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TREATMENT OF MINE TAILINGS THROUGH TWO METHODS OF CALCITE PRECIPITATION: (I) USING NATURAL MINERAL TRONA, (II) MICROBIALLY INDUCED CALCITE PRECIPITATION VIA DENITRIFICATION

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TREATMENT OF MINE TAILINGS THROUGH TWO METHODS OF CALCITE PRECIPITATION: (I) USING NATURAL MINERAL TRONA, (II) MICROBIALLY INDUCED CALCITE PRECIPITATION VIA DENITRIFICATION

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Inorganic Precipitation of Calcite in Mine Tailings using Trona

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Abstract

Mine tailings not only pose environmental risks such as dust emission and acid mine drainage but also present geotechnical risks in cases of tailings dam failures which could lead to loss of human life and severe damages to infrastructure. In-situ cementation of tailings has the potential to reduce the risks associated with such failures as well as the related environmental problems. Over the years, different methods of chemically and microbially induced cementation of tailings have been examined many of which have shown promising results on a laboratory scale and some even on a field scale. In this paper, a new inorganic method of inducing tailings cementation is introduced which uses a naturally occurring mineral called trona ($Na_2CO_3 \cdot NaHCO_3 \cdot 2H_2O$). Three methods of introducing dissolved trona, i.e., injections, slurry mixing, and infiltration, were tested and the results were compared. All methods were proven successful on a laboratory scale but with different amounts of precipitated calcite at different depths of the samples. The distinctions in precipitation profile could have applications in solving common problems associated with tailings including dust emission and liquefaction. Additionally, even distribution of precipitated calcite in the slurry mixing method shows potential in acid mine drainage prevention.

Keywords Mine Tailings; Trona; Cementation; Carbonate; Liquefaction; Acid Mine Drainage.

Introduction

Precipitation of calcium carbonate in soils is becoming an increasingly common method of soil stabilization that has been shown to increase the strength and liquefaction resistance of sand and fine-grained soils (Pham et al. 2017). These applications could prove very beneficial in the mining industry, particularly in the design of tailings dams where precipitated carbonates could help mitigate geotechnical and environmental hazards by acting as a pH buffer, dust control agent, and/or a physical or chemical stabilizer (Catalan and Yin 2003). For example, liquefaction of tailings is a major geotechnical concern in the mining industry as it can trigger tailings dam failure (Power et al. 2021; Petkovšek et al. 2021) by exerting larger horizontal stresses on the dam, i.e., stresses beyond the at-rest pressure that is applied on the dam by the tailings before the liquefaction (Martí et al. 2021). Such failures result in larger volumes of debris flows with lengthier runouts affecting larger areas (Ghahramani et al. 2020). Cementation due to calcium carbonate precipitation can increase the liquefaction resistance of tailings before dam failure occurs. Additionally, tailings can present a significant environmental hazard as they often contain acid generating minerals such as pyrite, pyrrhotite, or arsenopyrite. When exposed, these sulfide minerals react with oxygen and water to generate sulphuric acid which significantly lowers pH and increases the dissolved concentration of toxic metals in runoff that often contaminates nearby bodies of water (Park et al. 2019). Calcite in turn acts as a buffering agent by consuming sulfuric acid to increase the pH and precipitate out some of the dissolved metals. It is worth mentioning that the reaction of calcite with sulfuric acid could negate the mechanical improvements of calcite precipitation and should be considered if increasing the liquefaction resistance of tailings is the main goal of precipitating calcite. The use of calcite and other calcium salts in treating tailings is currently practiced in industry and typically involves microbially induced calcite precipitation (MICP), oxide cementing agents, or direct introduction of calcite.

MICP, also known as microbially induced calcium carbonate precipitation (MICCP), has shown promising results as a method of soil stabilization (e.g., O'Donnell et al. 2017c, a; Riveros and Sadrekarimi 2020; Portugal et al. 2020). However, some of the major setbacks of MICP methods include the availability of nutrients needed for microbial growth, the relatively slow process when compared to other chemical methods, and decreased microbial growth due to the presence of toxins in the tailings (Dhami et al. 2013a). Copper concentration in some tailings were shown to slow down the cell growth rate of *Sporosarcina pasteurii*, one of the more common microbes used for MICP via urea hydrolysis (de Oliveira et al. 2021). In addition, MICP methods that employ urea may create high concentrations of ammonium and certain trace elements in groundwater (Proudfoot et al. 2021).

Most inorganic methods of soil treatment using calcium oxide salts i.e., lime, cement, and fly ash, functionally perform mechanical or chemical treatment but rarely both. Lime is commonly used in the chemical treatment of acid mine drainage as it increases pH in the same way calcite does but without releasing CO_2 (Akcil and Koldas 2006). This however does not provide a significant environmental advantage since the production of lime releases CO_2. Cements are commonly used in the mechanical treatment of clays especially in shallow soils (Alireza et al. 2017). While chemically effective, the methods for introducing cement into soils are limited by viscosity even in thinner grouts that present diminishing returns due to lower concentrations of cement.

In this paper, we are introducing a new inorganic method of tailings cementation using trona ($Na_2CO_3 \cdot NaHCO_3 \cdot 2H_2O$). Trona is a mixed carbonate/bicarbonate mineral that forms in evaporating lakes under a restricted set of chemical conditions (Eugster 1980). One of the largest trona deposits in the world is located in the Eocene Green River Basin of southwest Wyoming (Bradley and Eugster 1969). The major benefit of using trona to precipitate calcite is its relatively high solubility in water, i.e., 200g/L at 25°C (Ozdemir et al. 2010), compared to other inorganic compounds commonly used in the stabilization of tailings such as lime with a solubility of 1.59 g/L at 25°C (National Lime Association 2007). This means a higher concentration of carbonate ions required for calcite precipitation will be available in a trona solution compared to other inorganic reactants. Furthermore, the significant difference in solubility of calcite (0.013g/L at 25°C, Blackman and Gahan 2014) and trona results in almost immediate precipitation of calcite upon the introduction of a calcium salt e.g., $CaCl_2$. This very high rate of calcite precipitation compared to the MICP and established inorganic methods could be important when moving from laboratory scale to field scale ground improvement methods. Another possible advantage of using trona is that an additional mole of bicarbonate ion is available in solution (Equation 1) which is proven to reduce the concentration of sulfate (Equation 2) in mine tailings (DiFonzo and Bordia 1998; Maziuk 2007).

$$Na_2CO_3 \cdot NaHCO_3 \cdot 2H_2O + CaCl_2 \rightarrow CaCO_3 + 3Na^+ + 2Cl^- + HCO_3^- + 2H_2O \quad (1)$$

$$2NaHCO_3 + 2H^+ + SO_4^{2-} \rightarrow Na_2SO_4 + 2H_2O + 2CO_2 \quad (2)$$

Furthermore, one of the challenges associated with carbonate precipitation is the reduction in pore space caused by the formation of calcite. While the cementing effect of calcite and the reduction in pore space increases the strength of soil, it also decreases the permeability of the soil creating a barrier for delivering nutrients or chemical constituents to the lower layers of a soil column (Buikema 2015). Therefore, when considering ground improvement methods for tailings, it is important to determine the best time and most suitable method to introduce the cementing agents to the tailings. A common method of transporting tailings from the mill to the tailings pond is to pump them as a slurry through large pipes. Tailings are discharged at the end of the pipes and deposited fluvially with fines traveling further from the point of discharge than the coarse particles (i.e, sand-size). This creates a particle size gradient in the tailings ponds which renders different permeabilities at different distances from the end of the pipe (Wielinga et al. 1999; Kossoff et al. 2014). Therefore, different methods of introducing the cementing agents might be required at different locations. Hence, this paper will also discuss different methods of introducing the cementing agents, i.e., trona and calcium salt solutions, into tailings samples and how it affects the precipitation process.

Materials and Methods

Mill tailings used for this experiment were taken from an open-pit copper mine. Samples were collected immediately after the milling process, i.e., before it was pumped to the tailings pond, to have a representative sample without the segregation of particles due to fluvial-like deposition at the pond. Sieve analyses and Atterberg limits tests were conducted on the samples according to ASTM D6913 and (ASTM D4318 2005), respectively (Fig. 1). Using the results of these tests, the tailings were classified according to the Unified Soils Classification System (ASTM D2487 2013), to be SM-Silty Sand. The mineralogy of the tailings is the same as the rock being mined, hydrothermally altered granite, and includes quartz, variably altered feldspars (both plagioclase and alkali feldspar), biotite, sericite, and clays. Further analysis using a shortwave-infrared (SWIR) handheld spectrometer (TerraSpec Halo) indicated that the tailings fine particles are best classified as illite. The tailings have a relatively low pyrite content (< 2%) and contain small amounts of calcite from the hydrothermal alteration. All tailings used in the subsequent experiments were air dried. When deionized water was added back into the tailings the pH was near neutral (7.07). The carbonate content of the tailings before and after treatment were measured using a rapid carbonate content chamber based on ASTM D4373. According to ASTM D4373, the results of this test should be reported to the nearest 1%, and any variations in the data are either due to variations of specimens or due to the operator/equipment differences. As all the tests in this study were conducted by the same operator (the first author) using the same equipment, the variations in the results were assumed to be caused only by specimen variations. Also, for each sample, multiple tests were conducted, and the averages were used for comparison. Therefore, it was deemed appropriate to report the average results to two decimal places. All testing was conducted in a laboratory with an average ambient temperature of 23.7° C.

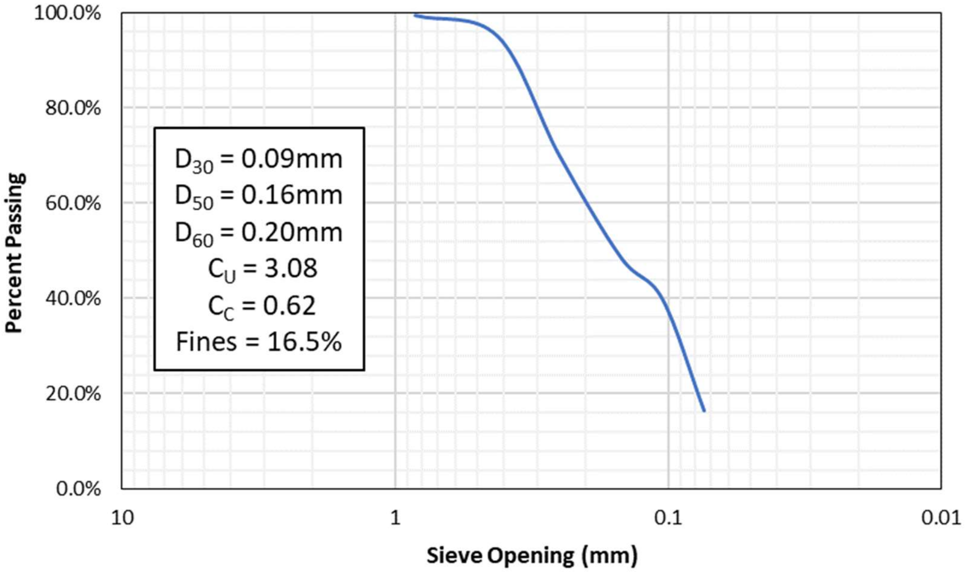

Fig. 1 Grain Size Distribution for Mill Tailings

The trona used for the experiments was obtained from a trona mine in Green River, Wyoming. The ore was not treated to increase purity before use for the following experiments. The carbonate content of the trona rock samples was determined using two methods, rapid carbonate content test as described above and X-ray diffraction. These combined methods confirmed the sample to be 85.8% (±0.2%) pure trona.

Concentration and Precipitate Crystal Size Experiment

According to O'Donnell et al. (2017b), even a 0.4% increase in calcium carbonate can significantly increase the cyclic shear resistance of treated soils. This resistance increase is dependent both on the concentration and the size of the crystals of the precipitated calcite (Dhami et al. 2013b). Therefore, the effects of the concentration of trona and $CaCl_2$ on the size of precipitated calcite crystals were first investigated. Secondly, the stoichiometric production of calcite had to be determined since trona contains both a carbonate and bicarbonate ion. The stoichiometry for this reaction (equation 1) shows a 1:2:2 molar ratio between trona, $CaCl_2$, and the precipitated $CaCO_3$ which assumes that trona will provide the carbonate and the bicarbonate to create an additional mole of calcite. At low pHs, off gassing of CO2 from the bicarbonate may present a concern. However, given the required discharge pH of most tailings this will likely present no concern for the applications of these experiments. The molecular weight of trona and $CaCl_2$ are 226g/mol and 111g/mol, respectively. Therefore, the mass ratio of trona, $CaCl_2$, and $CaCO_3$ to produce one part calcite in a sample is approximately 1:1:1. It is worth noting that when this experiment is applied to tailings a surplus of pore water calcium ions could eliminate the need for calcium salts however in order to ensure maximum efficiency, this ratio will be used for all experiments in this study. To determine the effects of concentration on crystal size in solution, three experiments were performed with logarithmically related concentrations. Three beakers were filled with 100ml of DI water and trona sample to create concentrations of 0.4g/L, 4g/L, and 40g/L. To make sure that all the trona in each beaker dissolved completely, each solution was stirred for 15 minutes using a magnet spinning at 600 rpm in the solution. After all the trona was dissolved, an equivalent mass of $CaCl_2$ was added to each beaker respectively to precipitate calcite. Five minutes after adding the $CaCl_2$, each sample was filtered using a 0.2 µm filter paper. The retained material on each filter was then oven-dried before comparing the sizes of precipitated crystals under a microscope.

Methods of Introduction Experiments

Three methods of introducing the chemical constituents, i.e., trona and $CaCl_2$, into the tailings were devised to mimic potential field applications. These methods are: 1) injection into the tailings at several different levels via a syringe; 2) infiltration of the solution from the surface and 3) slurry mixing the tailings and then introducing the constituents. Four beakers were filled with 180g of tailings placed loosely to a height of 4.5cm from the inside base of the beaker. The first sample, with a sample ID of T5.C, was used as the control experiment where no chemicals were added to the tailings. For this control sample, 110 g of DI water was added to the tailings. Given the desired increase of roughly 1% calcite in each sample and the 55g DI water mass to reach saturation in the sample, a 40g/L concentration was selected for the trona and $CaCl_2$ solutions used in these experiments.

Details of the chemicals used in the four samples are shown in Table 1. Each sample had an equivalent final mass and equal mass of chemical constituents even though the chemicals and DI water were added in different portions of the procedure for each experiment. Each experiment started by filling a beaker with 180g of tailings and 55g of trona solution. The trona solution was given five minutes to infiltrate the entire sample and settle to the saturation point before introducing $CaCl_2$ via the varied methods described below.

Table 1 Mass of the chemical constituents used in each experiment.

Sample ID	Tailings (g)	Trona (40g/L) Sol. (g)	$CaCl_2$ (40g/L) Sol. (g)	$CaCl_2$ (g)	DI Water (g)
T5.1	180	55	55	0	0
T5.2	180	55	0	2.2	52.8
T5.3	180	55	55	0	0
Control	180	0	0	0	110

The Injection Method (T5.1) was performed to evaluate the use of a probe to inject treatment solution into the tailings. In the injection method (Fig. 2), a needle was lowered near to the bottom of the sample, i.e., 0.75 cm from the base of the beaker, where 18.3 ml of $CaCl_2$ solution was injected. This process was then repeated at a height of 2.25cm and 3.75cm for a total injected volume of 55ml across three intervals. This method was tested because if the chemical solution is introduced from one point, the precipitated calcite around the point of introduction reduces the porosity of the sample which in turn inhibits the flow of the chemical solution to other areas of the tailings. Therefore, calcite precipitation would likely be limited to an area around the point of introduction. This method was an attempt to achieve a more evenly distributed precipitation.

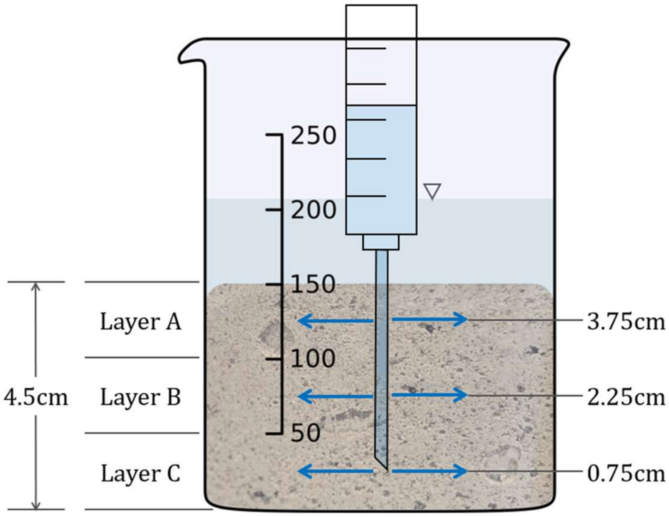

Fig. 2 Injection methods using a syringe at three variable depths

The next introduction method was the Slurry Mixing Method (T5.2). In this method, rather than using the $CaCl_2$ solution, all the DI water for the experiment was added at the beginning (107.8g) so tailings in a beaker could be properly suspended in solution (Fig. 3). This mixture was stirred constantly by using a magnet rotating at 600 rpm to keep solids suspended. Once all the particles were in suspension, 2.2g of $CaCl_2$ were added to the solution over a period of one minute after which the solution was stirred for five minutes. The magnet was then removed, and the sample was allowed to settle. When the magnet was removed from the sample approximately 0.5g of magnetic sediments (mostly magnetite) were removed from the sample leading to a small change in overall mass. This method was meant to simulate the introduction of chemicals into tailings at the end of the milling process or during pumping to the pond.

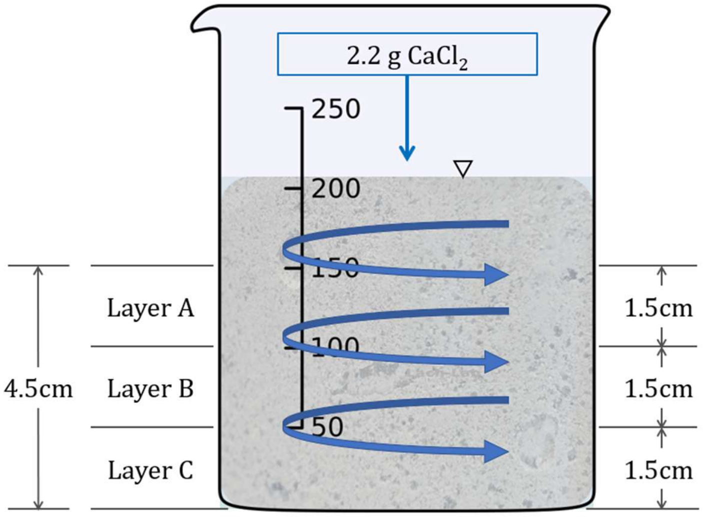

Fig. 3 Tailings layers for carbonate content measurements.

The third beaker consisted of an Infiltration Method (T5.3) meant to simulate the introduction of solution at the surface of existing mine tailings, i.e., fluvially deposited tailings. The tailings saturated with trona solution had 55g of $CaCl_2$ solution added (poured) on the top of the sample (Fig. 4).

After the procedures described above were completed, each sample was left to rest for 15 minutes at room temperature before oven drying. All four samples were oven dried at 57°C for 48 hours after which they were tested for strength using a Vane Shear device and then separated into layers A-C (as seen on the left side of Fig. 4) for carbonate content testing. Overall, 12 samples were tested using a carbonate content chamber providing a three-layered depth profile to determine the variation in precipitation over depth in each sample.

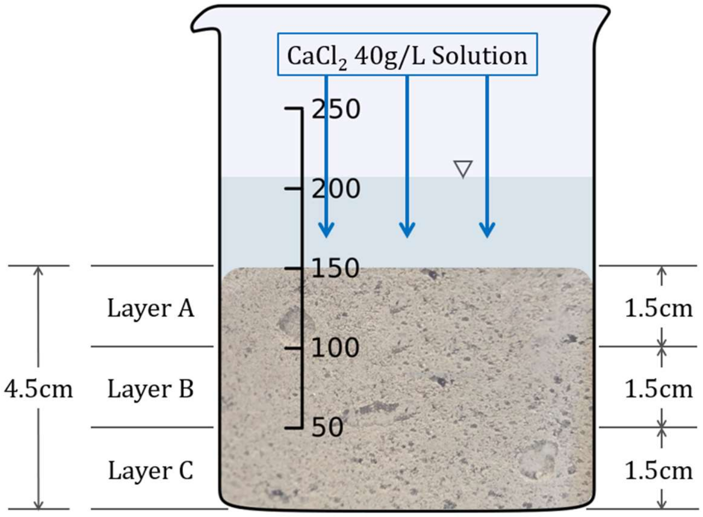

Fig. 4 Infiltration of $CaCl_2$ solution into tailings saturated with trona solution.

Stochiometric Efficiency and pH Experiment

To determine the rate of precipitation and the effects these experiments had on overall alkalinity, the slurry method was repeated a second time with a pH probe placed in the slurry. The pH was measured at 5-second intervals for the entire period that the $CaCl_2$ was added to the slurry. After the slurry was allowed to settle the pore water was flushed multiple times to remove any dissolved carbonate or bicarbonate that may have contributed to the carbonate content of the oven dried sample.

Results and Discussions

Concentration and Precipitate Crystal Size

The results of the microscopic imaging of the three samples used in the concentration and precipitate crystal size experiments are shown in Figure 5. As can be seen from the figure, the variable concentrations used in this study have no significant effect on the size of the precipitated crystals. $CaCO_3$ can often precipitate as a variety of polymorphs, i.e., vaterite, aragonite, and calcite, but given the rhombohedral structure of the precipitate seen in Figure 5, it can be concluded that the $CaCO_3$ precipitate will form as calcite (this was also confirmed by XRD analyses). The average size of calcite grains impacts cementation within tailings which could have a significant impact on the cyclic resistance increase. This experiment revealed that at these current concentrations one can expect to see calcite grains averaging 5.9µm in diameter.

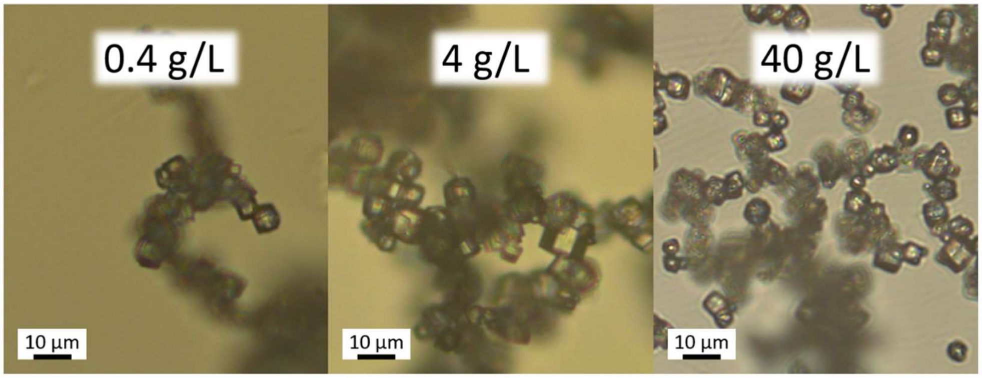

Fig. 5 Calcite precipitate at three different concentrations

Method of Introduction Impacts on the Precipitated Calcite Concentration over Depth

The carbonate content of the control sample was measured, and the results are presented in Table 2.

Table 2 Average Carbonate Content for Mine Tailings Sample

Sample ID	Mass (g)	CO_2 (kPa)	Time (min)	Equivalent Calcite Content (g)	Percent Carbonate (%)
Control a	9.981	10.5	30	0.147	1.47%
Control b	10.006	10.5	30	0.147	1.47%
Control c	10.005	10.0	30	0.138	1.38%
Control (avg)	-	-	-	0.144	**1.44% (±0.05)**

Given the solution concentrations, the mass of tailings, and the 1:1:1 ratio of trona, $CaCl_2$, and $CaCO_3$ used in our experiments, 0.54% carbonate content increase was expected due to carbonate precipitation with the potential for another 0.54% carbonate increase due to precipitation of $CaCO_3$ from bicarbonate (Cho et al. 2008). At a relatively high pH (>9) under lab conditions, bicarbonate will deprotonate to release carbonate which will likely bond to form additional calcite (Elamin 2011). Therefore, the expected total increase in samples assuming bicarbonate also contributes to the increase is 1.08%.

To determine the carbonate content increase in each sample, the average in-situ equivalent calcite content (1.44%) was subtracted from the measured equivalent calcite content of each treated sample (Table 3).

Table 3 Carbonate Increase Data across each Sample Layer

Method (Sample ID)	Height (cm)[1]	Carbonate Content (g)	Carbonate (%)	Carbonate Increase (%)
Injection (T5.1a)	3.75	0.289	2.89%	1.48%
Injection (T5.1b)	2.25	0.244	2.44%	1.04%
Injection (T5.1c)	0.75	0.209	2.09%	0.69%
Slurry Mix (T5.2a)	3.75	0.249	2.49%	1.09%
Slurry Mix (T5.2b)	2.25	0.244	2.44%	1.04%
Slurry Mix (T5.2c)	0.75	0.253	2.53%	1.13%
Infiltration (T5.3a)	3.75	0.271	2.71%	1.31%
Infiltration (T5.3b)	2.25	0.209	2.09%	0.69%
Infiltration (T5.3c)	0.75	0.200	2.00%	0.60%

[1] average height measured from the base of the tailings in the beakers representing each 1.5cm interval

The achieved increase in the carbonate content in treated samples reveals the carbonate increase must have also included the bicarbonate as the average carbonate increase exceeded the expected 0.54% due to calcite precipitation. The average increase in each sample was calculated as 1.07%, 1.09%, and 0.86% for the Injection (T5.1), Slurry Mixing (T5.2), and Infiltration (T5.3) methods, respectively. Apart from the last sample (T5.3), the precipitation appears to have formed at near 100% stoichiometric efficiency. The possible explanation for the discrepancy observed in the last sample is discussed later.

The calcite increase versus the depth in the specimens is plotted in Figure 6. The results show that each method of introduction followed a different precipitation trend that appears consistent with the visual characteristics of each sample (Fig 7). The sample treated with the injection method followed a linear path with carbonate decreasing with depth. This is likely the result of greater hydraulic conductivity at shallower depths which may be a combination of the immediate precipitation of calcite at lower depths, the small confining volume of the beaker, or the natural tendency for permeability to decrease with depth. It is also worth mentioning that a significant number of tension cracks were observed in the uppermost layer. This is consistent with the higher increase in the carbonate content at the surface layer. For all treated samples the cementation caused by the carbonates leads to apparent cohesion in the material which in turn causes tension cracks during the drying process. The slurry mix method resulted in a consistent concentration of added carbonate over depth (Fig 6) which was expected due to the homogeneity caused by the continuous stirring of the tailings and the chemical solutions.

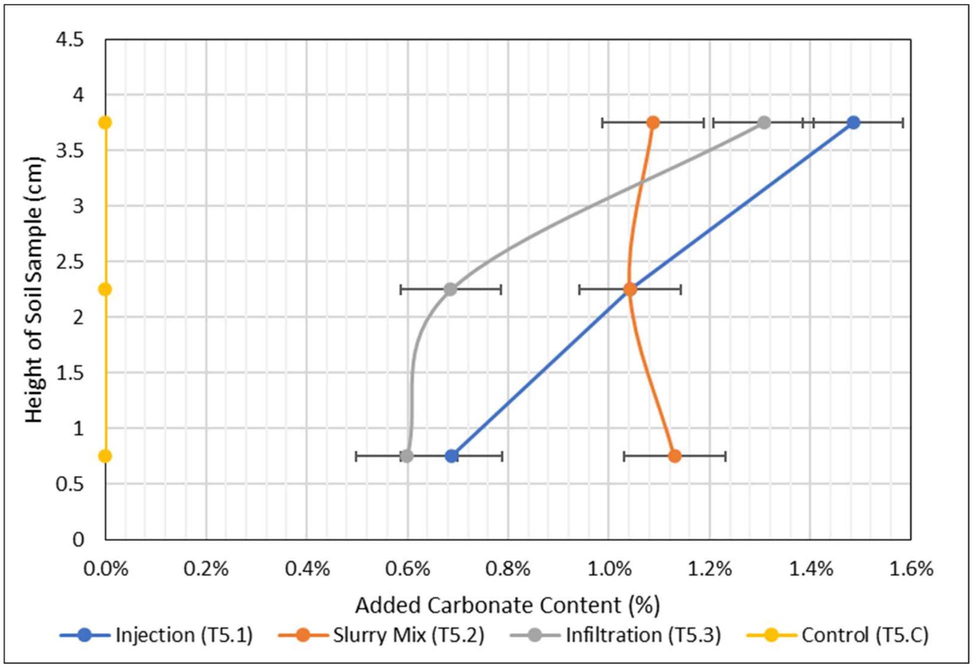

Fig. 6 Profiles for each method of introduction.

Visual inspection of the slurry sample also revealed very few tension cracks from local shrinkage in the sample which is consistent with the homogenous precipitation of calcite in this method. In the sample treated by the infiltration method, the carbonate increased exponentially towards the surface. This was also apparent from a dense precipitate layer seen at the surface of the treated sample (Fig. 7). Unlike the samples treated with the other two methods, this specimen only had a measured calcite content increase of 0.86% which is 80% of the expected increase. This is most likely due to sampling intervals used in this study. To be consistent among all the experiments, samples for calcite content measurements were taken from three layers of equal thickness, i.e., 1.5 cm, for each specimen. The measured calcite content increase for each interval is therefore an average increase over the whole 1.5 cm of the sampled layer. From the exponential calcite content increase seen in the T5.3 sample and the visual inspection of the treated specimen, most of the calcite has precipitated at the upper 2mm of the specimen. Therefore, the measured average calcite content increase for the top 1.5 cm layer underrepresents this top 2 mm of high concentration. Hence, adding the average calcite content increase measured for the three 1.5 cm layers does not equal the anticipated amount.

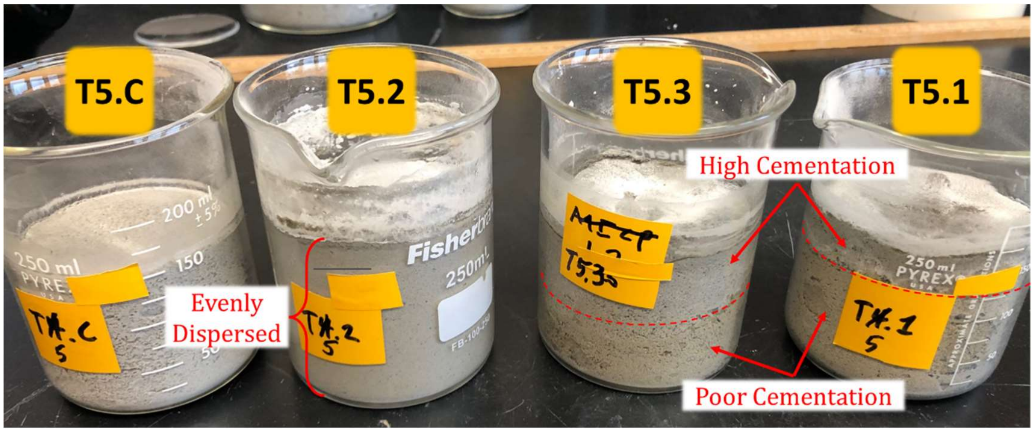

Fig. 7 Oven-dried samples after treatment before layer sampling.

The Vane Shear test showed an increase in strength at the surface of each sample when compared to the control sample (Table 4). Vane Shear is field equipment designed to estimate the strength of granular material on an undisturbed surface. As removing the top layers disturb the surface of the bottom layers, the Vane Shear test results would be unreliable for the lower layers. Therefore only the change in the strength at the top (surface) layers was compared. All samples showed some increase in strength compared to the control indicating a degree of strength increase due to calcite precipitation. More investigations and more precise strength testing are required to potentially correlate the increase in strength and the amount of precipitated carbonate.

Table 4 Strength (Vane Shear) Increase in the Sample Surface Layers

Sample ID	Vane Shear (kPa)	Strength Increase	Added Calcite (%)	Method of Introduction
T5.1a	368	88%	1.48%	Injection (T5.1)
T5.2a	662	238%	1.09%	Slurry Mix (T5.2)
T5.3a	260	33%	1.31%	Infiltration (T5.3)
T5.Ca	196	0%	0%	Control (T5.C)

Comparative Methods of Calcite Increase

The most common alternative method for focused calcium carbonate increase found in the literature is MICP. When comparing the applications of this technology to Trona the most apparent distinction is the rate of cementation, distribution, and mechanical improvement. Table 5 shows that the trona methods precipitate calcite far more rapidly than MICP. For MICP experiment calcite precipitated closer to the point of introduction following a trend similar to the infiltration method. Further studies comparing these methods will likely focus on the relationship between method of precipitation and strength increase.

Table 5 Precipitation Rates for MICP and Trona

Experiment	T5 Experiments (avg)	Pham et al. 2017	O'Donnell et al. 2017a
Total Increase (%)	1.29	0.93	1.94
Increase Period (hr)	0.033	1560	9600
Increase Rate (%/hr)	39.09	5.96E-04	2.02E-04

Stochiometric Efficiency and pH Results

Flushing the pore water from the slurry sample did not result in a reduction in added carbonate content (1.08%) when compared to the initial slurry mix added content (1.09%) meaning the entire carbonate component in the starting trona was precipitated before oven drying the sample. The high efficiency of precipitation in these experiments is likely due to a small confining space for experiments. When applied at a larger field scale the efficiency may drop significantly due to common challenges associated with mixing solutions in a porous media (Piscopo et al. 2013). Figure 8 shows that the precipitate formed almost immediately given the rapid change in pH which stabilized at 6.38 after all the $CaCl_2$ was added to the slurry mix. The drop in overall pH from 7.07 to 6.38 is likely due to the H^+ ions lost from the bicarbonate that precipitated calcite.

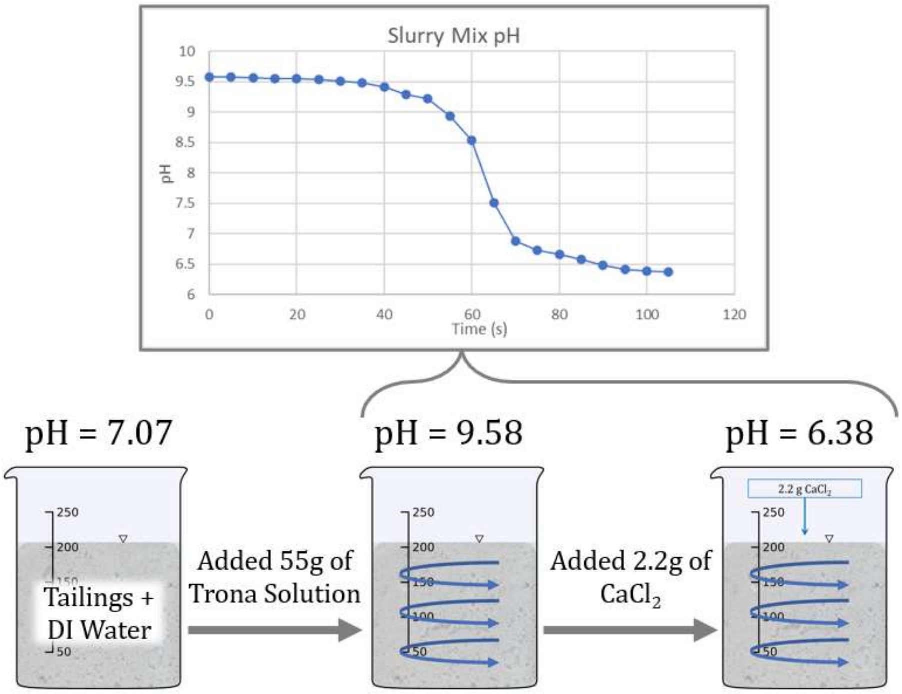

Fig. 8 Calcite forms rapidly after mixing trona and $CaCl_2$

Conclusions

The results of this study show that trona can be used as an inorganic substitute for other chemical agents typically used in ground improvement methods. Tailings treatment via trona and calcium salt is fast and efficient and shows potential as a method for increasing the strength of tailings. When considering the use of carbonate precipitation as a treatment method, the method of introduction could contribute significantly to leveraging resources to create efficient engineering solutions. For example, the infiltration method could be an effective dust control measure but appears less effective for stabilizing existing tailings at significant depths. The injection method appears to be less effective at depth as the injected chemical solutions tend to flow upward and precipitate more calcite at shallower depths. This was speculated to be the result of decreasing the hydraulic conductivity of tailing with depth. This may be exacerbated in the field where larger differences in hydraulic conductivity exist. However, this method would have to be repeated with a larger lateral area to solidify this assertion. The slurry method can be very effective in improving the overall cementation of new tailings if the chemical solutions are added to the tailings at the end of the milling process or while it is being pumped to the pond. Current slurry methods often use added alkaline materials at this stage to balance pH but the use of trona may prove more effective due to its higher alkalinity and solubility when compared to calcite and lime Regardless of the method of introduction, an increase of 1% calcite was observed across the entire media of tailings which significantly improves the neutralizing potential of the tailings. This increase could prove invaluable for the mitigation of acid mine drainage by decreasing the net acid-generating capacity of the tailings.

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Research Article

Effectiveness of Denitrification MICP Treatment on Copper Mine Tailings

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Abstract

Mine tailings present several geo-environmental hazards, among which are acid generation, leaching of heavy metals, and liquefaction. Microbially induced calcite precipitation (MICP) has the potential to mitigate these problems by increasing the calcite content of the tailings to improve shear strength and reduce acidic leachate. MICP via denitrification has proven successful in soils with grain size distributions comparable to that of most tailings (silty sand). Few studies have investigated the efficiency of the denitrification method in mine tailings. This study explores the effectiveness of MICP via denitrification on a copper mine's tailings using both in-situ bacteria and an inoculum bacteria culture. Furthermore, the presence and quantity of the 16s bacterial DNA within the tailings before and after MICP treatment was investigated. The results showed that while calcite did properly form in the upper portions of the tailings, the persistence of calcite with depth was significantly limited. The mine tailings used in this study contained high concentrations of heavy metals such as Cadmium, Zinc, Copper, and Lead. The limited precipitation depth is most likely due to the toxicity of these heavy metals in the tailings. Despite this limitation, denitrifying bacteria was shown to have the capacity to raise the pH of the leachate which could prove significant in mitigating the contaminant transport problems associated with mine tailings. Additionally, the results revealed that the overall hydraulic conductivity of the tailings was reduced after treatment by approximately 30 percent. The results also indicated the presence of 16s bacterial DNA in the tailings, and increased gene copy number following the MICP treatment.

Keywords: Microbially induced calcite precipitation (MICP), Denitrification, Mine tailings, Heavy Metals, Toxicity, Liquefaction

Introduction

While a key aspect of the mining industry, tailings impoundments create significant environmental and geotechnical hazards the foremost being acid mine drainage (AMD) and liquefaction. Some of the most common conventions for addressing these challenges consist of controlling surface and underground drainage (Klohn 1979) while simultaneously eliminating one or more of the components necessary for AMD to occur. Geofabrics, for example, are used to prevent the introduction of water or pit lakes to prevent the oxidation of sulfide minerals (Lange et al. 2010). While effective for controlling pollution, these methods do not eliminate the long-term potential for acid generation or liquefaction as the tailings pose an indefinite problem unless the in-situ conditions are chemically or mechanically treated. Some of the most common forms of chemical treatment are stabilization by the introduction of lime, cement, fly ash, and other cementing agents which increase the strength of the tailings. Several of these materials also provide alkalinity to the tailings which reduces the potential for acid generation. The major setback of using these alkaline materials is the environmental (CO_2 emission) and financial costs associated with acquiring and utilizing these materials. Therefore, an alternative to these traditional chemical treatments, i.e., microbially induced calcite precipitation (MICP), has attracted the attention of a lot of researchers.

The two primary metabolic pathways used to form calcite through MICP are urea hydrolysis and denitrification. The MICP method using the urea hydrolysis path requires certain bacteria and produces calcite along with ammonia which presents its own environmental concerns. The advantage of the alternative, the denitrification pathway, is that an acute strain of bacteria is rarely required, and an effective culture can be created using bacteria found in most native soils. While harmful by-products are of little concern in denitrification, the production of nitrogen gas in this method will influence the location and rate of precipitation which can lead to challenges in field application of this technology. On the other hand, the production of nitrogen gas results in desaturation of the soil which leads to a temporary strength increase (O'Donnell et al. 2017b).

Both MICP pathways have been proven successful in increasing the strength of sandy soils (Liu et al. 2021, Gui et al. 2018, Zhang et al. 2020a, Zhang et al. 2020b, & Erşan et al. 2015). Recently, in an attempt to utilize MICP treatment for mine tailings, de Oliveira et al. (2021) investigated the effectiveness of the MICP via the urea hydrolysis pathway. Their results showed that toxicity limited bacterial growth and consequently limited calcite precipitation was observed. The denitrification MICP method, however, has not been examined extensively for treatment of mine tailings. The denitrification pathway utilizes a greater variety of bacteria which may prove more effective in the treatment of tailings. Previous studies (Pham et al. 2018 & Van Paassen et al. 2010) have cultured denitrifying bacteria found in natural topsoil and utilized it for MICP treatment of sands. While these bacteria have proven effective, utilization of such in-situ bacteria needs to be investigated in tailings as these cultures may be more likely to acclimate to the harsh environment of tailings when given a nutrient broth. Bacteria that may reside in tailings could also be of use in field applications where optimized nutrient broth would be the only requirement for performing economically viable MICP in mine tailings. Furthermore, if in-situ bacteria are present, they may be able to grow faster than an introduced culture.

While resilient bacteria can often survive in harsh environments like those of mine tailings, the growth rate can be greatly inhibited by a variety of factors such as pH, the particle size of soil medium, the concentration of toxic metals, permeability, and the ratio of nutrients to cell count. Understanding the effects of these factors is essential for optimizing this remediation technology for use in a variety of soil and tailings environments. This paper explores the use of MICP via the denitrification pathway for tailings of a copper mine. Laboratory test procedures that have proven successful on controlled sandy soil columns were used in the experiments conducted in this study, to examine the efficiency of the MICP via denitrification pathway in mine tailings and identify any possible limiting factors. An introduced culture of denitrifying bacteria was used in the experiments. To examine the possibility of using the bacteria that might reside in the tailings, a separate experiment was also conducted by only introducing a nutrient broth optimized for maximum bacterial growth.

Materials and Methods

MICP has been studied significantly in both natural soils and in artificial soils where properties like grain size and chemical composition are carefully controlled. To better understand the effects of mine tailings on the efficiency of MICP, similar column experiments with periodic flushing of pore water were conducted in this study. Pham et al. 2017 demonstrated the capacity for MICP to occur via denitrification in a sand column with a D_{50} of 0.17mm and bulk density of 1.62g/cm^3. They conducted their experiments over a period of 65 days where an average calcite increase of 0.93% was achieved in their overall sample. Given the properties of the tailings used in this paper (discussed later), a similar procedure and method of inoculation were followed for experiments performed in this study. Furthermore, the results of the experiments performed on Ottawa Sand 20–30 by O'Donnell et al. (2017) were compared to the results of the current study to understand the influence of bulk density on the successful application of this technology.

2.1 Tailings Characteristics. Tailings from a copper-molybdenum mine in Montana were used as a sample medium to investigate the efficiency of the MICP via the denitrification pathway. Samples were taken directly from the mill where grain size would be more representative of the overall tailings in the tailings pond. The grain size distribution data was determined according to ASTM D6913 (Fig. 1) and Atterburg Limits followed ASTM D4318. These standards were used to classify the tailings according to Unified Soils Classification System (ASTM D2487 2013) as SM-Silty Sand. Based on previous studies (Zamani et al. 2019), silty sands show small amounts of bacterial filtration limiting the precipitation of calcite when compared to clean sand but can still be easily infiltrated for the precipitation of significant calcite (>2%).

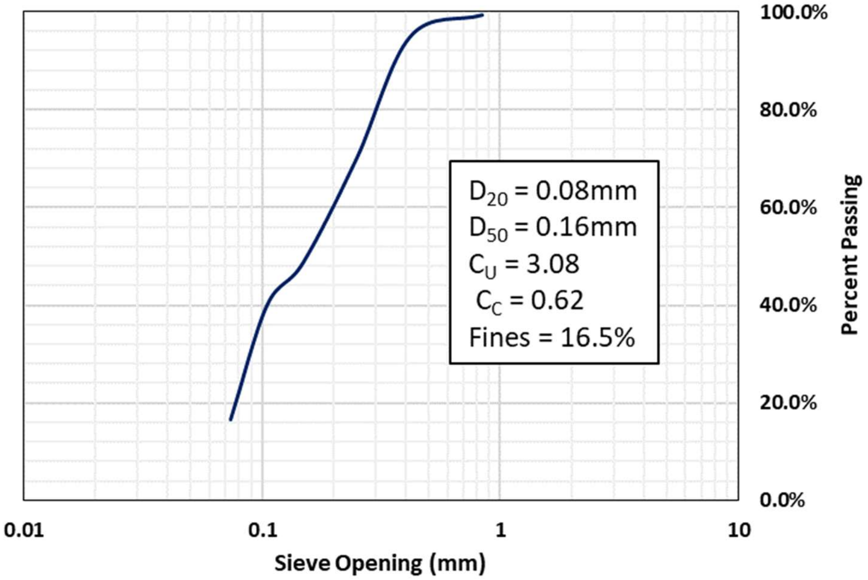

Figure 1: Grain Size Distribution for tailings classified as SM-Silty Sand.

XRF and XRD tests conducted on the samples revealed that the tailings are comprised primarily of quartz, biotite, sericites, feldspars, clay minerals, and a collection of metals that could act as inhibiting agents for some bacterial growth. Copper, lead, zinc, and cadmium are toxic to calcite precipitating bacteria (Mugwar and Harbottle 2016) and were observed in noteworthy concentrations in the tailings used in this study. The copper, lead, zinc, and cadmium concentrations in millimolar were 2.46, 0.08, 1.01, and 0.96 respectively. The pyrite content which is a common indicator of acid generating potential was shown to be below 2%. In turn, the alkaline or acid consuming minerals were approximated to be 1.4% through a carbonate content test. The overall pH of the tailings was 7.07 after samples were filled with DI water to the point of saturation. The average carbonate content in the tailings was evaluated according to ASTM D4373. This standard recommends reporting all content percentage values to 1% but given each test was carried out by the same operator (first author) and the significantly low standard deviation of readings among untreated samples (±0.04%), readings to the hundredth were considered appropriate for subsequent tests. For the purposes of this paper, the average room temperature was determined to be 23±2° C. for all experiments.

2.2 Nutrient Broth and Inoculum. The nutrient broth used for all experiments contained 60mM of calcium acetate and 50mM of calcium nitrate along will several micronutrients with concentrations following the procedures of the reference experiment (Pham et al. 2017). This ratio of nutrients and concentration (55:60:50 Ca–Ac–N) was selected for optimal bacteria growth. The inoculum used for this experiment was cultured from activated sludge taken from a wastewater treatment plant in Montana, USA. The activated sludge was stirred thoroughly and then mixed with nutrient broth at a 1:1 ratio. This concoction was incubated at room temperature for 48 hours.

2.3 Column Experiments. To determine the influence of an introduced bacteria culture vs in-situ bacteria on the efficiency of MICP two test were performed on two tailings columns. The first test involved the cycling of a nutrient broth through the tailings without any added bacteria. The second test followed the same procedure with the only exception that the first pore flush contained a mixture of 500ml of inoculum and 1000 ml of nutrient broth. The tailings column tests' setup shown in Figure 2, allowed new nutrient broth to be added through the top of the column which percolated through the tailings under an average pressure head of 45cm until a full pore volume of fluid was cycled.

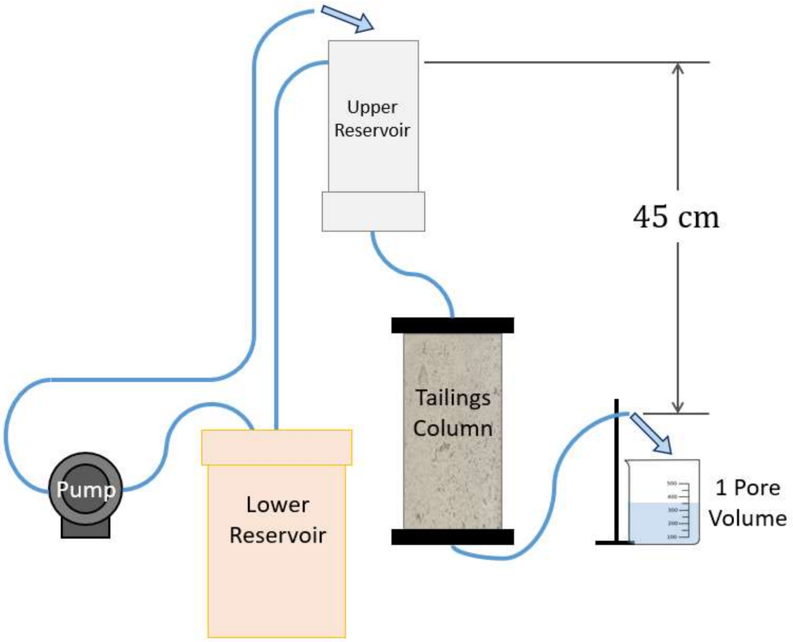

Figure 2: Experimental set up followed a typical constant head test with some modification.

Saturated tailings were placed loosely in an acrylic test chamber with an internal diameter of 11.4 cm to the initial target height of 16cm for the tailings column. After inserting the upper porous stone, sealing the cell, and bleeding the remaining air out of the system, an initial flow rate was measured for each test. The time required for a single pore flush was estimated using this flow rate in congruence with the porosity along with a small factor of safety (1.25) to ensure complete refresh of nutrients in the pore volume for each cycle. The first cycle removed the pore water and replaced it with a pore volume of nutrient broth and in the second experiment inoculum and nutrient broth. The pore volumes were then cycled every 7 days for 6 consecutive weeks during which the flow rate and pH were measured. After the final cycle, each column was separated into seven tailings layers of equivalent height and tested for calcite content using ASTM D4373.

2.4 DNA Extraction, Polymerase Chain Reactions, and Quantification. Total genomic DNA was extracted from 0.48g of tailings samples using a PowerSoil DNA Isolation kit according to the manufacturer's protocol and stored at -80°C. Tailings samples were taken in triplicate for each experiment both before and after treatment. Extracted DNA served as template DNA for subsequent polymerase chain reactions (PCR). Endpoint PCR was used to amplify 16s rRNA DNA present in each sample using bacterial/universal primer set 1100F and 1492R (Boyd et al. 2010). DNA was amplified in 20μL reactions containing 10μL of Qiagen HotStarTaq Plus Master Mix, 4μL nuclease-free water, 0.5 μM of each primer, and 2 μL of DNA extract. PCR parameters consisted of a 5-minute denaturation step at 95°C, 35 thermal cycles (30 seconds at 95°C, 2 minutes at 58°C, 2 minutes at 72°C), and a 7 minute final extension step at 72°C. All PCR experiments were accompanied by a no-template control containing 1μL of nuclease-free distilled water instead of DNA template to detect any possible reagent contamination. Following amplification, 12μL of each PCR product was loaded into a 2% agarose gel stained with 4μL of ethidium bromide. Gels were then subjected to electrophoresis for 25 minutes at 110 V. Following electrophoresis, results were visualized, and the fluorescent intensity of bands quantified using the Biorad Gel Doc XR System. The amplification of extracted DNA produced ~500bp bands of varying intensities. The presence, size, and intensity of PCR products are indicative of the presence of 16s microbial DNA, and a semi-quantitative measure of gene copy number.

Results and Discussion

3.1 Overview of Experimental Parameters. Table 1 shows the major differences between the tests conducted by Pham et al. 2017, O'Donnell et al. 2017, and the two experiments conducted in this study, i.e., the test without introduced bacteria and the inoculum bacteria treated test.

Table 1: Key parameters in each test potentially impacting the efficiency of MICP.

MICP Experiment	Pham et.al. Sand Column	O'Donnell OS-ACR-1	In-Situ Bacteria	Inoculum Bacteria
Period (days)	65	400	42	40
D50 Soil Part. Size (mm)	0.17	0.6	0.15	0.15
Total Nutrient Conc. (mM)	123.5	75	110	110
Nutrient Broth Avg Ac/N	1.3	2	1.2	1.2
Flush Frequency (days)	8	17.5	7	7
Average Calcite Increase*	0.93%	1.94%	0.02%	0.05%
Maximum Calcite Increase	1.53%	3.00%	0.16%	0.35%
Percent Increase /month	0.45%	0.15%	0.02%	0.04%
Avg Hydraulic Conductivity	5.8E-03	Not Stated	1.0E-04	2.9E-04

*This value represents the average calcite increase across the entire soil column.

3.2 Calcite Precipitation. Figure 3 shows the percent by weight of calcite precipitation in each test relative to the distance from the inlet of the nutrients/inoculum. For both MICP experiments conducted in this study, the depth of persistence for precipitated calcite did not reach beyond the top 2.2cm layer of tailings. However, the inoculum treated experiment precipitated over twice the calcite of the untreated sample in the same region of the sample. When compared to the Pham et al 2017 experiment, both persistence and overall calcite increase were significantly reduced in the tailings samples. The overall time of the experiment, concentration of nutrient broth, and hydraulic conductivity were similar (with some minor variations) in the experiments conducted in this study and the experiment conducted by Pham et al 2017. The major limiting factor for lower concentrations of precipitated calcite and persistence observed in tailings, therefore, can most likely be attributed to the toxicity of the tailings. The toxicity of the tailings is due primarily to the presence of copper, lead, zinc, and cadmium which are discussed in greater detail in the next section. These assertions are further strengthened by the apparent visual precipitation of calcite in the upper porous stone where there are no toxic metals (Figure 4). The concentration of calcium minerals in the porous stones were estimated using XRF data after rinsing both porous stones thoroughly with deionized water. The calcium minerals concentration in the upper porous stone was estimated at 6.68%. The 0.68% calcite in the lower porous stone is likely due to the leaching of the nominal concentration (1.4%) of calcite native to the tailings.

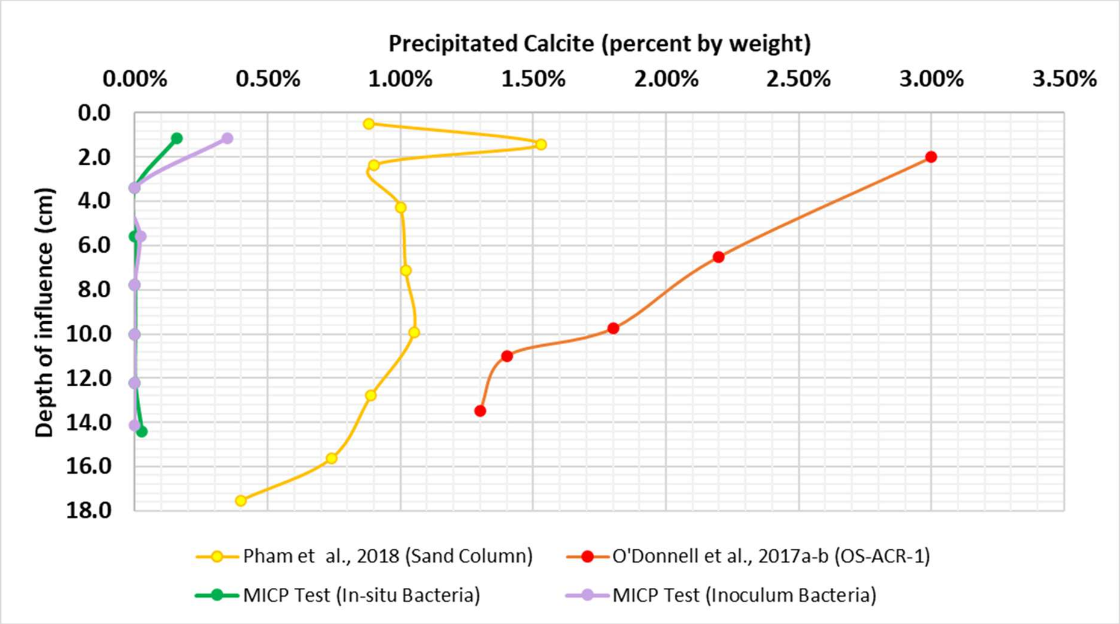

Figure 3: Calcite precipitation for various tests where depth of influence is measured from the infiltration point.

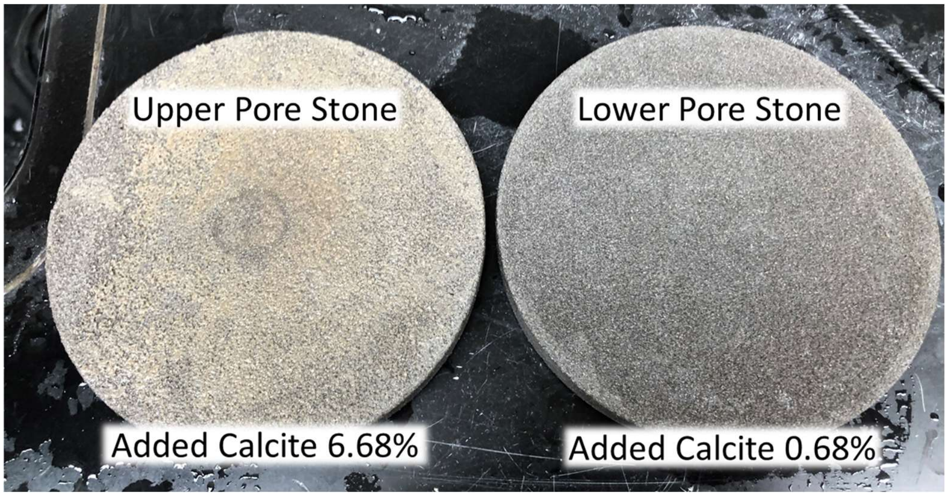

Figure 4: Upper and lower porous stones after the second MICP with Inoculum Treatment.

3.3 Hydraulic Conductivity. In both experiments conducted in this study, the overall hydraulic conductivity decreased by 33% and in each case the rate of reduction decreased after the first 2-3 weeks of treatment. It is worth noting that the inoculum treated test had a slightly higher initial hydraulic conductivity compared to the test without the introduced inoculum (Fig 5.). The inoculum treated test appears to show a rapid drop in conductivity followed by a plateau indicating the precipitation of calcite at the top of the system could be acting as a plug, particularly in the porous stone that saw significant precipitation of calcite. The sample used in the test without the introduced inoculum seemed to follow a more linear trend after the initial drop which could be indicative of the natural settling in the tailings column which would occur in both experiments. In both experiments, the calcite precipitation did not occur past the first layer of tailings despite a noteworthy difference in initial hydraulic conductivity. This supports the speculation that toxicology played a more significant role in the inhibition of MICP in mine tailings than low hydraulic conductivity.

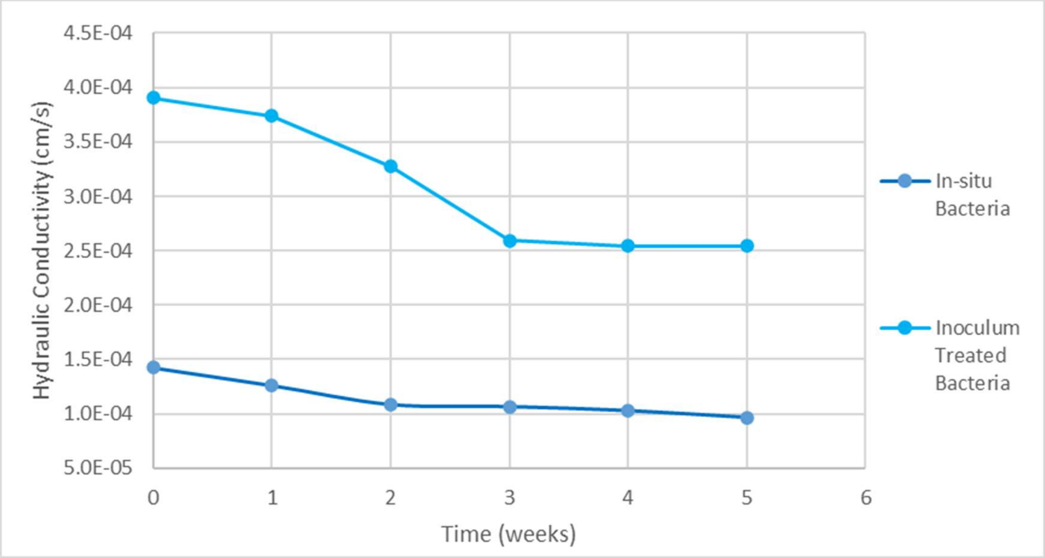

Figure 5: Hydraulic conductivity of each experiment over a period of five weeks.

3.4 Impacts on pH. As described earlier in this paper, the remaining sulfides present in the tailings have the capacity to generate acid when oxygenated water is introduced to the column. For this reason, the effluent had a continued decrease in pH over the experiment (Fig. 6) with the exception of the inoculum treated experiment. The last two weeks of this experiment saw an increase in pH which implies that while the precipitated calcite may not be persisting through the sample, the pore water is being treated by the increase in calcite in the upper layers of the system. This increase cannot be solely attributed to the actual composition of the nutrient broth as the same trend was not apparent in the in-situ sample. A further implication of this speculation is that the in-situ bacteria may with time increase the pH as seen in the inoculum treated sample.

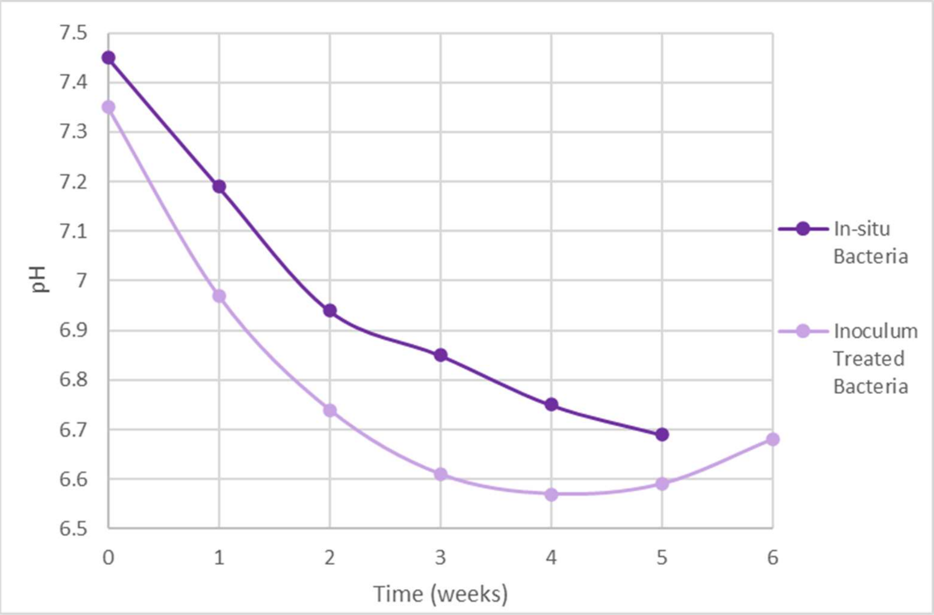

Figure 6: pH of the effluent for each week of the experiment over a period of five weeks.

3.5 Toxicity. Four heavy metals commonly associated with bacteria growth inhibition are copper, lead, zinc, and cadmium (Cordos et al. 2007). Table 2 shows average concentrations measured in the tailings used in our experiments via XRF.The minimum inhibitory concentration (MIC) of the metals, representing a 30% reduction in cell density of the bacteria *Sporosarcina pasteurii* as reported by Mugwar and Harbottle (2016) are also reported in table 2. It is worth noting that *Sporosarcina pasteurii* performs MICP through the urea hydrolysis pathway and these inhibitory concentrations could be different for denitrifying bacteria. These concentrations, therefore, are only used here as a guide for determining whether the present concentrations could prove inhibitory towards other bacteria. Further research is required to investigate the exact inhibitory concentrations for various denitrifying bacteria. Furthermore, the metals concentrations in pore water will differ significantly from the concentrations in the tailings and leachability depends on several factors including, pH, dissolved oxygen, and speciation of the heavy metals (Wang et al. 2019). Assuming a leachate of roughly 10% (values are usually lower) for a factor of safety these heavy metals are ranked by potential toxicity concern in table 2.

Table 2: Toxicity rating and concentrations for heavy metals limiting MICP performing bacteria.

Metal Ions	Pb	Zn	Cu	Cd
Tailings Concentration (ppm)	17	66	156	108
Mugwar and Harbottle (MIC) for *S. pasteurii* (ppm)	207	10	32	3
Ratio of COC* to 10% Leachate	0.01	0.68	0.49	3.21
Toxicity Rating	Negligible	Low	Low	High

*Contaminant of Concern

A common approach to understanding toxicity especially in MICP via urea hydrolysis is to isolate and identify the primary bacteria and then quantify the minimum inhibitory concentration of various toxins (mostly heavy metals). After identifying an MIC for major contaminants, bacteria acclimatization can create a culture that is more resilient under higher concentrations of toxic metals (de Oliveira et al. 2021). The challenge with this approach is that tailings are highly varied in composition and an isolated strain of bacteria that may work effectively against certain contaminants may prove entirely ineffective given small changes in tailing composition. An alternative to this method that may prove more effective for long term field applications would be to use a local culture of denitrifying bacteria found in or near the tailings to be remediated that could be acclimated using sequential dilutions of pore wate. The pore water dilutions would ensure that the bacteria culture was being acclimated to overcome the exact environmental contaminants instead of testing specific bacterium against isolated toxins. While this method would make quantifying the exact limitation of this technology more difficult to identify it would also be far more applicable as the inoculum created by exposing a local culture to increasing concentrations of contaminants would ensure that any bacteria that survived this process would be able to exist inside the tailings.

3.6 DNA Extraction and Relative Quantification. The analysis of end-point PCR product fluorescence can be used to semi-quantitatively analyse differences in DNA concentrations of distinct samples as the intensity of PCR bands is dependent on the initial gene copy number. Therefore, the analysis of end-point PCR band fluorescence provides a preliminary alternative to quantitative PCR (qPCR) in investigations of differences in the presence and abundance of DNA found in different sample types (Schmittgen et al. 2000). Figure 7 shows DNA extracted from tailings samples both before and after treatment in each column experiment. The successful amplification of DNA extracted from each sample indicates amplifiable 16s bacterial DNA was obtainable from all tailings samples using the methods articulated in this paper. The fluorescence intensity increase ratio (Table 3) from the analysis of amplified PCR products may be an indication of increased bacterial load in treated samples. As end-point PCR fluorescence is only semi-quantitative, further analysis of DNA extracted from tailings using qPCR is necessary to fully understand the differences in microbial communities found in tailings environments.

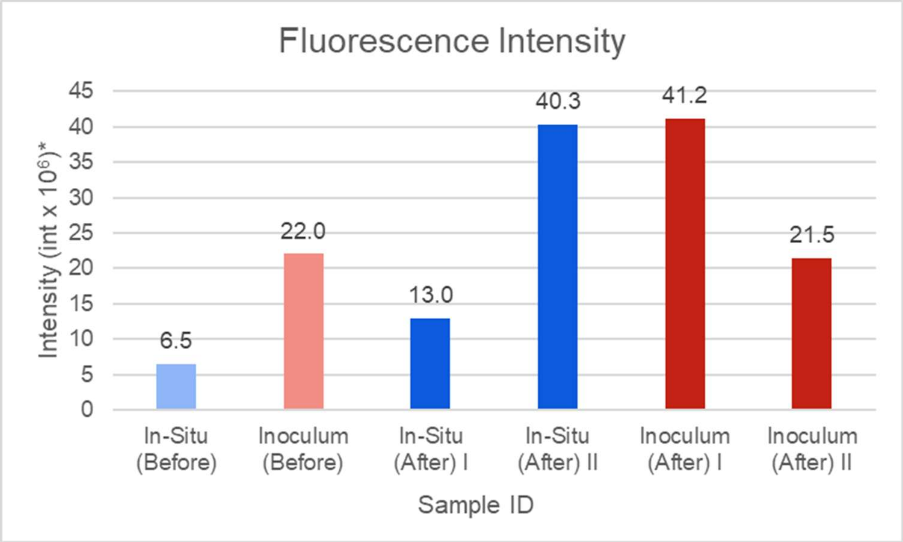

Figure 7: Relative fluorescence data for PCR bands produced through the amplification of 16s bacterial DNA extracted from tailings samples. *The Biorad Gel Doc XR System provided the empirical unit (int) for all intensity values.

Table 3: Fluorescence intensity data before and after treatment for in-situ and inoculum experiments.

Experiment ID	Intensity (Int)*	Intensity Increase Ratio (IIR)	Avg IIR
In-Situ (Before)	6538090	-	-
In-Situ (After)**	26672003	4.08	-

Inoculum (Before)	22027850	-	-
Inoculum (After)**	31335493	1.42	2.75

*The Biorad Gel Doc XR System provided the empirical unit (int) for all intensity values.

**Intensity values represent the average of the two values presented in Figure 7.

Conclusions

The results of this study indicate that in-situ bacteria can perform MICP via denitrification within tailings, but the depth of influence is limited significantly by toxicity. Using an inoculum bacteria culture generated twice the precipitation in the same depth interval, indicating the capacity for MICP in tailings is limited primarily by environmental factors. The results showed that immediate exposure to treatment solution caused a drop in pH likely due to the supply of oxygen and H_2O required for acid generation in sulfide minerals in the tailings. The pH eventually increased after continuous treatment. The increased pH of water infiltrating the tailings reduces leaching of heavy metals and acid generation in the effluent. Therefore, while precipitation was limited to the surface, the increase in leachate pH reveals the potential for treatment of pore water. Additionally, creating a thick surface crust of calcite has significant potential as a dust control agent in tailings. The results also showed a 33% reduction in the permeability of the tailings columns. The reduction in hydraulic conductivity revealed no significant limitations in the performance of MICP. Tailings can vary significantly across various mines, however poor MICP performance in tailings classified under USCS as silty sand can likely be attributed to toxicity as a primary limiting factor.

Successful amplification of 16s bacterial DNA found in mine tailings using the methods described in this paper presents an affordable, intuitive, and streamlined approach for detection and relative quantification of potential MICP performing bacteria. Apparent increases in fluorescence intensity of end-point PCR samples suggest that emphasis on PCR may be useful in further optimization of MICP methods. The results also showed that although the MICP was limited due to the existence of toxic metals in the tailings calcite precipitation was not entirely inhibited. To overcome these limitations, the utilization of bio-acclimation alongside microbial molecular analysis will likely provide valuable insight into microbial community dynamics. Ultimately, this may assist in the culturing of denitrifying microbes that can survive and thrive in the conditions of pore water found within tailings.

Data Availability

Data may be accessed by contacting the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest

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