Mine tailings consist of fine-grained particles that are deposited into large impoundments. Seasonal temperature and moisture fluctuations can result in dust emissions, an environmental hazard. Accordingly, there is a need for efficient and economical means for controlling dust emissions. Biogeotechnics provides one innovative approach to modifying soil properties. For example, *Sporosarcina pasteurii* has been studied for its ability to promote biocalcification in sand-sized particles by way of ureolysis. The application of this process to fine-grained materials, however, poses unique challenges. The goal of this work was to demonstrate biocalcification in fine-grained mine tailings to promote the formation of a crust that increased the surface strength of the tailings. Soil box experiments coupled with multiple lines of evidence collected using novel analytical techniques were used to confirm (a) the successful formation of the surface crust by way of ureolysis mediated by inoculated *S. pasteurii* and native urea-degrading microbes and (b) the impact of the crust on the surface strength of tailings. The crust formation and increased surface strength demonstrated in tailings inoculated with *S. pasteurii* and with native microbes provide a foundation for further research to advance the application of biogeotechnics in fine-grained materials for purposes such as dust mitigation.

**Introduction**

Mining operations result in the production of massive volumes of waste materials, including the mine tailings that result from the beneficiation of the ore (US EPA, 2010). These fine-grained tailings, with an average size range around 20 μm and a significant portion in the 1–10 μm range, are disposed of in slurry form into large-scale permanent structures called mine tailings impoundments (Vick, 1983). Impoundments can cover several square kilometres and are largely void of vegetation during active filling, which can last many years. The coupling of cold and warm weather temperature fluctuations, moisture variations and wind-induced shear stresses can result in dusting of the tailings particles into the atmosphere. Such storms can have a number of negative consequences, including human respiratory health problems, damage to local ecosystems and vehicle accidents due to poor visibility (Washington State Department of Ecology, 2016).

Although many mining operations apply a number of dust prevention controls, the treatments tend to be expensive and difficult to apply due to the large size of the impoundments and variable trafficability conditions across the impoundment. In addition, conventional approaches used for controlling dust, including application of surface penetrants, admixtures or surface blankets and the use of agronomic techniques (US Army and Air Force, 1987), themselves have several limitations with regard to mine tailings impoundments (Chen et al., 2015). For example, common surface penetration methods such as watering and salt solutions provide only a temporary respite and are less effective in warmer, drier climates. In addition, surface blanket and agronomic approaches are more feasible for an ‘end-of-life’ goal, where the system is either in a restorative phase or is no longer in use. However, active mine tailings impoundments are dynamic systems because mine tailings are discharged in the impoundment constantly until the tailings impoundment use is complete, and so approaches to controlling dust emissions during the operation of the impoundment must also be dynamic. Thus, there is a need for economical, effective and sustainable techniques for controlling dusting in mine tailings impoundments.

There is increasing interest in the use of biologically mediated processes for in situ site improvement (DeJong et al., 2013; Seagren...
and Aydilek, 2009). These approaches may have less impact on the environment and may be less expensive to implement compared with conventional engineering technologies. For example, microbiologically induced (CaCO₃) calcium carbonate precipitation (MICP) has been proposed as an approach for creating a light temporary surface crust that is permeable to water and could be used for dust control on heavy haul roads (e.g. at mining operations), construction sites and railroad corridors, particularly when dust is an environmental concern (Bang et al., 2009). Specifically, Bang et al. (2009, 2011) and Meyer et al. (2011) examined promoting MICP for dust suppression by way of urea hydrolysis (or ureolysis), using a poorly graded sand and/or a well-graded sand. Calcium carbonate precipitation was promoted in their experiments by adding urea and calcium chloride (CaCl₂), along with the urease enzyme for catalysing ureolysis, and/or a soil microorganism, Sporosarcina pasteurii, which produces the urease enzyme. As discussed elsewhere (e.g. Stocks-Fischer et al., 1999), during ureolysis, urea is hydrolysed by way of intracellular urease, producing carbamate and ammonia. The carbamate is subsequently further degraded into ammonia and carbonic acid. Ammonia is expelled from the cell and hydrolysed, which raises the external pH of the system and shifts the carbonate system towards bicarbonate (HCO₃⁻) and carbonate anions. Calcium cations present in the system are then able to react with carbonate anions, resulting in calcium carbonate precipitation due to the low solubility of calcite. Bang et al. (2009, 2011) and Meyer et al. (2011) demonstrated the potential of ureolytic MICP for dust control with sand-sized particles; however, finer grained materials pose challenges such as limitations to the treatment depth and the integration of the soil crust with the native material. These complications associated with fine-grained materials such as mine tailings have not been addressed.

In this study, it was hypothesised that, under controlled environmental conditions, ureolytic MICP could be induced using S. pasteurii and native microbes and that the resulting crust would result in the stabilisation of the surface mine tailings layer at the air–soil interface, thereby providing a means to reduce fugitive dust emissions. These hypotheses were investigated using bench-scale laboratory soil box experiments, the objectives of which were to demonstrate that (a) MICP can be stimulated in fine-grained iron mine tailings and the composition of the resulting crust quantified and (b) the resulting improvements to the surface strength of the tailings due to biocalcification can be quantified. To perform this investigation, several novel experimental techniques were used (e.g. pressure calcimeter) and/or developed (e.g. specific gravity determination and steel ball bearing drop test) for quantification of MICP and its effect on the mine tailings being treated.

Materials and methods

Tailings

Iron mine tailings (henceforth referred to as ‘tailings’) were obtained from a North American magnetite iron ore mine. Samples were extracted using a shovel and placed in 5 gallon (18.9 l) plastic buckets for transport and storage. The pH of the tailings was determined to be in the range 8·2–8·7, utilising ASTM method D 4972-01 (ASTM, 2001), and using calcium chloride solution and distilled water. This alkaline nature of the tailings makes them an excellent candidate for MICP.

To characterise the tailings’ particle size distribution, the grain size distribution was determined following the hydrometer analysis of ASTM method D 422 (ASTM, 2007). Based on the particle size distributions (Buikema, 2015), the tailings used in the tests reported here consisted of 89% silt-sized particles (0·002–0·075 mm), 7% sand and 4% clay-sized particles (uniformity coefficient Cu = 4·5; coefficient of gradation Cc = 2). Environmental scanning electron microscopy (ESEM) imaging was conducted and confirmed the angular shape of the tailings particles (Figure 1). In preparation for the experiments described in the following, the tailings were placed in storage containers open to the atmosphere to air dry.

S. pasteurii

A dehydrated sample of S. pasteurii culture (American Type Culture Association (ATCC) 11859) was obtained and rehydrated using the recommended ATCC 1376 Bacillus pasteurii ammonium (NH₄)–yeast extract (YE) medium (referred to here as Tris–YE medium) (Stocks-Fischer et al., 1999) and recommended ATCC protocol. Subsequently, S. pasteurii colonies were maintained on B. pasteurii urease (BPU) medium (Bang et al., 2001) agar streak plates. The colonies were restreaked onto fresh BPU plates monthly to ensure a supply of viable cells, and stored in a refrigerator at 4°C. These plates were used as the source of inoculum for the experiments described in the following.

S. pasteurii concentrations in aqueous solutions were estimated on the basis of a correlation curve between culture absorbance in Tris–YE medium at 600 nm and cellular density in solution, based on spread-plate counts by using Tris–YE medium agar
plates, performed as described in the following. The correlation curve was conducted in duplicate and covered a range of absorbance values corresponding to a range of $10^2$–$10^3$ colony-forming units (CFU)/ml.

**Soil box bioreactors**
The experiments were performed in soil box bioreactors (inside dimensions of 75 mm × 75 mm × 75 mm) that were constructed using 6·35 mm polycarbonate, adhered using acrylic cement (Scigrip 16). Brass hose barbs (6·35 mm) were threaded into the bottom of each soil box for drainage. Before use, the soil boxes were disinfected with 95% ethanol. Whatman 41 filter paper (catalogue number 1441-150) was cut to size and placed in the bottom of the soil box and then a thin layer (<1 mm) of well-graded sand was placed on top to help facilitate capillary rise. Subsequently, the soil boxes were packed with mine tailings.

To prepare the tailings for packing into the soil box, the air-dried tailings were abraded using a Micro-Deval apparatus (ASTM, 2015). One-third of the Micro-Deval cylinder was filled with tailings and steel balls, and the device was run for 7 min. After initial de-aggregation, the remaining large aggregates were hand crushed using a steel cylinder underneath a ventilation snorkel, to control dusting. The processed tailings were then sifted through a flour sifter and brought up to 5 wt% moisture content by using sterilised distilled water. The tailings were well mixed with a trowel and any aggregates broken up by the flour sifter, with the objective of producing a fine powder that had the same grain size distribution as the original material. Tailings were then applied to the soil box by way of pluviation, achieved using a flour sifter attached to a funnel and hose that allowed the tailings to be distributed evenly. Approximately 25 mm of tailings was deposited at the bottom of the soil box and compacted using a large hand tamp (50 mm dia.) followed by a small hand tamp (19 mm dia.) both of which were disinfected with a 95% ethanol solution. This process was repeated for seven to ten lifts until the soil box was nearly full of compacted tailings. The soil boxes were packed to a void ratio of approximately 1·1, which was similar to the void ratio determined in situ (Price, 1998). After the tailings were packed into the soil box, the contents were saturated overnight with sterile distilled water using a Mariotte tube connected to the brass hose barb by way of polypropylene tubing.

Three treatments of the mine tailings were prepared using the procedure described, each in duplicate soil boxes. The first treatment was performed using boxes packed with tailings that were inoculated with *S. pasteurii* to demonstrate the effect of bioaugmentation (denoted R1 and R1*). Approximately 207 ml per box of a stationary-phase *S. pasteurii* culture grown at 30°C in Tris-YE medium to $10^8$ CFU/ml was used to inoculate R1 and R1*. This was completed through four sequential applications of 50 ml of the inoculum to the top of the bioreactor, with a final application of 7 ml. The inoculum displaced the pore water, which drained out from the bottom. For the second treatment, the soil boxes (denoted R2 and R2*) were packed with unmodified tailings (i.e. without inoculation) to demonstrate the impact of the native microbial population during treatment. Finally, the third treatment was prepared in soil boxes (denoted R3 and R3*) packed with tailings that were autoclaved for 1 h at 15 pounds per square inch (psi) (103 kPa) and 121°C once per day for 3 d. The goal of this treatment was to eliminate or reduce the population of native microbes and demonstrate the role of any non-biologically mediated reactions. The pore water was not displaced in R2, R2*, R3 or R3* during the set-up.

After inoculation, the treatment phase of 23 d began in which a urea-calcium chloride solution was applied to the soil boxes twice daily at room temperature. The urea–calcium chloride medium (pH = 8·0) containing nutrient broth (3 g/l), urea (20 g/l), ammonium chloride (NH₄Cl) (10 g/l), sodium bicarbonate (NaHCO₃) (2·12 g/l) and calcium chloride (2·8 g/l) was prepared aseptically as described by Stocks-Fisher et al. (1999). This urea–calcium chloride solution was applied using a squirt bottle onto the top of the bioreactors and allowed to percolate through the packed tailings. The application interval was selected to allow the urea–calcium chloride medium to percolate through the soil medium sufficiently so that more fluid could be added in the second application, as the fluid did not readily penetrate through the soil. Effluent exited through the brass hose barb in the bottom of the bioreactors and into a 25 ml graduated cylinder.

**Monitoring and analysis of the soil box bioreactors**
Several measurements were made twice daily throughout the duration of the treatment phase, including (a) the volume of medium added to each bioreactor, (b) the volume of effluent collected from each bioreactor, (c) the pH of the influent and (d) the pH of the effluent. The trends in the measured percolation rate based on the volume balance were generally inconclusive and are not reported here (Buikema, 2015).

After the 23 d treatment phase, a well-defined surface crust was observable in each of the bioreactors. Subsequently, the soil boxes were allowed to air dry in the laboratory for 22 d before analysing the treatments. After drying, each soil box was cored using a sterile 60 ml syringe barrel with the tip cut off. The soil core was split into five layers: the top white crust, three 1 cm deep layers and a 3 cm deep layer. Based on previous experiments, it was determined that most of the biological activity occurred in the upper half of the soil horizon, and additional soil layers below 3 cm deep could be characterised into a single soil layer of 3 cm depth. The soil in each layer was pushed out of the syringe barrel by using the plunger and placed into pre-stereilised soil tins to allow analysis for cell numbers, specific gravity, calcium carbonate, ESEM and energy-dispersive spectroscopy (EDS), as described in the following.

**Analytical methods**
**pH**
Influent and effluent samples from the soil boxes were analysed for pH using a Thermo Scientific Orion Dual Star pH meter.
(catalogue number 2115001). The meter and probe were calibrated using three-point calibration.

**Soil cell counts**

Soil cell counts for each soil layer were conducted by way of serial dilution. First, a 1:100 dilution was made by placing 1 g of soil from each soil layer into an autoclaved blender containing 99 ml of phosphate buffer solution (Gerhardt et al., 1994) and mixed at medium to high speed for 2 min (Lindahl and Bakken, 1995). After blending, the slurry was allowed to settle for 2 min, after which 0.1 ml was aseptically extracted from the middle fraction with an automatic pipette and then serially diluted in tubes containing 9.9 ml of phosphate buffer solution to achieve a dilution range from 10^{-3} to 10^{-7}. Dilutions were spread on Tris-YE plates that were then inverted and incubated for 36 h at 30°C. After incubation, the colonies were counted to determine the cellular density of that soil layer, as CFU/g. Counts within the range of 30–300 were used for analysis.

**Urea agar slants**

BD urea agar slants (catalogue number 221096) were used to determine the presence of urea degraders in the soil samples. The slants were conducted in triplicate for each soil box and inoculated by using a small portion of the top layer of the white precipitate from each bioreactor that had been scraped off using a flame-sterilised loop. After inoculation, the agar slants were incubated at 35°C. A colour change was observed at 6, 24, 48, 72, 96, 120 and 148 h during incubation (Brink, 2010). A change in colour from orange to magenta indicated the presence of urea degraders.

**Specific gravity**

To provide a measure of the amount of calcium carbonate produced in the tailings, the specific gravity of the treated soil layers was measured using a Micromeritics AccuPyc 1330 helium pycnometer. Because the specific gravity of calcium carbonate (e.g. 2.54 for vaterite, 2.71 for calcite (Anthony et al., 2003)) is lower than that of the iron mine tailings (2.9–3.1 measured in this study), the biologically treated tailings were expected to have a lower specific gravity than the untreated tailings. This method provides a novel approach to characterising an increase in the amount of calcium carbonate due to biological activity. Before performing the test, soil samples were dried at 40°C for 3 d. After drying, 10 g of soil was placed into the pycnometer cup. The helium pycnometer measured the specific gravity of the soil sample five times, with the final value measured used for further analysis.

A calibration curve was developed to relate specific gravity to calcium carbonate content in the soil layers (Buikema, 2015). To develop the calibration curve, tailings were oven-dried (110°C) and then separated using a microcrusher to obtain six representative homogeneous samples. Oven-dried calcium carbonate was added by weight to the tailings to achieve various ratios of calcium carbonate to tailings content. The samples were mixed with a vortex mixer and analysed with the helium pycnometer. A six-point relationship between specific gravity and per cent by weight calcium carbonate was linear ($R^2 = 1.0$).

**Calcium carbonate by carbon dioxide pressure determination**

A second method, calcium carbonate by carbon dioxide (CO₂) pressure, was also used to determine the amount of calcium carbonate created by microbial activity in the tailings. In this method, hydrochloric acid was added to the tailings and the pressure induced by the reaction of the acid and the calcium carbonate measured. The method used was adapted from Fonnesbeck et al. (2013). An Omega PX309-015GV 0–103 kPa pressure transducer was attached by way of brass fittings to 3.13 mm (outside diameter) tubing, which was connected to a Luer lock fitting with an attached 18-gauge stainless steel 50 mm long non-corning needle. The pressure vessel consisted of a 125 ml serum bottle. Soil (1 g) was placed in the vessel with 5 ml of distilled water. The vessel was sealed with a rubber septum and aluminium crimp cap; 4 ml of 6 M hydrochloric acid HCl (with) 3 wt% ferric chloride tetrahydrate (FeCl₃·4H₂O) were then injected into the sealed vessel by using a 10 ml syringe with 50 mm long 22-gauge stainless steel non-corning needle. Ferric chloride tetrahydrate was added to prevent decarboxylation from organic material (Fonnesbeck et al., 2013; Sherrod et al., 2002). After injection, the pressure vessel was shaken every half hour (for 15 s) for 6 h to facilitate acid–soil interaction to ensure complete reaction. After 6 h, the 18-gauge needle was inserted into the septum of the pressure vessel to allow a pressure reading. The bottle was then swirled for 15 s, followed by a 10s stagnant period, to ensure that pressure measurements were consistent. Three measurements were recorded, 7 s apart, and then averaged. The pressure increase from the injection of acid was subtracted from the final pressure measurement. This was determined through six tests of injections into a serum bottle, which resulted in a 0.11 psi (0.758 kPa) increase per millilitre added with a linear regression fit of $R^2 = 0.98$ (Buikema, 2015). The pressure measurements were correlated with the calcium carbonate content of a tailings sample by way of a calibration curve conducted using serum bottles prepared with different mass ratios of tailings to calcium carbonate and a linear regression fit of $R^2 = 1.0$ (Buikema, 2015).

**Steel bearing drop test**

The strength of the mine tailings’ surface was characterised using a steel ball bearing drop test developed during this study, which was inspired by the method of Li et al. (2010) in which a ball bearing was shot at the sample using an air rifle. To perform the test, a 3.4 g steel ball bearing with a diameter of 9.4 mm was dropped from a height of 1.5 m onto the surface of the tailings sample. The diameter of the impact crater was measured in two orthogonal directions using a digital calliper and the two values were averaged to represent the average diameter of the impact crater. Five drops were conducted for each soil box. In addition, the moisture content was determined for each test box, because the strength of the tailings’ surface is also moisture dependent.
(Zwissler et al., 2017). The test results from the treated tailings samples were plotted on a calibration curve developed using an untreated tailings sample that related the impact crater diameter to the moisture content of the sample.

**ESEM and EDS analysis**
Treated and untreated samples were analysed using ESEM and EDS. All samples were first oven-dried at 40°C for 3 d. Subsequently, samples were prepared for ESEM analysis in two ways. The first sample type involved extracting a solid piece of white surface precipitate and placing it on the sample button. Carbon tape was used to secure the soil specimen onto the sample. The second sample involved extracting a piece of tailings directly from under the white surface crust to observe the tailings better. Samples were placed on the sample button and had a light dusting with an air canister to remove any loose sample particles. The prepared samples were then coated with a 10 µm thick carbon coating and placed in a sample holder. The samples were next wrapped in Parafilm and stored in a desiccator until ESEM analysis. EDS analysis was conducted simultaneously with the ESEM imaging to assess the relative ratio of calcium in the samples.

**Results and discussion**
At the conclusion of the treatment phase, each of the soil boxes, with the exception of R2*, exhibited a significant white surface crust, as shown in Figures 2(a)–2(f). As demonstrated by the top (Figure 3(a)) and side views of the crust formed in R1 (Figure 3(b)), the crust was clearly adhered to the underlying tailings. The thickness of the crust was approximately 0·75 mm, similar to the 1 mm crust developed by Stabnikov et al. (2011) in their study using sand. The development of the calcium carbonate crust by way of stimulation of MICP was expected to also result in an increase in the system pH and increased numbers of urea-degrading bacteria. These trends, as well as the measurements made after the treatment phase to quantify the microbial numbers (plate counts, urea agar slants), extent of calcium carbonate precipitation (specific gravity, carbon dioxide pressure measurements, ESEM, EDS) and strength of the surface of the treated tailings (ball drop test), are described in the following.

**pH of bioreactors**
The biological activity in the bioreactors during the treatment phase was monitored by measuring the pH of the effluent. In all
of the bioreactors, the pH ultimately converged to approximately pH 9 (Figure 4), which is consistent with the theoretical equilibrium pH of the growth medium used with ureolysis (Mortensen et al., 2011) and similar to what other researchers have observed in systems promoting urease activity with the same medium (Chou et al., 2011; DeJong et al., 2006; Stocks-Fischer et al., 1999). These data indicate that not only was S. pasteurii active in the bioaugmented reactors (R1, R1*), but that native urea degraders were also present and active in all of the bioreactors. The results also demonstrate that the mine tailings are a suitable environment for ureolytic activity to occur.

Note that, initially, the effluent pH increased above the pH of the growth medium (pH = 8.0), presumably due to the production of ammonium ions during ureolysis and the natural pH of the mine tailings (8.2–8.7). Subsequently, with the exception of R2*, which appeared to exhibit a clogging problem, the pH in all of the bioreactors decreased, probably due to bicarbonate deprotonation and carbonate precipitation, which was later followed by another pH increase that occurred presumably as precipitation slowed down and the pH increase due to ureolysis exceeded the pH decrease due to precipitation (Millo et al., 2012). The bioaugmented reactors R1 and R1* had a relatively short period...
of about 3 d before ureolysis became sufficiently dominant again to cause the final pH increase (Figure 4), whereas reactors R2, R3 and R3* had a longer periods of reduced pH, with the longest average low pH period for the autoclaved tailings in R3 and R3* (Figure 4). These results probably reflect the numbers of urea-degrading organisms present and the corresponding rates of the microbially mediated ureolysis, with the highest initial concentration of urea degraders in the bioaugmented reactors R1 and R1* and the fewest urea degraders in the reactors with autoclaved tailings R3 and R3*.

Cell counts and urea agar slants
To confirm the presence of urea degraders suggested by the pH trends and visual observations in the bioreactors, urea agar slants were conducted using the crust material. All urea agar slants exhibited colour change throughout from orange to magenta after the 24 h incubation period, indicative of the pH change associated with ureolysis. Thus, urea degraders were present in all the soil boxes, whether inoculated with S. pasteurii or not.

The addition of the urea–calcium chloride growth medium was expected to increase the cell density in the reactors. As shown in Figure 5, after the treatment phase, all bioreactors had a high cellular density near the surface of the bioreactors, decreasing with depth. Very low cell counts were observed below 2·5 cm of the surface. This was expected in R1 and R1* because the inoculum was applied to the surface and the physical straining of the S. pasteurii inoculum by the tailings would block movement of the bacteria. Straining is generally an important mechanism when the limiting dimension of the bacteria is >5% of the mean diameter of the media particles (Herzig et al., 1970) and is possible here given the typical size of S. pasteurii (average length 2·8 µm (Tobler et al., 2014), which indicates that it is very likely that the limiting dimension might be ≥1·3 µm = 5% of the d50 of the tailings (=26 µm). Interestingly, the cell counts in the surface crusts were similarly high in the reactors that were not inoculated (R2, R2*) and the autoclaved iron mine tailings (R3, R3*), confirming that native microbial populations were present and not completely destroyed by autoclaving. However, the colony morphologies on the agar plates differed for each set of conditions, indicating different bacterial communities were dominant in each case.

In all of the bioreactors, it was expected that native ureolytic microbes might be presented in deeper soil horizons, but low cell counts were observed with depth in all of the reactors (Figure 5). This could be attributed to substrate limitations (e.g. carbon and energy source, oxygen) with depth due to utilisation by bacteria near the surface. However, ureolytic activity is not negatively impacted by anoxic or aerobic conditions (Mortensen et al., 2011), so oxygen supply may not be the key factor impacting ureolytic activity, but it may impact cellular growth in deeper soil horizons (Martin et al., 2012).

The tailings used in these soil box experiments were not collected or stored aseptically, so the origin of the native microbes is not certain. However, Reasoner’s 2A agar plates (Apha et al., 1998) and urea agar plates (Burbank et al., 2012) were also performed using serial dilutions of magnetite tailings samples that were aseptically collected from five different locations in the same iron mine tailings impoundment. These spread-plate counts demonstrated the presence of heterotrophic organisms (4·4 × 10^5–4·7 × 10^6 CFU/ml), including urea degraders, in the aseptically collected samples.

Specific gravity
If MICP occurred in the soil box bioreactors, as suggested by the effluent pH and microbiological measurements, then the white surface crust should be calcium carbonate. One technique used to demonstrate the presence of calcium carbonate was changes in the specific gravity of the tailings, because untreated iron mine tailings have a measured specific gravity of 2·9–3·1,
while calcium carbonate in the form of calcite has a specific gravity of 2.71. In fact, the specific gravity decreased significantly near the surface of each bioreactors (Figure 6), with decreasing changes observed in deeper soil layers. The greatest decrease in specific gravity occurred in the reactors inoculated with *S. pasteurii*, with R1 and R1* having specific gravities of 2.59 and 2.55, respectively, at the surface. Because the measured values were below 2.71, it is hypothesised that a calcium carbonate mineral other than calcite was formed. For example, vaterite is an unstable form of calcium carbonate with a specific gravity of 2.54, and spherical structures that are typical of vaterite (Shen et al., 2006) were observed with the ESEM, as discussed in the following text.

Given that the bacterial cells mediate the changes in the solution chemistry that promote calcium carbonate precipitation and cell numbers were greatest where the largest change in specific gravity occurred, the correlation between specific gravity and cell numbers was evaluated. A strong correlation was observed (Figure 7), with $R^2 = 0.80$ for R1 and R1* inoculated with *S. pasteurii*.

![Figure 6](image1.png)

**Figure 6.** Specific gravity measurements with depth in the soil box bioreactors inoculated with *S. pasteurii* (R1 and R1*), with native microorganisms (R2 and R2*) and using autoclaved tailings (R3 and R3*)

![Figure 7](image2.png)

**Figure 7.** Relationship between cell density and specific gravity measurements for *S. pasteurii*-inoculated reactors and the reactors containing both native microorganisms and autoclaved tailings
Pasteurii and $R^2 = 0.84$ for R2, R2*, R3 and R3*. A greater specific gravity change per change in cell numbers was observed in R1 and R1*, suggesting that S. pasteurii is more effective at calcium carbonate precipitation than the native urea degraders.

Calcium carbonate
Calcium carbonate was also measured using a pressure calcimeter apparatus, with which higher pressures indicate a higher calcium carbonate content. As illustrated in Figure 8, the greatest pressures generated in each bioreactor occurred with the upper crust samples. The deeper soil layers (0.5–5 cm depth) resulted in pressure measurements of approximately 3.2 psi (22.1 kPa), which is a result of background carbonates already present in the soil sample. The greatest pressures were measured in the surface crust samples of R1 and R3, with 14.4 and 14.6 psi (99.3 and 101 kPa), respectively. Surface crust samples from R1* and R2 produced the second highest pressures at 10.5 and 9.3 psi (72.3 and 64.1 kPa), respectively, while the surface crust samples from R2* and R3* resulted in the lowest pressures at 6.6 and 9.3 psi (45.5 and 64.1 kPa). Based on the calibration curve developed in this study (Buikema, 2015), these pressures correspond to 18.3–61.6% calcium carbonate by weight in the surface crusts. These values are much higher than the calcium carbonate contents observed in some experiments with sand columns. For example, in sand column experiments using a treatment medium similar to the Stocks-Fischer et al. (1999) recipe used in this experiment, but with a higher ammonium chloride level, it was observed that the per cent calcite by mass values ranged from about 2 to 3% near the medium injection point (Mortensen et al., 2011). This is much lower than the values observed in this work with the iron tailings, but those high values were observed only at the surface.

Given that the specific gravity estimates and the pressure calcimeter measurements are both representative of the mass of calcium carbonate formed, the correlation between the two measurements was examined (Buikema, 2015). Although there was some bias in the best-fit linear regression due to the large number of data points at or near the background levels, there was a relatively strong linear correlation between the two values ($R^2 = 0.85$).

ESEM
The white surface crusts of R1, R2 and R3 were also observed using ESEM, as well as the mine tailings directly underneath the surface crust (Figure 9, with additional images in Buikema et al. (2017)). Precipitates observed in the surface crust layers exhibited a variety of forms, including amorphous solids and small rhombohedral conglomerations, along with spherical morphology. The latter morphology has been observed in other studies (e.g. Al-Thawadi and Cord-Ruwisch, 2012; Millo et al., 2012; Lian et al., 2006) and is consistent with the structure of vaterite (Shen et al., 2006). Significant rod-shaped pitting was observed, particularly in the amorphous precipitates, with less pitting noted in the spherical and rhombohedral structures, indicating that the bacteria were completely encapsulated in calcium carbonate. Interparticle bridging between tailings particles by calcium carbonate was not readily evident in the tailings directly underneath the white surface crust. However, small cubic crystals along with amorphous solids were observed in the tailings samples (Figure 9), indicating that those particles were cemented together as a result of MICP.

Energy-dispersive spectroscopy
The surface crusts and mine tailings directly under the surface crust were also analysed using EDS analysis and at the same time the ESEM observations were made. These measurements are relative ratios that depend greatly on the sample being analysed; thus, these measurements simply provide more qualitative evidence of the extent of calcium carbonate formation. As summarised in Table 1, all of the surface crusts had high
percentages of calcium, indicating significant calcium carbonate formation, which correlates with the other analytical measurements conducted on the crust samples. Consistent with the pressure calcimeter results, R1 and R2 had the highest relative percentage of calcium in the crust layer at 65 and 49.5%, respectively, followed by R3 at 44%. The tailings directly beneath the white surface crusts had lower percentages of calcium carbonate and a higher relative percentage of iron, further indicating that most biological activity occurred in the upper 1 mm of soil depth.

Figure 9. ESEM imaging of the surface crust in soil box reactors inoculated (a) with *S. pasteurii* (×1000 magnification), (c) with native microorganisms (×2000 magnification) and (e) using autoclaved tailings (×1500), along with the iron mine tailings directly underneath the corresponding surface crust: (b) ×1000, (d) ×1500 and (f) ×1500.
The final analysis was the ball drop test, which was used to test whether the surface crust formed by way of MICP resulted in an increase in surface strength of the treated tailings compared with untreated tailings at the same moisture content. Specimens were tested at different moisture contents because the soil boxes dried at different rates after the treatment phase. As illustrated in Figure 10, the surfaces of all of the soil box reactors resulted in a reduction in the diameter of the impact crater from the steel ball compared with the untreated tailings at the same moisture content. These results indicate that MICP promoted by *S. pasteurii* and the native microorganisms was able to contribute to an increase in the strength of the treated tailings surfaces. Additionally, the results suggest that the ball bearing drop test is a potentially useful technique for evaluating the surface strength of engineered and natural biological surface crusts. Many previous studies have used the needle penetrometer test to evaluate the surface strength of engineered and natural biological surface crusts. Many previous studies have used the needle penetrometer test to evaluate the surface strength of biological soil crusts (e.g. Langston et al., 2005; Rice et al., 1997), but in preliminary tests performed during this research, the needle penetrometer was inconclusive in determining increases in surface strength generated by MICP on the iron tailings.

**Practical relevance and potential applications**

Fine-grained mine tailings pose significant hazards to the environment when emitted into the atmosphere. Biomediated approaches such as MICP represent a possible solution, but most MICP studies to date have focused on coarse-grained soils, so little information is available regarding application to fine-grained soils. This research suggests that the bacterium *S. pasteurii* can effectively improve the surface strength of fine-grained, alkaline soil through the process of MICP, resulting in the production of a surface crust that improves soil strength at the atmosphere–soil surface interface. Nevertheless, application of MICP to fine-grained materials such as iron mine tailings does pose challenges. For example, the results presented earlier indicate that fewer added cells were able to penetrate into the deeper soil horizons, and/or the inoculated or native cells were less likely to be stimulated in the deeper soil horizons, due to the substrates being consumed in the upper soil layers where biological activity was highest. This is expected as the effectiveness of MICP depends on the soil being sufficiently permeable to allow adequate flow of injected chemicals to the bacteria. The effectiveness of MICP also depends on the number of particle contacts available within the soil matrix because, the more particle contacts there are, the greater the impact of precipitation on the soil matrix (Mitchell and Santamarina, 2005). Dense, well-graded soils such as iron tailings should have a higher number of particle contacts per unit volume compared with loose, poorly graded soils (Mortensen et al., 2011); however, the transport of cells and amendments is limited. For example, Mortensen et al. (2011) found that coarser and well-graded sands had a faster rate of precipitation than finer and more poorly

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**Table 1. EDS analysis of surface crust layer from the soil box bioreactor experiment**

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Sample type</th>
<th>Carbon</th>
<th>Oxygen</th>
<th>Silicon</th>
<th>Chlorine</th>
<th>Calcium</th>
<th>Iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>Crust</td>
<td>6.32</td>
<td>27.24</td>
<td>0.25</td>
<td>0.65</td>
<td>64.63</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>Tailings</td>
<td>10.74</td>
<td>45.82</td>
<td>23.89</td>
<td>1.69</td>
<td>1.41</td>
<td>16.45</td>
</tr>
<tr>
<td>R2</td>
<td>Crust</td>
<td>—</td>
<td>48.93</td>
<td>0.78</td>
<td>0.24</td>
<td>49.52</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>Tailings</td>
<td>42.11</td>
<td>11.88</td>
<td>1.03</td>
<td>24.79</td>
<td>20.19</td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td>Crust</td>
<td>—</td>
<td>54.69</td>
<td>0.09</td>
<td>1.06</td>
<td>43.94</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Tailings</td>
<td>—</td>
<td>52.42</td>
<td>9.18</td>
<td>0.52</td>
<td>32.03</td>
<td>5.85</td>
</tr>
</tbody>
</table>

The crust sample refers to the white surface crust; the powder sample refers to the mine tailings directly under the white crust.

---

**Figure 10.** Results of crater diameter from ball drop tests on biotreated and untreated iron tailings as a function of the sample moisture content. 1 inch = 25.4 mm. Each symbol represents the average of five measurements and the error bars represent ± one standard deviation. Note that a high standard deviation was calculated at a moisture content of 0%, due to some ball drops not creating a crater.
graded soils. Very coarse soils and very fine soils had the slowest rates of precipitation.

Relative costs for such bacterial treatment on a large scale have not been examined. Nevertheless, the application to mine tailings impoundments may provide a better alternative to conventional dust mitigation practices, due to the frequent addition of fresh mine tailings to the mine tailings impoundment.

Conclusions

A key environmental hazard associated with fine-grained iron mine tailings is the potential for the generation of dust storms due to the shear stresses introduced from wind. One potentially innovative approach to modifying the surface properties of mine tailings, and thereby providing dust mitigation, is to apply biomediated methods such as bioaugmentation. In this study, inoculated *S. pasteurii* was demonstrated to be able to stimulate MICP by way of ureolysis, producing a surface crust on the tailings surface, as demonstrated through a variety of analyses, including changes in pH, microbiological measurements (i.e. by way of cell plate counts and urea degrader slants), calcium carbonate measurements (i.e. by way of specific gravity, pressure calcimeter) and ESEM and EDS. Importantly, native urea degraders were discovered to be present in the tailings during the treatment phase of the experiment. Ureolysis by the native microbes also resulted in measurable changes in the composition and properties of the surface crust on the tailings that were similar in magnitude to the changes observed in the *S. pasteurii*-bioaugmented reactors. This discovery opens the possibility that MICP could potentially be applied to iron tailings by biostimulating native microorganisms, rather than by bioaugmentation with non-native urea-degrading microbes.

The native and inoculated microorganisms were also able to produce effectively a surface crust that contributed to a measurable surface strength increase. These results indicate that naturally present urea degraders could effectively achieve similar surface strength increases as *S. pasteurii*, which would reduce the potential costs and environmental impact of inoculating a large surface area. The ball bearing drop test proved useful in preliminary strength testing of the surface crusts and, with further development, may be a useful tool for assessing surface crust strength of biologically mediated crust formation. These preliminary results provide a foundation for further research to advance the application of MICP in fine-grained materials for geotechnical purposes such as dust mitigation.

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Stabilisation of iron mine tailings
through biocalcification
Buikema, Zwisssler, Seagren, Oommen and Vitton

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