M. Abubakar, 2026, 14:2
ISSN (Online): 2348-4098
ISSN (Print): 2395-4752

International Journal of Science, Engineering and Technology
An Open Access Journal

Effects of mode of application of brevibacillus brevis on calcite precipitation of bio-treated lateritic soil.

M. Abubakar[1*], M. A. Garba[1], K. J. Osinubi[2], F.ASCE A. O. Eberemu[3], M.ASCE

[1]Professor, Ahmadu Bello University Zaria, Kaduna State, Nigeria

[3]Professor, Ahmadu Bello University and Africa Center of Excellence on New Pedagogies in Engineering, Education (ACENPEE), Kaduna State, Nigeria

[1*]Lecturer Federal Polytechnic Daura, Katsina State and Graduate student, Ahmadu Bello University Zaria, Kaduna State, Nigeria,

[1]Graduate student, Ahmadu Bello University Zaria, Kaduna State, Nigeria

Abstract- Sustainable method of soil improvement called Microbial Induced Calcite Precipitation (MICP) has received significant interest recently. In this technique, the behaviours of bio-treated soil are controlled by the amount of calcite content (CC) produced. Various methods have been used in the literature to determine the CC in soil using injection technique. The quantity of calcite precipitation is affected by the mode of application of microbes used and this effect hasn't been studied extensively. There is no preferred mode of application only the most commonly used method documented. In this paper, the CC of lateritic soil bio-treated at stepped Brevibacillus brevis (B. brevis) suspension density (SD) and cementation reagent (CR) concentration using three mode of treatment (i.e. mixing, injection and spraying method) and mix ratio (i.e., 25B–75C, 50B–50C and 75B–25C) were determined. The B. brevis SD and CR used to trigger the MICP process are based on McFarland Standards. Based on this result, the mixing mode produced the best result then injection mode and the least result was for the spraying mode of treatment. The highest CC values of 12, 8.72 and 6.4 % were recorded at $24E^8$ cells/ml - 1M using mixing, injection and spraying mode of treatment respectively. The recorded CC values based on the mode of treatment were in the order mixing > injection > spraying method of application respectively.

Keywords: Microbial Induced Calcite Precipitation (MICP), calcite content, treatment method, mixing, injection and spraying.

I. INTRODUCTION

Soil improvement using biocement has been studied for the last decade (Mitchell and Santamarina 2005; Van Paassen et al. 2010; Chou et al. 2011; Chu et al. 2013; Martinez et al. 2013; Montoya and Dejong 2015; O'Donnell and Kavazanjian 2015). Using the microbially induced calcium carbonate precipitation (MICP) process, calcium carbonate crystals are precipitated in soil and act as a cementing agent for soil particles in a way similar to cement to increase the shear strength and reduce the permeability (Whiffin et al. 2007; Burbank et al. 2012; Park et al. 2014). The common MICP process uses urease-producing bacteria (UPB) to decompose urea to increase pH. With the presence of calcium ions, calcium

carbonate is precipitated through the following reaction [Eq. (1)]:

$CO(NH_2)_2 + 2H_2O + Ca^{2+} + \rightarrow CaCO_3 + 2NH^{+4}$ (i)

The amount of improvement is much related to the amount of microbially induced calcium carbonate content (CCC) in soil [1, 11]. Therefore, CCC is an important parameter to be measured for biocemented soil. Several methods have been adopted by different researchers to determine CCC as depicted in figure 1.

II. METHODS

2.1 Bacteria isolation, culture/growth medium Isolation of B. brevis

The isolation of B. brevis from soil was carried out by the tyrosine selection method as described by Edwards and Seddon [15]. For each sample, 10g soil was added to 90mL of distilled water and incubated for 45 minutes at 28°C on a rotatory shaker (B. Bran scientific & instrument company, England) at 250 rpm. Thereafter, 2 mL of the broth culture was added to 20 mL of sterile Tyrosine broth that contained (g/l) in distilled water: 6.5 nutrient broth and 5.0 tyrosine, after autoclaving at 121oC for 15 min in 50 mL Erlenmeyer flask. The mixture was incubated for 4 hours at 28°C on a rotatory shaker at 250 rpm. After incubation, 5 mL aliquots from each culture were placed in hot water bath operating at 80oC for 3 minutes. And then 0.1 mL was spread on Tyrosine agar (Nutrient broth 6.5g/L, tyrosine 5g/L and Agar 15g/L) and incubated at 28oC for 24 h. After 24 hours of incubation, bacterial colonies on the plates were visually examined. Colonies showing morphological features such as being light brown, non-spreading, serrated edges and clear halos typical for the B. brevis, were subcultured on Tyrosine agar.

2.1.1 Identification of B. brevis

The cultural and biochemical characteristics of the isolates were used for a presumptive characterization of the B. brevis [6, 17] and then confirmed based on the retention factor (RF) on the thin layer chromatography plate as compared to the standard (Gramicidin).

2.2 Methods of treatment

Numerous researchers have used various methods in introducing bacteria to soil specimens. The most common methods used to introduce bacteria solution into the soil are grouting, spraying and mixing method [12].

2.2.1 Mixing method

In this method, the lateritic soil samples were mixed using spatula with a volume of each B. brevis suspension and cementation reagent in a bowl (plate Ia). Three trial mixes were adopted for the computation of the volume of each of the bacterial suspension density (0, 1.5 x 10^8, 6.0 x 10^8, 12 x 10^8, 18 x 10^8 and 24 x 10^8 cell/ml respectively) and cementation reagent was added to the soil. After mixing the soil sample with the three trial mixes for the various bacterial suspensions; the treated samples were left air-dried before being pulverized and passed through BS No. 40 sieve (425 µm aperture) for calcite contents test procedures.

2.2.2 Injection method

In this method 60ml the syringe was used to inject the required bacteria (0, 1.5 x 10^8, 6.0 x 10^8, 12 x 10^8, 18 x 10^8 and 24 x 10^8 cell/ml respectively) and cementation reagent to the soil samples (plate Ib). The lateritic soil sample was treated in this method, both bacteria and cementation solution was grouted alternately by specify volume, from top to bottom, following the vertical flow path regulated by a peristaltic pump. Primarily, this is attained by allowing a retention period (normally 3 hours after introducing bacteria culture to the sand column) [18].

After injecting the soil sample with the three trial mixes for the various bacterial suspensions, the treated samples were left to air-dry, before being pulverized and passed through BS No.40 sieve

(425 µm aperture) for calcite contents test procedures.

2.2.3 Spraying method

In this method ordinary pump (air freshener spraying can) is used to spray the required bacterial solution into the lateritic soil sample in a bowl (plate Ic). After spraying the bacterial solution (0, 1.5 x 10^8, 6.0 x 10^8, 12 x 10^8, 18 x 10^8 and 24 x 10^8 cell/ml respectively), the cementation reagent was added to the soil. The soil samples were left undisturbed for a minimum of 4 hours, after which the nutrient cycles was supplied to feed on the microorganisms.

After spraying, the soil sample with the three trial mixes for the various bacterial suspensions, were treated and left to air-dry before being pulverized and passed through BS No. 40 sieve (425 µm aperture) for calcite contents test procedures.

Plate I: schematic diagram for Modes of treatments considered: (a) mixing method (b) grouting method (c) spraying method]

2.3 Acid Washing Method

Several methods have been proposed for calcite quantification as depicted in plate II. Acid washing method was utilized in this paper due to its promising performance accurately in calcite content determination over other methods.

In using this method, 5 g sample was mixed with 20 mL of 1-M HCl acid to dissolve calcium carbonate. Then all the solution and insoluble solid were washed by distilled water on filter paper with a coarse pore size in a No. 200 sieve for 10 min. This washing process was able to remove all soluble` calcium from the sand particles.

Then all solid particles remaining on the sieve were oven-dried and weighed. The weight difference between the original sand sample (A) and post washing sample (B) was the mass of calcium carbonate. The CCC was calculated as

$$CCC\ (\%) = 100 - \left(\frac{B}{A}\right) X\ 100 \quad (i)$$

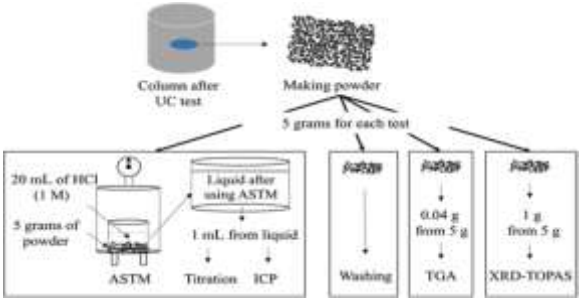

Plate II: Procedure for sampling and testing for CCC determination (source: [11]

III. RESULTS AND DISCUSSION

Calcium carbonate content

One of the controlling factors in microbial induced calcite soil improvement system is the improvement of binding material called calcite which hardens the soil and improves its workability. The variation of calcite content formed by treatment with B. brevis for different cementation reagent concentration for the three modes of treatment considered and different mix ratios is shown in Figure 1-3.

For 25 % B. brevis (B) : 75 % cementation reagent concentration (C) mix ratio treatment of lateritic soil, the peak calcium carbonate content (CCC) of 6.4 % was recorded for the mixing mode of treatment at B. brevis suspension density 24 x 10^8 cells/ml and 1.0 M cementation reagent concentration. The injection mode of treatment recorded CCC value of 6.2 % at B (1.8 x 10^9 cells/ml) and C (1.0 M). The lowest CCC value of 3.0 % was obtained at B (1.8 x 10^9 cells/ml) and C (0.50 M) using the spraying mode of treatment

(see Figure 1). It was observed that the CCC values recorded for the different modes of treatment were in the order mixing > injection > spraying mode which could probably be attributed to the intensity of the penetration and homogeneity of the microbes and cementation reagent into the soil sample for the various treatment modes considered [12].

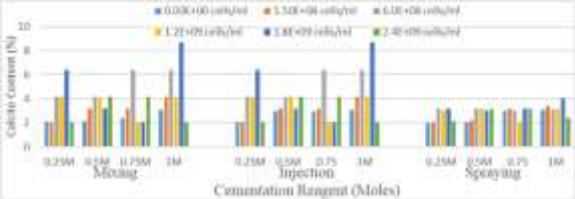

Figure 1: Variation of calcite content of lateritic soil - B. brevis (B) mixtures with cementation reagent concentration (C) for the three modes of application when prepared with 25 % B : 75 % mix ratios

The 50 % B. brevis (B) : 50 % cementation reagent concentration (C) mix ratio treatment of lateritic soil recorded the highest CCC value of 8.72 %, at B. (1.8 x 10^9 cells/ml) and C (1.0 M) for mixing mode of application. For the injection mode of application, peak CCC value of 8.70 % was recorded 24 x 10^8 cells/ml and 1.0 M. The lowest CCC value of 4.03 % was obtained at 1.8 x 10^9 cells/ml and 1.0 M for spraying mode of treatment (see Figure 2). The peak CCC values recorded for the modes of treatment considered were in the order mixing > injection > spraying. This could probably be attributed to the intensity of the penetration of microbes into the soil sample for the various treatment modes considered [12].

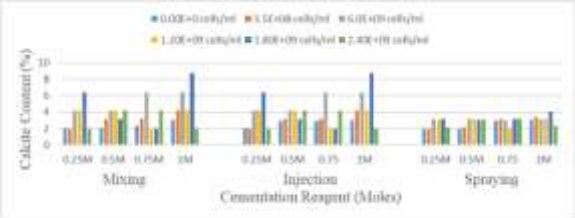

Figure 2: Variation of calcite content of lateritic soil - B. brevis (B) mixtures with cementation reagent concentration (C) for the three modes of application when prepared with) 50 % B : 50 % C mix ratios

The 75 % B. brevis (B) : 25 % cementation reagent concentration (C) mix ratio treatment of lateritic soil recorded peak CCC value of 12.0 % for the mixing mode of application at 24 x 10^8 cells/ml and 1.0 M. For the injection mode of application peak CCC value of 5.38 % was recorded at 24 x 10^8 cells/ml and 1.0 M. The least CCC value of 3.5 % at B. brevis suspension density 24 x 10^8 cells/ml and 1.0 M was recorded using the spraying mode of treatment (see Figure 3). The recorded CCC values based the mode of treatment were in the order mixing > injection > spraying could probably be attributed to the intensity of the penetration of microbes into the soil sample for the various treatment modes considered [12].

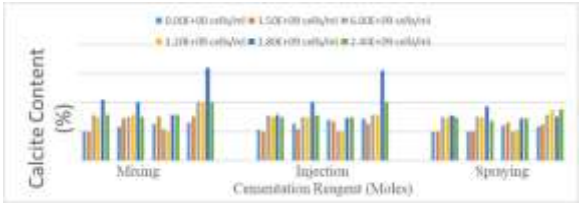

Figure 3: Variation of calcite content of lateritic soil - B. brevis (B) mixtures with cementation reagent concentration (C) for the three modes of application when prepared with 75 % B : 25 % C mix ratios

The marginal increases in CCC values recorded for the various B. brevis suspension densities and cementation reagent concentrations for the various modes of treatment might be related to the increase in the quantity of urease enzymes formed by B. brevis. As B. brevis suspension density and cementation reagent concentration increased additional urease enzyme was freed by the bacteria to facilitate the production of calcium carbonate ($CaCO_3$). [13] and [14] reported that increased bacteria suspension density resulted in better enzyme actions as the surfaces of the

microorganisms enhanced the induced calcite precipitation.

Generally, considering the result recorded for all the three modes of treatment, the mixing mode produced the best result followed by the injection mode and the least result recorded was for the spraying mode of treatment. The CCC can thus be ranked based of the mode of application in the order mixing > injection > spraying. The probable explanation for this trend is not unconnected to the retention capacity and the mobilisation of the urease enzyme, because of the introduction of ureolytic bacteria into the soil matrix, which is an important factor responsible for the successful use of MICP in soil improvement [12].

Furthermore, in the mixing mode of application both bacteria and cementation reagents are evenly distributed, thus minimized the filtering issues common found in injection mode. The homogeneity in bacteria and cementation reagents make this mode the best among the others considered. The major drawback associated with this mode is it can alter the soil matrix and is less-practical for large-scale applications due to mechanical mixing challenges that is energy and time consuming. Grouting/Injection mode of treatment is Suitable for larger volumes and can target specific areas effectively. It has been widely used in field applications [18]. This mode is Prone to bacterial filtering, leading to uneven distribution and reduced effectiveness of the process over long distance. Spraying mode of treatment Allows for surface treatment and is easier to implement in certain contexts. This mode is less effective for deeper soil layers compared to mixing or injection modes. While mixing provides uniformity, injection offers targeted applications [12].

IV. CONCLUSION

The following conclusion were from the result recorded:

1. Modes of treatment

The CCC can thus be ranked based of the mode of application in the order mixing > injection > spraying. The choice depends on project scale, soil type and desired outcomes.

2. Mixing ratio

The CCC can thus be ranked based on the mix ratio considered in the order 25 % B : 75 % C > 50 % B : 50 % C > 75 % B : 25 % C respectively.

REFERENCES

[1] Al Qabany, A. A., and Soga, K. (2013). "Effect of chemical treatment used in MICP on engineering properties of cemented soils." Géotechnique, 63(4), 331–339.

[2] van Paassen, L. A., Ghose, R., van der Linden, T. J. M., van der Star, W. R. L., and van Loosdrecht, M. C. M. (2010). "Quantifying biomediated ground improvement by ureolysis: Large-scale biogrout experiment." J. Geotech. Geoenviron. Eng., 10.1061/(ASCE)GT.1943-5606 .0000382, 1721–1728.

[3] Chou, C., Seagren, E., Aydilek, A., and Lai, M. (2011). "Biocalcification of sand through ureolysis." J. Geotech. Geoenviron. Eng., 10.1061 /(ASCE)GT.1943-5606.0000532, 1179–1189.

[4] Chu, J., Ivanov, V., Stabnikov, V., and Li, B. (2013). "Microbial method for construction of an aquaculture pond in sand." Géotechnique, 63(10), 871–875.

[5] Martinez, B., et al. (2013). "Experimental optimization of microbialinduced carbonate precipitation for soil improvement." J. Geotech. Geoenviron. Eng., 10.1061/(ASCE)GT.1943-5606.0000787, 587–598.

[6] Montoya, B., and DeJong, J. (2015). "Stress-strain behavior of sands cemented by microbially induced calcite precipitation." J. Geotech.

Geoenviron. Eng., 10.1061/(ASCE)GT.1943-5606.0001302, 04015019.

[7] O'Donnell, T., and Kavazanjian, E., Jr. (2015). "Stiffness and dilatancy improvements in uncemented sands treated through MICP." J. Geotech. Geoenviron. Eng., 10.1061/(ASCE)GT.1943-5606.0001407, 02815004.

[8] Whiffin, V. S., van Paassen, L. A., and Harkes, M. P. (2007). "Microbial carbonate precipitation as a soil improvement technique." Geomicrobiol. J., 24(5), 417–423

[9] Burbank, M. B.,Weaver, T. J.,Williams, B. C., and Crawford, R. L. (2012). "Urease activity of ureolytic bacteria isolated from six soils in which calcite was precipitated by indigenous bacteria." Geomicrobiol. J., 29(4), 389–395.

[10] Park, S. S., Choi, S. G., and Nam, I. H. (2014). "Effect of plant-inducedb calcite precipitation on the strength of sand." J. Mater. Civ. Eng., 10.1061/(ASCE)MT.1943-5533.0001029, 06014017.

[11]Choi, s., Park, S., Wu, S. and Chu, J. (2018). '' Methods for Calcium Carbonate Content Measurement of Biocemented Soils.'' J. Mater. Civ. Eng., 2017, 29(11): 06017015

[12] Bernard, L. D. M. A. (2019). "Biologically Induced Cementation for Soil Stabilisation." Unpublished Master's thesis at Curtin University, 1:16

[13] Chi, L., De Y, Shihui, L., Tuanjie Z, Siriguleng, B., Yu, G. and Lin, L. (2017). "Improvement of geomechanical properties of bio-remediated aeolian sand." Geomicrobiology Journal. Doi: 10.1080/01490451.2017.1338798

[14] Osinubi, K. J., Gadzama, E.W., Eberemu, A. O., Ijimdiya, T.S. and Yakubu, S. E.(2019c) Evaluation of the Strength of Compacted Lateritic Soil Treated with Sporosarcina Pasteurii.Proceedings of the 8th International Congress on Environmental Geotechnics (ICEG 2018), "Towards a Sustainable Geoenvironment" Edited by Liangtong Zhan, Yunmin Chen and Abdelmalek Bouazza, 28th October – 1st November, Hangzhou, China, © Springer Nature Singapore Pte Ltd., 3:419–428, On-line: https://doi.org/10.1007/978-981-13-2227- 3_50.

[15] Edwards, S. G. and Seddon, B. (2000). Selective medium based on tyrosine metabolism for the isolation and enumeration of Brevibacillus brevis (Bacillus brevis). Letters in Applied Microbiology, **31**: 395-399

[16] Cowan T. B and Steel S. A. (2003) District laboratory practice in tropical countries part 2, 2nd edn. Cambridge University Press, New York.

[17] Bergey K. (2004) Soil Improvement Using Microbial: A Review." Indian Geotechnical Conference IGC2016, 15-17 December 2016, IIT Madras, Chennai, India.

[18] Whiffin, V.S., Paassen L.A.v., Harkes M.P. (2007). "Microbial carbonate precipitation as a soil improvement technique," Geomicrobiology Journal, 24: 417–423.