Journal of Basics and Applied Sciences Research (JOBASR)
ISSN print: 3026-9091
Volume 2(1) March 2024
DOI: https://doi.org/10.33003/jobasr-2024-v2i1-41

The Effect of Bacillus *Pumilus* on the Free Swell and Atterberg Limits of Black Cotton Soil for use in Road Subgrades

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ABSTRACT
The study evaluated the potential utilisation of Bacillus *pumilus* (B. *pumilus*) induced calcite precipitate for the improvement of the engineering properties of black cotton soil in road construction which is an eco-friendly technique and forms part of what is referred to as green engineering. The effect of B. *pumilus* on the free swell and Atterberg limits of black cotton soil for use in road subgrades was studied using microbial induced calcite precipitation (MICP) method. Five stepped B. *pumilus* suspension density of 0, 1.5 x 10^8, 6.0 x 10^8, 1.2 x 10^9, 1.8 x 10^9 and 2.4 x 10^9 cells/ml, respectively, were utilized for the treated specimens, while only cementitious reagent acted as the control specimen. Three (3) mix ratios of % Bacteria suspension (B) - %Cementitious reagent (C) (i.e., 25B - 75C, 50B - 50C and 75B - 25C) were adopted. It was observed that free swell treated with 25B - 75C decreased from 70 % and 50 % for the natural and control specimens, respectively, to minimum value of 46 %, with increasing B. *pumilus* suspension density at 2.4 × 10^9 cells/ml. Atterberg limits results showed liquid limit values decreased from 53 % and 62 % for the natural and control samples, respectively, to 43 % for samples treated with 25B - 75C at B. *pumilus* suspension density of 2.4 x 10^9 cells/ml, while the plastic limit increased from 28.2 % for the control soil sample to 30 % for the 75B - 25C mix ratio at B. *pumilus* suspension density of 2.4 x 10^9 cells/ml. Tests results indicated a considerable improvement with 25B - 75C mix ratio at B. *pumilus* suspension density of 2.4 × 10^9 cells/ml which significantly reduced the free swell, while the Atterberg limits fell below Nigerian General Specifications requirements of not greater than 35% Liquid limit, 12% Plasticity index and 35% passing No.200 sieve for utilization as road subgrades. However, it could be used as a subgrade material in low-volume roads.

Keywords:
Bacillus *pumilus*,
Free swell,
Atterberg limits,
Green engineering.

INTRODUCTION

The property of a soil influences the nature and design of the structure built on it. Consequently, problematic soils have the potential of causing serious damages, if they are not identified and stabilized (Mishra, 2015). Black cotton soils (BCS) regarded as problematic soils display characteristics of increased volume on soaking up water and reduced volume and contract when moisture is lost (Sadjadi *et al*., 2014). Therefore, buildings in this type of soil have always been a problem for engineers as the structure supported by it often distorts and cracks unexpectedly (Prashantha and Anupam, 2017).

In order to employ expansive soils successfully, it is essential that proper treatment of the soil is carried out. There are several soil improvement techniques such as stabilization, soil reinforcement, removal of the existing weak soil as well as substituting it with a non-expansive soil etc. (Mohamed, 2021). The stabilization of soils to improve the mechanical properties are commonly used to replace deficient materials on site during construction (Seyed *et al*., 2012; Afrin, 2017)

In engineering today, non-traditional chemical stabilizers are also used to improve soils (Guruprasad *et al*., 2016). Additionally, the application of agricultural or industrial waste ashes to increase the engineering applications of BCS have proven successful in soil improvement (Ijimdiya, 2010; Amruta *et al.*, 2016). However, the use of chemical and cement stabilization methods in geotechnical engineering projects affects the underground pH level, contributes to soil and groundwater contamination due to their toxic and hazardous attributes (DeJong *et al*., 2006; Armand *et al.*, 2021).

In recent years, geotechnical engineers have advanced a sustainable way of improving soil properties, which is based on utilizing microorganisms and related biological processes (Ijimdiya and Musa, 2017). This draws the attention to the use of environmentally friendly soil improvement technologies which have comparative advantages over conventional soil improvement methods. The concept behind this emerging eco-friendly method is the ability of microorganism in the soil to react chemically and yield deposits that can cover or/and unite the soil particles (Murtala *et al*., 2016). The calcite precipitation from the sediments produced in the process of the reaction is known as Microbial Induced Calcite Precipitation (MICP). MICP as an evolving microbiological method of soil stabilization is environmentally friendly and can be used in a sustainable way. The study aimed at evaluating the potentials of B. *pumilus* on the free swell and Atterberg limits of BCS for use in road subgrade applying MICP technology. The objective of the study was to determine the variations in free swell and Atterberg limits of BCS when treated with stepped Bacillus *pumilus* suspension density.

MATERIALS AND METHODS

Materials

Black cotton soil

The BCS used in the study was collected by disturbed sampling method from a borrow pit located in Gombe state (Latitude $10^0$19'N and Longitude $11^0$30'E), Nigeria. The top soil was removed to a depth of 0.5 m before samples were collected.

Microorganism

The urease-producing microorganism used was Bacillus *pumilus* classified according to the American Type Culture Collection, ATCC 27142. Stepped concentration of Bacillus *pumilus* solution of 0 /ml, 1.5 x 10^8 cells/ml (0.5MFS) , 6.0 x 10^8 cells/ml (2.0 MFS), 1.2 x 10^9 cells/ml (4.0 MFS), 1.8 x 10^9 cells/ml (6.0 MFS) and 2.4 x 10^9 cells/ml (8.0 MFS), respectively, were mixed with the soil and a cementitious reagent. The media, which is liquid, was sterilized in an autoclave for a duration of 20 minutes at a temperature of 121°C.

Cementitious reagent

The composition of the cementitious reagent used in this study include 3g of nutrient broth, 20g of urea ($CO(NH_2)_2$), 10 g of ammonium chloride (NH_4Cl), 2.12g of sodium bicarbonate ($NaHCO_3$) and 2.8g of calcium chloride ($CaCl_2$) per litre of distilled water (Stocks-Fischer *et al*., 1999; Stoner *et al*., 2005; Osinubi *et al.*, 2019 a,b). The cementitious reagent stimulated the urea hydrolysis process.

Methods

Free swell

The test was carried out in accordance with the United States Bureau of Reclamation (USBR) method (Holtz and Gibbs, 1956). Free swell was calculated using:

$$\text{Free Swell} = \frac{\text{Final Volume} - \text{Initial Volume}}{\text{Initial Volume}} \times 100\% \quad (1)$$

The same process was then replicated for various percentages of bacteria suspension and cementation reagents.

Atterberg limits

The Atterberg limits test includes the determination of liquid limits, plastic limits and the plasticity index for the natural and treated soils. Test procedures were carried out in line with Test 1(A) BS 1377 (1990) Part 2 for the natural and treated soil samples.

Preparation of samples and MICP procedures

To calculate the amount of B. *pumilus* for varying cells/ml and cementitious reagent for free swell and Atterberg limits MICP technique was performed. 400g of the natural soil specimen going through BS Sieve No.40 (425μm aperture) was used for this procedure. The soil was mixed with each bacterial suspension and cementitious reagent in a bowl. The volume of B. *pumilus* solution of 1.5×10^8, 6×10^8, 1.2×10^9, 1.8×10^9 and 2.4×10^9cells/ml and cementitious reagent calculated based on three mixes as follows:

For the first mix ratio:

The soil sample was mixed with 25% bacterial suspension and 75% cementation reagent (i.e., 25B - 75C) of the total volume which was obtained from the equations;

Total Volume =

$$\frac{\text{Liquid Limit of Natural Sample}}{100} \times \text{Weight of soil sample} \quad (2)$$

Volume of Bacteria =

$$\frac{25\%\ of\ the\ Liquid\ Limit\ of\ Natural\ Soil}{100}\ x\ 400g \quad (3)$$

Volume of Cementation Reagent =

$$\frac{75\%\ of\ the\ Liquid\ Limit\ of\ Natural\ Soil}{100}\ x\ 400g \quad (4)$$

For the second mix ratio; the natural soil sample was mixed with 50% bacterial suspension and 50% cementation reagent (i.e., 50B - 50C) of the total volume which was obtained from the equations.

For the third mix ratio; the natural soil sample was mixed with 75% bacterial suspension and 25% cementation reagent (i.e. 75B - 25C) of the total volume obtained from the equations;

After the three test mix ratios, the treated soil specimen were air-dried and thereafter crushed then sieved with BS No.40 sieve (425 μm aperture) for free swell and Atterberg limits tests.

RESULTS AND DISCUSSION

Natural Black Cotton Soil

Table 1 shows the index properties of the natural black cotton soil, while Table 2 summarized its oxide composition.

Table 1: Properties of the natural black cotton soil

Property	Quantity
Natural moisture content, %	15.1
Percentage passing No. 200 sieve	82.0
Free Swell, %	66.7
Liquid Limit, %	53.0
Plastic Limit, %	26.0
Plasticity Index, %	27.0
Linear Shrinkage, %	11.4
AASHTO Classification	A-7-6 (24)
Unified Soil Classification System (USCS)	CH
Specific Gravity	2.42
Maximum Dry Density, Mg/m^3	1.66
Optimum Moisture Content, %	17.5
Colour	Greyish black
Dominant clay mineral	Montmorillonite

Table 2: Oxide Composition of Black Cotton Soil

Oxide	Concentration (%)
CaO	3.58
SiO_2	49
Fe_2O_3	14.23
Al_2O_3	15.1
MnO	0.23
TiO_2	2.09
K_2O	2.25
V_2O_5	0.1
Cr_2O_3	0.022
CuO	0.022
Ag_2O	2.17
Eu_2O_3	0.16
LOI	11.1

Effect of Bacillus pumilus on Black Cotton Soil

Free swell

Figure 1 shows the variation of free swell (FS) of BCS with B. *pumilus* suspension density

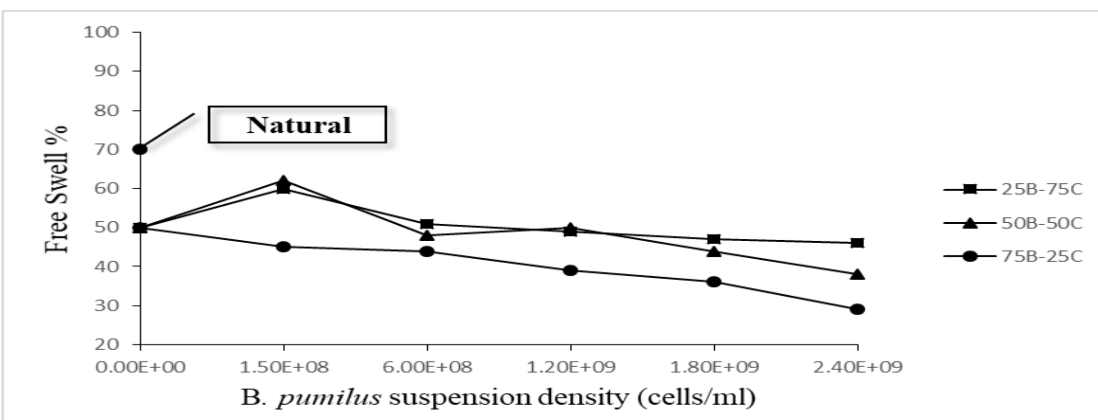

Figure 1: Variation of free swell of black cotton soil with B. *pumilus* suspension density

Free swell values recorded for the natural and control specimens were 70.0% and 50.0%. Generally, it was observed that FS decreased with increasing B. *pumilus* suspension density. The lowest FS values of 46, 38 and 29 % at B. *pumilus* suspension density of 2.4 x 10^9 cells/ml were recorded at treatment of 25B – 75C, 50B – 50C and 75B – 25C mix ratios, respectively. The drop in FS with increasing B. *pumilus* suspension density could be linked to the high population of bacteria (from 0 to 10^9 cells) which influenced the amount of calcite precipitation during MICP process that helped to neutralize the swelling effect of clay particles in the soil (Anbu *et al*., 2016; Eberemu *et al*., 2021).

Atterberg limits

Liquid limit

Figure 2(a) shows the variation of liquid limit (LL) of BCS with B. p*umilus* suspension density

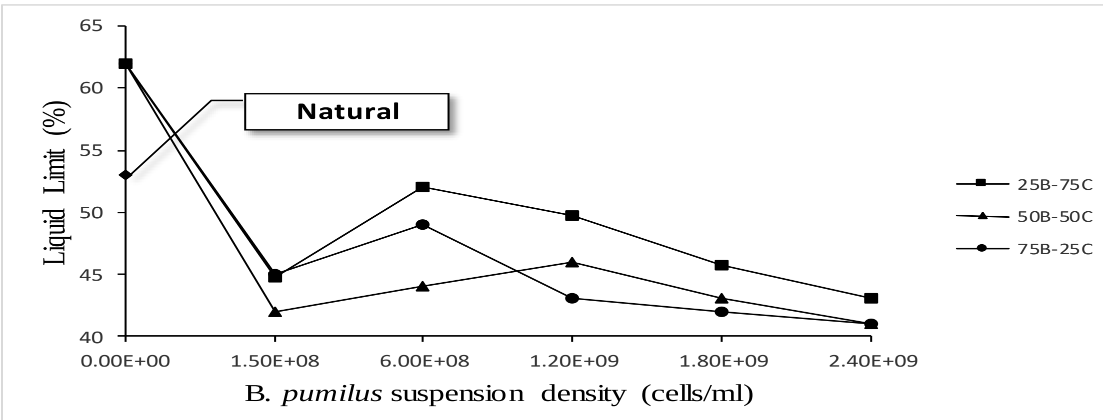

Figure 2(a): The variation of liquid limit of black cotton soil with B. *pumilus* suspension density

Generally, LL values dropped with higher B. *pumilus* suspension density for all the mix ratios studied. The natural and control specimens recorded LL values of 53.0 and 62.0 %, respectively. For 25 B – 75 C, 50 B – 50 C and 75 B – 25 C mix ratios, minimum LL values of 43.0, 41.0 and 41.0 %, respectively, were obtained at B. p*umilus* treatment of 2.4 x 10^9 cells/ml. The decreasing trend likely due to precipitation of larger quantity of calcite at higher B. p*umilus* suspension density. In a biogeochemical reaction, the formation of calcite precipitate occurs from the nucleation of bacteria cells. Therefore, the quantity of calcite precipitated increased with higher bacteria cell concentration (Li *et al.*, 2015; Osinubi *et al*., 2017a; Tiwari *et al.*, 2021).

Plastic limit

Figure 2(b) shows the variation of Plastic Limit (PL) of BCS with B. *pumilus* suspension density.

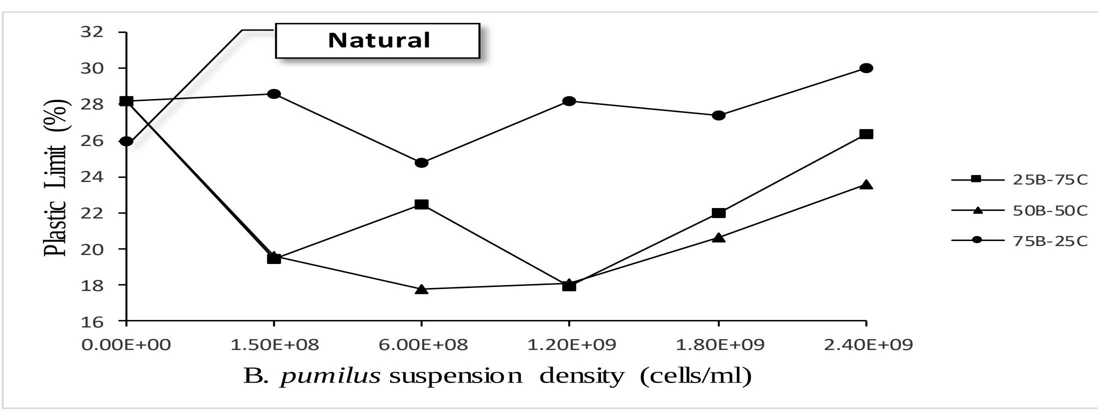

Figure 2(b): Variation of plastic limit of black cotton soil with B. *pumilus* suspension density

PL values for natural and control samples are 26.0 and 28.2 %, respectively. The PL values initially decreased to minimum values at B. *pumilus* treatment of 6.0 x 10^8 cells/ml and thereafter increased to 26.4, 23.6 and 30.0 % for 25 B – 75 C, 50 B – 50 C and 75 B – 25 C test mixes accordingly at B. *pumilus* teatment of 2.4 x 10^9 cells/ml. The gradual increase in PL can be attributed to the cation exchange reaction resulting from precipitated calcites in the reaction of cementation fluid involving carbonate ions generated during the process of urea hydrolysis and calcium ions which led to the bonding of the soil grains (Neupane, 2016; Osinubi *et al.*, 2017).

Plasticity index

Plasticity index (PI) is the mathematical difference between the liquid limit (LL) and plastic limit (PL). Figure 2(c) shows the variation of PI of BCS with B. *pumilus* suspension density

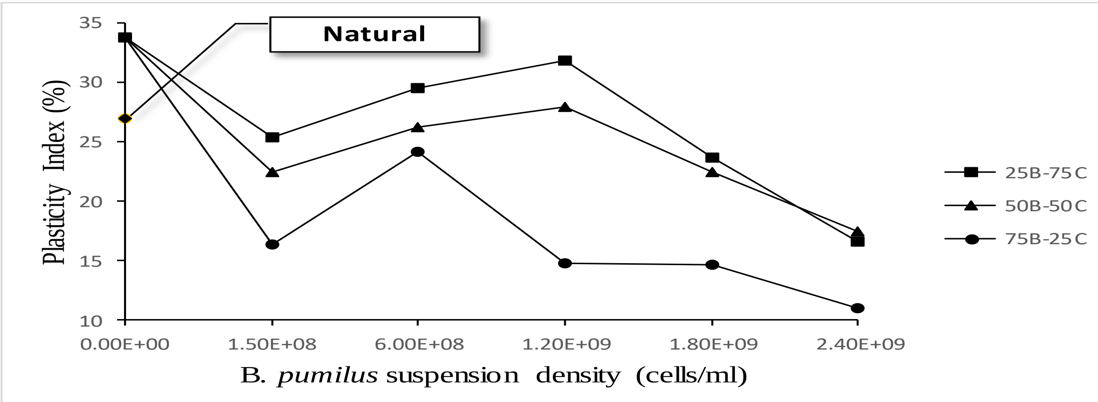

Figure 2(c): Variation of plasticity index of black cotton soil with *B. pumilus* suspension density

The PI of the natural BCS and control samples were 27.0 and 33.8 %, respectively. Peak PI values of 29.5 and 26.2 % were documented for treatment with 25 B – 75 C and 50 B – 50 C mix ratios, respectively, at B. *pumilus* treatment of 1.2 x 10^9 cells/ml and 24.2 % for 75B-25C mix ratio at 6.0 x 10^8 cells/ml, before dropping to 16.6, 17.4 and 11.0 % at B. *pumilus* suspension density of 2.4 x 10^9 cells/ml. Generally, these values indicate that the PI decreased as the bacteria concentration increased. The decrease in PI value of the treated specimens indicates enhancement of the natural soil because of the calcite precipitate that cemented the soil particles and clogged the voids (Miller and Azad,2002; Salahudeen *et al*.,2014).

CONCLUSION

Black cotton soil classified as A–7-6 (24) and CH group according to AASHTO and USCS, respectively. The soil was modified with B. *pumilus* suspension densities of 0, 1.5 x 10^8, 6.0 x 10^8, 1.2 x 10^9, 1.8 x 10^9 and 2.4 x 10^9 cells/ml. 3 mix ratios of 25 B – 75 C, 50 B – 50 C and 75 B – 25 C were chosen to ascertain the influence of bacteria (B) -cementation (C) reagent mixture treatment on the soil specimens. The index properties (free swell and Atterberg limits) increased with upper B. *pumilus* suspension density. The best values recorded for BCS treated with 25 B – 75 C mix ratio did not satisfy Nigerian General Specifications requirements of not more than 35 % liquid limit, 12 % maximum plasticity index value and 35 % passing sieve No. 200 (0.075mm aperture) for use in road subgrades.

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