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Assessment of Embankment Erosion of Lateritic Soil Bio-treated with *Bacillus Thuringiensis*

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Abstract: *The research evaluated the potential of ureolytic organism (Bacillus thuringiensis, Bt) through microbial induced carbonate precipitation technique in mitigating lateritic soil erosion. The urease activity and the pH of the varying bacterial (0, 1.5 × 10^8, 6.0 × 10^8, 1.2 × 10^9, 1.8 × 10^9, and 2.4 × 10^9 cells/ml) and cementation solution, C_s(0.25 M, 0.5 M, 0.75 M and 1 M) mixtures was evaluated. The rate of soil detachment of natural and bio-treated modelled embankment slopes was tested using a fabricated jet erosion device in the laboratory. The results indicated that the urease activity evaluated using electrical conductivity method increased with increase in C_s and Bt density in solutions. The increase in EC values for the C_s is in the order: 1 M >0.75 M > 0.5 M > 0.25 M regardless of the bacterial population in solution mixtures. The recorded pH values fall within the range (i.e., 6 - 9) that reportedly favours an effective MICP process. The rate of soil detachment as well as total erosion mass decreased with higher bio-treatment with Bt - C_s. Therefore, lateritic soil bio-treated with Bacillusthuringiensis (Bt) (2.4 × 10^9 cells/ml) - cementation solution concentration (C_s) (0.75 M) using the mixing method can be used to mitigate embankment erosion rate of lateritic soil.*

KEYWORDS: *Bio-treatment,Embankment, Erosion, Lateritic soil, Bacillusthuringiensis*

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1. INTRODUCTION

The field of bio-inspired and bio-mediated geotechnics has experienced sustained research interest among geotechnical/geo-environmental engineers in the last two decades. The motivation driving the technology stems from the unique way nature offers efficient and resilient solutions to problems akin to real-time geotechnical engineering problems. This is particularly interesting and provokes curiosity because the key components of the natural biome (i.e., plants, animals, and microorganisms) lack access to materials and energy but are able to develop techniques to provide foundation support and anchorage, dig and tunnel through soft and loose soils. They are able to develop these solutions by adopting a trial and error process of natural selection during their evolution history (Martinez and Frost, 2023).

The solutions achieved over time by these natural systems have motivated the novel ideas behind the bio-inspired and bio-mediated geotechnical engineering solutions to address today's engineering problems (Gadzama et al., 2023). The soil microbes over the years have found useful applications in the area of waste management through bio-degradation and composting, bio-energy through microbial fuel cells, bio-construction through bio-cementation and premeditation through the use of organisms such as fungi, algae bacteria and plants to immobilise, breakdown, detoxify, change or remove undesirable chemical species or pollutants in an environment (Ganguly et al., 2023).

A lot of research involving chemical compounds produced from different combinations, such as liquid polymers, silicates, enzymes, resins, lignin derivatives, and acids, which are generally referred to as non-traditional stabilisers is documented in scholarly literature (Basha *et al*., 2005; Liu *et al*., 2011; Gadzama*et al*., 2023; Amadi*et al*., 2023). However, more studies have been carried out with respect to the application of chemical additives such as lime and cement also known as traditional stabilisers(Karol, 2003; Liu *et al*., 2011; Etim*et al*., 2017; Idhame*t al*., 2023). The associated problems involving the use of the traditional stabilisers including the undesirable emissions (carbon dioxide, CO_2) which put the environment in harm's way have prompted interest in resilient and sustainable alternative soil strengthening methods.

Worthy of note is emerging multi-disciplinary research aimed at harnessing the biogeochemical activities to augment the engineering attributes of marginal soils. The research field is generally recognised as microbial-induced calcite precipitation (MICP). Calcite mineral precipitation (a by-product of metabolic activities of urease-positive soil micro-organism present in a vicinity) is deposited within an enclave provided the right conditions exist (Rusznyk*et al*., 2012; Omoregie*et al*., 2023; Jain, 2021; 2023). Soil microbes capable of initiating urease activity have been identified and classified; also, research has shown that such bacteria are bound in the soil (Nasser *et al*., 2018; Jain, 2021). Studies have shown that the MICP technique improves significantly the mechanical characteristics of permeable materials and is also effectively employed in erosion mitigation (Devrani*et al*., 2021; Dubey *et al*., 2022; Naeimi*et al*., 2023; Gadzama*et al*., 2022; Yohanna*et al*., 2022; Montoya, 2023).

In this study, the ability of *Bacillus thuringiensis* (Bt) induced calcite precipitate to improve the erosionresistance of lateritic soil modelled embankment using a fabricated jet erosion test apparatus in the laboratory was evaluated.

II. MATERIALS AND METHOD

2.1 Materials

Soil: Soil samples were obtained by disturbed sampling technique at depths of 0.5 m - 3 m at a location with coordinates of 6° 12'15"N and 7°0'40"E on latitude and longitude, respectively, at Abagana, in Anambra State, Nigeria. The soils in the study area are basically ultisolsand alfisols,which are naturally prone to erosion (Okorafor *et al.,* 2017).

Microorganism:*Bacillus thuringiensis* (Bt) was isolated from the soil and cultured using biochemical confirmatory test kits to identify and characterise Bt in the laboratory. It was used throughout the research.

Cementation solution**:** The solution is made of equimolar urea and calcium chloride in addition to constituents recommended by Stocks-Fischer et al., 1999which include ammonium chloride (10 g), sodium bicarbonate (2.12 g) and nutrient broth (3 g) dissolved per dm^3 of de-ionised water for optimal cementation solution during MICP protocols. Four varying concentrations of cementation solutions (C_s) (i.e., 0.25, 0.5, 0.75 and 1 M) consisting of nutrient broth, ammonium chloride (NH_4Cl), urea ($CO(NH_2)_2$), sodium bicarbonate ($NaHCO_3$) and calcium chloride ($CaCl_2$) were used.

2.2 Methods

2.2.1 Urease activity and pH test

Urease activity was evaluated indirectly by employing the electrical conductivity (EC) approach suggested by Whiffin (2004). This was determined immediately following sample preparation under standard conditions. In this method, 1 ml of Bt solution was blended with 9 ml of cementation mixture and the EC results were recorded for a period of 5 minutes at 26° C. A unit urease activity refers to the required number of urease enzymes to hydrolysed 1 mM of urea present in the cementation solution in one minute (Whiffin, 2004; Lauchnor*et al.,* 2015; Sun *et al.,* 2018). During hydrolysis, for any 1 mole of urea produced, 2 moles of NH^{4+} (ammonium ions) is liberated. Lauchnor*et al.* (2015) reported a correlation between EC and urease activity (the amount of hydrolysed urea during the process) as presented in equation (1):

$$Urea\ hydrolysed\ (mM) = \Delta EC\left(\frac{mS}{m}\right) \times 0.1111 \quad (1)$$

As reported in literature (e.g., Stocks-Fischer *et al.,* 1999; Sun *et al.,* 2018), urease activity is common in weak and warm acid/alkaline medium relative to harsher or extreme media. Also, there is rarely noticeable change in urease activity for pH range of 6 - 9 of a given medium (Lauchnor*et al.,* 2015; Lai *et al.*, 2022; Vinay *et al*., 2023).

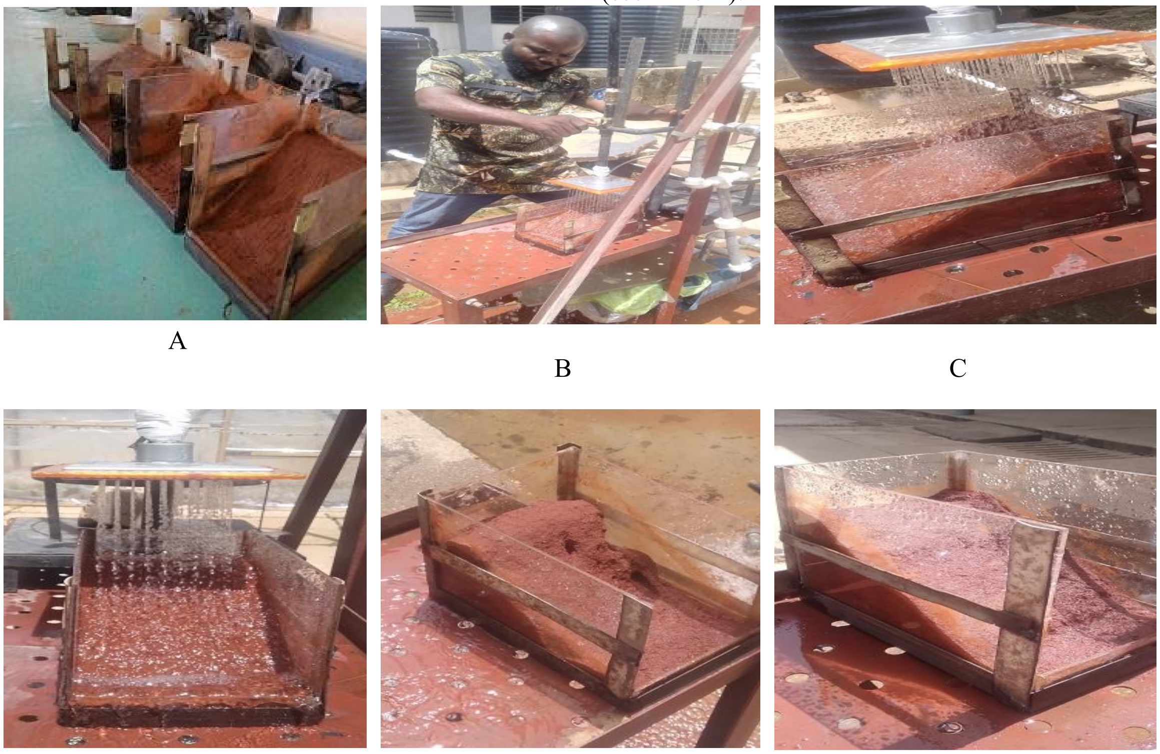
Plate I depict the formation of calcite precipitated during enzymatic reaction involving the bacterial solution and the cementation reagent (see Plate IA). Plates IB and IC show the set-ups for EC and pH tests, respectively.

A B C

Plate I: (A) Precipitated calcite (B) Electrical conductivity test set-up (C) pH test set-up.

2.2.2 Embankment erosion test

The experimental setup is made up of a water reservoir tank, a surface pump, polyvinyl chloride (PVC)/steel pipes, water sprinkle system, and a pressure gauge. The pressure gauge was connected to a surface pump that draws out water from the reservoir tank. During the experiment, the water sprinkles downwards and falls onto the prepared embankment slope placed at a specified height below the water sprinkle system to initiate an erosion condition similar to a surface runoff on the model embankment slope (see Plate II).



A B C

D E F

Plate II: (A) Model lateritic soil slopes prepared for erosion test (B) Embankment erosion test set-up (C – D) Erosion tests (E) Natural lateritic soil slope after erosion test (F) Bio-treated lateritic soil slope after erosion test

Before the commencement of the test on the model embankment slope, the side wall at the toe was removed to allow for free passage of detached soil during experimentation.The surface pump regulatory valve was opened to the calibrated constant pressure headset on the pressure gauge (in unit of bar) to achieve the desired dripping water intensity for the intended test duration (1 hour). During the tests, soil detachment occurred and washed off the embankment slope through the toe end of the model embankment. The rate, as well as the mass of the eroded soil, was noted at the end of the test.

2.2.3 Preparation of bio-treated sample and determination of erosion rate of embankment

A predetermined soil mass was first blended with 50 % of Bt solution density (established optimum bacteria-cementation reagent combination) based on natural soil OMC and placed in a sealed polythene bag for bacterial attachment period of six (6) hours. Stepped bacterial population density in solution of 0, 1.5 × 10^8, 6.0 × 10^8, 1.2 × 10^9, 1.8 × 10^9, and 2.4 × 10^9 cells/ml were used in the study. At the end of the retention time, 50 % of cementation solution, C_s (based on OMC) was added to the bacterial attached soil and thoroughly mixed. The bio-treated soil was placed in an acrylic container with Length × Width × Height dimensions of 200 mm × 120 mm × 150 mm, respectively, to form a laboratory-scaled embankment slope. The angle of the slope of the embanked soil in the acrylic container was 34^o with the dimensions 200 mm × 120 mm × 98 mm, representing a slope ratio of 1: 1.5 = Horizontal: Vertical. When the desired embankment slope was achieved, the bio-treated soil was air-cured at an ambient temperature of 25° ± 2° C before carrying out the erosion test. During curing, mass of specimens was observed until the mass loss became constant or negligible for at least three days. When the change in mass was negligible, the corresponding mass of the specimen was recorded as m_1 . The constant weighted soil was placed on the erosion test equipment to commence the erosion test. At the end of the test, the specimen was allowed to equilibrate with the prevailing laboratory conditions until it achieved similar moisture content as before erosion test. This mass was recorded as m_2. The total mass of eroded soil was calculated using equation (2):

$$M_E = M_1 - M_2 \qquad (2)$$

Where: $M_E$mass (g) of eroded soil, M_1 and M_2 is the mass (g) of dry soil before and after erosion test.

The rate of soil detachment on the slope embankment was determined as the ratio of the total eroded mass per unit area per unit time (Zhaoyu*et al.*, 2020) expressed as:

$$E_r = \frac{M_E}{B \times L \times T} \qquad (3)$$

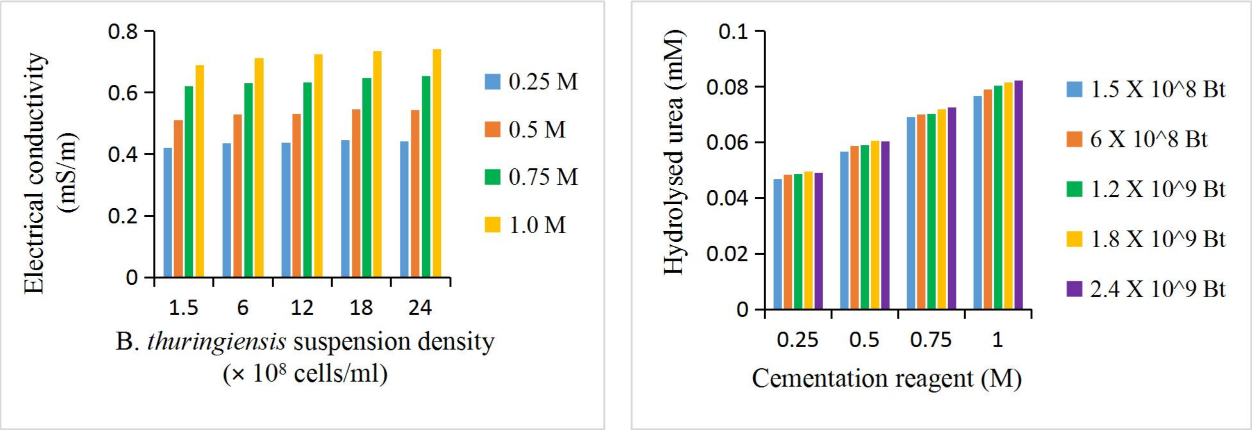
Where: E_r = rate of soil detachment g/(m^2.s), M_E = mass (g) of eroded soil, B = embankment width (m), L = slope length (m), and T = duration (s) of the erosion experiment.

III. RESULTS AND DISCUSSION

.3.1 Urease Activity and pH of Bacterial and Cementation Solution Mixtures

One popular method of determining urease activity is by the addition of colometric assays, such as Nessler's reagent to a hydrolysed urea solution to detect for ammonia. This measures the spectral absorbance of ammonium species in the solution using a spectrophotometer at a specific wavelength. However, the test procedure is strenuous and unable to provide sufficient data set for evaluation at the initial rate of urea hydrolysis process (Vinay *et al*., 2023). On the other hand, urease activity measurement using electrical conductivity (EC) method provides sufficient data points at the early stage of ureolytic hydrolysis thereby accounting for the rate of urea hydrolysis at the initial stage (McCleskey *et al*., 2012; Liu *et al.,* 2021; Vinay *et al*., 2023). The study therefore, adopted the EC method to evaluate the urease activity of the varying Bt density in solution and the various C_s concentration mixtures.

The urease activity of the various solution mixtures determined using EC at temperature of 26^o C is shown in Fig.s 1A and B. The results obtained indicate an increase in EC values with an increase in C_s and *Bt* density in solutions. The EC values were observed for a period of 5 – 10 minutes for each category of *Bt* density in solution and the varying C_s mixtures. The initial EC value of 0.42 mS/m recorded for the reaction involving *Bt* (1.5 × 10^8 cell/ml), and 0.25 M Cs increased to 0.441 mS/m when the *Bt* density in solution was increased to 2.4 × 10^9 cell/ml in the same C_s (0.25 M). Similarly, the EC value of 0.653 mS/m recorded for the mixture of 2.4 × 10^9 cell/ml and 0.75 M C_s increased to 0.741 mS/m for the mixture containing 2.4 × 10^9 cell/ml and 1 M C_s. In the same vein, similar results were recorded when 0.5 M C_s was admixed with varying *Bt* densities in solution (see Fig. 1). The observed variation in the EC values is indicative of urease activity that resulted in the ureolytic hydrolysis of urea in the mixture and consequently calcite was precipitateed (Eryürük, 2022). The increase in EC values for the Cs is in the order: 1 M > 0.75 M > 0.5 M > 0.25 M regardless of the bacterial population in solution mixtures.



A B

Fig. 1: Urease activity of the various solution mixtures: (A) Variation of electrical conductivity of cementation solution with *Bacillus thuringiensis* suspension density (B) Variation of hydrolysed urea (rate of urease activity) of Bacillus *thuringiensis* suspension density with cementation solution concentration.

The high EC values recorded at higher cementation concentrations could be due to higher amount of calcium (Ca^{2+}), carbonate (CO_3^{2-}), and ammonium (NH_4^+) ions released in the mixture as a result of enzymatic urease produced by *Bt* present in the solution. The result is consistent with the findings reported by Whiffin (2004), Sun *et al.* (2018), Liu *et al.* (2021), and Vinay *et al*. (2023).

The increase in EC of the mixtures depicted the increased rate of urea hydrolysed with higher *Bt* suspension density and C_s concentration in the mixture (see Fig. 1B). It implies that the greater the *Bt* cells in the mixture, the higher the EC values and, by extension, the higher the rate of urease activity. The urease activity values recorded were in the range 0.0466 – 0.0823 mM with peak value of 0.0823 mM recorded for at 2.4 × 10^9 cell/ml - 1 M C_s mixture. Therefore, it can be deduced that the rate of urease activity increased with higher density of *Bt* cells in the medium. This was also the case when C_s concentration increased. The results obtained agree with the findings reported by Eryürük (2022).

The quantified pH values for the various bacterial and cementation solutions are presented in Fig. 2. It was observed that the pH values are in the range 8.17 - 8.45. The alkalinity of these solution mixtures could be traced to the amount of sodium bicarbonate in the cementation solution. The recorded pH values fall within the range (i.e., 6 - 9) that reportedly favours effective MICP process (Lauchnor*et al.,* 2015; Lai *et al*., 2022).

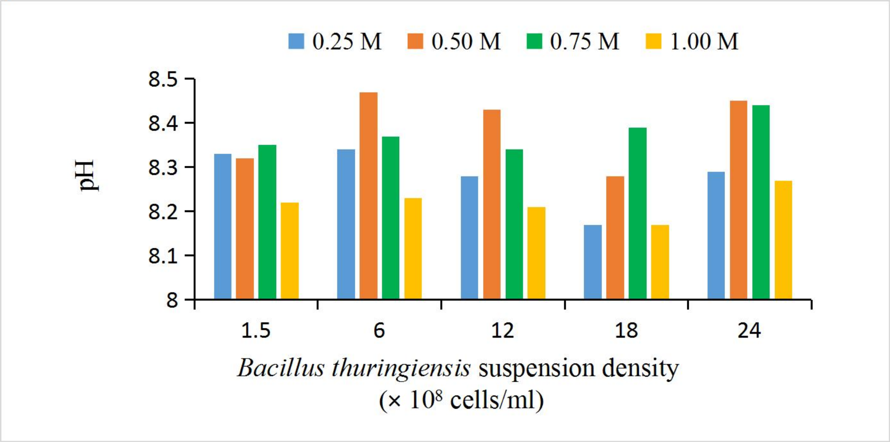


Fig. 2: Variation of pH of cementation solution concentration with *Bacillus thuringiensis* suspension density in solution.

3.2 Effect of Bt Suspension Density on Rate of Embankment Erosion

The variations in total erosion mass and soil erosion rate values of the prepared lateritic soil embankment slope with *Bacillus thuringiensis* suspension density are shown in Fig.s 3 and 4, respectively.

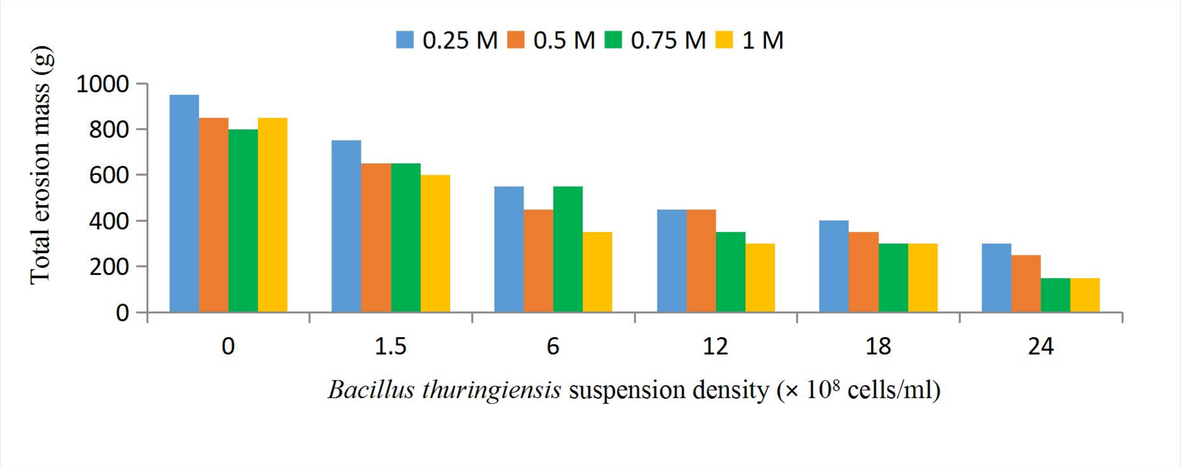


Fig. 3: Variation of total erosion mass of lateritic soil with *Bacillusthuringiensis* suspension density after 60 minutes.

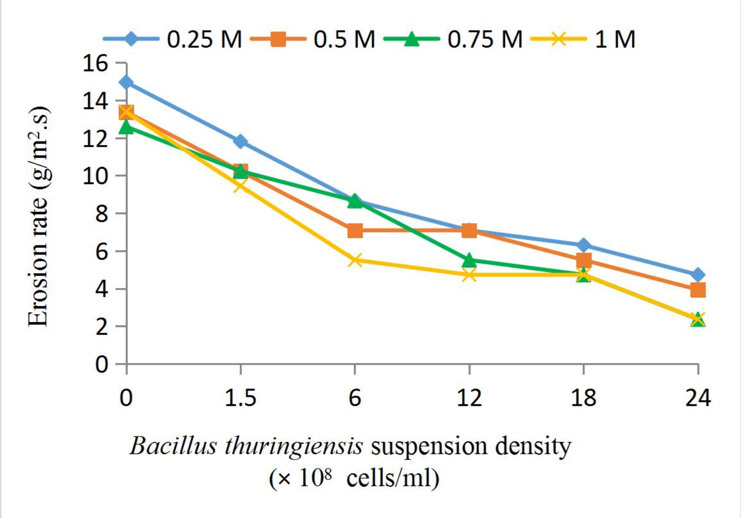


Fig. 4: Variation of erosion of embankment lateritic soil with *Bacillusthuringiensis* suspension density after 60 minutes.

The rate of soil detachment, as well as total erosion mass, decreased with higher bio-treatment with *Bt* - C_s. For the natural lateritic soil embankment, the detachment of soil particles commenced after 3 minutes and gradually increased throughout the duration of the experiment. However, the bio-treated soil embankment exhibited higher resistance to grain detachment, especially at higher *Bt* - C_s bio-treatments.

The resistance of the bio-treated soil to hydrodynamic forces may be due to calcification resulting from the bio-geochemical reaction involving enzymatic urease from Bt and urea in the cementation solution within the soil matrix that improved soil grains bonding forces thereby improving strength against detachment (Dubey *et al*., 2022). Similar findings were reported by (Zhaoyu*et al*., 2020).

IV. CONCLUSION

The following deductions can be made from the results of the research:

i. The urease activity recorded electrical conductivity (EC) values for C_s in the order 1 M > 0.75 M > 0.5 M > 0.25 M regardless of *Bt* suspension density used. The recorded pH values (8.17 - 8.45) fall within the range (6-9) conducive for effective MICP process.

ii. The bio-treated lateritic soil slope recorded better erosion resistance performance than the natural soil slope. Also, the rate of soil particle detachment and the total eroded soil mass decreased with increase in *Bt* - C_s bio-treatment of the slopes with least soil detachment rate and total eroded mass of 150 g and 2.36 g/m^2.s, respectively, recorded for 2.4 x 10^9 cells/ml - 0.75 M bio-treatment after 1 hour of erosion test.

Lateritic soil bio-treated with *Bacillusthuringiensis* (*Bt*) (2.4 × 10^9 cells/ml) - cementation solution concentration(C_s) (0.75 M) using the mixing method can be used to mitigate embankment erosion rate of lateritic soil

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