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Influence of Soil Microbial Inoculation on the Cohesion of Soil

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Abstract - *This study investigates the influence of microbial inoculation on the cohesion of different soil types using Bacillus cereus MK202350.1(B_c) and Alcaligenes faecalis DQ110882.1(A_f) admixed with cementation solution (1M Urea and 1M $CaCl_2$). Four soil samples—three sandy (USCS: SW) and one silty clay (USCS: ML)—were characterised and treated using microbial-induced calcite precipitation (MICP) at varying concentrations v/v nutrient broth to cementation solution (1:100, 1:200, 1:300). Cohesion was monitored over a 364-day period using standard direct shear tests. Prior to treatment, the sandy soils had no cohesiveness, according to the initial classification of the soils, while the silty clay had natural cohesive qualities. Significant increases in cohesion were observed in all samples following inoculation, with B_c treatments routinely outperform A_f. The sandy soils treated with B_c showed the greatest improvement. Cohesion rose from 0.00 kN/m² to over 30.00 kN/m² in B_c treated soils at a concentration of 1:100. Although at a slower pace, the silty clay sample also showed progressive increases in cohesiveness. These results support the viability of microbial inoculation as a soil stabilisation method, especially for cohesionless soils. The treatment's is dose-dependent and B_c had better performance. MICP can be tailored to meet engineering needs and soil conditions.*
Keywords: *Microbial inoculation, Cohesion, Bacillus cereus, Alcaligenes faecalis*

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I. INTRODUCTION

Soil cohesion is a fundamental property influencing the stability, strength, and load-bearing capacity of soils, particularly in geotechnical and agricultural applications. Soil cohesion refers to the component of shear strength that is independent of interparticle friction. It is the force that holds soil particles together, even in the absence of external pressure (Roy and Bhalla, 2017; *et al.,* 2023). Cohesion arises from the electrochemical and physicochemical interactions among soil particles, as well as the presence of organic matter and moisture (Tunçal and Mujumdar, 2023; Kumari and Mohan, 2021; Hamdi *et al.,* 2024). In many regions, especially those with sandy or degraded soils, low cohesion can lead to challenges such as erosion, reduced structural integrity, and poor root anchorage (Dahanayake*et al.,* 2024; Stachew*et al.,* 2021). Traditional methods to enhance soil cohesion often involve mechanical compaction or the addition of chemical stabilisers (Chatrabhuj, 2024; Zada, *et al.,* 2024; Fondjo*et al.,* 2021). While effective, these approaches can be resource-intensive and may have environmental drawbacks (Shukla, *et al.,*2024; Mizanur*et al.,* 2020). As a sustainable alternative, microbial soil treatment has garnered attention for its potential to improve soil properties through natural biological processes (Samantaray*et al.,* 2023; Pandey, and Saharan, 2025).

MICP is a biogeochemical process where certain microbes induce the precipitation of calcium carbonate (calcite) within the soil matrix (Liu *et al.,* 2024; Wilcox *et al.,* 2024; Rahman *et al.,* 2020). This calcite acts as a natural cement, binding soil particles together and thereby increasing soil strength and cohesion (Payan, *et al.,* 2024; Liu *et al.,* 2024; Zhang *et al.,* 2024; Zhang *et al.,* 2023). Microbial inoculants can modify soil properties, leading to improved cohesion through mechanisms such as microbial-induced calcite precipitation (MICP) and the formation of stable soil aggregates (Liu *et al.,* 2024; Jiang *et al.,* 2023). Microbial inoculants, particularly fungi and actinomycetes, play a vital role in soil aggregation by producing extracellular polysaccharides that bind soil particles into stable aggregates (Edwards and Arancon, 2022; Chen *et al.,* 2024). The presence of diverse microbial communities enhances soil structure, as mixed cultures can lead to better aggregation compared to pure cultures (Edwards and Arancon, 2022; Laishram *et al.,* 2024). Improved soil aggregation contributes to increased water retention and nutrient availability, further enhancing soil cohesion (Zhu &Yang, 2019; Sekaran *et al.,* 2020).

MICP enhances the strength and stability of low-cohesion soils by precipitating calcium carbonate, which binds soil particles together. This process involves biogeochemical reactions such as urea hydrolysis and

denitrification, which contribute to the formation of calcite crystals that reinforce soil structure (Liu et al., 2024). MICP-treated soils exhibit improved macroscopic properties, including increased strength and reduced permeability, which are crucial for construction and environmental applications (Liu et al., 2024). Certain microorganisms, notably species like *Bacillus cereus* and *Alcaligenes faecalis*, have demonstrated capabilities to influence soil structure positively. These bacteria can produce extracellular polymeric substances (EPS), which act as natural binders, promoting the aggregation of soil particles and thereby enhancing cohesion (Bolan *et al.,* 2023). Additionally, microbial activities can lead to the precipitation of minerals, further contributing to soil particle bonding and structural integrity.

Despite the promising potential of microbial inoculation in improving soil cohesion, there remains a need for comprehensive studies that evaluate the long-term effects of such treatments across different soil types. This study aims to address this gap by investigating the influence of microbial inoculation using *Bacillus cereus MK202350.1(B_c) and Alcaligenes faecalis DQ110882.1(A_f)* admixed with cementation solution on the cohesion of soil over a 364-day period.

II. METHODLOGY

Study Design and Duration: This experimental study evaluated the impact of microbial inoculation on soil cohesion over a 364-day period. Four soil types were selected: Soils A, B, and D (sandy soils) and Soil C (plastic soil). Each soil type underwent treatment with *Bacillus cereus* MK202350.1 and *Alcaligenes faecalis* DQ110882.1 admixed with cementation solution (1M Urea and 1M $CaCl_2$), individually, alongside untreated controls.

Soil Sample Collection: Soil samples were collected from the designated sites, ensuring minimal disturbance. The study area for soil samples collection is Ibadan, a metropolis in southwest Nigeria with a complex geological basis comprising weathered soil profiles, basement rocks, and sedimentary layers. The four sites under investigation were Ologuneru (7° 28ʾ 50.2″ N, 3° 48ʾ 48.1″ E), Apete (7° 26ʾ 20.9″ N, 3° 52ʾ 47.4″ E), the Toll-Gate area (7° 19ʾ 29.8″ N, 3° 52ʾ 52.3″ E), and Amuloko (7° 20ʾ 29.0″ N, 3° 58ʾ 56.5″ E), therefore offering a cross-section of Ibadan's geological and environmental scene. These sites together allow for diverse geological regions within Ibadan, from weathered basement complexes to sedimentary layers and lateritic soils to be considered, therefore offering a complete knowledge of soil and environmental variance over urban, peri-urban, and rural settings.

Soil Preparation: Samples were collected at depths of 2–3 meters, air-dried, and sieved through a 2 mm mesh to remove debris. Each soil sample was labeled and subdivided for laboratory analysis and microbial treatment.

Figure 1: Air drying soil samples

Soil Characterisation: Before microbial inoculation, the soil samples were geotechnically characterised to determine baseline characteristics. The moisture content and particle sieve analysis were determined to characterise the soil.

Particle Sieve Analysis: Particle size distribution of the soil samples was determined using mechanical sieve analysis as specified in ASTM D422. This test provided insight into the textural composition of the soils (i.e., the proportions of sand, silt, and clay) and was critical in interpreting the cohesion behavior of treated and untreated soils.

The apparatus used included a mechanical sieve shaker, a set of standard sieves ranging from 4.75 mm to 0.075 mm, a balance accurate to 0.1 g, a brush, and a set of clean collection pans. For each soil sample, approximately 500 grams of oven-dried soil was placed in the top sieve of the arranged stack. The stack was mechanically shaken for 10–15 minutes to ensure thorough separation of particles. The mass of soil retained on each sieve

was then recorded. The percentage of the total sample retained on each sieve was then calculated and used to develop a grain size distribution curve.

Bacterial Strain and Cultivation: *Bacillus cereus* MK202350.1 and *Alcaligenes faecalis* DQ110882.1 admixed with cementation solution were obtained from certified microbial culture collections. Each strain was cultured separately in nutrient broth at 30°C for 24 hours under aerobic conditions. The bacterial strains B_c and A_f were isolated from the native soil samples, screened for calcite precipitation potential, and identified through classical biochemical tests and molecular techniques (16S rDNA sequencing). Cultures were grown in Nutrient Broth at 37°C for 24–48 hours and to concentrations of 4.9×10^6 and 3.7×10^6 CFU/mL (OD_{600} = 0.433 and 0.402), respectively.

Figure 2: Bacteria strain and cultivation

Inoculum Preparation: Cultures were centrifuged, and the cell pellets were resuspended in sterile saline to achieve a concentration of approximately 10^6 CFU/mL Following culture preparation, 50 mL of each bacterial suspension was mixed thoroughly with the designated soil samples. A cementation solution containing 1 M urea and 1 M calcium chloride was applied to promote microbial-induced calcite precipitation (MICP). Moisture content was maintained by weekly monitoring and reapplication of sterile distilled water if necessary.

Figure 3: Inoculum preparation

Treatment Application: For each soil type, 100 g samples were placed in sterile containers and inoculated with 10 mL of the prepared bacterial suspensions. Treatments includes B_c only, A_f only and Control (sterile saline). Bacterial concentrations were examined using serial dilution and colony-forming unit (CFU) counts using spread plate techniques. Three distinct concentrations (1:100, 1:200, and 1:300) were experimentally designed for soil inoculation. A cementation solution consisting of 1M Urea and 1M Calcium Chloride in distilled water was applied to the soil samples. Application volume depended on the saturation moisture content (1 Pore volume) of each soil to provide uniform conditions. Every 100 kg containerised soil sample received between 13-20litres single dosage of the cementation solution and 45-150 mL of nutrient-bacteria at varying concentrations. The soils were left at ambient temperature and investigated for 364 days.

Cohesion Measurement: Soil cohesion was assessed at 0, 7, 14, 28, 56,112,196, 280, and 364-days post-inoculation. Unconfined compressive strength (UCS) tests were conducted on the treated and untreated soil specimens prepared from each treatment group to assess the impact of microbial inoculation on the cohesion of the soil. The peak stress at failure was recorded as the measure of soil cohesion. In accordance with British Standard BS 1377-7:1990, the unconfined compressive strength (UCS) test was used to assess each sample's cohesiveness. Under carefully monitored conditions, cylindrical specimens measuring roughly 38 mm in

diameter and 76 mm in height were made and allowed to cure. Until failure, the specimens were subjected to axial loading at a steady strain rate. Assuming that the internal angle of friction is zero in unconfined conditions, cohesion was computed as half of the unconfined compressive strength and the maximum axial load was noted. This made it possible to compare the effects of microbial activity on bonding and interparticle cohesion in the soil matrix during the 364-day treatment period.

Figure 4: Determination of the cohesion of soil samples on the direct shear box

III. RESULTS AND DISCUSSION

Initial Characterisation of Soil Samples

The four samples A-D were classified in accordance with the British Soil Classification System (BSCS). The parameters used were the soils grading curves and the liquid and plastic limit for sample C. Sample A, B & D are sandy soil (SM) with little proportion of gravel while Sample C is clayey sand (SC) with little proportion of gravel.

The smooth gradation curves having a wide range of particle sizes and low fines content demonstrate that Samples A, B, and D are primarily well-graded sands (Figures 1-4). These characteristics (Table 1) confirm their classification as SW (Well-Graded Sand) under the Unified Soil Classification System (USCS). Although these soils lack cohesiveness and finer particles that affect specific gravity in silty or clayey soils, this gradation promotes good drainage and strength, making them appropriate for engineering applications.

However, Sample C had a narrower gradation and a greater percentage of fine particles, which is a sign of subpar (poor) grading. This is consistent with its USCS classification as ML (Silt with low plasticity). The curve's comparatively steep gradient indicated a preponderance of silt-sized particles with little sand content, which frequently results in lower strength and permeability but higher water retention and compressibility.

Table 1: Classification of Soil Samples

Soil Sample	**USCS**	**Plasticity Classification**
	Group Symbol	
Sample 1	SW	Nil
Sample 2	SW	Nil
Sample 3	ML	Inorganic clays of low plasticity
Sample 4	SW	Nil

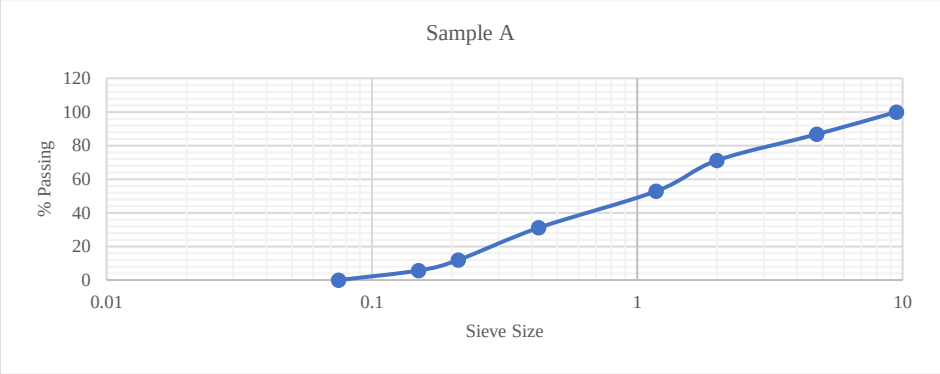

Figure 5: Particle sieve distribution of Sample A

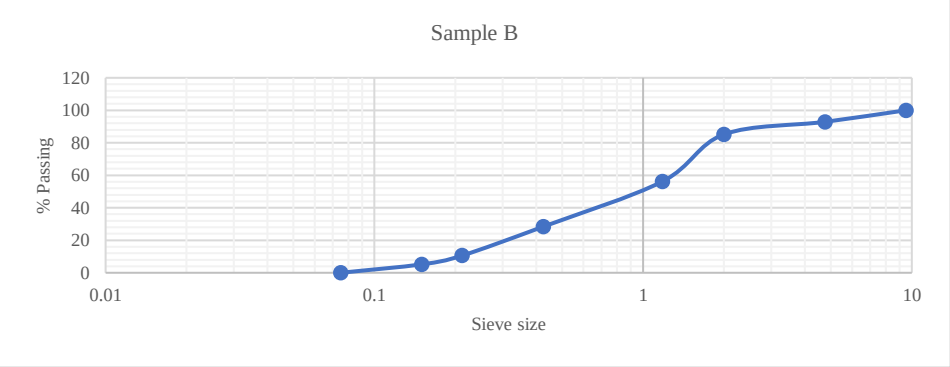

Figure 6: Particle sieve distribution of sample B

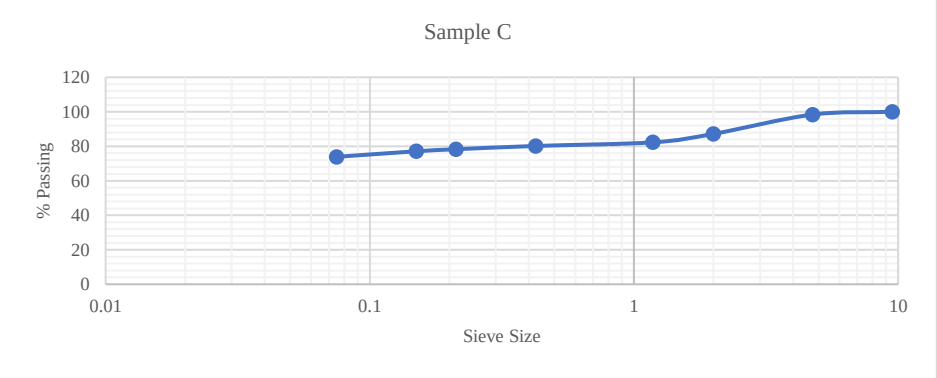

Figure 7: Particle sieve distribution of Sample C

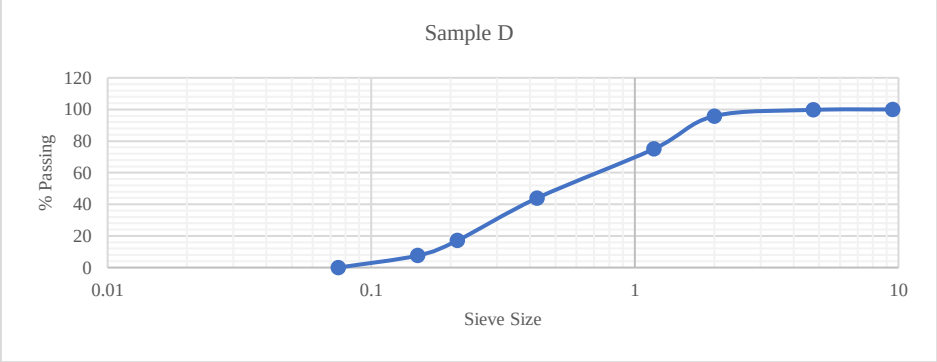

Figure 8: Particle sieve distribution of sample D

Influence of Microbial Inoculation on Soil Cohesion

For all soil sample, the development of soil cohesion after microbial treatment with B_c and A_f was assessed over a period of 364 days. Table 1 and Figures 5–8 present the results, which show a consistent increase in cohesion across all treatment levels and treatment days when compared to untreated controls, which was 0 kN/m^2 (for sandy soils) and 8.20 kN/m^2 (for cohesive soil, Sample C).

Cohesion, a measure of the bonding between soil particles, significantly increased due to the influence of microbial inoculation. In Sample C, B_c combined with the cementation solution increased cohesion from an initial control value of 8.20 kN/m² to 23.62, 22.53, and 21.35 kN/m² after 364 days. For Samples A, B, and D treated with B_c at concentrations of 1:100, 1:200, and 1:300 respectively, cohesion values rose from 0 kN/m² (control) to (30.52, 28.50, and 25.20 kN/m²), (30.60, 30.50, and 28.70 kN/m²) and (24.32, 21.52, and 20.43 kN/m²), respectively within 364 days of treatment. Similarly, soils treated with A_f also showed improved cohesion, Sample A increased to (29.40, 26.45, and 24.20 kN/m²), Sample B (29.53, 27.60, and 25.60 kN/m²), Sample C (22.32, 21.22, and 20.54 kN/m²) and Sample D (21.23, 20.62, and 19.13 kN/m²). Across all concentrations and samples, B_c treated soils demonstrated comparatively higher cohesion gains than A_f treated soils.

Table 2: Cohesion of soil sample A induced with B_c and A_f

Testing Days	**Cohesion of soil sample**					
	Sample A					
	1:100		**1:200**		**1:300**	
	Bc	***Af***	***Bc***	***Af***	***Bc***	***Af***
Control	0.00	0.00	0.00	0.00	0.00	0.00
7	16.80	14.93	15.00	13.67	14.30	12.70
14	18.72	16.32	17.63	14.31	16.40	14.23
28	21.40	17.83	20.54	16.71	18.70	16.70
56	23.40	22.50	22.50	21.40	18.90	19.30
112	24.50	24.60	23.70	22.50	19.50	21.60
196	27.20	25.57	25.30	24.20	21.20	22.90
280	28.40	26.02	27.80	24.90	24.80	23.72
364	30.52	29.40	28.50	26.45	25.20	24.20
	Sample B					
	1:100		**1:200**		**1:300**	
	Bc	***Af***	***Bc***	***Af***	***Bc***	***Af***
Control	0.00	0.00	0.00	0.00	0.00	0.00
7	18.16	17.42	15.00	12.70	14.60	10.40
14	18.30	17.81	16.31	13.50	16.30	11.50
28	21.00	18.20	18.90	17.32	18.91	15.10
56	24.24	22.52	24.35	18.10	22.40	16.22
112	28.30	24.90	26.30	20.31	23.62	18.13
196	29.72	26.51	28.91	23.80	24.90	21.60
280	29.41	27.80	30.34	24.62	25.60	22.91
364	30.60	29.53	30.50	27.60	28.70	25.60
	Sample C					
	1:100		**1:200**		**1:300**	
	Bc	***Af***	***Bc***	***Af***	***Bc***	***Af***
Control	8.20	8.20	8.20	8.20	8.20	8.20
7	11.57	10.63	10.90	10.23	10.00	9.90
14	12.32	11.32	11.62	10.81	11.23	10.22
28	12.91	11.82	12.45	11.42	11.82	10.71
56	13.52	12.52	12.53	11.91	12.07	11.23
112	16.23	15.42	15.61	14.82	14.92	13.93
196	17.31	16.43	16.81	15.82	16.23	15.22
280	20.32	18.52	19.11	17.32	18.72	16.62
364	23.62	22.32	22.53	21.22	21.35	20.54
	Sample D					
	1:100		**1:200**		**1:300**	
	Bc	***Af***	***Bc***	***Af***	***Bc***	***Af***
Control	0.00	0.00	0.00	0.00	0.00	0.00
7	10.20	9.90	9.30	8.20	7.80	6.50
14	11.71	10.82	10.52	9.73	8.72	8.61
28	12.42	11.31	10.91	10.41	9.82	9.62
56	16.32	14.92	14.82	12.82	11.82	11.32
112	18.42	16.92	16.71	15.72	15.52	14.34
196	20.42	17.32	18.72	16.71	17.92	15.45
280	21.62	19.32	20.34	18.51	18.34	17.13
364	24.32	21.23	21.52	20.62	20.43	19.13

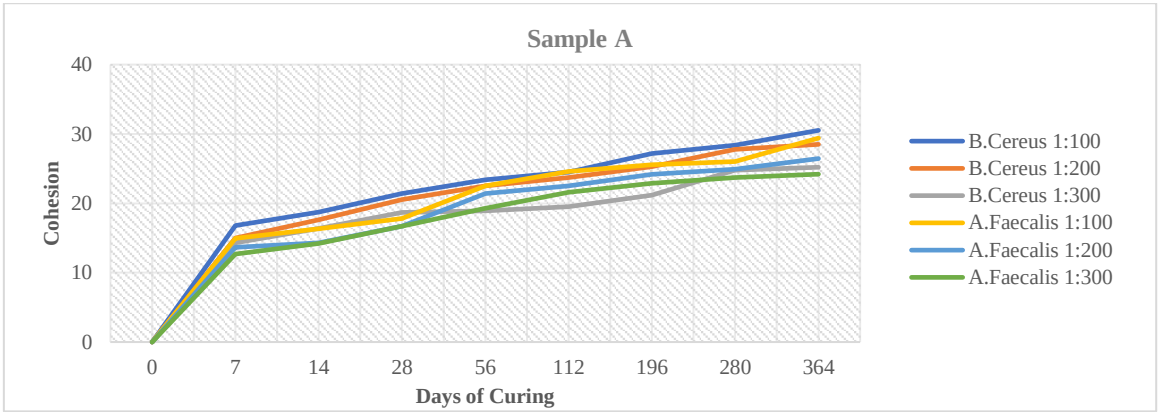

Figure 9: Cohesion of soil sample A treated with B_c and A_f at different dosages

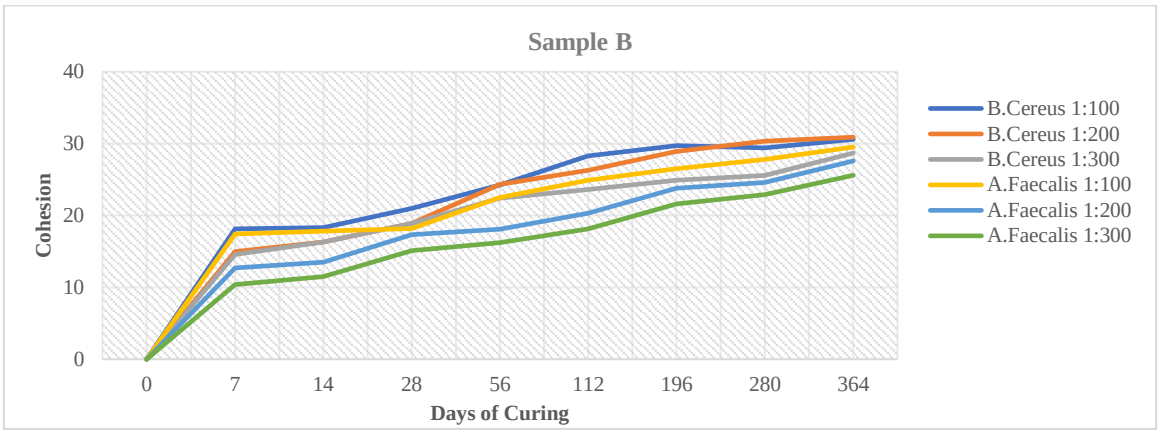

Figure 10: Cohesion of soil sample B treated with B_c and A_f different dosages

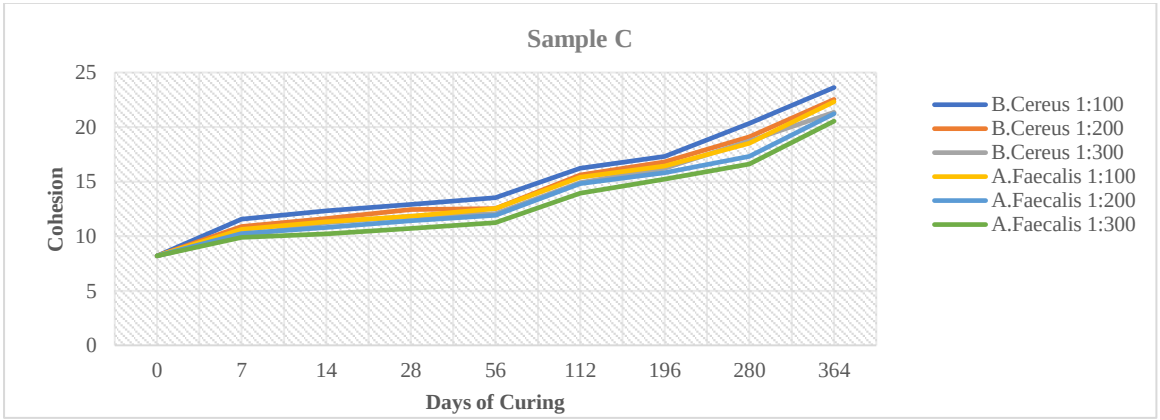

Figure 11: Cohesion of soil sample C treated with B_c and A_f at different dosages

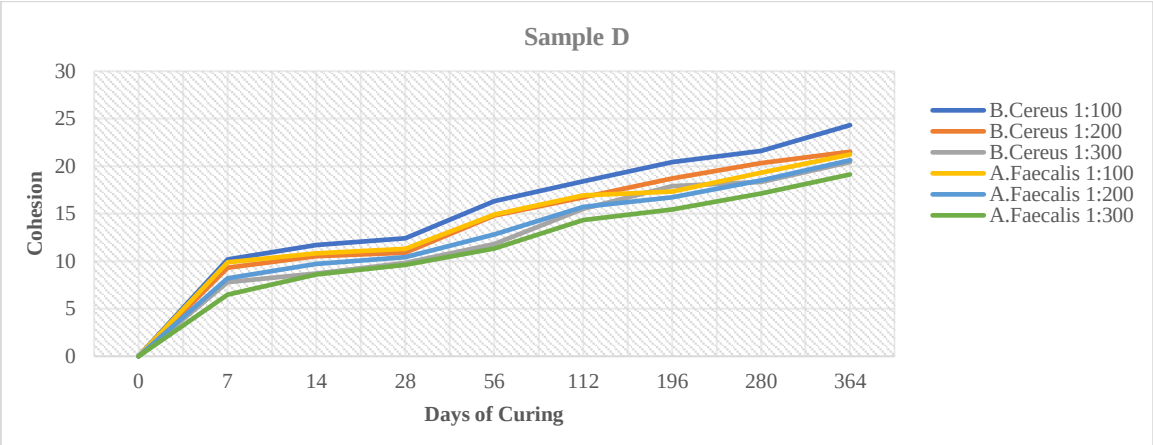

Figure 12: Cohesion of soil sample D treated with B_c and A_f at different dosages

In sample A, all treatments with B_c and A_f significantly improved cohesion as compared to the control (Fig. 5). The 1:100 concentration of B_c produced the highest cohesion value (30.52 kN/m^2), on Day 364, while A_f had a cohesion of 29.40 kN/m². It was also noted that cohesion values decreased with decreasing concentration. At a concentration of 1:300, Sample A had cohesion values of 25.20 kN/m² and 24.20 kN/m² for B_c and A_f, respectively. This trend indicates a dose-dependent relationship, with higher bacterial densities promoting a more robust microbial-induced calcite precipitation (MICP), which strengthens inter-particle bonding. Although Sample B's cohesion improvements were marginally greater at later stages, they still followed a similar pattern to Sample A (Fig. 6). At a concentration of 1:100, B_c and A_f achieved peak cohesion values of 30.60 and 29.53 kN/m², respectively at Day 364. Likewise, despite lower bacterial concentrations, the 1:300 concentration treatment had cohesion values above 25 kN/m² suggesting strong microbial activity. This implies that calcium carbonate bridging and microbial inoculation was favorable for Sample B (well-graded sandy matrix).

In contrast to the sandy samples, Sample C, a fine-grained plastic soil, had an initial cohesion value of 8.20 kN/m² (Fig. 7). Cohesion increased gradually with microbial inoculation in all treatments, reaching 23.62 kN/m² and 22.32 kN/m² for B_c and A_f (1:100 dosage) respectively by Day 364. Calcite cementation was able to accumulate gradually because the finer matrix likely retained bacterial cells and metabolic byproducts better. However, in the early stages, the rate of cohesion increase was slower, which could be because bacterial movement was restricted by lower permeability.

Similar to Samples A and B, Sample D (a well graded sand soil) had no measurable cohesiveness before treatment (Fig. 8). But over time, microbial treatment caused a noticeable rise in the cohesion of the soil. At a dosage of 1:100, B_c achieved 24.32 kN/m² by Day 364, while A_f reached 21.23 kN/m². Significant improvements (up to 20.43 and 19.13 kN/m^2) were still observed at lower dosages (1:300), demonstrating the potential of MICP even at lower microbial loads.

Comparative Evaluation of Different Samples and Bacterial Strains

In every sample, B_f consistently performed better than A_f in terms of improved cohesion, this is as a result of calcite precipitation and more effective urease activity. The ability of microbial treatment to convert non-cohesive soils into materials with a moderate level of cohesiveness was demonstrated by the most notable cohesion increases observed in sandy soils (Samples A and B). However, even though the final cohesion values were still significant, Sample C, which is naturally cohesive, showed the lowest relative increase, the microbial treatment had less room to improve it.

Additionally, the results show a distinct dose-dependent response, with the most effective concentration being 1:100. However, both bacterial strains demonstrated significant cohesion improvements even at 1:300, which could be helpful for field applications where cost is a concern.

IV. CONCLUSION

This study demonstrated the potential of *Bacillus cereus* MK202350.1and*Alcaligenes faecalis*DQ110882.1 in enhancing soil cohesion through microbial-induced calcite precipitation (MICP).

Across all four soil samples, both bacterial strains significantly improved cohesion over the 364-day monitoring period, with B_c generally yielding higher values than A_f. The effect was more pronounced in well-graded sandy soils (SW), which initially exhibited no cohesion, than in the silty clay (ML) soil that possessed inherent cohesive properties. In general, cereus produces higher values than A. Faecalis. Compared to the silty

clay (ML) soil, which had natural cohesive qualities, the effect was more noticeable in well-graded sandy soils (SW), which at first showed no cohesion.

Although detectable improvements were still seen at lower dosages (1:300), cohesion gains were consistently greater at higher bacterial concentrations (1:100). The effectiveness of microbial treatment in converting loose, non-cohesive soils into more structurally stable matrices is demonstrated by this dose-responsive behavior.

V. RECOMMENDATIONS

Based on the findings of this study, the following recommendations are given

i. In geotechnical projects involving weak or cohesionless soils, microbial soil improvement using *Bacillus cereus* MK202350.1 and *Alcaligenes faecalis* DQ110882.1 is advised, especially when traditional stabilisation techniques are prohibitively expensive or environmentally unsustainable.

ii. *Bacillus cereus* MK202350.1 should be given preference because of its better ability to induce cohesion, which is probably related to its higher urease activity and capacity to form calcite.

iii. In order to assess the stability and longevity of bio-cemented soils under various loading and environmental circumstances, future field-scale applications should include long-term monitoring (beyond 12 months).

iv. To improve treatment procedures, more investigation should be done into how various soil types, pH values, moisture content, and nutrient amendments interact with microbial treatments.

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