MICROBIAL INDUCED CALCIUM CARBONATE PRECIPITATION (MICCP) FOR ROAD CONSTRUCTION

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ABSTRACT

The growing concern over climate change has led the drive for the development of alternative building materials in several industries, including road construction. Bio-based construction, using Microbial Induced Calcium Carbonate Precipitation (MICCP) has been investigated in recent years as a potential cost-effective and environmentally friendly alternative engineering approach. The Council for Scientific and Industrial Research (CSIR) developed a research program looking at MICCP. Several barriers to using MICCP in road construction was found, a potential biohazard using exotic bacteria and the current technique used for treatment. In this paper, *in situ* cultivation of indigenous urease positive bacteria was investigated and compared to a CSIR designed biological prototype. The objective of this paper is to present the results of Unconfined Compressive Strength (UCS) tests performed on a marginal G5 (COLTO, 1985) material treated with the prototype and *in situ* cultivated bacteria.

The work showed that it was possible to cultivate urease positive bacteria present within the G5 material. It was found that the cementation solution could act as a stimulation and cementation media when the pH is reduced to give the bacteria time to cultivate and buffer the pH upward for Calcium Carbonate Precipitation to take place. Lastly, the CSIR prototype performed better in terms of UCS and treatment technique. The treatment consists of only one application of the prototype, which is more consistent with current road construction practice, as compared with the multiple application needed for *in situ* cultivation.

Keywords: Microbial Induced Calcium Carbonate Precipitation (MICCP), UCS, alternative pavement materials, bio-stabilisation.

1. INTRODUCTION

The growing concern over climate change has led the drive for the development of alternative building materials in several industries, including road construction. The condition of a countries' road network is closely related to its economic and social well-being and several methods of stabilizing road building materials exist, with cement stabilization using Ordinary Portland Cement (OPC) being one (Smit, *et al.,* 2021). The production of OPC, however, is both energy consuming and environmentally unfriendly (Ramdas *et al.*, 2021b). Twelve to fifteen percent of global industrial energy consumption and 7-9% of gross anthropogenic CO_2 release is attributed to the cement industry (Ahmad *et al.*, 2021). For this reason, bio-based construction, using microbial induced calcium carbonate precipitation (MICCP) has been investigated in recent years as a potential cost-effective and environmentally friendly alternative engineering approach (Ramdas *et al.*, 2021b).

MICCP binds material through the formation of calcium carbonate bridges between soil grains. Under specific environmental conditions, urease positive bacteria use urea as enginery source, producing ammonia and carbon dioxides which in turn increase the pH of the surrounding environment. Bicarbonate becomes carbonate promoting the influx of calcium ions and proton expulsion. The calcium irons react with the carbonate and hydroxide ions due to the net pH increase, forming calcium carbonate bonds (Figure 1) (DeJong et al., 2010; Canakci et al., 2015; Portugal et al., 2020). These calcium carbonate bonds are present in three crystalline forms in nature – aragonite, valerite and calcite. The most stable form is calcite, which presents under the trigonal or rhombohedral crystalline structure (Portugal et al., 2020).

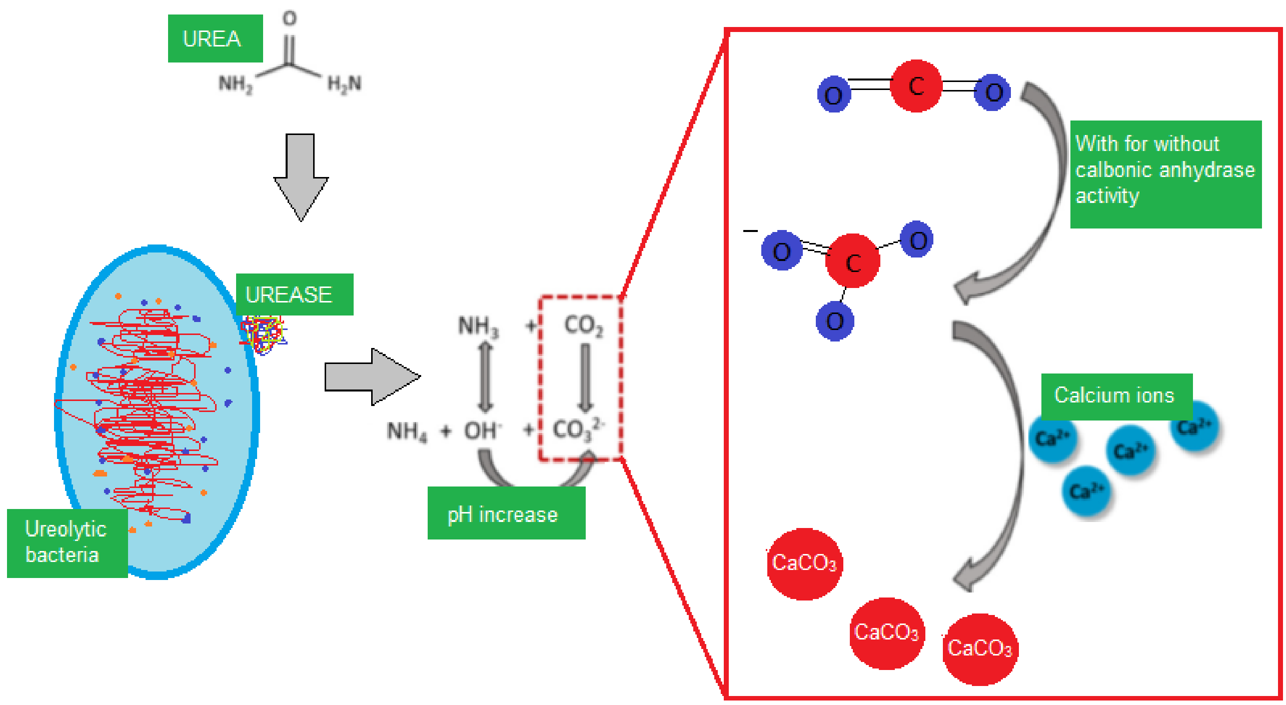

Figure 1: Process of Calcium Carbonate Precipitation by Urease Positive Bacteria

MICCP is novel, natural, environmentally friendly and durable (De Muynck *et al.*, 2010; Akyol *et al.*, 2017). This biogenic technology has been used as early as 1996 to decrease hydraulic conductivity in highly permeable water channels (Ferris *et al.*, 1996) and has since been used for several engineering applications aimed at improving the engineering properties of soil. Some of these include:

- Improved unconfined compressive strength (Dove *et al.*, 2011; Stabnikov *et al.*, 2013; Gomez *et al.*, 2014; Putra *et al.*, 2016; Salifu *et al.*, 2016; Aamir *et al.*, 2018; Jijian *et al.*, 2019; Osinubi *et al.*, 2019).
- Increased shear strength characteristics (DeJong *et al.*, 2006).
- Decreased hydraulic conductivity (Ferris *et al.*, 1996; Yasuhara *et al.*, 2011; Soon *et al.*, 2013; M Gomez *et al.*, 2014; Carrel *et al.*, 2018).

1.1 Advantages of MICCP

MICCP, compared to other chemical treatments, requires less energy, can be carried out beneath existing structures, works at ambient temperatures (Osinubi *et al.*, 2020), allows for the treatment of large areas (Akyol *et al.*, 2017) and is considered to be environmentally friendly compared to OPC.

Pascal *et al.* (2009) and Naeimi and Haddad (2018) compared the cost of producing the materials needed for bio-cementation with the cost of cement production and found it to be cheaper. MICCP cost can be reduced even more since up to 30% of the production cost is made up of the chemical substrates, equipment and manpower needed for the sterilization of the growth medium (Yasodian *et al.*, 2012). Therefore, cultivating the bacteria in nonsterile conditions may reduce the price of MICCP treatment further. In addition, Yasodian *et al.* (2012) reported that unsterilized specimens showed a greater reduction in plasticity characteristics compared with sterilized specimens, suggesting that the other microbial species might be taking part in the MICCP process.

1.2 Barriers to the Use of MICCP in Road Construction

The following barriers to the use of MICCP in road construction have been identified:

- Non-homogenous treatment – Microorganisms, cementation reagent and sufficient nutrients are required for proper MICCP soil treatment (Zhu and Dittrich, 2016). Cementation reagent and nutrients can be rapidly exhausted due to: (1) nutrients being depleted over time, (2) not providing enough nutrients initially, (3) the flow rate of cementation reagent through the soil being too fast for a reaction to take place (Gomez *et al.*, 2014) or (4) the flow of the reagent is to slow resulting in clogging (Cheng *et al.*, 2017). This leads to an uneven distribution of nucleation sites and non-homogenous layers through the treated area.

- Potential biohazard from exotic bacteria introduction – *Bacillus spp* and *Sporosaricina spp* is the most common bacteria used in MICCP and may be exotic to the location being treated. Bacterium death due to lack of nutrients and physical isolation may reduce the risk of gene escape. This should be looked at on a case-to-case basis as it involves several variables such as competition and predation of the microorganisms already in the soil. Using native bacteria may reduce the cost as the bacteria doesn't have to be imported and cultivated in sterile environments, but no analyses about the risk-evaluation of potential biohazard contamination from the introduction of bacteria has been developed (Portugal et al., 2020). Burbank *et al.* (2011) developed a method of *in situ* cultivation of indigenous soil urease positive bacteria prior to treatment thus reducing the price and potential biohazard.

- Lack of testing with road building materials and incompatible treatment techniques – Most of the testing conducted to date has focused on sand stabilization and only recently on road base material (Porter *et al.*, 2018). MICCP treatment techniques have focused on improving or replacing the conventional technique of *in situ* grouting. Traditional *in situ* grouting techniques are expensive, time consuming, require heavy machinery and is not good for the environment (Cheng, 2012). MICCP *in situ* grouting is a good alternative to the traditional method, but is not practical for use in road construction, due to the cost, use of injection wells, the repeated application of the cementation reagent and the time it takes which can be anything between 20 to 27 days. Road construction consists of mechanically mixing cement or lime into the soil and compacting it. Thus, a one-step process like traditional construction methods is required if MICCP is to be used in future.

MICCP has shown promise and warrants further investigation for use in road construction. For this reason, the Council for Scientific and Industrial Research (CSIR) developed a research program looking at MICCP. The CSIR has a long history of product and process development using *Bacillus spp* organisms (Lalloo *et al*., 2007) and past research serves as the foundation for this study, which aims to provide sustainable solutions to address the challenges faced by road construction. In this paper, a novel method of *in situ* cultivation of indigenous urease positive bacteria present in road building material was investigated and the results are compared with a prototype bio-stabilizer developed by Ramdas *et al.* (2020) in the Chemicals Cluster of the CSIR. The objective of this the paper is to present the results of Unconfined Compressive Strength (UCS) tests performed on a G5 (COLTO, 1985) material treated with the prototype and *in situ* cultivated bacteria. This work forms part of an alternative pavement materials programme at the CSIR that also investigates the use of nano-modified emulsions (Akhalwaya *et al*., 2020; Rust *et al*., 2020; Smit *et al.*, 2021).

2. METHODOLOGY

2.1 Soil Samples

It is anticipated that MICCP will ideally be used for subbase/subgrade stabilization, hence G5 (COLTO, 1985) soil samples obtained from a test site located near Diepsloot were used in the laboratory evaluation. This specific G5 material was previously used by Akhalwaya & Rust (2018) and was therefore similarly used for this laboratory evaluation in order to understand current laboratory results comparatively with previous results on the G5 material stabilized with nano-modified emulsions. X-Ray Diffraction (XRD) scans of the G5 materials (COLTO, 1985) was conducted by the CSIR (Akhalwaya & Rust, 2018; Jordaan et al. 2017) and indicate that the material may be classified as marginal due to the high contents of problem minerals. This is due to the approximately 17-20% Mica (Muscovite) and 7-43% clay minerals (Smectite/Kaolinite) present in the soil. Table 1 contains further properties and descriptions of the untreated soil samples. Although some of these properties exceed the maximum criteria stipulated for a G5 material, the aim of this laboratory study is to serve as a proof-of-concept for granular material stabilization and MICCP will be used on G6-G8 materials in the near future.

Table 1: Summary of classification results for K46 G5 untreated material (Akhalwaya & Rust, 2018)

Sample Description, Information and Properties		Atterberg Limits (TMH1, 1986: Methods A2&A3)	
Sample Name	K46 Diepsloot	Liquid Limit %	19.37
Material Classification	G5 (COLTO, 1985)	Plasticity Limit %	16.11
Stabilising Agent	Untreated	Plasticity Index%	3.26
pH Value	8.07	Electrical Conductivity	0.01

Table 1: Cont'd

Sieve Analysis - % of material passing sieves (TMH1, 1986: Method A1a)		Compactions (TMH1, 1986: Method A7)	
Sieve Size (mm)	% passing each sieve		
75.0 mm	100.00	MOD AASHTO: Max Dry Density (MDD) (kg/m^3)	2096
53.0mm	100.00	Optimum Moisture Content (OMC) (%)	7.4
37.5 mm	100.00	Dry Density achieved (kg/m^3)	2099
26.5 mm	97.5	% of Max Dry Density (MDD)	100
19.0 mm	92.2	Moulding Moisture Cont. (%)	7.8
13.2 mm	86.1	% Swell	0.20
9.5 mm	79.2	**Soaked California Bearing Ratio (CBR) (TMH1, 1986: Method A8)**	
6.7 mm	71.4		
4.75 mm	69.4	100 % Mod AASHTO	102
2.00 mm	49.4	98 % Mod AASHTO	82
0.850 mm	31.1	95 % Mod AASHTO	65
0.425 mm	20.1	93 % Mod AASHTO	39
0.250 mm	14.0		
0.150 mm	9.9		
0.075 mm	6.6		

2.2 Bacteria Samples and Soil Treatment

2.2.1 Prototype

Bacillus licheniformis from the Netherlands Culture Collection of Bacteria, NCCB 100133 was prepared as described by Ramdas *et al*. (2020) and developed into a prototype. The prototype was added as a bio-based stabiliser to the UCS samples at a concentration of 0.025l/kg of soil (Mgangira, 2009) (Table 2).

Table 2: Details of samples

Identifier	Description	Treatment	Curing Time
Prototype	G5 material + prototype	One application of prototype	3 days
SC	G5 material + Stimulation + Cementation	10 applications of Stimulation solution 10 applications of Cementation solution (pH 6)	3 days
C	G5 material + Cementation	20 application of Cementation solution (pH 6)	3 days

2.2.2 *In Situ* Cultivation

Two cultivation methods were used during this study, however all UCS samples were prepared by replacing the water with the stimulation solution at OMC shown in Table 3. The samples were kept at ambient temperature for 20 days. Depending on the cultivation method, each day, one pore volume of a solution was added to the top of the samples and allowed to filter through by means of gravity.

The first set of samples (SC) were cultivated using an adjusted method suggested by Gomez *et al.* (2014) (Table 2). For the first 10 days, one pore volume of stimulation solution was added followed by cementation solution the next 10 days (Table 3).

The stimulation solution is used to cultivate urease positive bacteria already present in the soil samples. The cementation solution allows calcium carbonate precipitation to take place. The role of the bacteria during calcium carbonate precipitation is to increase the pH, as discussed in the introduction. At a pH of 8, the cementation solution will result in immediate precipitation, resulting in clogging and non-homogeneous treatment. To prevent this Gomez *et al.* (2014) used a transition solution applied on day 11 consisting of Tris Base (0.01 mol/L). Cheng *et al.* (2019), however, showed that decreasing the pH of the cementation solution gave the bacteria more time to increase the pH thus allowing the cementation solution to penetrate the sample before calcium carbonate precipitation could take place, resulting in a homogeneous treatment. For this reason, the pH of the cementation solution was reduced to 6 with HCL solution.

The second set of samples (C) received one pore volume of cementation solution every day for 20 days, and no stimulation solution after UCS samples were prepared (Table 2). Again, at a pH of 8, the addition of cementation solution will result in immediate precipitation of calcium carbonate. Since the stimulation solution and cementation solution differ only with the addition of calcium chloride, as shown in Table 3, the cementation solution can act as both cultivation and cementation medium if immediate precipitation can be prevented. Thus, by lowering the pH of the cementation solution, the bacteria have time to cultivate in addition to increasing the pH for calcium carbonate precipitation to take place. The pH of the cementation solution was reduced to a pH of 6 with HCL solution. This method was based on the one-phase low pH injection method suggested by Chen e*t al.* (2019).

Table 3: Summary of Treatment Solution Constituents

Constituent	Stimulation Solution	Cementation Solution
Urea (mol/L)	0.5	0.5
Ammonium Chloride (mol/L)	0.0125	0.0125
Sodium Acetate (mol/L)	0.17	0.17
Yeast Extract (g/L)	0.1	0.1
Calcium Chloride (mol/L)	-	0.25
Initial Solution pH	7.6	8.0

After all the test samples were prepared, they were cured for 24 hours in an oven at 30°C before being subjected to a rapid curing process in an oven for 48 hours at 40°C - 45°C (temperatures in the oven did not exceed 50°C). The pH of the effluent was also monitored during the 20-day treatment phase to determine the ureolytic activity.

2.3 Strength Testing

Three UCS test samples of each treatment technique were prepared and tested. UCS (dry) tests were conducted after curing in accordance with the SANS 3001-GR53 and samples were mixed and compacted accordance to SANS 3001-GR50. The OMC was 6% and compaction effort was 5 layers 55 blows (SANS 3001-GR50).

Cement treated samples were also prepared to compare the results to a more traditional stabilizing agent. The samples were stabilized using 0.7% cement and cured as described above to keep the variables the same.

The test results were also compared with results obtained from a study by Porter *et al.* (2018) that looked at road base material treated with MICCP. It should be noted that the UCS samples used by Porter *et al.* (2018) was smaller than standard UCS samples to prevent non-homogeneous treatment due to immediate calcite precipitation resulting in clogging. This was not a problem in this study due to the decrease in pH, allowing the cementation solution to penetrate the sample before Calcium Carbonate Precipitation could take place.

3. EXPERIMENTAL RESULTS AND ANALYSIS

3.1 Unconfined Compressive Strength (UCS)

The UCS results for the samples are shown in Figure 2. The CSIR prototype treated samples performed better than the *in situ* cultivated bacteria samples. The CSIR prototype performance differs due to its multicomponent mode of action (consisting of metabolites, enzymes, and/or polymeric substances) (Ramdas *et al.* 2021a) compared with samples where *in situ* bacteria was cultivated. The UCS of the *in situ* cultivated samples may also be lower due to the saturation levels affecting the compaction of the samples. The amount of moisture applied to the samples is much higher than the OMC.

The samples that only received cementation solution (C) had a higher UCS compared with the SC samples (Figure 2). Researchers have found that strength gain was proportional to the amount of cementation treatments received, an increase in the number of treatments result in an increase in calcite deposition (Burbank *et al.*, 2013; Cheng *et al.*, 2017; Gomez *et al.*, 2018). Since the cementation solution acts as both cultivation and cementation medium, the C samples in effect received a higher number of treatments.

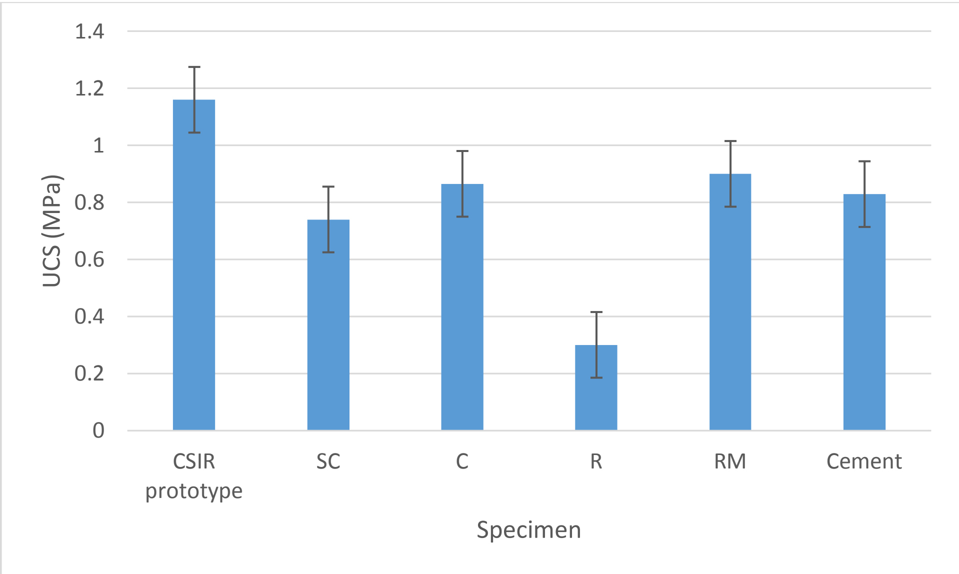

Figure 2: UCS results for MICCP treated samples and 0.7% cement treated. R and RM data obtained from Porter *et al.* (2018)

The samples that only received cementation solution (C) had a UCS comparable to that of the samples treated with cement (Figure 2). Indicating that MICCP may be viable alternative to cement stabilization. More tests and a full life cycle cost analysis is however needed.

Figure 3 shows the grading distribution of the Porter et al. (2018) road base material (R) and the G5 used during this study. The Porter et al. (2018) paper did not report any other aggregate tests conducted on the untreated road base material. Thus, based on the grading alone the road base material could possibly be classified between a G5 and G10 (COLTO, 1985).

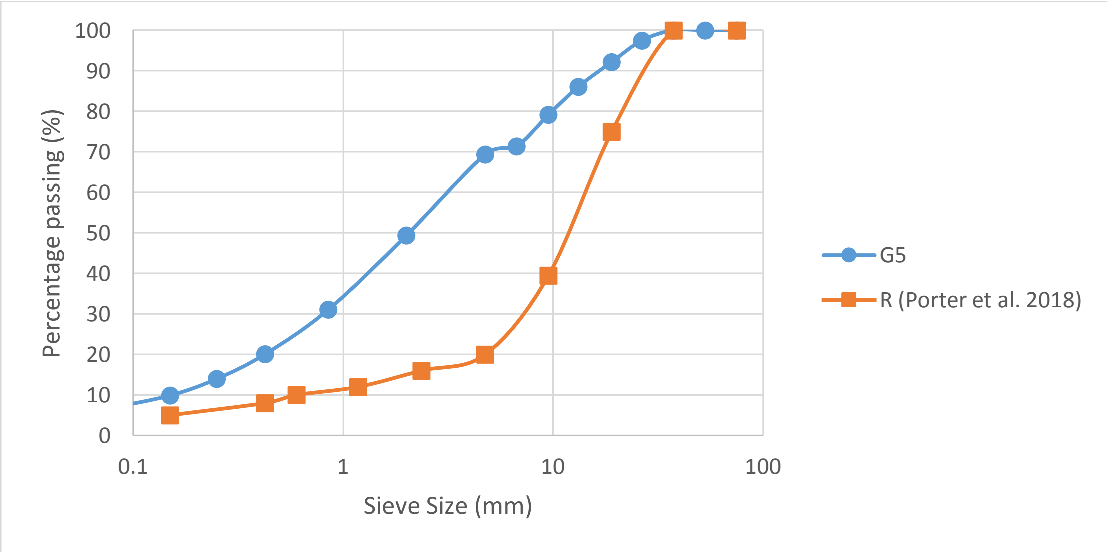

Figure 3: Grading analysis of the G5 and Porter et al. (2018) untreated material

UCS results for untreated road base samples (R) and MICCP treated samples (RM) performed by Porter et al. (2018) are also shown in Figure 2. MICCP road base samples were treated with a mixture of urea positive bacteria before compaction, after which 20 cementation solution treatments were added. The CSIR prototype samples had a higher UCS compared with the RM and the C samples had a slightly lower strength.

3.2 Effluent pH

Ureolytic activity was determined by measuring the effluent pH. The pH gives an indication whether the bacteria completed ureolysis, thus preparing the samples for calcite precipitation. A pH of 9 or above has been found to indicated active urea hydrolysis (Gomez *et al*., 2014). After 2 days of cementation solution (pH6) application the C sample effluent pH was 9 and remained constant for the remainder of the treatment process. The SC samples also had a pH of 9 after one day of treatment which remained constant for the stimulation phase. At day 11 (start of the cementation phase) the pH dropped to 8, increase to 9 by day 12 and remained constant for the remainder of the cementation phase.

CSIR prototype samples had no effluent as the treatment consists of one application at or close to OMC.

3.3 Other Observations

After about the second treatment, the C samples showed white residue at the bottom of the collection containers and along the side of the samples that is assumed to be calcite deposits (Figure 4). The SC samples only showed this residue after 12 days when the stimulation solution was switched to the cementation solution.

There was a strong smell (assumed to be ammonia) present after one day of treatment, indicating that the bacteria completed ureolysis.

(a) (b)

Figure 4: In situ cultivated C UCS samples (a) after two applications of cementation solution (b) after 20 days and curing

4. CONCLUSIONS AND RECOMMENDATIONS

Based on the results and discussions, the following conclusions and recommendations are made regarding the MICCP treated samples:

- It was possible to cultivate urease positive bacteria present within the G5 material that is a marginal material. This opens the possibility of using this technology to upgrade marginal material already present on the construction site, resulting in a possible cost saving as good quality material is not hauled over long distances.
- The cementation solution can act as a stimulation and cementation media when the pH is reduced to give the bacteria time to cultivate and buffer the pH upward for Calcium Carbonate Precipitation to take place.
- The CSIR prototype performed better in terms of UCS and treatment technique. The treatment consists of only one application of the prototype, which is more consistent with current road construction, as compared with the multiple application needed for *in situ* cultivation.
- During *in situ* cultivation the saturation and UCS of the samples are affected. An *in situ* cultivation technique that allows the reduction of moisture to OMC level would be beneficial.
- The samples that only received cementation solution (C) had a UCS comparable to that of the samples stabilized with 0.7% cement. And the CSIR prototype performed better. Indicating that MICCP may be viable alternative to cement stabilization. More tests and a full life cycle cost analysis is however needed.
- MICCP treatment on its own may not be sufficient, but supplementing cement stabilization, reducing the overall requirement for cement, may be a better phased approach to MICCP introduction into construction. Thus, more investigations into this combination are needed.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

Aamir, M, Abdelmalek, B & Will, P, 2018. 'Improvement of Coarse Sand Engineering Properties by Microbially Induced Calcite Precipitation', *Geomicrobiology Journal*, 35(10):887-897.

Ahmad, MR, Chen, B & Ali Shah, SF, 2021. 'Mechanical and microstructural characterization of bio-concrete prepared with optimized alternative green binders', *Construction and Building Materials*, 281:122533.
doi: 10.1016/j.conbuildmat.2021.122533.

Akhalwaya, I & Rust, FC, 2018. 'Laboratory evaluation of road construction materials enhanced with Nano-Modified Emulsions (NMEs)', Southern African Transport Conference, CSIR, Pretoria, South Africa.

Akhalwaya, I, Smit, MA, Rust, FC & Du Plessis, L, 2020. 'Heavy Vehicle Simulator (HVS) Testing of Innovative Pavement Structures in South Africa'. Chapter in: Accelerated Pavement Testing to Transport Infrastructure Innovation, Chabot A, Hornrych P, Harvey J and Loria-Salazar LG eds. Springer, pp 251-260.

Akyol, E, Bozkaya, Ö & Dogan, NM, 2017. 'Strengthening sandy soils by microbial methods', *Arabian Journal of Geosciences*, 10(15):1-8. doi: 10.1007/s12517-017-3123-9.

Burbank, MB, Weaver, TJ, Green, TL, Williams, B & Crawford, RL, 2011. 'Precipitation of calcite by indigenous microorganisms to strengthen liquefiable soils', *Geomicrobiology Journal*, 28(4):301-312. doi: 10.1080/01490451.2010.499929.

Burbank, M, Weaver, T, Lewis, R, Crawford, R & Williams, B, 2013. 'Geotechnical tests of sands following bioinduced calcite precipitation catalyzed by indigenous bacteria', *J Geotech Geoenviron Engng*, 139:928-936.

Canakci, H, Sidik, W & Halil Kilic, I, 2015. 'Effect of bacterial calcium carbonate precipitation on compressibility and shear strength of organic soil', *Soils and Foundations*, 55(5):1211-1221. doi: 10.1016/j.sandf.2015.09.020.

Carrel, M, Morales, V, Beltran, M, Derlon, N, Kaufmann, R, Morgenroth, E & Holzner, M, 2018. 'Biofilms in 3D porous media: delineating the influence of the pore network geometry, flow and mass transfer on biofilm development.', *Water Research*, 134:280-291.

Cheng, L, 2012. *Innovative Ground Enhancement by Improved Microbially Induced CaCO3 Precipitation Technology*. Murdoch University.

Cheng, L, Shahin, MA & Chu, J, 2019. 'Soil bio-cementation using a new one-phase low-pH injection method', *Acta Geotechnica*, 14(3):615-626. doi: 10.1007/s11440-018-0738-2.

Cheng, L, Shahin, M & Cord-Ruwisch, R, 2017. 'Surface percolation for soil improvement by biocementation utilising in-situ enriched indigenous aerobic and anaerobic ureolytic soil microorganisms', *Geomicrobiol J*, 34:546-556.

Committee of Land Transport Officials (COLTO), 1985. TRH14 - Guidelines for Road Construction Materials, Pretoria, South Africa.

DeJong, JT, Fritzges, MB & Nüsslein, K, 2006. ‘Particle shape effects on packing density, stiffness, and strength: natural and crushed sands’, *Journal of Geotechnical and Geoenvironmental Engineering*, 132(5):591-602.
doi: 10.1061/(ASCE)1090-0241(2006)132.

DeJong, JT, Mortensen, BM, Martinez, BC & Nelson, DC, 2010. ‘Bio-mediated soil improvement’, *Ecological Engineering*, 36(2):197-210. doi: 10.1016/j.ecoleng.2008.12.029.

De Muynck, W, De Belie, N & Verstraete, W, 2010. ‘Microbial carbonate precipitation in construction materials: A review’, *Ecological Engineering*, 36(2):118-136. doi: 10.1016/j.ecoleng.2009.02.006.

Dove, J, Shillaber, C, Becker, T, Wallace, A & Dove, P, 2011. ‘Biologically Inspired Silicification Process for Improving Mechanical Properties of Sand’, *Journal of Geotechnical and Geoenvironmental Engineering*, 137(10):949-957.

Ferris, FG, Stehmeier, LG, Kantzas, A & Mourits, FM, 1996. ‘Bacteriogenic mineral plugging’, *Journal of Canadian Petroleum Technology*, 35(8):56-61.

Gomez, M, Anderson, CM, Dejong, JT, Nelson, DC & Lau, XH, 2014. ‘Bacteria, bio-films and invertebrates… the next generation of geotechnical engineers. In: Proceedings of geo-congress 2014: technical papers, ASCE’, *Geotechnical Special Publication*, 234:1674-1682.

Gomez, MG, Anderson, C, DeJong, J, Nelson, D & Lau, X, 2014. ‘Stimulating In-Situ Soil Bacteria for Bio-Cementation of Sands’, *Geo-Congress*, pp. 1674-1682.

Gomez, MG, Graddy, CMR, DeJong, JT, Nelson, DC & Tsesarsky, M, 2018. ‘Stimulation of Native Microorganisms for Biocementation in Samples Recovered from Field-Scale Treatment Depths’, *Journal of Geotechnical and Geoenvironmental Engineering*, 144(1):04017098. doi: 10.1061/(asce)gt.1943-5606.0001804.

Jijian, L, Hongyin, X, Xiaoqing, H, Yue, Y, Dengfeng, F, Shuwang, Y & Hao, Q, 2019. ‘Biogrouting of hydraulic fill fine sands for reclamation projects’, *Marine Georesources & Geotechnology*, 37(2):212-222.

Jordaan, G, Kilian, A, Muthivelli, N & Dlamini, D, 2017. 'Practical Application of Nano-Technology in Roads in Southern Africa', Proceedings for the 8th Africa Transportation Technology Transfer Conference, Livingston, Zambia.

Lalloo, R, Ramchuran, S, Ramduth, D, Görgens, J & Gardiner, N, 2007. 'Isolation and selection of Bacillus spp. as potential biological agents for enhancement of water quality in culture of ornamental fish' Journal of Applied Microbiology, 103(5):1471-1479.

Mgangira, MB, 2009. ‘Evaluation of the Effects of Enzyme-based Liquid Chemical Stabilizers on Subgrade Soils’, *Proceedings of the 28th Southern African Transport Conference (SATC 2009)*, (July), pp. 192-199.

Naeimi, M & Haddad, A, 2018. ‘Investigation on the environmental impact of soil improvement techniques: Comparison of cement grouting and biocement.’, in *Farid A, Chen H (eds) Proceedings of GeoShanghai 2018 International Conference: Geo-environment and geohazard (GSIC). Springer, Singapore*, pp. 483-490.

Osinubi, K, Gadzama, E, Eberemu, A, Ijimdiya, T & Yakubu, S, 2019. ‘Evaluation of the Strength of Compacted Lateritic Soil Treated with Sporosarcina Pasteurii’, in *Zhan L., Chen Y, Bouazza A. (eds) Proceedings of the 8th International Congress on Environmental Geotechnics Volume 3. ICEG 2018. Environmental Science and Engineering. Springer, Singapore*. Available at: https://doi.org/10.1007/978-981-13-2227-3_52.

Osinubi, KJ, Eberemu, AO, Ijimdiya, TS, Yakubu, SE, Gadzama, EW, Sani, JE & Yohanna, P, 2020. ‘Review of the use of microorganisms in geotechnical engineering applications’, *SN Applied Sciences*, 2(2):1-19. doi: 10.1007/s42452-020-1974-2.

Pascal, S, Niklas, H, Christel, C, David, B & Göran, H, 2009. ‘Biogrouting compared to jet grouting: environmental (LCA) and economical assessment.’, *Environ Sci Health Part A*, 44(4):346-353.

Porter, H, Dhami, NK & Mukherjee, A, 2018. ‘Sustainable road bases with microbial precipitation’, *Proceedings of Institution of Civil Engineers: Construction Materials*, 171(3):95-108. doi: 10.1680/jcoma.16.00075.

Portugal, CRME, Fonyo, C, Machado, CC, Meganck, R & Jarvis, T, 2020. ‘Microbiologically Induced Calcite Precipitation biocementation, green alternative for roads – is this the breakthrough? A critical review’, *Journal of Cleaner Production*, 262:121372. doi: 10.1016/j.jclepro.2020.121372.

Putra, H, Yasuhara, H, Kinoshita, N, Neupane, D & Lu, C, 2016. ‘Effect of Magnesium as Substitute Material in Enzyme-Mediated Calcite Precipitation for Soil-Improvement Technique’, *Frontiers in Bioengineering and Biotechnology*, 4:37.

Ramdas, VM, Mandree, P, Mgangira, M, Mukaratirwa, S, Lalloo, R & Ramchuran, S, 2020. ‘Establishing miniaturised structural testing techniques to enable high-throughput screening of microorganisms and microbial components for unpaved road stabilisation application’, *Journal of Advanced Research*, 21:151-159. doi: 10.1016/j.jare.2019.11.002.

Ramdas, VM, Lalloo, R, Lekha, P, Mgangira, M, Muniyasamy, S, Mukaratirwa, S & Ramchuran, S, 2021a. 'Understanding the mechanism of interaction of candidate soil stabilizing prototypes by using microscopy and spectroscopy techniques'. Microscopy Research and Technique.

Ramdas, VM, Mandree, P, Mgangira, M, Mukaratirwa, S, Lalloo, R & Ramchuran, S, 2021b. ‘Review of current and future bio-based stabilisation products (enzymatic and polymeric) for road construction materials’, *Transportation Geotechnics*, 27 (May 2020):100458. doi: 10.1016/j.trgeo.2020.100458.

Rust, FC, Smit, MA, Akhalwaya, I, Jordaan, GJ & du Plessis, L, 2020. Evaluation of two nano-silane-modified emulsion stabilised pavements using Accelerated Pavement Testing. International Journal for Pavement Engineering: 21(9).

Salifu, E, MacLachlan, E, Iyer, K, Knapp, C & Tarantino, A, 2016. 'Application of microbially induced calcite precipitation in erosion mitigation and stabilisation of sandy soil foreshore slopes: A preliminary investigation', *Engineering Geology*, 201:96-105.

Smit, M, Akhalwaya, I & Rust, F, 2021. 'Laboratory evaluation of alternative cost-effective pavement materials', in *South African Transport Conference*.

Soon, NW, Lee, LM, Khun, TC & Ling, HS, 2013. 'Improvements in engineering properties of soils through microbial-induced calcite precipitation', *KSCE Journal of Civil Engineering*, 17:718-728.

South African National Standard (SANS), 2010. Civil engineering test methods Part GR53: Determination of the unconfined compressive strength of compacted and cured specimens of cementitiously stabilized materials. SANS 3001-GR53. Pretoria, South Africa.

South African National Standard (SANS), 2010. Civil engineering test methods Part GR50: Preparation, compaction and curing of specimens of laboratory mixed cementitiously stabilized materials. SANS 3001-GR50. Pretoria, South Africa.

Stabnikov, V, Jian, C, Ivanov, V & Li, Y, 2013. 'Halotolerant, alkaliphilic urease-producing bacteria from different climate zones and their application for biocementation of sand', *World Journal of Microbiology and Biotechnology*, 29:1453-1460.

Yasodian, S, Dutta, R, Mathew, L, Anima, T & Seena, S, 2012. 'Effect of microorganism on engineering properties of cohesive soils', *Geomechanics & Engineering*, 4(2):135.

Yasuhara, H, Hayashi, K & Okamura, M, 2011. 'Evolution in mechanical and hydraulic properties of calcite-cemented sand mediated by biocatalyst. In: Proceedings of geo frontiers 2011: Advances in geotechnical engineering, Dallas TX, ASCE', *Geotechnical Special Publication*, 211:3984-3992.

Zhu, T & Dittrich, M, 2016. 'Carbonate precipitation through microbial activities in natural environment, and their potential in biotechnology: A review', *Front Bioeng Biotechnol*, 4, p. 4.