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Effect of Microbial Induced Calcite Precipitation on the Classification and Strength Properties of Lateritic Soil as a Subgrade Material

Olapoju P. O., Ige, J. A. & Oluremi J. R.
Department of Civil Engineering
Ladoke AKintola University of Technology
Ogbomoso, Nigeria
E-mail: jroluremi@lautech.edu.ng
Phone: +2348062328259

ABSTRACT

With the unavoidable use of weak lateritic soil as subgrade materials and the environmental concerns of the chemical stabilization of soils, there is a need to investigate a more sustainable and environmental friendly solution, the use of Microbial Induced Calcite Precipitation (MICP) to improve the mechanical properties of the soil. Hence, this study investigated the potential of the application of *Bacillus Mycoides*, a bacteria to improve the geotechnical properties of lateritic soil for a subgrade application. Some geotechnical tests on atterberg limits, compaction, California bearing ratio organic matter and calcium carbonate contents were performed on the soil samples before and after treatment. The compaction and CBR tests were performed for 7, 14, and 28 days after treatment. The findings showed that the fineness of soil, liquid limit (LL), plastic limit (PL), plasticity index (PI), and organic matter decreased with the application of microbial treatment, whilst the calcium carbonate content increased after treatment. After the application of *Bacillus Mycoides*, the maximum dry density (MDD) and CBR increased with a corresponding decrease in OMC for both soaked and unsoaked condition. Hence, there were significant improvement on the geotechnical features of the lateritic soil, indicating that Microbial Induced Calcite Precipitation (MICP) is a potential stabilization method for soil improvement for use in road construction.

Keywords: Lateritic soil, Microbial Induced Calcite Precipitation (MICP), Microbial Treatment, Geotechnical properties and tests, *Bacillus Mycoides*

1. INTRODUCTION

Over the years, Nigerian government has spent huge amounts in the routine maintenance of roads due to noticeable pavement failures sometimes even few weeks into the usage of some newly constructed roads. Investigations from various researchers have revealed that most of these failures occurred as a result of weak subgrade soil (Osuolale, 1997).

A number of conventional approaches have been used in geotechnical engineering applications to improve the engineering properties (p^H , particle size distribution, consistency, strength) of soil over the years including the use of chemical additives such as cement, blast furnace slags, and agricultural residue such as cassava peels, ground nut shell ash, coconut shell ash, biagasse etc. (Osinubi et al, 2019).

However, due to the concerns of cost, eco-friendliness, possibility of contamination of groundwater, and threats to human environment regarding these methods, there is a lingering challenge to provide a more sustainable solution to this problem (Osinubi et al, 2017). One of these viable solutions is the use of microbial induced calcite precipitate (MICP) (Osinubi, 2020, Delong, et al, 2013), which consists of a biological process through which micro-organism (naturally occurring non-pathogenic bacteria) react with minerals (calcium source and cementation reagent) to produce calcite as a by-product to modify and improve the engineering properties of soil (Wei-Soon et al, 2012).

Apart from improving the geotechnical properties of soil, microbial induced calcite precipitate (MICP) has also been used to solve various engineering problems such as to repair cracks in concrete (Ramachadran et al, 2001), reduce permeability (Nemati and Voordoum 2003), repair of calcareous monuments (Dick et al, 2006), improve the compressive strength of concrete (Ramachadran et al, 2001), improve concrete durability (De Muynck et al, 2008), select enhanced oil recovery site (Stewart and Fogled, 2001), waste water treatment (Hammes et al, 2003), increase bioconcrete, cementation of porous media, improvement in strength and stiffness of engineering soils, and hydraulic control of engineering facilities (Chi et al, 2017). Moreover, the performance of microbial induced calcite precipitate is found to be adequate in soil enhancement for road construction and waste containment application (Neville 2000, Nwaiwu 2004, Eberemu 2008, Ijimaliya 2010, Oluremi, 2015, Osinubi et al, 2015, Osim 2017).

The basic group of micro-organisms of importance in the study of microbial grouting are the bacteria which come in three shapes (i) rods (Bacillus) (ii) sphere (Cocus) and (iii) spirals (Spirillum). The micro-organism used in the MICP technique using ureolysis (organisms that reach with urea) can be from any of the general Bacillus, Sporosarcins, Sporobactobacillus, Clostridium and Desulfotomeculum (Karim, et. al 2016). Microbial activities can change the appearance of soil and increase its ability to withstand applied forces by precipitating calcite, which binds soil particles. Microbes constitute between 70% and 85% of the living component within soil systems, but a good understanding of their metabolic rate is essential to forecast correctly how microorganisms will act under different condition (Yargicoglu, 2015). The number of organisms in a single kilogram of soil at the surface is between 10^9 and 10^{12} (Mitchell, 2015 and Umar et al, 2016) and the presence of microorganism may not cause any harm to the soil environment as the majority are native species of the soil.

The desired product of MICP is calcite and its function is to improve the geotechnical properties of soil. MICP can occur through any of the following processes. Urea hydrolysis, photosynthesis, sulfate reduction, denitrification (nitrate reduction), ammonification, or methane oxidation. Among all these processes through which calcite are formed, ureolysis is the most preferred because of its straightforwardness for the precipitation of calcite (Zhu, 2016). Also, it can achieve 90% chemical conversion efficiency of calcite in less than 2hours, followed by photosynthesis (Mujah, 2017).

Osinubi et al, (2019) found that the UCS values satisfied the regulatory minimum value of 200KN/m² for all suspension density considered as a result of its investigation of the unconfined compressive strength of lateritic soil treated with bacillus coagulans for use as liner containment system. Pahala et al, (2019) had shown that MICP is one step closer to in-situ application. Jian Chu (2014) also agreed that MICP could improve the geotechnical properties of soil and the most suitable micro-organism is the facultative and microgeophilic bacteria.

Various factors affect MICP such as nutrients (Umar, et al, 2016), bacteria type (Wei-Soon et al, 2012), geometric compatibility of bacteria (Rodriquez et al, 2012), bacteria cell concentration (Anbu et al, 2016), fixation and distribution of bacteria in soil, temperature (Godlead, et al, 2015 & Whiffin, 2004), reagents concentration (Dawoud, et al, 2014, LaRock, 2001, Burbank et al, 2011 and Al Qabany et al, 2012), pH (Osinubi et al, 2020), and injection method. Hence, keeping all factors except geometric compatibility constant, this study evaluated the performance of microbial induced calcite precipitate as a possible alternative to the conventional method of soil improvement. It focused on assessing the changes in the geotechnical properties (p^H, particle size distribution, compaction, California bearing ratio) of the lateritic soil after treatment with *Bacillus mycoides* MT047265.

2. METHODOLOGY

2.1 Material Sampling

Lateritic soils were obtained from laterite mass deposit a Toll-gate along Lagos-Ibadan express way with the geographical coordinate (A) 7° 19' 28" N and 3° 52' 27" E, (B) 7° 19' 32" N and 3° 52' 29" E, (C) 7° 19' 33" N and 3° 52' 38" E in Oluyole Local Government Area, Oyo State.

2.2 MICP Treatments

The microorganism used in this study is *Bacillus mycoides* MT047265 and it was collected from Civil Engineering Department, The Polytechnic, Ibadan. The organism was collected on Petri dish and sub-cultured in order to re-affirm its identity before it was applied. The bacteria was selected because of the performance history in Durojaye et al., (2022).

2.3 Geotechnical Tests

The tests carried out include p^H, organic content, specific gravity, particle size distribution, Atterberg limits, compaction, California bearing ratio and calcium carbonate content.

2.3.1 Particle Size Distribution

Particle size distribution analysis was carried out using both the mechanical sieving techniques and hydrometer with conformity to BS 1377 (1990). The soil that passed through the 425 µm sieve was used for the Atterberg Limits tests.

2.3.2 Atterberg Limits

Atterberg limits such as liquid limit, plastic limit, shrinkage limit and plasticity index were conducted in accordance to the BS 1377 (1990) before and after treatment with *Bacillus mycoides* MT047265.

2.3.3 Compaction

The objective of this test was to establish the existing link between moisture and density for a specific compactive energy. The least compactive effort (Proctor compaction) according to the BS 1377 (1990) was used.

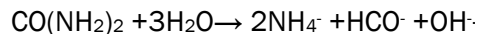
4000 g of soil sample that passed through the 4.75 mm sieve was mixed with 5% water content relative to the mass of soil, then divided into three layers in the mould, each receiving 27 blows from a 2.5 kg rammer. The moisture content was determined from both the surface and bottom of the mould. The moisture-density relationship was used to determine the maximum dry density (MDD) and optimum moisture content (OMC). The compaction tests were carried out for the treated soil samples after 7, 14, 28 days of the application of microbial treatment.

2.3.4 California Bearing Ratio (CBR)

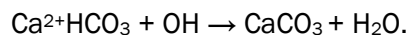
California bearing ratio tests were conducted for the natural and treated soil samples in accordance to the BS 1377 (1990). Two sets of soil sample were compacted in a CBR mould using proctor compactive effort that is, 56 blows of 2.5 kg rammer dropped from 300mm height and allowed to saturate for 7 days. For the unsoaked sample, the compacted soil was taken to the CBR machine to determine the strength and the stiffness of the compacted soil sample at both the top and the bottom of the sample respectively. However, for the soaked sample, the compacted specimen was immersed in a curing tank to soak for 72 hours before a similar approach to determine the strength and stiffness. The CBR tests were repeated for the treated soil samples after 7, 14, 28 days of the application of *Bacillus mycoides* MTO47265.

2.4 Treatment of Sample for Microbial Induced Calcite Precipitation

The *Bacillus mycoides* is a ureas producing bacteria which was added to the lateritic soil. The bacillus mycoides was grown in a nutrient broth at temperature 37°C under the aerobic condition. The cementation fluid included 3g of nutrient broth, 10g of NH₄Cl, 2.12g of NaHCO₃, 20g of urea and 2.80g of CaCl₂ per litre of distilled water. The role of *Bacillus Mycoides* was to secret enzyme urease which will aid MICP process by urea hydrolysis as shown in the reaction below.



The pH increased as a result of NH₄⁺ and thus calcium chloride supplied calcium ion and hence calcium calcite (CaCO₃) is formed.



The calcite formed acts as a binder within the soil specimen and the process of bio-clogging and bio-cementation was activated.

2.5 Calcium Carbonate Content

Acid wash method was used to determine the Calcium Carbonate Contents (CCC) of the soil samples. 5 g of natural soil and treated soil with *Bacillus mycoides* were mixed with 20 ml 1-M Hydrochloric acid (HCl) to dissolve calcium carbonate. The solution and insoluble solids were washed by diluted water on filter paper with a coarse pore size in a 75µm sieve for 10min. The remaining soil on sieve 75 µm were oven-dried and weighed.

$$CCC = \frac{100 - EX100}{F}$$

Where E= weight of post washing sample
 F= original soil sample

The calcium carbonate content were determined at 0, 7, 14 and 28 days respectively.

3. RESULTS AND DISCUSSION

The pH value of the untreated soil was found to be 4.5, thus the soil was acidic and increased to 8.5, indicating alkalinity upon the treatment with *Bacillus mycoides* MT047265 and cementation reagents.

3.1 Particle Size Distribution

Figure 1a indicated the grading curve of the samples A, B & C before treatment. Since more than 35% of the soil sample in the three natural soil samples passed through sieve no 200 (0.0075 mm) i.e, 41.46%, 36.12% and 37.12% respectively.

Figure 1b indicated the grading curve of samples A, B & C after the application of *Bacillus mycoides* MT047265 showing that we have less than 35% of the soil passing through sieve no 200 (0.0075 um) that is 28.62%, 29.04% and 28.78% respectively. Thus, the reduction in fine particles indicate an improvement in the classification properties of the soil after treatment with the bacteria.

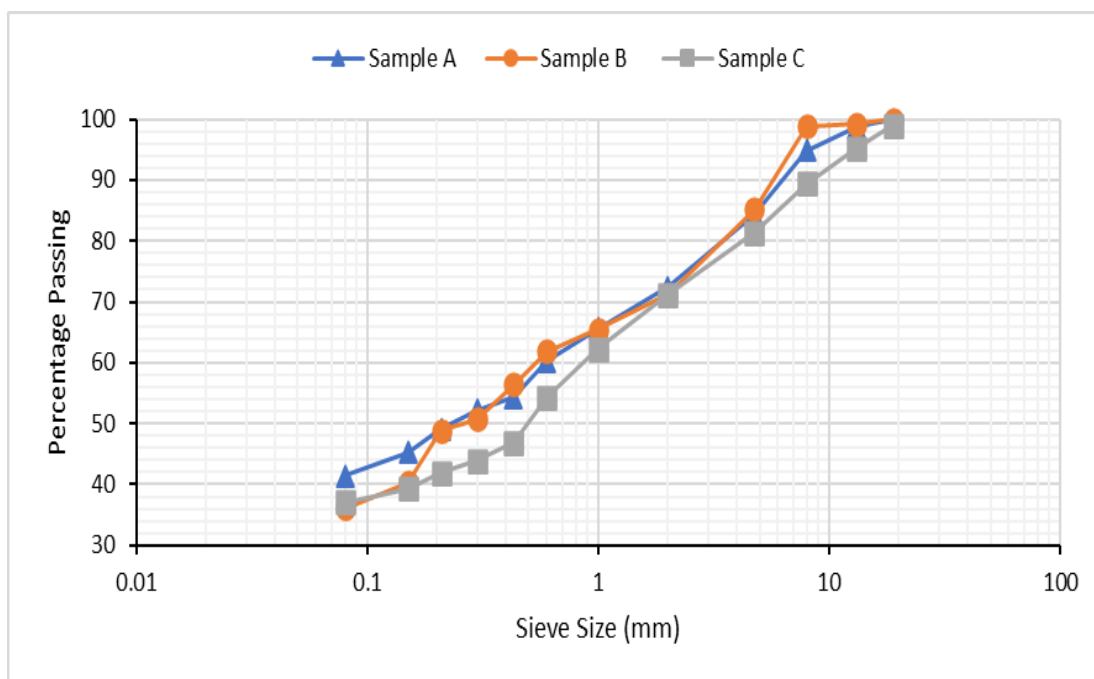


Figure 1a: Particle size distribution of all samples before treatment

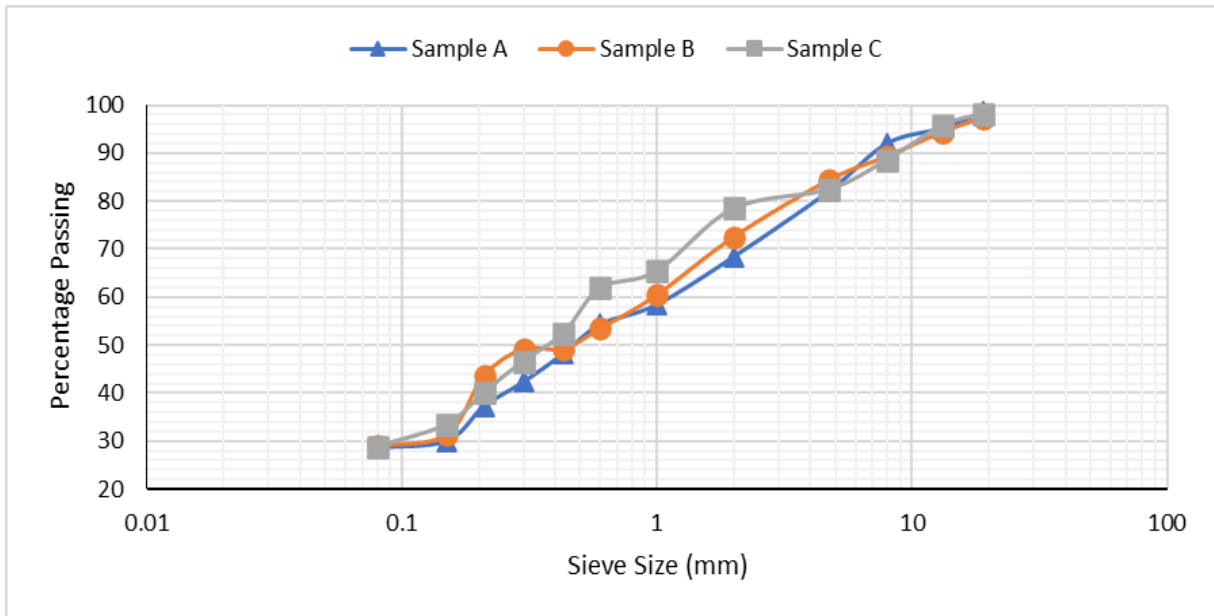


Figure 1b: Particle size distribution of all samples after treatment with *Bacillus Mycoides* MT047265. Table 1 shows the summary of the index properties of all the soil samples before and after treatment. The average specific gravity of the soil is 2.63.

Table 1: Index Properties of the natural and MICP stabilized lateritic soil samples

Property	Untreated Samples			Treated Samples		
	A	B	C	A	B	C
Percentage passing No. 200 sieve	41.6	36.12	37.12	28.62	29.04	28.78
Natural Moisture content (%)	15.6	16.5	14.2	9.8	15.8	12.3
Liquid Limit%	55.6	45	34.48	21	20	24
Plastic limit %	39	24.75	18	11.25	7.6	12.9
Plasticity index %	16.6	20.25	16.48	9.75	12.4	11.1
Specific gravity	2.68	2.62	2.58	2.65	2.6	2.63
AASHTO classification	A-4 (2)	A-4 (2)	A -4 (2)	A-4 (2)	A-4 (2)	A-4 (2)
USCS	SC	SC	SC	SC	SC	SC
Organic content	3.75	4.8	4.49	2.28	2.83	2.5
Colour	Reddish	Reddish	Reddish	Reddish	Reddish	Reddish
Dominant clay mineral	Kaolinite	Kaolinite	Kaolinite	Kaolinite	Kaolinite	Kaolinite
Group index	1.3	1.95	0.7	0.7	0.49	0.25

3.2 Atterberg Limits

From Table 1, it is shown that the plasticity index of Sample A, B, and C reduced from 16.6 to 9.75, 20.25 to 12.4, and 16.48 to 11.1 respectively. The reduction of the atterberg limits after 28 days the application of *Bacillus mycoides* MT047625 indicates soil improvement in terms of performance as a construction material.

3.3 Organic Matter Content

The organic matter content of the natural soil samples A, B and C were found to be 3.75%, 4.8% & 4.49% with an average of 4.35% organic matter before microbial treatment. After treatment with *Bacillus mycoides* MT047265, the organic matter content reduced for all samples A, B and C to 2.28%, 2.83% and 2.51% respectively, producing an average of 2.54% after the microbial treatment. This reduction also indicates an improvement in the geotechnical properties of the soil under consideration.

3.4 Compaction Test

Figure 2a and 2b shows the variation of the optimum moisture content and maximum dry density respectively, of the lateritic soil samples with days of stabilization at soaked and unsoaked condition.

The trend showed an increase in maximum dry density values with a corresponding reduction in optimum moisture content with time after the treatment of the soil with *Bacillus mycoides* MT047625 for all the samples.

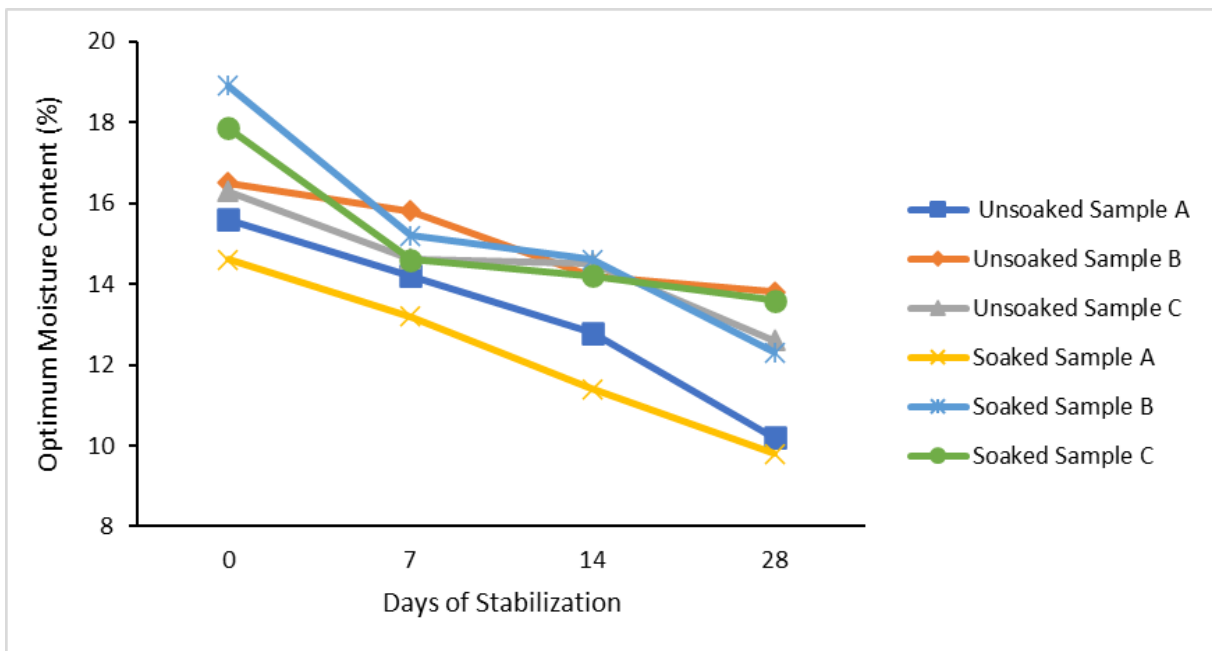


Figure 2a: Variation of OMC with days of stabilization at soaked and unsoaked condition for all the samples.

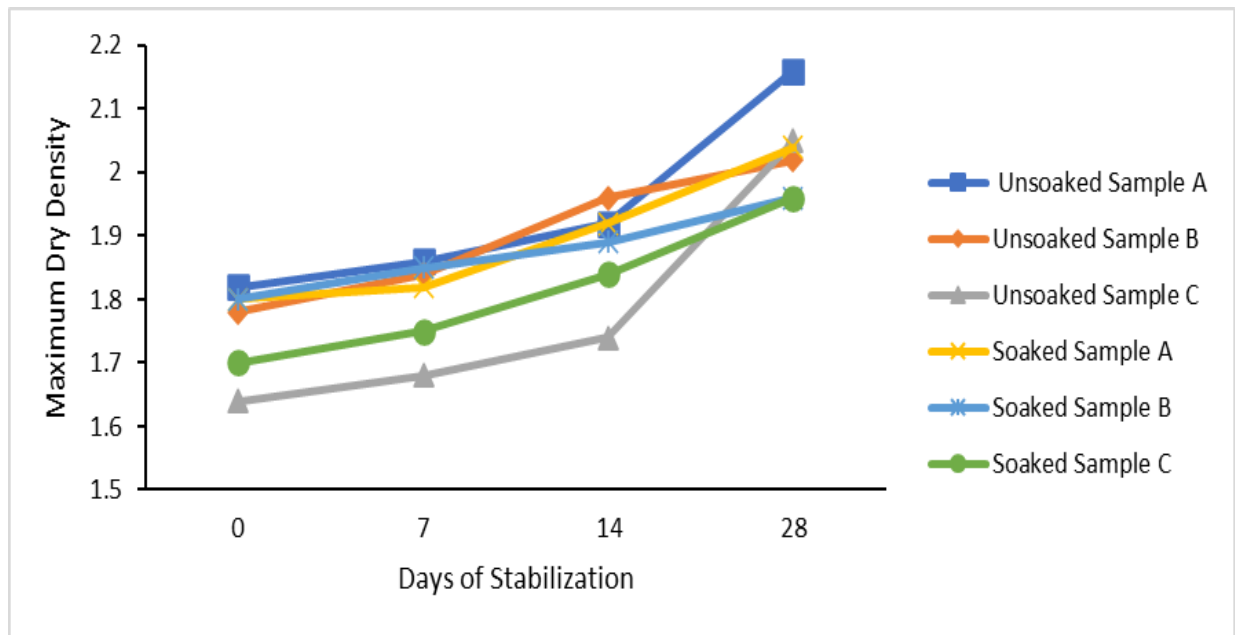


Figure 2b: Variation of MDD with days of stabilization at soaked and unsoaked condition for all the samples.

3.5 California Bearing Ratio

Figure 3a and 3b shows the result of California Bearing Ratio for the three soil sample A, B & C for both the unsoaked and soaked conditions respectively. This shows an increasing trend in the CBR values for both conditions for all the samples. In the soaked condition from day 0 to day 28 of microbial treatment, the CBR values of Sample A increased from 6.75% to 10.4%, Sample B increased from 6.05% to 10%, and Sample C from 5.91% to 11.2%. Additionally, for the unsoaked condition, the CBR values increased from 53% to 90.5%, 55.5% to 81.3%, 48.1% to 81.3% for Samples A, B, and C respectively before and after 28 days of microbial treatment.

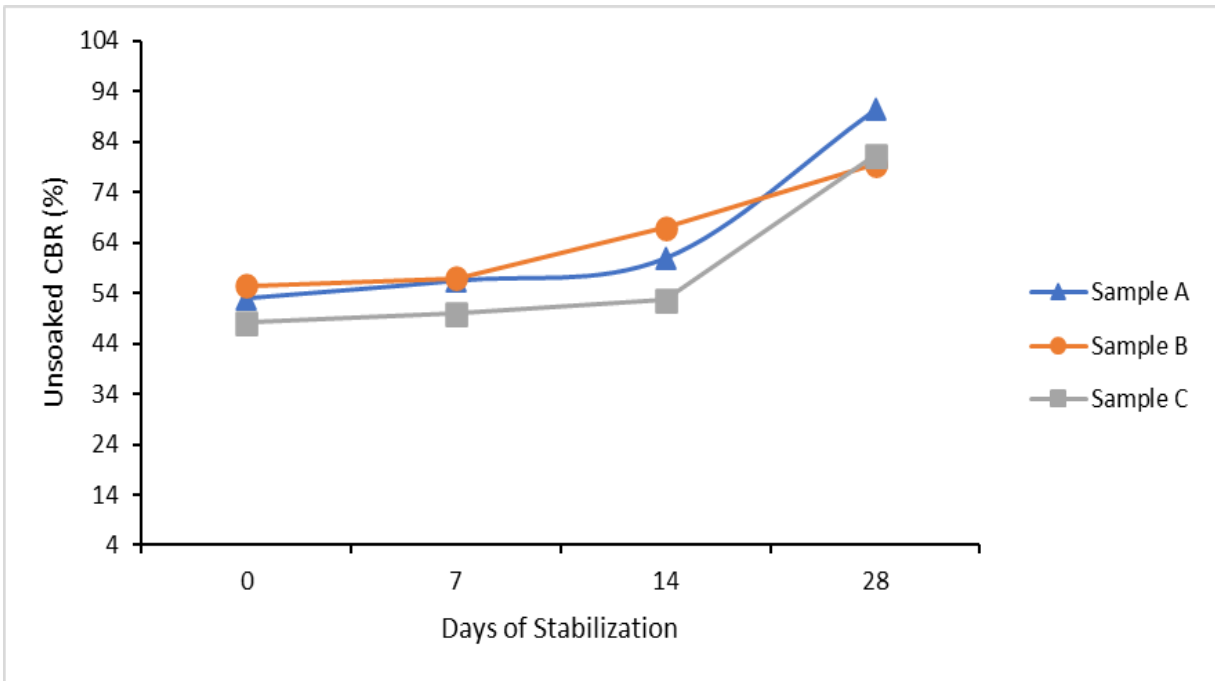


Figure 3b: Variation of CBR with days of stabilization at unsoaked condition for all the samples.

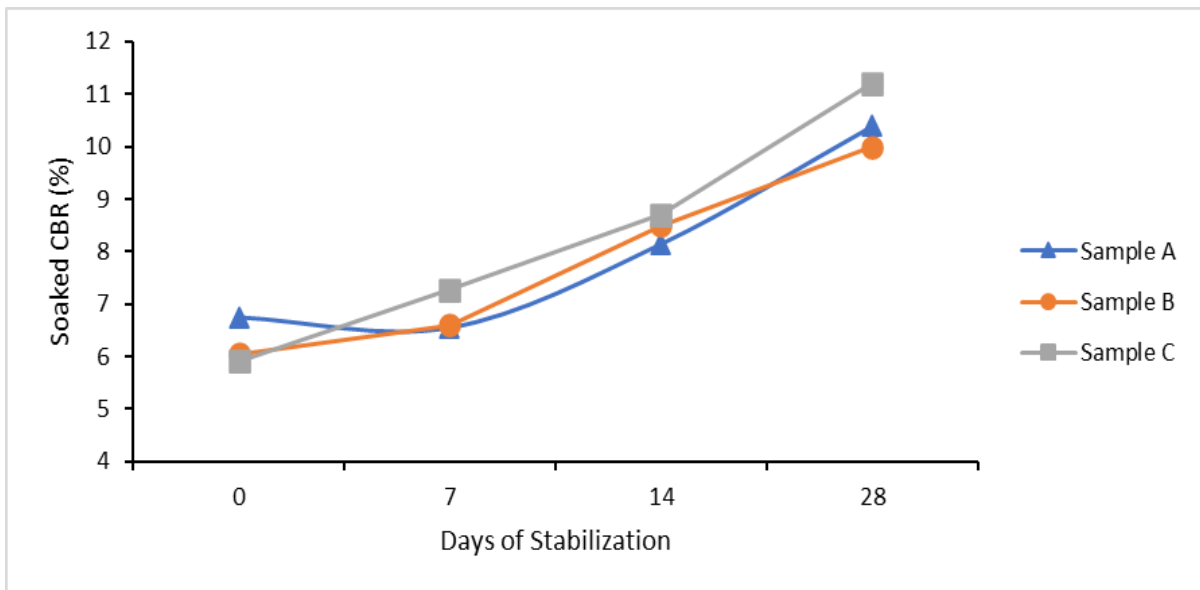


Figure 3b: Variation of CBR with days of stabilization at soaked condition for all the samples.

3.6 Calcium Carbonate Content

Figure 4 shows that the calcium carbonate precipitation increases with the ages of treatment. The calcite precipitate of Sample A increased from 2.6% at day 0 to 6% after 28 days of treatment with *Bacillus mycoides* MT047625, sample B increased from 3.6% to 5.6% and that of sample C increased from 3.2% to 5.2%. T

he higher the calcium carbonate contents, the higher the pozzolanic effect of the microbial induce calcite precipitation, thus, the bio-clogging (reduction in permeability of the soil) and bio-cementation (increase in shear strength) had been successfully activated in the soil samples under consideration.

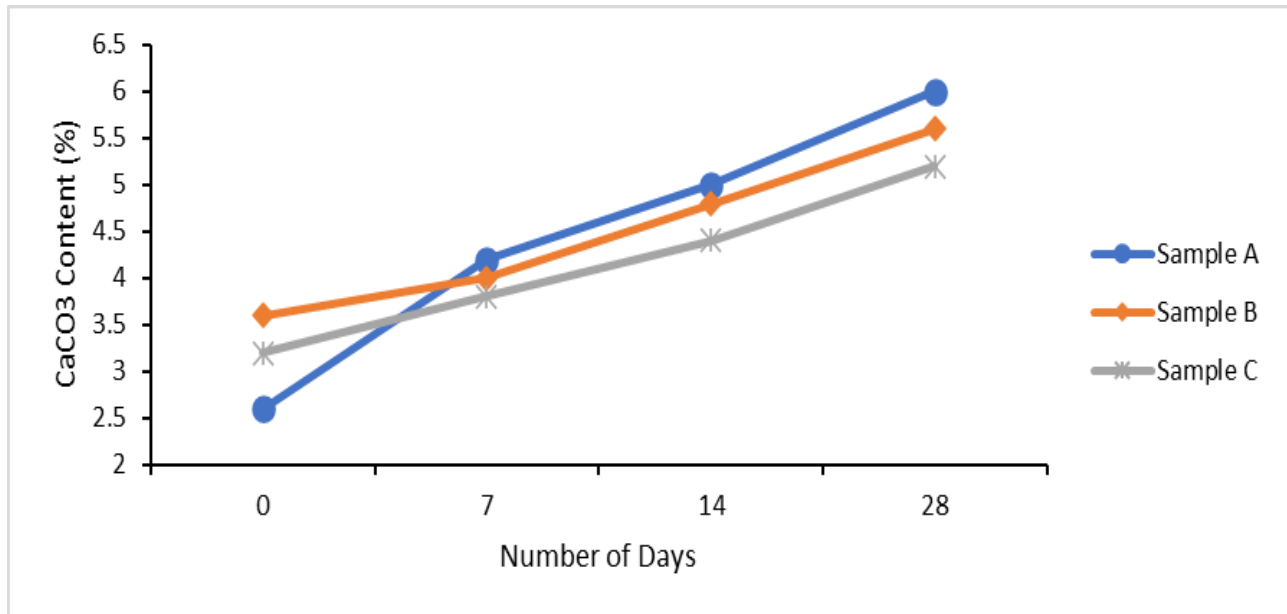


Figure 4: Variation of CaCO_3 content with days for all the samples.

4. CONCLUSION

Based on the results of the microbial treatment of weak lateritic soil with *Bacillus mycoides* MT047625 and various geotechnical tests conducted on the lateritic soil in accordance with the BS 1377 (1990), the following conclusions were made:

- i. The reduction in fine particles, atterberg limits (liquid limit, plastic limit, plasticity index) and organic matter content of the soils after 28 days the application of *Bacillus mycoides* MT047625 shows an improvement in the classification properties of the soil after treatment with the bacteria.
- ii. There was an increase in the calcium carbonate content of the soils after microbial treatment which is an indication of improvement of the mechanical properties of the soil.
- iii. The CBR and MDD values of the lateritic soil increased upon treatment and steadily increased with increasing number of days of stabilization with a corresponding decrease in OMC for both the unsoaked and soaked conditions. This indicates of improved strength and reduction in hydraulic conductivity of the soil
- iv. Overall, there were significant improvement in the classification properties of the soil after the MICP treatment with *Bacillus mycoides* MT047625. This implies that native urease forming bacteria can be utilized in MICP soil improvement and that this green technique can be employed effectively and efficiently in the improvement of mechanical properties of soil.

However, since factors that affect MICP such as nutrients, types of bacteria, bacteria cell concentration, temperature, pH, reactant concentration, injection method, fixation and distribution of bacteria in soil were all kept constant in this study except geometric compatibility of the bacteria, it is recommended that further investigations on the effect of each factor on the application of MICP on soil improvement should be carried out.

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