COMBINING BIO-BASED CEMENTATION WITH CEMENT STABILISATION FOR ROAD CONSTRUCTION

MA SMIT¹ and FC RUST²

 ¹CSIR Smart Mobility, PO Box 395, Pretoria 0001; Tel: 012 8412665; Email: <u>msmit3@csir.co.za</u>
 ²Pavement Engineering Research Consultancy, 25 Oker St, Vermont 7201; Tel: 082 4476098; Email: <u>chris@perc.co.za</u>

ABSTRACT

The growing concerns over climate change and the move towards sustainable, costeffective road development have resulted in the development of bio-based construction methods. Microbial induced calcium carbonate precipitation (MICP) binds material through the formation of calcite bridges between soil grains. Current MICP treatment techniques, however, are not compatible with road construction processes. Due to this the Council for Scientific and Industrial Research (CSIR) has started investigating bio-stabiliser treatment techniques for road construction. The objective of this paper is to present Unconfinded compressive strength (UCS) results for a G8 material stabilised using in-situ bacteria present in the soil. After 10 days of cementation solution treatments a UCS dry result of 1.16MPa was achieved. No wet UCS results were, however, produced as all the samples disintegrated. The decision was made to supplement MICP with 0.7% cement. Not only did UCS wet results show a 100% increase compared with MICP only treated samples, but the UCS dry results were comparable to adding 2.5% cement to the G8 material. Partial replacement of cement with MICP during stabilisation could lead to an overall reduction in the amount of cement used in road construction without compromising strength, which will have a positive environmental and economic impact.

Keywords: Microbial induced calcite precipitation (MICP), UCS, Alternative pavement materials, Bio-stabilisation.

1. INTRODUCTION

Road construction is expensive, and, in many instances, the local in-situ materials are unable to provide the strength needed for the roads they are used in. Engineers usually have to import good quality material at a high cost or, alternativity, treat the in-situ material or a lower quality material with some form of mechanical or chemical stabilisation. Chemical stabilisation with cement, lime, fly ash and bitumen is not a new concept in pavement construction and have been used successfully over many decades. Cement production, however is both energy consuming (12-15% global industrial energy consumption) and environmentally unfriendly (7-9% of gross anthropogenic CO2 release) (Ahmad *et al.*, 2021). With the growing concerns over climate change in recent years, the global focus has shifted to more environmentally sustainable alternatives for traditional chemical stabilization, bio-based building materials being one (Smit *et al.*, 2022). Biobased alternatives mimic or manipulate biomineralization to fabricate functional materials to solve engineering problems (Xiao *et al.*, 2022). The biomineralization of calcium carbonate (CaCO₃) for examples has been investigated as an alternative to Portland Cement stabilisation of granular materials in pavements (Ramdas *et al.*, 2021).

Additionally, biomineralization may compliment mechanical stabilisation of low quality materials.

Biomineralization of CaCO₃ has been found to improve the unconfined compressive strength (UCS) of soil (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), increase shear strength characteristics (DeJong et al., 2006) and decrease hydraulic conductivity (Ferris et al., 1996; Yasuhara et al., 2011; Soon et al., 2013; Gomez et al., 2014; Carrel et al., 2018) by binding soil grains through the formation of calcium carbonate bridges. The biomineralization of CaCO₃ is achieved through a process called Microbial Induced Calcite Precipitation (MICP). Under optimal conditions, bacteria species containing the urease enzyme use urea as a food source and excrete ammonia and carbon dioxide. Ammonia reacts with water present in the soil producing ammonium and hydroxide, resulting in an increase in the pH of the surrounding environment. The carbon dioxide in aqueous media from bicarbonate which then results in the formation carbonate. When carbonate is formed it promotes the absorption of calcium ions on the surface of the bacteria cells. The bacteria cells are negatively charged, driving calcium irons to accumulated around the cell walls. The calcium irons react with the carbonate and hydroxide ions due to pH increase to form calcium carbonate bonds (Porugal et al. (2020). The calcium carbonate bonds fill pores between soil particles and glue soil particles to the bacteria cells and each other (Sheng et al., 2020). Once the bonds harden, they form calcium carbonate crystals in the calcite crystalline form (Porugal et al., 2020). The main role of the bacteria during the MICP process is to increase the pH of the surrounding environment for calcite precipitation to take place (Gomez et al., 2014).

Due to MICP's environmentally friendly and durable nature (De Muynck *et al.*, 2010; Akyol *et al.*, 2017) it has been a subject of investigation at the Council for Scientific and Industrial Research (CSIR) as an alternative to cement stabilization of road sub-base materials (Mgangira, 2009; Ramdas *et al.*, 2020, 2021; Smit *et al.*, 2022). The work done by Mgangira, (2009) and Ramdas *et al.* (2020, 2021) looked at the development of an enzyme-based stabiliser. Previous work done by Smit *et al.* (2022) focused on the use of bacterial already present in the soil for MICP.

Soil stabilization techniques using MICP differ depending on the goal of the treatment, the soil type, the size and species of the bacteria used and its compatibility with the soil, the source of the bacteria, environmental conditions and chemical reagents. There, however, exist common steps that can be summarised as follows:

- Step 1: Obtain urease positive bacteria. Bacteria can be obtained or isolated and identified then cultivated to increase the number of organisms.
- Step 2: Cultivate the bacteria. This can occur under sterile or none-sterile conditions in a laboratory.
- Step 3: Treatment of soil. The most common sequence of the soil treatment starts with the application of concentrated bacterial solution consisting of bacteria cultures and a chemical solution containing nutrients to promote bacteria growth and movement. Then several applications of cementation solution consisting of urea and a source of calcium to initiate calcite deposition are made. The application mechanisms used range from grouting (injecting), immersion, spraying and gravity (surface percolation).

Buying, importing or identifying microorganisms and growing them in sterile conditions is expensive and make up about 30% of the cost of MICP stabilization (Yasodian *et al.*,

2012). To reduce the cost of importing microorganisms and limiting the potential negative environmental effect of introducing foreign microorganisms to the local environment, Smit et al. (2022) suggested using urea positive bacteria already present in the soil, thus also solving to guestion of bacteria compatibility with the soil. One kilogram of soil has nearly 10¹² microbes (Mitchell and Santamarina, 2005) and the bacteria capable of MICP comprise between 17-30% of cultivatable aerophilic and anaerobic microorganisms (Burbank et al., 2011). Manipulation of the in-situ bacteria for MICP is not common and from the research review at the time of the Smit et al. (2022) study had only been used by a few researchers which included Burbank et al. (2011), Burbank et al. (2013), Gomez et al. (2014), Cheng et al. (2017), Gomez et al. (2017), and Gomez et al. (2018). All these tests were conducted only on sand and only Cheng et al. (2017) used a treatment technique other than jet grouting. In the Smit et al. (2022) study the researchers achieved in-situ cultivation of indigenous bacteria already present in the soil and CaCO3 precipitation using an adjusted method suggested by Gomez et al. (2014). The method also called the two-phase method consists of compacted soil samples being treated with several applications of a cultivation solution used to selectively grow urease positive bacteria present in the soil. Followed by several applications of cementation solution used to activate the cementation reaction (see Table 1 for description).

Constituent	Cultivation 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

Table 1: Summary of Treatment Solution Constituents (Smit et al., 2022)

In the Smit *et al.* (2022) study the total treatment time for the first samples was 23 days including curing, after which UCS tests were performed. Not only was $CaCO_3$ precipitation achieved, but the UCS test results also looked promising. It was suggested however that the number of treatments be reduced to be more compatible with large scale construction application. Multiple application during construction projects is impractical, however, strength gain is proportional to the number of cementation treatments due to increase in calcite deposition (Burbank *et al.*, 2013; Cheng *et al.*, 2017; Gomez *et al.*, 2018).

To reduce the treatment time a one-phase method was suggested, but first the problem of immediate precipitation had to be addressed. Smit *et al.* (2022) observed that the cultivation solution and cementation solution differ only by the addition of calcium chloride thus the cementation solution can act as both cultivation and cementation medium (reducing the number of treatments needed to achieve the same strength) if immediate precipitation can be prevented. At a pH of 8, the cementation solution will result in immediate precipitation, resulting in clogging and non-homogeneous nature of the samples (Cheng *et al.*, 2019). To prevent this the pH of the cementation solution described by Smit *et al.* (2022) was reduced to a pH of 6 giving the bacteria time to grow and gradually increases the pH to 8 so precipitation can take place. UCS samples were treated for 20 days with cementation solution (pH 6) only and showed a strength improvement of about 17% compared to the two-phase treatment samples.

This paper builds on the findings the Smit *et al.* (2022) paper and presents the strength results for samples with a reduced number of MICP treatments using the one-phase method. To maintain strength, the samples were cured for a longer time. This study also

tested UCS wet samples and found that the samples were unstable. To improve the wet UCS results the suggestion was to supplement MICP with cement. According to Porter *et al.*, (2018) this will also reduce the overall requirement for cement and may be a better phased approach to MICP introduction into road construction.

2. METHODOLOGY

2.1 Soil Samples

The same material used in the Smit *et al.* (2022) study was used for this investigation. The material contains 17-20% Mica and 7-43% clay (Jordaan *et al.*, 2017; Akhalwaya and Rust, 2018). Table 2 contains general properties and descriptions of the material which can be classified as a G8-G9 materials according to COTO (Committee of Land transport Officials, 2020).

Sample description, Information and properties		Atterberg Limits (TMH1, 1986 : Methods A2&A3)	
Sample Name	K46 Diepsloot	Liquid Limit %	19.4
Co-ordinates	26.059784,27.8579501	Plastic Limit %	16.1
Material Classification	G8-G9 (COTO, 2020)	Plasticity Index %	3.3
pH Value	8.07	Electrical Conductivity (S/m)	0.01
Sieve Analysis - % of material passing sieves		Compactions (TMH1, 1986: Method A7)	
Sieve Size (mm)	% passing	MOD AASHTO: Max Dry Density (MDD) (kg/m ³)	2096
50	100	Optimum Moisture Content (OMC) (%)	7.4
37.5	100	Dry Density achieved (kg/m ³)	2099
20	93	% of Max Dry Density (MDD)	100
14	87	Moulding Moisture Cont. (%)	7.8
5	70	% Swell	0.20
2	49	Soaked California Bearing Ratio (CBR) (TMH1, 1986: Method A8)	
0.425	20	100 % Mod AASHTO	102
0.075	7	98 % Mod AASHTO	82
Grading Modulus	0.76	95 % Mod AASHTO	65
Initial Consumption of stabiliser	2.5%	93 % Mod AASHTO	39

Table 2: Summary of classification results for K46 G8-9 untreated material

2.2 Bacteria Samples and Soil Treatment

MICP 5-day (MICP_{5-day}) and 10-day (MICP_{10-day}) UCS dry and wet samples were prepared by replacing the water with the cementation solution at OMC shown in Table 3 and compacting. The samples were kept at ambient temperature for 5 and 10 days respectively. Each day one pore volume of cementation solution was added to the top of the samples and allowed to filter through by means of gravity (Table 4). The pH of the cementation solution was reduced to a pH of 6 with HCL solution.

MICP supplemented with cement (MICP_{0.7%cement}) UCS samples were prepared by adding 0.7% cement and water at OMC to the G8 soil samples and compacting. A 0.7% cement content was used as a proof-of-concept to show that MICP with a low percentage of added cement could achieve high UCS dry results and assist with UCS wet results. In future, the design of MICP with cement stabilised materials will have to consider the initial

consumption of stabiliser (ICS) test to determine to optimum amount of cement to add, this will be investigated further.

The MICP_{0.7%cement} samples were kept at ambient temperature for 10 days. Each day one pore volume of cementation solution was added to the top of the samples and allowed to filter through by means of gravity (Table 4). The pH of the cementation solution was reduced to a pH of 6 with HCL solution.

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

Table 3: Summary of Treatment Solution Constituents

Samples stabilised with only cement were also prepared. The Initial Consumption of stabiliser test was conducted in accordance with SANS3001-GR57 and the result was 2,5%. Then the samples were stabilised using 0.7%, 2%, 2.5% and 3% cement and cured in accordance with SANS 3001-GR53 (2010). An SABS approved extended common cement with a strength class of 32.5N was used (Table 4).

The test results were compared with UCS results obtained from the Smit *et al.* (2022) study. The UCS samples from the Smit *et al.* (2022) study was treated for 20-days as opposed to 5-days and 10-days. In the Smit *et al.* (2022) study the MICP_{20-day} UCS dry and wet samples were prepared using the same G8 material presented in this investigation. Each day for 20 days one pore volume of cementation solution (Table 3) was added to the top of the samples and allowed to filter through by means of gravity (Table 4). The pH of the cementation solution was also reduced to a pH of 6 with HCL solution. It should be noted however that the 20 day treated samples were rapid cured for 3 days whereas the UCS samples in this study was cured for 7 days in accordance with SANS 3001-GR53 (2010).

Identifier	Description	Treatment	Curing time
MICP _{20-day}	G5 material Cementation solution (Smit <i>et al.,</i> 2022)	20 application of Cementation solution (pH6)	24 hours @ 30°C 48 hours @ 40°C – 45°C
MICP _{5-day}	G5 material Cementation solution	5 application of Cementation solution (pH6)	7 days 50°C
MICP _{10-day}	G5 material Cementation solution	10 application of Cementation solution (pH6)	7 days 50°C
MICP _{0.7%cement}	G5 material Cementation solution 0.7% cement	10 applications of Cementation solution (pH 6)	7 days 50°C
0.7% Cement	G5 material 0.7% cement	Only cement	7 days 50°C
2% Cement	G5 material 2% cement	Only cement	7 days 50°C
2.5% Cement	G5 material 2.5% cement	Only cement	7 days 50°C
3% Cement	G5 material 3% cement	Only cement	7 days 50°C

Table 4: Details of samples

2.3 Strength Testing

UCS samples were prepared and cured in accordance with SANS 3001-GR53 (2010) after being mixed and compacted in accordance with SANS 3001-GR50 (2010). Wet UCS samples were submerged in a water bath prior to testing in accordance with SANS 3001-GR53 (2010).

3. EXPERIMENTAL RESULTS AND DISCUSSION

The UCS (dry) results for MICP_{10-day} and MICP_{5-day} treatments are shown in Fig. 1 and is compared to the MICP_{20-day} UCS results from the Smit *et al.*, (2022) study (Table 5). There was a 9.4% increase in UCS for the MICP_{10-day} samples compared to the MICP_{5-day}. This was expected as strength gain is 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). The MICP_{5-day} and MICP_{10-day} samples, however had a 22.5% and 34.1% increase in strength gain respectively compared to the MICP_{20-day} samples. This may be due to curing, according to Porter *et al.* (2018) a longer period of curing results in optimum strength gain thus countering the strength loss with reduced number of treatments.

Identifier	Treatment	Curing Time
MICP _{20-day}	20 application of Cementation solution (pH6)	24 hours @ 30°C 48 hours @ 40°C – 45°C
MICP _{5-day}	5 application of Cementation solution (pH6)	7 days 50°C
MICP _{10-day}	10 application of Cementation solution (pH6)	7 days 50°C
MICP _{0.7%cement}	0.7% cement and 10 applications of Cementation solution (pH 6)	7 days 50°C

 Table 5: Samples details

No wet UCS results were obtained as the samples disintegrated within 2 minutes after being placed in the water. This was an unexpected result since MICP has been used to reduce hydraulic conductivity in several studies (Ferris *et al.*, 1996; Yasuhara *et al.*, 2011; Soon *et al.*, 2013; Gomez *et al.*, 2014; Carrel *et al.*, 2018), these studies were however mostly confined to sandy soils. To date no other studies which have conducted UCS wet tests on MICP treated samples could be found. Calcium carbonate has a low solubility in water, however in low quantities, low pressure and temperature it is sparingly soluble (Caciagili & Manning, 2003). Thus, increasing the calcium carbonate content without increasing the number of treatments needs to be investigated, to improve the wet strength of MICP stabilised materials without increasing the time and cost of stabilisation.

Thus to improve the wet UCS without increase the number of MICP treatments the decision was made to supplement the MICP treatment with a small amount of cement.

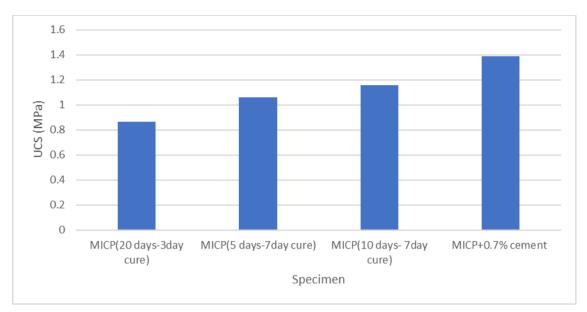


Figure 1: UCS results for 20-day, 10-day, 5-day and MICP supplemented with 0.7% cement. Please refer to Table 5 for sample description

Only 0.7% cement was added to the samples which resulted in a 19.8% increase in UCS compared to the C_{10} -^{day} samples (Fig. 1). The biggest improvement in UCS observed was in the wet state. The wet UCS measurement was 0.47Mpa, a 100% increase in strength compared to all MICP treated samples which disintegrated after 2 minutes in the water bath (Fig. 2). The wet results were compared with samples treated with 0.7% cement only. The samples containing MICP treatments and the 0.7% cement treated samples had a 46% lower wet UCS compared with the cement treated samples. It should be noted that the extender in the cement (slag GGBS or fly ash) may perform differently to limestone and should be investigated.

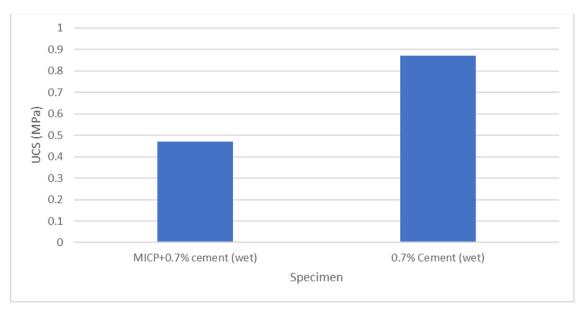


Figure 2: UCS wet results for MICP supplemented with 0.7% cement and 0.7% cement

MICP treatment was compared with traditional cement treated G8 material. Fig. 3 shows the UCS results for 0.7%, 2%, 2.5% and 3% cement treated samples compared to the 10 day MICP treated samples and MICP samples supplemented with 0.7% cement. Treating the samples with only MICP had a UCS comparable with the 2% cement treated samples.

The samples combining MICP and 0.7% cement added, resulted in a 67.7% increase in UCS which is comparable with 2.5% cement treated samples.

Since the ICS was 2.5% it should be noted that the samples may still have been susceptible to carbonation, thus further work is required to investigate this.

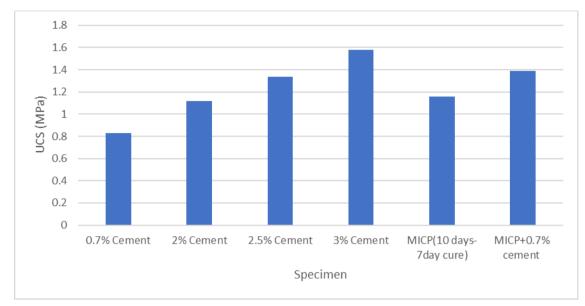


Figure 3: UCS results for 0.7, 2, 2.5 and 3% cement treated samples compared with the 10-day MICP treated samples and MICP supplemented with 0.7% cement

4. FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

The following findings, conclusions and recommendations are made regarding the MICP treated samples based on the results and discussions above:

- Longer curing times increase strength gain in MICP treated samples despite a 50% decrease in the number of treatments.
- No UCS wet results were obtained from samples only stabilised with MICP. Calcite has a low solubility in water, however in low quantities it is sparingly soluble. Thus, increasing the calcite content without increasing the number of cementation treatments needs to be investigated.
- Adding 0.7% cement resulted in a 16.5% increase in the unconfined compressive strength of the soil samples compared to the 10-day MICP treated samples.
- The biggest improvement when 0,7% cement is added with the MICP is however in the wet condition which showed an unconfined compressive strength of 0.47 MPa compared to no results for any of the other samples.
- It was shown that adding MICP treatments and 0.7 % cement resulted in a 40.4% increase in the UCS over soil samples stabilised with 0.7% cement only. The increase in UCS was comparable to samples stabilised with 2.5% cement.
- Partial replacement of cement with MICP could lead to an overall reduction in the amount of cement used without compromising strength, which will have a positive environmental and economic impact (if the cost of the cementation solution can be kept below the price of cement).
- Wet testing of the combination of MICP treatments and the 0.7% cement addition, resulted in a reduction in UCS of 46% compared to the 0.7% cement treated samples.

- Further investigation is needed to evaluate the type of extended used in the cement as slag GGBS and fly-ash extended cement may perform differently compared with lime.
- The initial consumption of stabiliser is important since the samples may still been susceptible to carbonation if sufficient cement is not provided, thus further work is required to determine the correct ration of cement and MICP.

5. ACKNOWLEDGEMENTS

The authors acknowledge the generous support of the CSIR Transport Infrastructure Engineering staff, Chemicals Cluster Researchers and laboratory technicians.

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, F. 2018. Laboratory evaluation of road construction materials enhanced with Nano-Modified Emulsions (NMEs), in *Southern African Transport Conference*. Pretoria, South Africa.

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 microorganiss 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.

Caciagli, NC & Manning, CE. 2003. The solubility of calcite in water at 6-16 kar and 500-800°C *Contibution to Mineraloy and Petrology*, 146:275-285.

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, 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 (COTO). 2020. Standard specifications for Road and Bridge works for South African Road Authorities, Pretoria, South Africa.

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.

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.

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, 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, Anderson, CM, Graddy, CMR, DeJong, JT, Nelson, DC & Ginn, TR. 2017. Large-Scale Comparison of Bioaugmentation and Biostimulation Approaches for Biocementation of Sands, *Journal of Geotechnical and Geoenvironmental Engineering*, 143(5):1-13. Doi: 10.1061/(asce)gt.1943-5606.0001640.

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, in *Proceedings for the 8th Africa Transportation Technology Transfer Conference*. Livingston, Zambia.

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.

Mitchell, J & Santamarina, J. 2005. Biological consideration in geotechnical engineering, *Journal of Geotechnical and Geoenvironmental Engineering*, 131(10):1222-1233.

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.

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

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, FC. 2021. Laboratory evaluation of alternative cost-effective pavement materials, in *South African Transport Conference*, (SATC 2021), Pretoria, South Africa.

Smit, M, Akhalwaya, I, Rust, FC & Ramdas, VM. 2022. Microbial induced calcium carbonate precipitation (MICCP) for road construction, in *South African Transport Conference*, (SATC 2022), Pretoria, South Africa..

Soon, NW, Lee, LM, Khun, TC & Ling, HS. 2013. Improvements in engineering properties of soil through microbial-induced calcite precipitation *KSCE Journal of Civil Engineering 2*, 17(4):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.

Xiao, Y, He, X, Zaman, M, Ma, G & Zhao, C. 2022. Review of Strength Improvements of Biocemented Soils, *International Journal of Geomechanics*, 22(11):1-23. Doi: 10.1061/(asce)gm.1943-5622.0002565.

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.