2 *Effect of treatment cycle on calcium carbonate content*

The influence of treatment cycles on calcium carbonate precipitation in MICP-treated sand samples was investigated through acid digestion and SEM analyses, with results presented in Figure 5 and Figure 6 respectively. The higher values in the top section across all treatments are expected in gravity-fed systems, where solution tends to accumulate and react near the injection point (van Paassen et al., 2010). The top layers consistently showed higher calcium carbonate content, reaching values above 6% for all treatment durations, consistent with the visible top cemented layer seen in demolded samples in Figure 3. In contrast, the bottom layers exhibited the lowest calcium carbonate content, likely due to insufficient transport of bacteria and cementation solution to deeper regions under gravity-driven flow. However, increasing the number of treatment cycles progressively enhanced cementation across all layers. This trend suggests that repeated injections may help push reactants further down, allowing more uniform distribution over time (Al Qabany & Soga, 2014; Whiffin et al., 2007). This may also indicate that bacterial retention improves with successive cycles, enhancing the likelihood of precipitation beyond the initial zone. Therefore, while each individual injection may have limited reach, the overall effect of increasing treatment volume is a more connected and strengthened soil matrix both in terms of strength and carbonate content.

SEM of samples treated for 2, 4, and 6 days, imaged at 100X and 1000X magnifications, shown in Figure 6, further illustrate the microstructural evolution with increasing treatment duration. After 2 days of treatment, calcium carbonate precipitation is minimal, with only sparse surface coatings observed on individual sand grains and no significant bridging between adjacent particles. This incomplete bonding is consistent with the lack of structural integrity observed in Figure 3. By 4 days of treatment, carbonate deposition became more pronounced, particularly at particle contacts, with initial development of calcite bridges between grains, indicating progressive bonding formation visible at higher magnification. The denser and more continuous precipitation contributed to strength gains to maintain structural integrity of the treated sand sample. After 6 days of treatment, extensive precipitation is observed throughout the particle surfaces. At higher magnification (1000X), continuous calcite bridges are clearly visible, potentially providing interparticle bonding. The morphological transition from dispersed surface coatings to dense interparticle bridging aligns with the measured increase in calcium carbonate content, confirming that prolonged treatment under cold conditions may enhance the efficiency of particle bonding (Al Qabany & Soga, 2014).

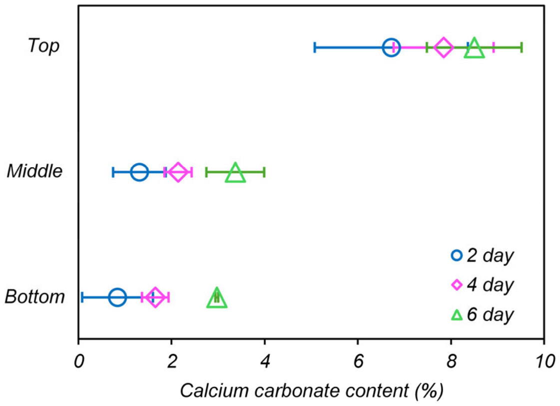

Figure 5. Calcium carbonate content measured at the top, middle, and bottom of MICP-treated samples after 2, 4, and 6 days of treatment. Results show increasing overall $CaCO_3$ content with treatment duration, with the highest accumulation consistently observed at the top due to gravity-driven infiltration. Cementation in the middle and bottom layers remains relatively similar across all treatment durations.

3.3 *Effects of Cold Temperature*

At low temperatures, the enzyme's catalytic efficiency decreases due to reduced molecular motion and lower collision rates between enzyme and substrate (Bachmeier et al., 2002; Illeová et al., 2003). As a result, the rate of carbonate ion production slows down, which in turn delays or limits calcium carbonate precipitation. This might explain why samples treated for only two days in this study did not develop enough cementation to support UCS testing. Several studies have also shown that at lower temperatures, calcium carbonate formation tends to be slower and sometimes less stable. For example, Wang et al. (2023b) found that ureolysis and precipitation were reduced at 4 °C compared to 20 °C and 35 °C. Similarly, Krajewska et al. (2012) reported that the rate of urea hydrolysis decreases with decreasing temperature. Despite these limitations, the 6-day treated samples in this study achieved UCS of 385 kPa and more than 3% calcium carbonate content, suggesting that extending the number of treatment cycles might compensate for the slower reaction rate at low temperatures.

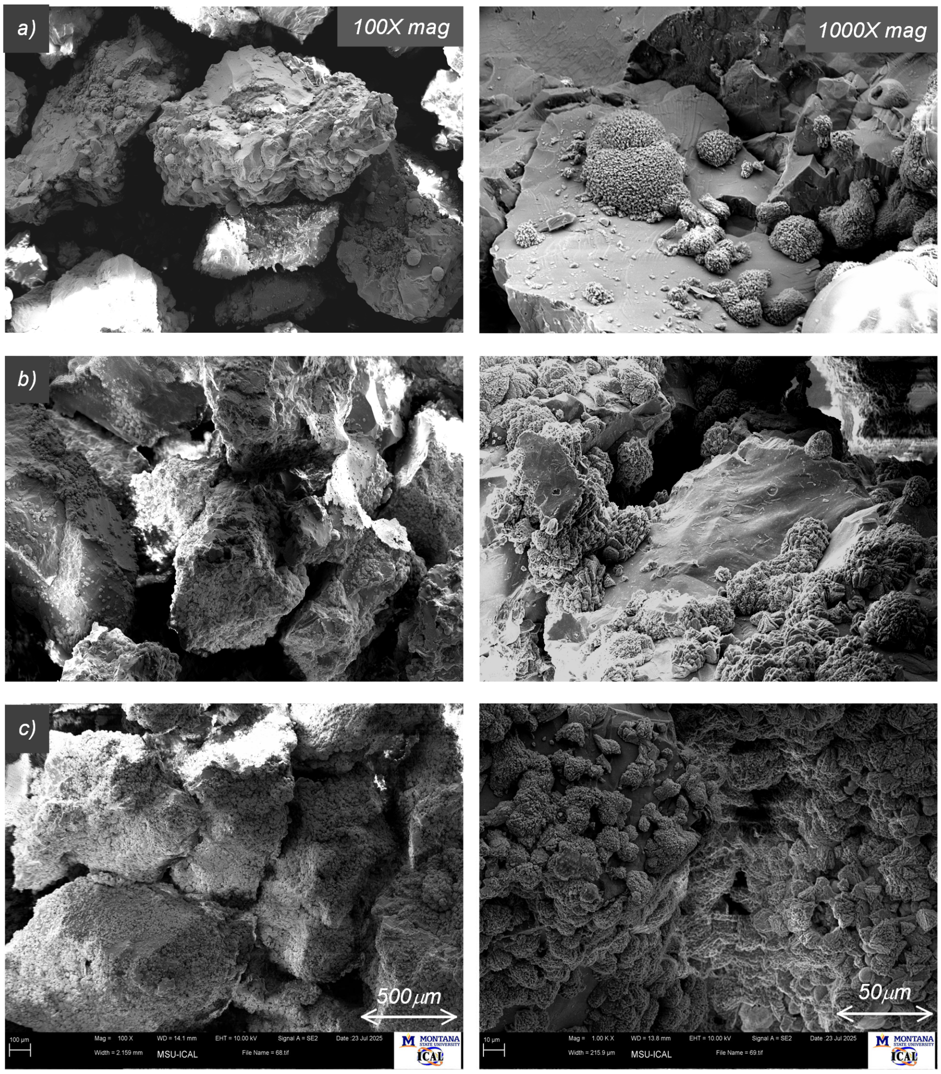

Figure 6. SEM images of MICP-treated sand samples after (a) 2 days, (b) 4 days, and (c) 6 days of treatment, captured at 100 X (left) and 1000 X (right) magnifications. The images illustrate increasing cementation with longer treatment durations. Minimal bonding is observed after 2 days, while samples treated for 4- and 6-days exhibit increased and more continuous cementation, with dense calcite clusters bridging particles after 6 days.

Another possibility for the observed improvement is that repeated treatments provide more time for bacterial attachment and crystal nucleation. As each cycle introduces fresh bacteria and reactants, even limited enzyme activity may accumulate into effective cementation over time. Although cold temperatures are known to reduce the efficiency of MICP due to slower ureolysis and precipitation rates, the findings of this study suggest that increasing the number of treatment cycles might help mitigate these limitations. By modifying the injection strategy to include more frequent and prolonged treatments, it might be possible to achieve effective cementation even under low-temperature conditions.

4 CONCLUSIONS

This study investigated the influence of treatment volume on the strength development and calcium carbonate precipitation in MICP-treated sand cured at 4 °C. A gravity-fed injection system was employed to deliver bacterial and cementation solutions over treatment durations of 2, 4, and 6 days. Cylindrical sand columns were prepared at 35% relative density using commercial Sakrete sand with a mean particle size (d_{50}) of 0.67 mm. The effects of treatment duration were evaluated through UCS testing, acid digestion for calcium carbonate quantification, and microscopic imaging. Visual inspection of the dried samples indicated a heavily cemented surface layer across all specimens, but only samples treated for 4 and 6 days retained sufficient integrity for mechanical testing. UCS results revealed that compressive strength increased with treatment duration, rising from untestable (2-day) to 260 kPa (4-day) and 385 kPa (6-day), indicating that prolonged treatment enhances bio-cementation effectiveness even under cold conditions. Stress–strain analysis confirmed that 6-day specimens not only achieved higher peak strength but also exhibited stiffer responses compared to 4-day specimens. Acid digestion results showed that calcium carbonate content increased with the number of treatments, with the top portion of each column consistently displaying the highest accumulation. This vertical gradient in cementation was attributed to preferential precipitation near the injection point, a known limitation of gravity-fed systems. SEM imaging supported these trends by showing progressively denser and more continuous calcite bridging between sand particles from 2 to 6 days of treatment. These findings demonstrate that treatment volume plays a critical role in the performance of MICP under cold curing. Even with the biochemical limitations imposed by low temperatures, increasing the number of bacterial and cementation cycles enhances calcium carbonate precipitation, ultimately leading to measurable improvements in strength.

5 ACKNOWLEDGEMENTS

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