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Recent Advances in Microbial Induced Carbonate Precipitation for Waste Management

Samantha Wilcox, **Catherine N. Mulligan**
Concordia University, Montreal, Canada, catherine.mulligan@concordia.ca

Carmen M. Neculita
University of Quebec in Abitibi-Témiscamingue, Rouyn-Noranda, *Canada*

ABSTRACT: Microbially induced carbonate precipitation (MICP) is an innovative bioremediation solution. The method uses microorganisms as a biochemical enhancement to the cementation process, where contaminants are contained and entrapped *in-situ*. The microorganisms facilitate precipitation of $CaCO_3$ crystals within the pore spaces of particles to cement the material forming a biocement matrix. Through urea hydrolysis, the bacteria increase the dissolved inorganic carbon and pH within the material causing favorable conditions for carbonate precipitation. Application of MICP was applied to 2 slag materials to evaluate the potential for remediation. A physiochemical characterization of the iron-rich materials was conducted using x-ray fluorescence (XRF). After 3-flush cycles and 3 weeks of curing, the orange sample (OS) exhibited strong cementation resisting slaking over an 18 h period. However, while the black sample (BS) initially appeared to have cemented, the particles quickly degraded during the first hour of the slake test. Both iron-rich slag materials showed a lack of remediation. Metal and metalloid (metal(loid)) concentrations in the control were lower than the MICP treated samples. Further, the pH of the leachate from the OS was outside acceptable limits, while the BS demonstrated higher pH of the leachate from the MICP treated samples in comparison to the controls. Additional flush cycles over a longer period are required to enhance $CaCO_3$ precipitation and, therefore, formation of the biocement matrix to entrap metal(loid) contamination.

KEYWORDS: MICP, urease, slag material, metalliferous waste, remediation.

1 INTRODUCTION

Microbial induced carbonate precipitation (MICP) has gained recent traction in the fields of civil engineering as an emerging technique for soil and cement strengthening. The method focuses on the precipitation of carbonate crystals through bacterial metabolic activity. The crystals can bind together to form a cement matrix that increases the unconfined comprehensive strength of a material. The same principles can be used for biological remediation of contaminated waste. The method can immobilize and/or sequester contaminants via precipitation, sorption, encapsulation, or detoxification (Wilcox et al., 2024).

Ureolytic MICP uses bacteria to facilitate carbonate precipitation through bacterial enzymatic activity. The bacteria secrete the enzyme urease, which acts as a catalyst for urea hydrolysis. Urea hydrolysis increases pH, ammonium concentration and dissolved inorganic carbon in the extracellular environment. In the presence of a calcium source the increased pH leads to precipitation of calcium carbonates ($CaCO_3$; calcite, aragonite, vaterite).

Metalliferous waste is a novel substrate for MICP application. These materials are derived from multiple mining activities including mineral extraction (waste stockpiles), mineral processing (tailings), and smelting and refining (slags). To date, most MICP studies involve sand, soil, or cement. The use of metalliferous waste is more complex as it can provide an inhospitable environment for bacterial growth and survival.

The goal of the present study is to evaluate the efficacy of MICP to metalliferous waste. The study characterizes the chemical composition of slag material using XRF. The study applies MICP using a top-bottom flush cycle approach to cement materials. Leaching is analyzed from the treated samples and compared to a control to establish the efficacy of MICP for waste management.

2 MATERIAL AND METHODS

2.1 *Materials*

Two slag materials were provided by a smelter facility located in Quebec. The slag materials are labelled according to their color: (i) orange sample (OS); (ii) black sample (BS). These materials were transported to the laboratory and refrigerated prior to testing.

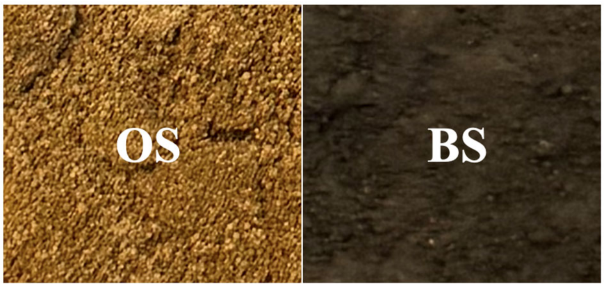

Figure 1. Slag materials OS (left) and BS (right).

2.2 *Sample characterization*

A physiochemical characterization was completed on the slag materials. The chemical composition of the samples was conducted using XRF with Malvern Panalytical Epsilon 1. The pH was measured according to (ASTM, 2019). The oxidation-reduction potential (ORP) and electrical conductivity (EC) was measured using the Aqua TROLL 600 Vented.

2.3 *Bacterial suspension*

Experiments used *Sporosarcina pasteurii* (ATCC 11859) purchased from the American Type Culture Collection. 5% v/v *S. pasteurii* was inoculated in 200 mL of sterile nutrient broth. The nutrient broth is composed of 13 g/L autoclaved nutrient broth (Oxoid CM0001) supplemented with 20 g/L filter sterilized urea (Cas No. 57-13-6) with 0.22 μm filters. Each flush cycle utilized overnight grown cultures with bacteria suspended to an optical density at 600 nm (OD_{600}) of approximately 0.8 with a pH of 9.5. The OD_{600} was measured with the Thermo Scientific Evolution 201 UV Visible Spectrophotometer. All autoclaving occurred at 121°C for 45 min.

The remaining bacterial suspension was centrifuged (8000 rpm, 10 min) then filter sterilized (0.22 µm) prior to test the supernatant for pH using the Thermo Scientific Orion 2 Star pH Benchtop.

2.4 *MICP treatment*

MICP application used a top-bottom flush cycle approach as shown in Figure 2. 50 mL centrifuge tubes were seared at the bottom and filled with cotton balls to block the hole prior to autoclaving. The sterile tubes were filled with 10 g of dry slag material. Flushing occurred 3 times every 12 h, before curing over 3 weeks. A 1:1 ratio of slag material to bacteria suspension (i.e., 10 mL) was applied. The bacterial suspension was permitted to drain from the columns prior to injection of a 1:1 ratio of sterile cementation solution composed of 13 g/L nutrient broth, 20 g/L urea and 2.8 g/L calcium chloride (Cas No. 10035-04-8). Experiments were compared to a control without bacterial suspension (i.e., application of sterile broths).

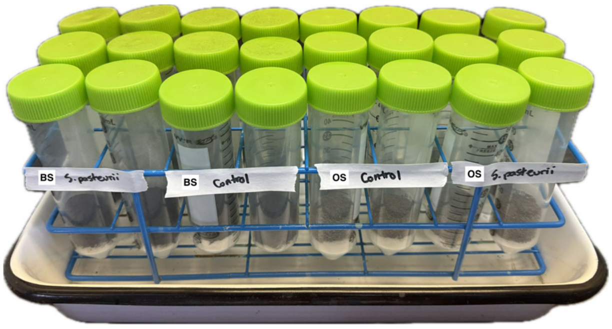

Figure 2. MICP column testing with to BS (left) and OS (right). Testing included *S. pasteurii* and controls.

2.5 *Slake Test*

The slake test was measured according to ASTM D4644-16 with slight modification (ASTM, 2016). In short, samples were submerged in DI water for 18 h. The samples were described at 1 h and 18 h according to 6 classifications described by (Mwandira et al., 2019). These include: (i) degrades to a pile of flakes or mud; (ii) forms many chips; (iii) forms few chips; (iv) develops several fractures; (v) develops few fractures; (vi) no change.

2.6 *Leaching*

Leaching was performed according to EPA Method 1315 with slight modifications (EPA, 2013). Samples were submerged in deionized (DI) water and the container was sealed to mitigate carbonation. The leaching was measured at 1, 3, 6, and 18 h. After each interval, the sample was inserted into a clean vessel with fresh DI water. The effluent was analyzed for metal(loid)s concentrations, pH, ORP, and EC. Metal(loid)s were analyzed using inductively coupled plasma mass spectroscopy (ICP-MS) Agilent Technologies ASX-500 Series Autosampler and 7700 Series. The pH was analyzed using the Thermo Scientific Orion 2 Star pH Benchtop. EC was measured with the Fisherbrand Traceable Conductivity tester and ORP was analyzed using the Oakton ORPTestr® 50.

3 RESULTS

3.1 *XRF results*

The chemical makeup of both samples is described by Table 1. From the XRF results, OS is composed of height weight percent (>5%) Al_2O and Fe_2O_3, while BS is composed of SiO_2, Ti, Fe_2O_3. Since both samples are primarily composed of Fe_2O_3 they are described as iron-rich slag materials. The pH, ORP, and EC of the metalliferous wastes are shown in Table 2.

Table 1. XRF results of slag materials

Parameter (%)	OS*	BS*
Al_2O_3	5.33 ± 0.57	4.05 ± 2.02
SiO_2	0.17 ± 0.24	8.34 ± 3.88
P_2O_5	0.21 ± 0.01	0.27 ± 0.01
Cl	1.29 ± 0.17	-
CaO	1.44 ± 0.06	1.76 ± 0.48
Ti	0.32 ± 0.01	11.73 ± 2.45
V	0.84 ± 0.05	0.18 ±0.02
Cr	0.35 ± 0.03	-
Mn	0.79 ± 0.05	0.19 ± 0.01
Fe_2O_3	37.53 ± 3.56	27.70 ± 3.62
SO_3	-	0.43 ± 0.21
K_2O	-	0.43 ± 0.11
Zn	-	0.12 ± 0.01
Zr	-	0.11 ± 0.01
Eu	-	0.11 ± 0.02

*Concentrations less than 0.1 % are not noted. Tests were run in duplicate.

Table 2. Chemical properties of slag materials

Time	OS	BS
pH	10.04 ± 0.06	8.27 ± 0.06
ORP	144.38 ± 6.04	197.12 ± 12.86
EC	39.86 ± 25.06	7.78 ± 0.04

3.2 *Slake test*

The slake test is used to qualitatively analyze the stability of the MICP treated samples to assess its resistance to erosion. After 3 weeks of curing both samples exhibited cementation in the controls and MICP treated samples (Figure 3). The OS exhibited more cementation in the treated samples, while the BS appeared the same.

Figure 3. OS (left) and BS (right) after 3 weeks of curing. The controls are shown on top and the MICP treated samples on bottom. The duplicate experiments are shown.

The classifications from the slake test are provided in Table 3. After 1 h submerged in DI water, the OS remained intact exhibiting no change in the control or MICP treated samples. However, the BS formed many chips in both the treated and control samples. After 18 h (Figure 4), the BS degraded, while the OS remaining as they were after curing.

Table 3. Slake test results of cemented slag materials

Sample	Slake Classification	
Time	1 h	18 h
OS Treated	VI	VI
OS Control	VI	VI
BS Treated	II	I
BS Control	II	I

Figure 5 illustrates the strong resistance of physical degradation of the MICP treated sample after 18 h submerged in water. The cementation remains intact, and the duplicate samples show high reproducibility. The carbonate precipitates at the surface create a crust that resists degradation and erosion, which is beneficial for physical degradation caused by rainfall and other weather conditions.

Figure 4. OS (left) and BS (right) after 18h submerged in DI water. The controls are shown on top and the MICP treated samples on bottom. The duplicate experiments are shown.

Figure 5. Treated OS after 18 h of slake test.

3.3 *Leaching*

The pH, ORP, and EC of the leachate are shown in Figure 6. The recorded values of these parameters for both samples are high with and without treatment. This is likely due to the high metal content of the samples, specifically Fe. The ORP of both samples decrease over time. The ORP of the BS slightly decreased with the MICP treated sample but increased with the OS in contrast to their control. The pH in the leachate from both the OS MICP treated and controls lie outside acceptable ranges indicating the treatment has not adequately treated the samples. Alternatively, the pH of the BS is within acceptable limits. However, the pH of the treated samples was slightly higher than the controls. This is likely due to the pH increase caused by application of ureolytic bacteria. Since the samples did not adequately biocement, the high pH in the leachate was caused biochemically by the bacteria. Further, the BS EC decreased over time, but the MICP treated sample EC was marginally higher at 18 h. Conversely, the MICP treated OS increased with time and again was higher than the control at 18 h.

The metal(loid) leaching is shown for the OS and BS in Figures 7 and 8, respectively. Mg is not shown in the graphs as the concentrations are higher than other metal(loid) ranges. Mg leachate reached a maximum of 112.4 and 100.3 mg/L for the OS treated and control samples, respectively. The Mg was marginally sequestered in the MICP treated samples. However, the BS showed maximum Mg leaching at 47.2 and 4.9 mg/L for the treated and control samples, respectively. Mg was not sequestered via MICP treatment.

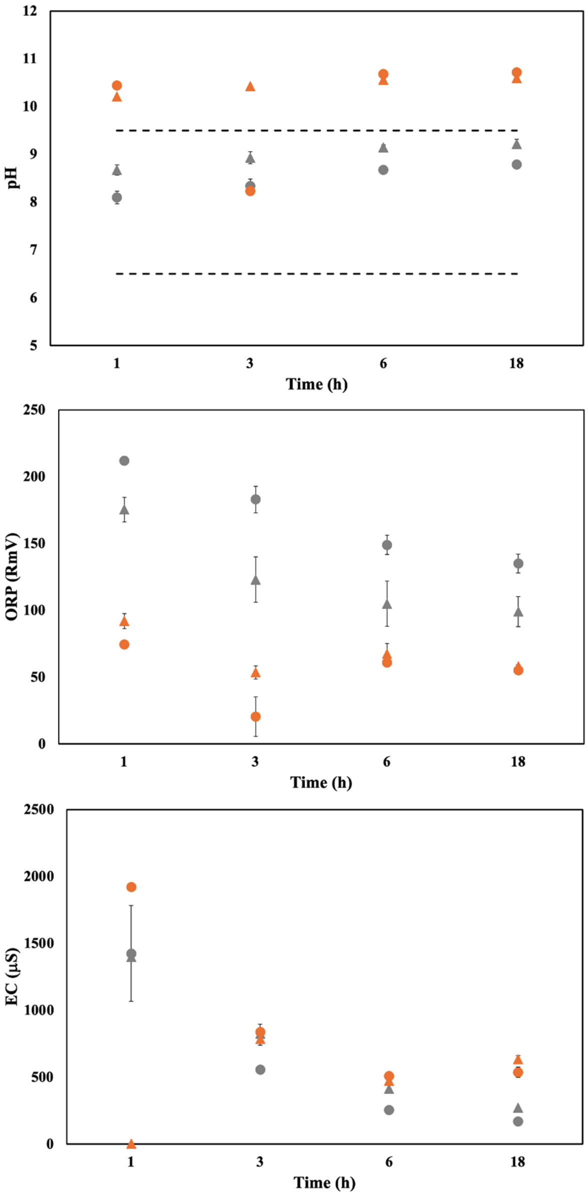

Figure 6. pH (top), ORP (middle), and EC (bottom) of leachate at time intervals. The legend includes: (i) controls are circles; (ii) MICP treated samples as triangles; (iii) OS as orange; (iv) BS as grey. The pH graph shows the acceptable ranges as dashed lines.

Both the BS and OS demonstrated higher metal(loid) concentrations in the leachate with the MICP treated samples in comparison to the control for K, V, Na, and P. This is likely attributed to the non-uniform characteristics of the waste material, which has a high variation in metal(loid) content. The biocement matrix was not suitable for entrapment and sequestration of metal(loid) leaching. This is particularly interesting for the OS, which demonstrated a stable cement matrix. Despite cementation, the $CaCO_3$ precipitation likely did not fill the void spaces of the sample allowing the mass transfer of contaminated leachate to flow from that sample.

MICP application to slag materials could be improved with increased flush cycles over longer periods, Flushing should occur at 24 h time intervals over a week to permit adequate bacterial precipitation. The MICP treatment over 12 h increments could have flushed the existing adsorbed bacteria and precipitated $CaCO_3$ crystals before the formation of the biocement matrix.

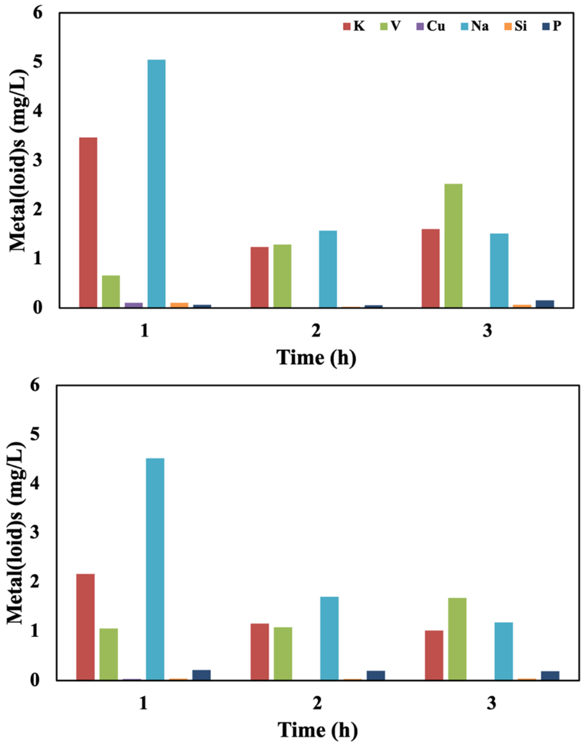

Figure 7. OS metal(loid) leaching from MICP treated sample (top) versus its control (bottom.

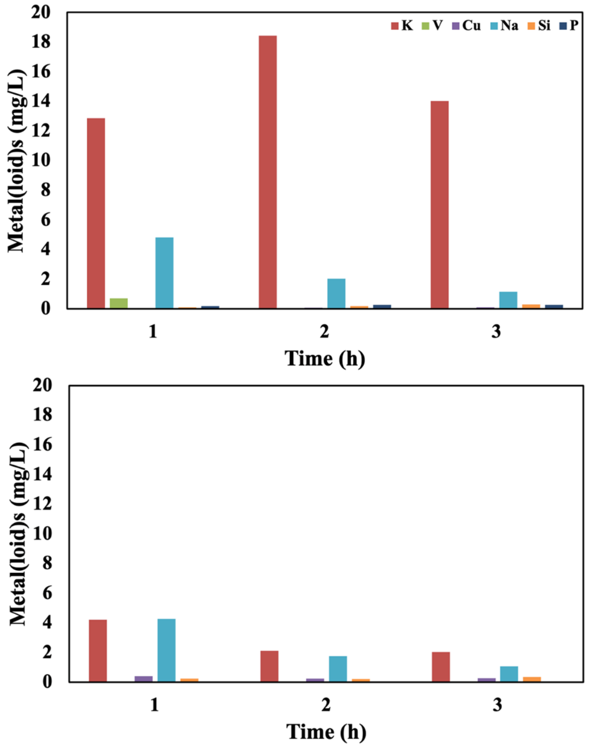

Figure 8. BS metal(loid) leaching from MICP treated sample (top) versus its control (bottom).

4 CONCLUSIONS

The aim of this study was to evaluate MICP treatment for two slag materials for waste management. MICP application included 3-flush cycles of bacterial suspension followed by cementation solution then 3 weeks of curing. The OS formed a biocement matrix that was able to withstand 18 h of slaking. However, short term MICP treatment to both iron-rich materials was unsuccessful. The pH and metal(loid) concentration in the leachate from the samples did not reduce leaching by the samples. Application of additional flush cycles over a longer time is required to promote $CaCO_3$ precipitation between solid particles promoting remediation.

5 ACKNOWLEDGEMENTS

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