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Research Paper

Microbial Treatment of Soil Using Calcite-Inducing Bacteria: Impact on Specific Gravity

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Abstract - *This study examines the impact of microbial inoculation on the specific gravity of various soil types using Bacillus cereus MK202350.1 (B_c) and Alcaligenes faecalis DQ110882.1 (A_f). Microbial cultures at concentrations v/v of 1:100, 1:200, and 1:300 was applied to four soil samples collected from different locations. The specific gravity of the soil samples was measured over a course of 364 days. All treated samples showed a consistent increase in specific gravity when compared to untreated controls, with sandy soils showing the most noticeable effects. In general, B_c outperformed A_f, particularly at higher concentrations. The results demonstrate that microbial-induced calcite precipitation (MICP) is a promising biotechnological method for increasing soil density and enhancing geotechnical characteristics. With potential uses in ground improvement and eco-geotechnical solutions, the study demonstrates that microbial treatment is a sustainable and efficient way to increase the density and engineering behavior of soils.*

Keywords: *Microbial treatment, Specific gravity, Bacillus cereus, Alcaligenes faecalis, Sandy soil, Silty soil.*

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I. INTRODUCTION

Natural soil, though critical in civil engineering and construction purposes, often exhibits limitations in its engineering properties, and these physical properties, including specific gravity are fundamental to determining its suitability for construction, agricultural use, and environmental applications. Natural soils often lack the ideal characteristics required for construction and other engineering purposes. For example, certain soils like clay are highly sensitive to moisture changes, leading to expansion and contraction that can compromise the stability of building foundations and cause structural damage (Pushpakumara and Mendis, 2022; Ashioba and Udom, 2023). The specific gravity of soil is a key physical parameter influencing soil behaviour, as it reflects the density and mineral composition of soil solids (Wu *et al.*, 2024). Specific gravity, defined as the ratio of the density of soil solids to that of water, influences the compaction, strength, and load-bearing capacity of soil. The specific gravity of soil can influence the effectiveness of these microbial treatments, as it affects the soil's physical properties and the microbial activity within it (Vu *et al.*, 2022; Zhao *et al.*, 2025; Hossain *et al.*, 2021; Umar, *et al.*, 2023). It influences its moisture retention and nutrient availability, which are critical in geotechnical improvement of soil (Rasheed *et al.*, 2022; Verma *et al.*, 2021; Hossain *et al.*, 2021). Low specific gravity in soils, particularly in sandy and loose-textured soils, can lead to reduced bearing capacity, poor compaction, and suboptimal structural support (Umar, *et al.*, 2023; Firoozi and Firoozi, 2024).

Conventional methods for enhancing soil properties often rely on mechanical compaction or chemical stabilisation, which can be resource-intensive and may pose long-term environmental risks (Verma *et al.*, 2021; Utkarsh and Jain, 2024; Zaini *et al.*, 2024; Bian, *et al.*, 2024; Almuaythir *et al.*, 2024).In response to these challenges, microbial-based soil treatment has emerged as a sustainable and cost-effective alternative (Samantaray *et al.*, 2024; Oro, *et al.*, 2024). Certain microorganisms can influence the physical and chemical characteristics of soil through biological processes such as bio-cementation, bio-clogging, and bio-solidification (Ayilara and Babalola, 2023; Omoregie *et al.*, 2023). According to Reddy *et. al.*, (2020), it is a recently evolved bacterial based soil-stabilisation technology that generates calcite precipitation all through the soil matrix by the metabolic mechanics of microorganism, thus improving the engineering characteristics of the soil. These

processes can alter the microstructure and mineral composition of soil, potentially affecting its specific gravity (Omoregie *et al.,* 2023). Among the promising candidates for microbial soil treatment are *Bacillus cereus* and *Alcaligenes faecalis*. These naturally occurring soil bacteria are known for their ability to produce extracellular polymers, induce mineral precipitation, and interact with soil particles in ways that may lead to denser, more cohesive soil structures.

The microbial treatment of soil using *Bacillus cereus* and *Alcaligenes faecalis* has shown significant potential in enhancing soil quality and degrading contaminants, particularly petroleum hydrocarbons. Studies has shown that *Bacillus species*, are known for their ability to precipitate calcium carbonate ($CaCO_3$), which can increase soil density and alter specific gravity by filling voids between soil particles (Soltani-Jigheh *et al.,* 2020; Zeb, 2023). The introduction of these bacteria can lead to improved soil aggregation, resulting in a denser soil matrix. This was observed in studies where bacterial treatment increased shear strength and reduced optimum moisture content, indicating a denser soil structure (Soltani-Jigheh *et al.,* 2020).

While the use of *Bacillus cereus* and *Alcaligenes faecalis* shows promise for improving soil properties, the effectiveness of these treatments can vary based on soil type and environmental conditions, necessitating further research to optimize application methods and understand long-term impacts on the specific gravity if the soil (Muhsin *et al.,* 2024). Additionally, while there has been growing interest in the use of such bacteria to improve soil strength and stability, there is limited research on the long-term effects of microbial activity on the specific gravity of different soil types. This study aims to fill that gap by evaluating the effect of *Bacillus cereus* and *Alcaligenes faecalis* on the specific gravity of different soil types over a 364-day treatment period.

II. METHODLOGY

This study was conducted to evaluate the influence of microbial-induced calcite precipitation (MICP) on the specific gravity of different soil types over a period of 364 days. The experimental design involved four main components: soil types, bacterial strains and growth media, substrate solutions, and geotechnical testing procedures.

Study Area and Soil Sampling

The study was conducted in Ibadan, a major urban center in southwest Nigeria, characterised by a complex geological setting consisting of weathered basement rocks, sedimentary formations, and lateritic soils. Soil samples were collected from four distinct locations: Ologuneru (7° 28′ 50.2″ N, 3° 48′ 48.1″ E) – a semi-urban area underlain by basement rocks and lateritic soils, Apete (7° 26′ 20.9″ N, 3° 52′ 47.4″ E) – a peri-urban site with sedimentary rock outcrops, Toll-Gate (7° 19′ 29.8″ N, 3° 52′ 52.3″ E) – an urban transit zone with disturbed soil profiles, Amuloko (7° 20′ 29.0″ N, 3° 58′ 56.5″ E) – a rural setting with relatively undisturbed soil development. At each site, subsoil samples were manually collected at depths of 2–3 meters. The samples were labeled A–D, air-dried, and securely covered to prevent moisture absorption before further analysis.

Soil Characterisation

Before the application of the MICP treatment, the soil samples were geotechnically characterised to determine baseline characteristics. This is to provide basis for evaluating how microbial activity affected the specific gravity of the soil. The particle size distribution of the soil samples was determined.

Particle Size Distribution: Soil particle size distribution was determined using sieve analysis after oven-drying and washing. The sieve analysis method was used to assess the particle size distribution. Following a thorough washing to get rid of any fines, the samples were oven-dried to get rid of any last traces of moisture. After drying, 500 grammes of each soil sample were put in a stack of standard sieves that were arranged from 4.75 mm to 0.075 mm in decreasing mesh size. For effective particle separation, the sieves were placed on a mechanical sieve shaker and shaken for ten to fifteen minutes. Weighing the amount of soil retained on each sieve allowed us to determine the percentage of the total mass. The grain size distribution curves were plotted using these values, allowing the soils to be classified and their textural groupings (such as sandy, silty, or clayey) identified.

Bacterial Isolation and Screening

The B_c and A_f were isolated from randomly collected soil samples in Ibadan using microbiological method. Bacterial cultures were grown in Nutrient Broth at 37°C, 130 rpm, concentrations of 4.9×10^6 and 3.7×10^6 CFU/mL (OD_{600} = 0.433 and 0.402) respectively. DNA extraction was conducted using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research). The 16S rDNA regions were amplified using universal primers (27F and 1492R). PCR products were sequenced using the ABI 3500xl Genetic Analyzer, and sequence data were processed using BioEdit v7.2.5 and compared via NCBI BLAST to confirm bacterial identity.

Fig.1: Cementation solution preparation

Fig. 2: Identification of the isolate

Bacterial Concentration and Application

Bacterial concentrations were verified through serial dilution and colony-forming unit (CFU) counts using spread plate techniques. The experimental design involved applying the nutrient broth to cementation solution (v/v) of 1:100, 1:200, and 1:300 to 100kg soil sample and stored.

Fig 3: Identified molecular bacterial broth

Cementation Solution and MICP Treatment

A cementation solution consisting of 1M Urea and 1M Calcium Chloride in distilled water was applied to the soil samples. Application volume was based on the saturation moisture content (1 pore volume) of each soil to ensure uniform conditions. Each containerised soil sample (100 kg) received 13-20 litres single dosage of the cementation solution and 45-150 mL of nutrient-bacteria at varying concentration. The soils were left at room temperature and monitored over 364 days.

Specific Gravity Evaluation

Post-treatment, the soil samples were re-analysed to assess changes in specific gravity. The specific gravity rest were conducted at regular intervals throughout the 12-month period (0, 7, 14, 28, 56,112, 196, 280, and 364-days post-inoculation) to track microbial effects on the specific gravity of soil under different conditions and treatment levels. In accordance with BS 1377-2:1990, the pycnometer method was used to measure the specific gravity of soil particles. This involved adding de-aired distilled water after a known mass of oven-dried soil (usually passing the 2 mm sieve) had been filled into a clean, dry pycnometer bottle. After gently shaking the bottle to get rid of any air bubbles, water was added until the bottle was full. Both the mass of the pycnometer filled with water and the mass of the pycnometer filled with soil and water were measured. The weight of the soil divided by the weight of an equivalent volume of water that the soil particles had displaced was used to determine specific gravity (Gs). This feature is crucial for analyzing the soil's volumetric behavior and compaction characteristics. It also acts as a predictor of possible alterations brought on by bio-mineralisation from microbial treatments.

Fig. 4: Specific Gravity Determination

III. RESULTS AND DISCUSSION

Soil Characterisation

The four soil samples' initial characterisation provided important information about their physical and classification characteristics, which served as the foundation for assessing how microbial treatment affected specific gravity. The classification parameters in Table 1, and particle size distribution in Figures 1 – 4 gave clear differences in soil type, gradation, and plasticity.

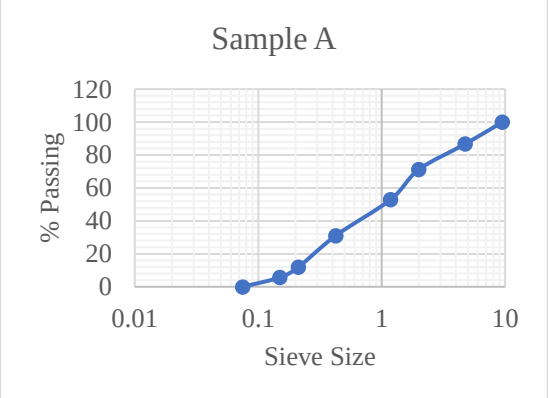

Fig. 5: Particle sieve distribution of sample A

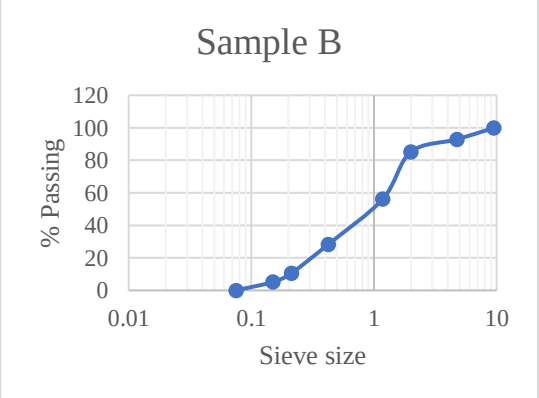

Fig. 6: Particle sieve distribution of sample B

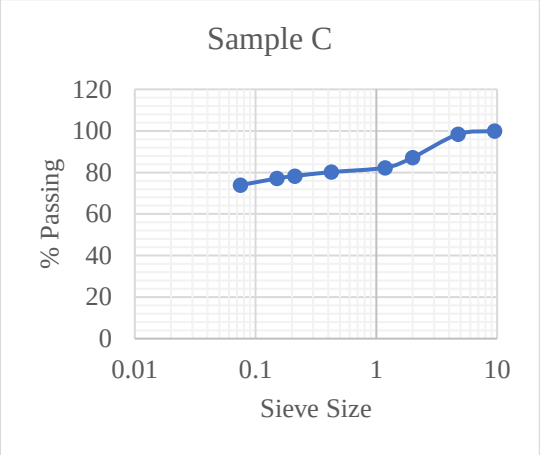

Fig. 7: Particle sieve distribution of sample C

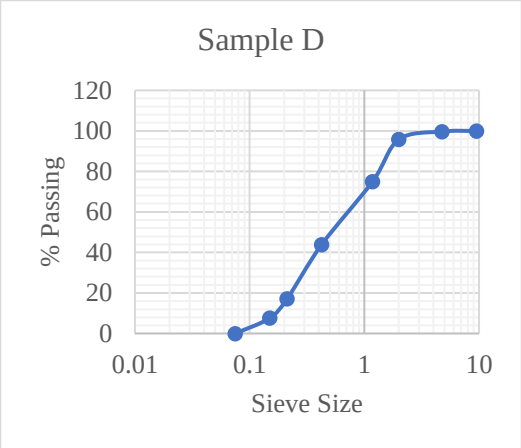

Fig. 8: Particle sieve distribution of sample D

Table 1: Classification of Soil Samples

Soil Sample	**USCS**	**AASHTO**		**Plasticity Classification**	**Group Name**
	Group Symbol	Classification	Remarks		
Sample A	SW	A-1-b	Excellent to good	Nil	Well graded sand
Sample B	SW	A-1-b	Excellent to good	Nil	Well graded sand
Sample C	ML	A-4	Fail to poor	Inorganic clays of low plasticity	---
Sample D	SW	A-1-b	Excellent to good	Nil	Well graded Sand

Table 1 summarises the classification results using the AASHTO and USCS systems According to AASHTO, samples A, B, and D (SW, A-1-b) are categorised as "Excellent to Good" soils because they lack detectable plasticity, which is characteristic of clean sands with good engineering qualities. Unless impacted by mineral precipitation or microbial activity, these samples should have minimal natural specific gravity variability.

Sample C is categorised as "Fair to Poor" under USCS and A-4 under AASHTO. It is classified as a low-plasticity, inorganic silty soil. This is due to the greater amount of fine-grained material, which influences specific gravity, compaction properties, and water retention, its behaviour is very different from that of the sand samples.

Samples A, B, and D's lack of plasticity emphasises their granular nature, whereas Sample D's low plasticity suggests some cohesion and capillarity brought on by fine particles, albeit not to the same degree as in clays.

The characterisation data is essential for interpreting the changes in specific gravity after microbial treatment. Well-graded sands (SW) are more likely to show changes in specific gravity as microbially induced calcite precipitation fills pore spaces and may bind grains. However, because of its finer particles and reduced permeability, silty soil (ML) might react differently, which could have an impact on calcite distribution and bacterial mobility.

Effect of Microbial Inoculation on Soil Specific Gravity

One important measure of soil density that affects soil stability and strength is specific gravity. The effect of microbial inoculation on the specific gravity of soil samples was evaluated over a 364-day period using varying concentrations of *Bacillus cereus* MK202350.1 (B_c) and *Alcaligenes faecalis* DQ110882.1 (A_f) admixed with cementation solution. The evolution of specific gravity for each soil sample (A–D) over time and treatment concentrations (1:100, 1:200, and 1:300) is shown in detail in Tables and Figures 5–8. The initial (control) values for all soil types served as benchmarks to assess microbial-induced changes. As the treatment days went on, the specific gravity values of the control samples for each soil gradually increased from their initial lower values. After 364 days, the specific gravity of sample A increased significantly from 2.65 (control) to 2.81 for the 1:100 concentration, while it also rose to 2.76 and 2.73 for the lower concentrations (1:200 and 1:300). This implies that the density of the soil is more significantly impacted by higher concentrations of Bacillus cereus

(MK202350.1). Similar patterns were observed in Sample B. For the 1:100 concentration, the specific gravity increased from its initial value of 2.60 to 2.74. Even lower concentrations can eventually increase soil density, as evidenced by the increases in the 1:200 and 1:300 concentrations, which reached 2.71 and 2.68, respectively. For the 1:100 concentration in Sample C, the initial value of 2.58 increased to 2.71, while the concentrations at 1:200 and 1:300 increased to 2.68 and 2.64, respectively. The specific gravity of Sample D increased from 2.61 (control) to 2.75 for the highest concentration and 2.71 and 2.69 for the lower concentrations.

Table 2: Specific Gravity of Soil Sample A induced with B_c and A_f

Testing Days	Specific Gravity of Soil Sample					
	Sample A					
	1:100		**1:200**		**1:300**	
	B.cereus	***A.faecalis***	***B.cereus***	***A.faecalis***	***B.cereus***	***A.faecalis***
Control	2.65	2.65	2.65	2.65	2.65	2.65
7	2.69	2.68	2.68	2.67	2.66	2.65
14	2.70	2.69	2.69	2.67	2.67	2.66
28	2.73	2.72	2.69	2.68	2.67	2.67
56	2.75	2.73	2.71	2.70	2.69	2.68
112	2.75	2.74	2.73	2.71	2.70	2.70
196	2.76	2.75	2.74	2.72	2.71	2.70
280	2.78	2.76	2.76	2.73	2.72	2.71
364	2.81	2.79	2.77	2.75	2.73	2.72
	Sample B					
	1:100		**1:200**		**1:300**	
	B.cereus	***A.faecalis***	***B.cereus***	***A.faecalis***	***B.cereus***	***A.faecalis***
Control	2.60	2.60	2.60	2.60	2.60	2.60
7	2.63	2.61	2.61	2.60	2.60	2.60
14	2.64	2.63	2.62	2.61	2.61	2.61
28	2.65	2.63	2.63	2.62	2.62	2.62
56	2.66	2.65	2.64	2.64	2.63	2.63
112	2.68	2.66	2.65	2.65	2.64	2.64
196	2.70	2.67	2.67	2.66	2.65	2.64
280	2.71	2.69	2.69	2.68	2.66	2.65
364	2.74	2.70	2.72	2.70	2.68	2.66
	Sample C					
	1:100		**1:200**		**1:300**	
	B.cereus	***A.faecalis***	***B.cereus***	***A.faecalis***	***B.cereus***	***A.faecalis***
Control	2.58	2.58	2.58	2.58	2.58	2.58
7	2.61	2.60	2.59	2.58	2.58	2.58
14	2.62	2.61	2.60	2.59	2.59	2.58
28	2.63	2.62	2.61	2.60	2.60	2.59
56	2.65	2.64	2.62	2.61	2.60	2.60
112	2.66	2.65	2.63	2.62	2.61	2.61
196	2.67	2.66	2.64	2.63	2.62	2.61
280	2.69	2.67	2.65	2.64	2.63	2.62
364	2.71	2.68	2.68	2.65	2.64	2.63
	Sample D					
	1:100		**1:200**		**1:300**	
	B.cereus	***A.faecalis***	***B.cereus***	***A.faecalis***	***B.cereus***	***A.faecalis***
Control	2.61	2.61	2.61	2.61	2.61	2.61
7	2.64	2.63	2.63	2.62	2.62	2.62
14	2.65	2.64	2.64	2.63	2.62	2.62
28	2.66	2.65	2.64	2.63	2.63	2.62
56	2.67	2.66	2.65	2.64	2.64	2.63
112	2.69	2.67	2.67	2.65	2.65	2.64
196	2.71	2.68	2.69	2.66	2.66	2.65
280	2.73	2.70	2.70	2.67	2.67	2.66
364	2.76	2.73	2.72	2.68	2.69	2.67

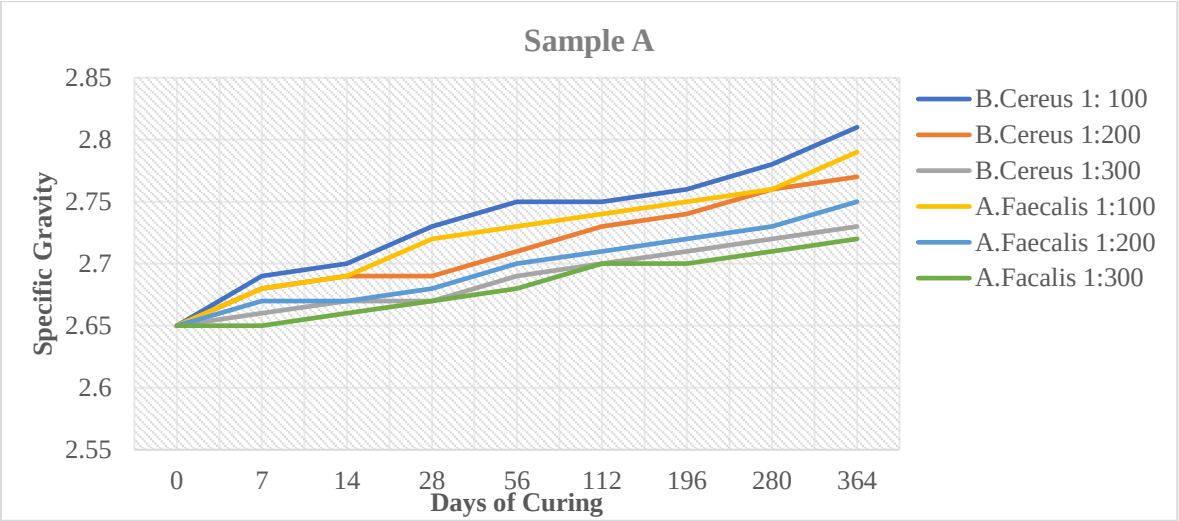

Fig. 9: Specific gravity of soil sample A treated with B_c and A_f at different dosages

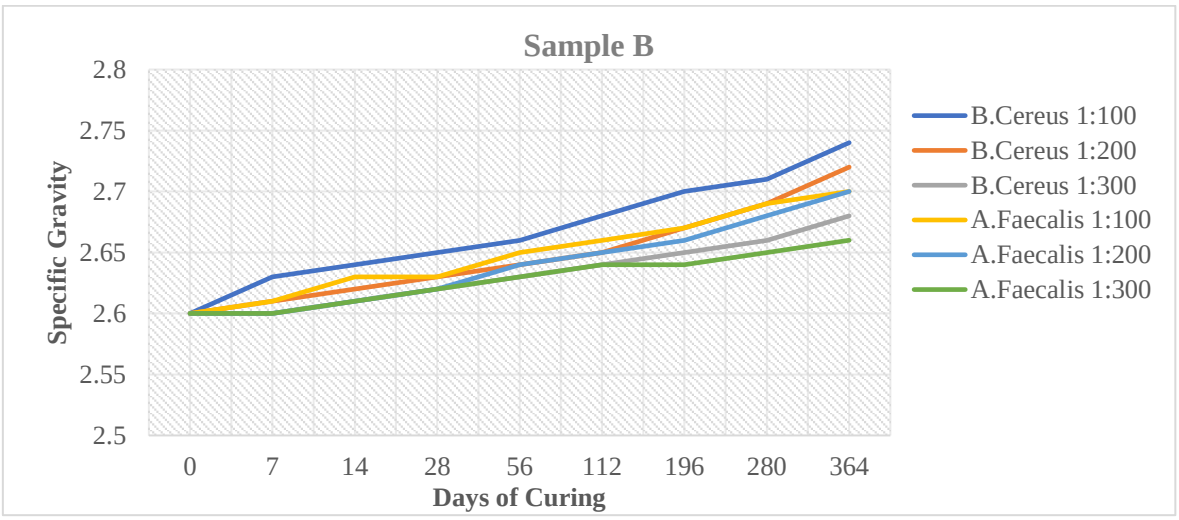

Fig. 10: Specific gravity of soil sample B treated with B_c and A_f at different dosages

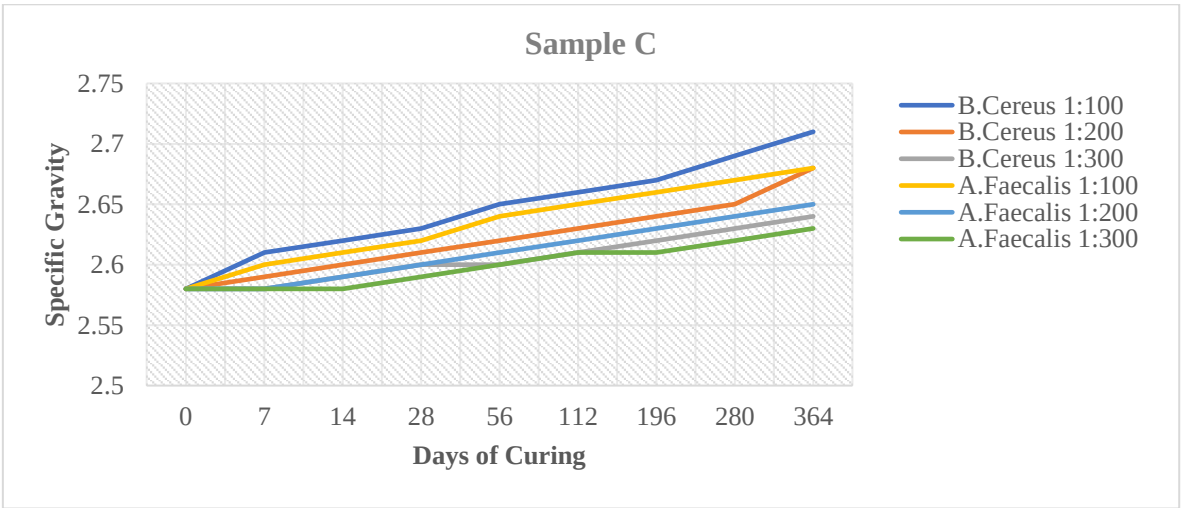

Fig. 11: Specific gravity of soil sample C treated with B_c and A_f at different dosages

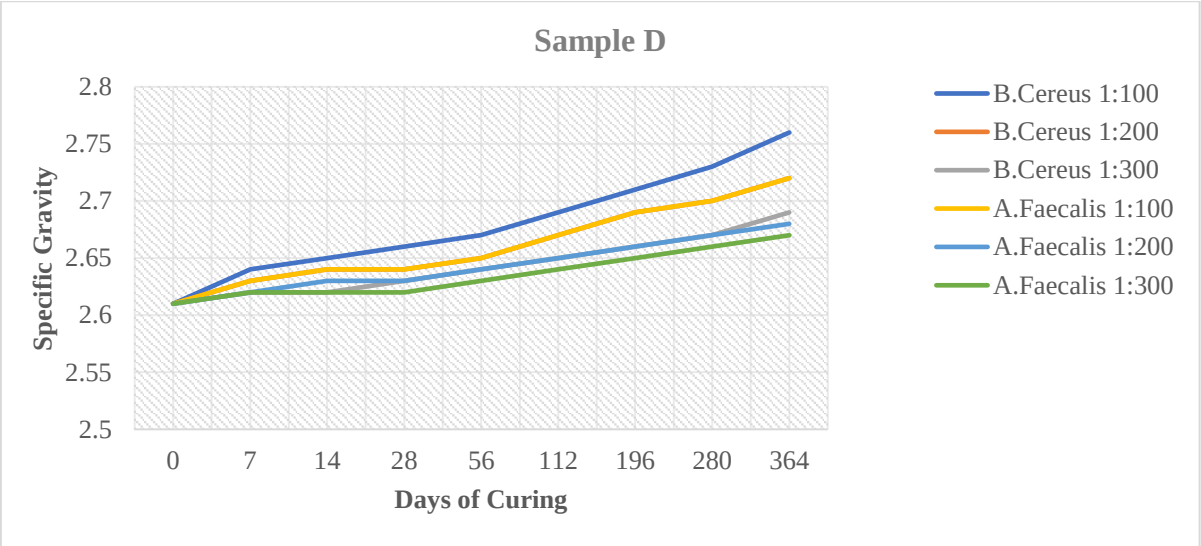

Fig. 12: Specific gravity of soil sample D treated with B_c and A_f at different dosages

Across all microbial dosages, Sample A (well-graded sand) showed a progressive increase in specific gravity. The most significant enhancement was seen at the 1:100 concentration of B_c, going from an initial value of 2.65 to a final value of 2.81 after 364 days. Similarly, A_f had final value of 2.79 at the same concentration after 364 days. At lower concentrations (1:200 and 1:300), the soil sample showed a more gradual increase, with final specific gravity values ranging from 2.72 to 2.77. This trend points to increased soil particle density and decreased pore space as a result of active microbial precipitation of minerals, especially calcium carbonate. B_c consistently performed better than A_f at all concentrations, suggesting that this well-graded sandy matrix has a greater capacity for biomineralisation.

Sample B (well-graded sand) showed a similar pattern, although it began with a slightly lower initial specific gravity (2.60). At a concentration of 1:100 of B_c, the specific gravity rose to 2.74 after inoculation, whereas A_f had a final specific gravity of 2.70. Once more, improvements in the specific gravity of the soil samples were marginally diminished at lower concentrations, ranging from 2.65 to 2.72. This trend confirms that soil mass density was successfully raised by microbial activity. Despite having the same USCS classification, Sample B's results were marginally worse than Sample A's, most likely because of slight textural or mineralogical variations.

In contrast to the sandy samples, Sample C (silty soil) displayed a totally different behavior. Although improvements were noted, they were less significant than the initial specific gravity of 2.58. At a concentration of 1:100, B_c increased the specific gravity of the soil sample to 2.71, while A_f increased the specific gravity of the soil sample to 2.68. The slow rate of increase could be due to the higher fines content, decreased permeability and unfavorable conditions for bacterial mobility and calcite dispersion. Nevertheless, the results shows that even silty soils benefit from microbial treatment, albeit more slowly and perhaps with longer treatment duration or inoculation optimisation.

Sample D (well- graded sand), mirrored the responses of Samples A and B. B_c increased the specific gravity from 2.61 to 2.76 at a concentration of 1:100, and A_f increased the specific gravity to 2.73 at the same concentration. Once again, the performance of the bacteria slightly declined at lower concentrations, but the steady increase in performance supports the ability of microbial treatment to increase soil density.

All samples showed an increase in specific gravity, which suggests that B_c-facilitated calcite precipitation has improved soil compaction and particle bonding. Greater increases in specific gravity are typically the result of higher concentrations, which could lead to better soil stabilisation, increased load-bearing capacity and increased resistance to erosion. Although less quickly, lower concentrations still have a beneficial effect on the soil. This is consistent with the soil's enhanced construction suitability and structural integrity.

For every sample and concentration, microbial activity caused a steady increase in specific gravity over time. The biggest improvements were obtained at higher concentrations (1:100), underscoring the dose-dependent character of microbial treatment. B_c outperformed A_f, presumably as a result of its higher calcite precipitation efficiency. Sandy soils responded more favorably than silty soil (Sample C), likely due to better microbial transport, distribution, and pore structure conducive to mineral deposition. These results indicate that specific gravity, a key geotechnical parameter can be successfully increased through biologically mediated processes and show the feasibility of microbial soil improvement techniques.

IV. CONCLUSIONS

This study showed that microbial treatment with *Bacillus cereus* MK202350.1 (B_c) and *Alcaligenes faecalis* DQ110882.1 (A_f) significantly affected the specific gravity of different soil types, over the course of 365 days. When compared to untreated controls, the specific gravity of all treated samples consistently increased; however, the most pronounced improvements were seen in sandy soils (classified as SW according to USCS). B_c consistently outperformed A_f in all samples and dosages, and the highest specific gravity values were obtained with a bacterial concentration of 1:100. The successful microbial-induced calcite precipitation (MICP), which successfully filled pore spaces, improved particle bonding and increased soil particle density, is indicated by the observed increase in specific gravity. The broad applicability of this biotechnological approach in geotechnical engineering is demonstrated by the fact that silty soils also showed measurable improvements, even though sandy soils responded more favorably.

V. RECOMMENDATIONS

The following recommendations are given in light of the research's findings.

i. To verify laboratory results in authentic environmental settings and evaluate scalability, controlled field applications ought to be carried out.

ii. Although a 1:100 bacterial concentration resulted in the greatest improvement, future research should examine the cost-benefit ratio between resource input and performance for the best field application.

iii. Research should be done on the potential synergistic effects of co-inoculation or the use of microbial consortia to further improve performance.

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