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Original Article

Evaluation of the Potential of Biocementation in Clayey and Sandy Soils for Foundations Using Fish Viscera Waste

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Abstract *- Faced with the dual challenges of rapid urbanization on unsuitable soils and the environmental burden of organic waste from the fishing industry, Peru urgently needs innovative and sustainable engineering solutions. A biotechnological alternative is introduced for soil improvement based on Microbial-Induced Calcite Precipitation (MICP), using Sporosarcina pasteurii bacteria nourished with nutrient-rich extracts from the viscera of horse mackerel, mullet, and bonito. Bacterial cultures were prepared in a solution of urea-ammonium, distilled water, and Tris buffer, with parameters such as bacterial concentration (1.10E7 to 1.30E7 CFU/ml), viscera extract volume (3–9 ml), and temperature (25–35 °C) optimized for maximum calcium carbonate precipitation. Under optimal conditions (1.30E7 CFU/ml, 9 ml, 35 °C),* $CaCO_3$ *production reached 0.0194 g. Soil treatment trials demonstrated significant engineering benefits: in low-plasticity clay, allowable bearing capacity increased by 80% and deformation was reduced by 68.5%; in poorly graded sand with gravel, capacity rose by 20% and deformation decreased by 35.1%. Clay-sand mixtures showed capacity gains up to 31% and deformability reductions of 55.7%. The results are attributed to the formation of calcium carbonate bridges, which enhance particle cohesion and stiffness. Technical and environmental feasibility of revalorizing fish viscera as a nutrient source for biocementation, offering a low-cost and sustainable strategy for urban soil stabilization.*

Keywords *- Microbial biocementation, Soil stabilization, Calcium carbonate precipitation, Organic waste valorization, Sustainable geotechnology.*

1. Introduction

Urban growth in Peru, with a current population of nearly 32 million inhabitants and a projection of 35.8 million by 2030, is steadily increasing the pressure on soils destined for housing and infrastructure [1]. In this context, unplanned urbanization has led to the occupation of land with low or medium bearing capacity in Peru, with a current population of nearly 32 million inhabitants and a projection of 35.8 million by 2030, which is steadily increasing the pressure on soils destined for housing and infrastructure capacity, raising vulnerability to settlement and seismic actions [2]. This issue has been highlighted in metropolitan geotechnical diagnoses and urban risk assessments [3]. In Lima, subsurface investigations identified in the Horacio Zevallos sector a distribution of 95% medium-capacity deposits and 5% low-capacity deposits, concentrated in Las Praderas de Pariachi [4] in Huaycán. The northwestern and southeastern extremes present 80% medium-capacity soils and 20% low-capacity soils [4]. The Historic Centre of Lima exhibits bearing capacities between 1 and 4 kg/cm², compromising historic and patrimonial buildings [5]. At the same time, the construction sector maintains a significant climate footprint; the cement industry contributes a considerable share of global CO_2 emissions, drawing attention in mitigation policies and the development of alternative materials [6]. In parallel, the fishing industry generates approximately 18% organic waste relative to the processed resource, mainly viscera, whose inadequate disposal creates local environmental and sanitary burdens [7, 8]. This dual challenge, improving weak soils and valorizing organic waste, demands sustainable geotechnical solutions with scientific grounding and economic feasibility.

An alternative with growing empirical support is Microbially Induced Calcite Precipitation (MICP). The central mechanism relies on ureolytic bacteria, particularly Sporosarcina pasteurii, which express urease, facilitating urea breakdown and increasing the alkalinity of the pore medium [9]. The process generates HCO_3^- and CO_3^{2-}, which in the presence of Ca^{2+} precipitate as calcium carbonate ($CaCO_3$), depositing within soil pores and grain contacts [10].

The development of interparticle cementing links together with partial pore filling leads to enhanced cohesion, stiffness, and shear strength and reductions in permeability [11]. Compared to conventional stabilizers (lime/cement), MICP can reduce energy requirements and emissions across the life cycle of the treated material [12]. Techno environmental evaluations in cementitious matrices report reductions in embodied energy and emissions when biogenic routes replace binder fractions or when in situ biocementation treatments are applied [13, 14].

The technical literature has documented robust results of MICP in granular soils. In China, in situ flocculation applied to sand columns of 300 mm and 1000 mm produced a uniform spatial distribution of $CaCO_3$ and notable enhancements in UCS values [15]. In lateritic soils, experiments with gradients of bacterial concentration identified an optimum of 1.20×10^9/CFU/ml, raising UCS to 2232 kN/m² [16]. In Malaysia, percolation treatments in sand columns, using low-cost solvents, achieved UCS 11,448 kPa and $CaCO_3$ precipitation 33.24%, demonstrating the process's effectiveness in reinforcing granular matrices with practical mixing and curing schemes [17]. Complementarily, the solidification of mining tailings sands optimized with calcium acetate as precursor promoted volumetric mineralization and associated geotechnical gains, aligned with circular economy strategies for industrial by-products [18].

In fine soils, the response depends on transport kinetics and nucleation. Studies report that under controlled conditions of temperature (25–30°C), pH (6.5–8), and urea (6–8%), treatment with S. pasteurii increases shear strength and cohesion in marginal soils [19]. Curing periods of about 56 days in plastic clays consolidate a calcitic matrix that bonds fine particles, resulting in stiffness gains and reduced deformability. Regarding microbial persistence and comparative performance, evaluations in Iran showed that S. pasteurii maintained activity for approximately 30 days and increased resistance up to 2.3 MPa, reducing hydraulic conductivity compared to other strains [20].

In Australia, it was demonstrated that S. pasteurii outperforms lytic bacteria in crystallization rate (6) and crystal size (5–40 µm) under controlled conditions [21]. In Canada, treated silica sand samples increased the shear resistance values, grew from 15.77 kPa to 135.8 kPa, maintaining integrity under freeze–thaw and flooding cycles, although a 29% reduction was recorded under acid rain exposure, highlighting durability limits in acidogenic environments [22]. Likewise, materials engineering studies report S. pasteurii variants with elevated urease activity and successful applications in concrete repair, reducing water absorption and improving adhesion under optimized parameters of $CaCl_2$, urea, temperature, and incubation time [23, 25]. Recent reviews emphasize that the efficiency of MICP is governed by multiple coupled factors, including bacterial viability, urease activity, nutrient composition, and operational conditions defined by pH, thermal level, and ionic strength [26]. In addition, the bacteria's physiological state, whether employing whole cells, cell fractions, or isolated urease enzymes, strongly influences the rate of urea breakdown and the resulting deposition of carbonate minerals [27]. The application method also plays a critical role; techniques such as injection, spraying, or direct mixing influence pore fluid transport, precipitation uniformity, and ultimately the mechanical performance of treated soils [28].

Despite these advances, most investigations still rely on synthetic culture media (yeast extract, tryptone), whose cost can represent a substantial fraction of the process [17]. From a biochemical standpoint, there is strong justification for exploring residual substrates as alternatives. Fish viscera contain approximately 15–20% proteins and abundant free amino acids that provide readily assimilable nitrogen and carbon, stimulating both bacterial growth and urease synthesis in ureolytic strains. They also include micronutrients such as potassium, magnesium, iron, phosphorus, and trace amounts of calcium, which act as enzymatic cofactors and essential metabolic nutrients, thereby reducing the need for costly commercial supplements [29]. Furthermore, the moderate lipid fraction can generate hydrophobic microenvironments at solid–liquid interfaces that favor $CaCO_3$ nucleation [30]. From a sustainability perspective, the use of fish viscera contributes to the valorization of abundant fishery waste in Peru while lowering the operational cost of MICP [31].

This methodological gap defines the experimental strategy of the present study. Before extending the approach to geotechnical applications, the research first validates through controlled experimentation that fish viscera extract can effectively induce and sustain biomineralization. Accordingly, Phase I consisted of 18 laboratory tests that systematically varied bacterial concentration (1.10×10^7–1.30×10^7 CFU/ml), extract volume (3–9 ml), and incubation temperature (25–35 °C) to quantify $CaCO_3$ production and determine the optimal conditions. This validation step is essential given the absence of direct precedents in the literature [15-31]. Once the optimum dosage was identified, corresponding to the combination that maximized $CaCO_3$ precipitation, Phase II was implemented to assess geotechnical performance. The optimized treatment was applied to fine-grained soils identified as low-plasticity clays (CL), coarse soils categorized as poorly graded sands with gravel (SP), and their respective blends (30% and 40%), selected for their regional importance and because they represent contrasting porous media commonly encountered in urban foundations in Lima and Huancayo [4, 5]. In CL, reduced plasticity and the formation of calcite bridges are expected to yield greater gains in cohesion and lower compressibility. In SP, filling voids and grain-to-grain cementation are expected to translate into increases in internal friction angle and stiffness. In mixtures, the permeability of the sandy fraction combined with the specific surface area of fines provides transport–nucleation synergies, offering a balanced pathway for precipitation and bonding that is particularly relevant to shallow foundation applications [15, 16, 22, 23]. Performance was evaluated through direct shear tests and oedometer consolidation tests, allowing quantification of bearing capacity and deformability, and establishing the causal relationship between $CaCO_3$ production validated in Phase I and the mechanical response measured in Phase II [19, 22, 23].

In summary, the contribution of this research lies in: (i) substituting synthetic culture media with a residual substrate supported by biochemical justification and local availability [29-31]; (ii) optimizing culture parameters with a quantitative metric of $CaCO_3$ production prior to geotechnical application [15-28]; and (iii) demonstrating measurable improvements in bearing capacity and consolidation behavior in CL, SP, and their mixtures, soils that are representative of urban contexts in Peru [4, 5, 22, 23]. This two-phase route first validates the biogenic input ($CaCO_3$) and subsequently applies it to soil systems, thereby closing the research gap identified in the literature and offering a low-cost, low-impact alternative for soil stabilization in urban environments.

2. Materials and Methods

2.1. Biocementation

Biocementation is a biogenic process whereby microorganisms cause the deposition of mineral compounds, predominantly calcium carbonate ($CaCO_3$), on a specific substrate. This technique takes advantage of the ability of certain microorganisms, notably Sporosarcina pasteurii, to hydrolyze urea, a phenomenon that causes an increase in pH in the surrounding phase and, consequently, promotes the initiation and enlargement of calcium carbonate crystallites [26, 27]. The representation shown in Figure 1 summarizes the route of calcium carbonate precipitation mediated by microorganisms. In the sequence described, a bacterium converts urea into metabolic products, generating carbonate ions (CO_3^{2-}) as a by-product. These ions, in turn, interact with calcium cations (Ca^{2+}) found in the medium, leading to the generation of $CaCO_3$ crystalline structures. This process is known as microorganism-induced biomineralisation (MICP) and has various applications in environmental and civil engineering, such as soil consolidation, crack sealing and bioremediation.

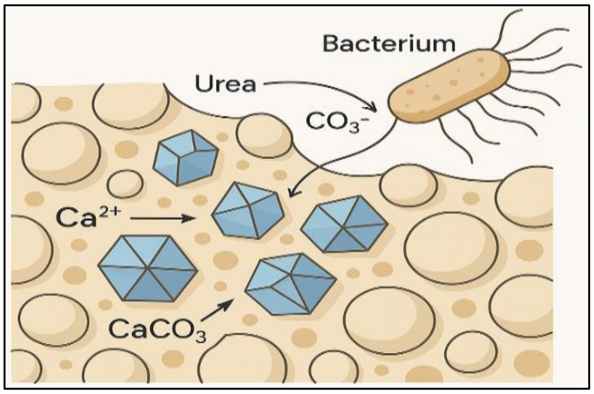

Fig. 1 Calcium carbonate

Figure 2 shows a diagram summarising the main parameters that influence the biocementation process. Among the most representative are environmental conditions, which include pH, temperature and nutrient medium, as well as bacterial conditions, determined by the use of live cells, cell fractions or bacterial enzymes. Also noteworthy is the type of bacteria, classified according to their urease activity (low, medium, or high). Finally, the application method is key to the process, considering techniques such as injection, spraying, or mixing, with variables such as the direction of application, flow rate, or use of support. These factors, taken together, determine the efficiency of the biocementation process in geotechnical and environmental applications.

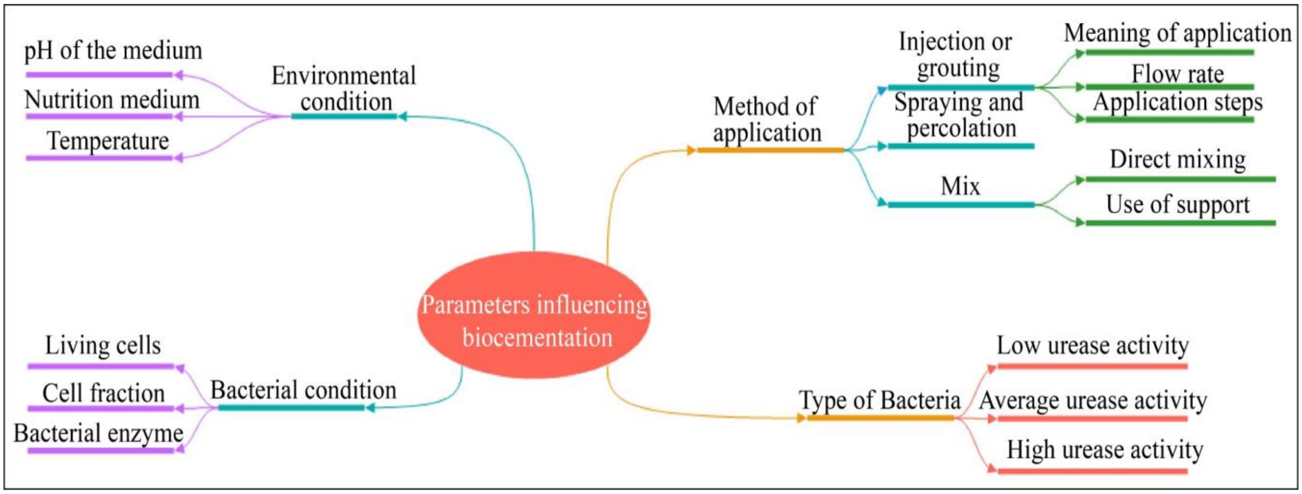

Fig. 1 Parameters for biocementation

In this research, specific parameters were used to evaluate the process of biocementation induced by microorganisms, using Sporosarcina pasteurii, whose bacterial condition as a living cell is key to ensuring the metabolic activity necessary to induce the biocementation process. The microorganism was chosen for its significant ureolytic capacity, which allows for the effective hydrolysis of urea and the consequent destabilization of the soil, favoring $CaCO_3$ deposition [28].

An unconventional culture medium, derived from fish viscera, was used to supply the nutrients necessary for the proliferation and biocementation activity of the strain. Critical parameters, specifically the alkalinity of the solution and the temperature used during incubation, were meticulously regulated to maintain bacterial activity and viability throughout the experimental period. A direct mixing approach was adopted, which required careful handling of the cells; Logistical support and methodological guidance were provided by the 'C3 Ingeniería Especializada' laboratory.

2.2. Fish Offal

Fish viscera, consisting of the remaining soft organs of the digestive tract, i.e., the stomach, intestines, and associated membranes, were the waste substrate used [29]. The substrate was naturally colonized by the strain then identified as S. pasteurii; a contemporary taxonomic review, based on comparative morphology and molecular phylogenetics, has repositioned the former Bacillus pasturii within the genus Sporosarcina, reflecting greater affinity in cell structure and phylogenetic markers [28]. These microorganisms are part of the intestinal microbiota of fish and have a high capacity to adapt to extreme environmental conditions [30].

Their presence in the viscera is due to their tolerance to the digestive system's pH, temperature and salinity, which favours their growth even in complex organic substrates [31]. In this context, the use of fish viscera represents an accessible and sustainable alternative for obtaining these bacteria, taking advantage of an abundant waste product from the fishing industry.

As part of the experimental procedure, Figure 3 shows the collection of the material in item (a), while item (b) shows the extract obtained, which will be used as a source of nutrients to promote the growth of the bacterium Sporosarcina pasteurii.

2.3. Sporosarcina Pasteurii

The species Sporosarcina pasteurii belongs to the group of Gram-positive, aerobic bacteria that are motile due to peritrichous flagella and capable of forming spores. It belongs to the phylum Firmicutes, class Bacilli, and family Planococcaceae [32]. It is characterized by its high ureolytic activity, producing the enzyme urease, which makes it relevant in biotechnology and civil engineering applications.

(a) Offal (b) Extract

Fig. 2 Fish guts

Figure 4 shows the procedure carried out to obtain Sporosarcina pasteurii, which was performed with the support of the "C3 Ingeniería Especializada" laboratory. This stage was fundamental in the experimental development, as it enabled the isolation of the bacterial strain required for subsequent tests.

Throughout the process, the safety measures established for handling chemical reagents were applied, using the Personal Protective Equipment (PPE) appropriate for the procedure, such as surgical gloves, lab coat, cap, and mask. Item (a) shows the Sporosarcina pasteurii bacterium in liquid suspension.

To initiate its growth, in item (b), a culture medium was prepared in a flask, consisting of 10 ml of urea-ammonium solution, 3 g of agar, 20 ml of distilled water, and 15.75 ml of Tris base buffer solution, used to maintain a stable pH during the process. In item (c), fish viscera were extracted and used as a natural nutrient supplement. Item (d) shows its incorporation into the culture medium in a volume of 1 ml of fish viscera extract. Item (e) shows the mixture of the extract with the culture medium.

To ensure homogeneous dissolution of the components, in item (f), a burner was used to bring the mixture to a boil, stirring constantly during the process. Once boiling was reached, the solution was carefully poured into Petri dishes (item g), where it was left to cool at room temperature for approximately 30 minutes (item h).

Item (i) shows the bacterial culture being seeded into the solidified medium using a sterile inoculating loop under controlled aseptic conditions. Item (j) shows the plates being transferred to the incubator, where they were kept at a constant temperature of 28°C for a period of 24 hours to promote bacterial growth.

Finally, item (k) shows the successful development of Sporosarcina pasteurii colonies on the Petri dishes, which was observed after the incubation period. The results were validated by counting the colonies.

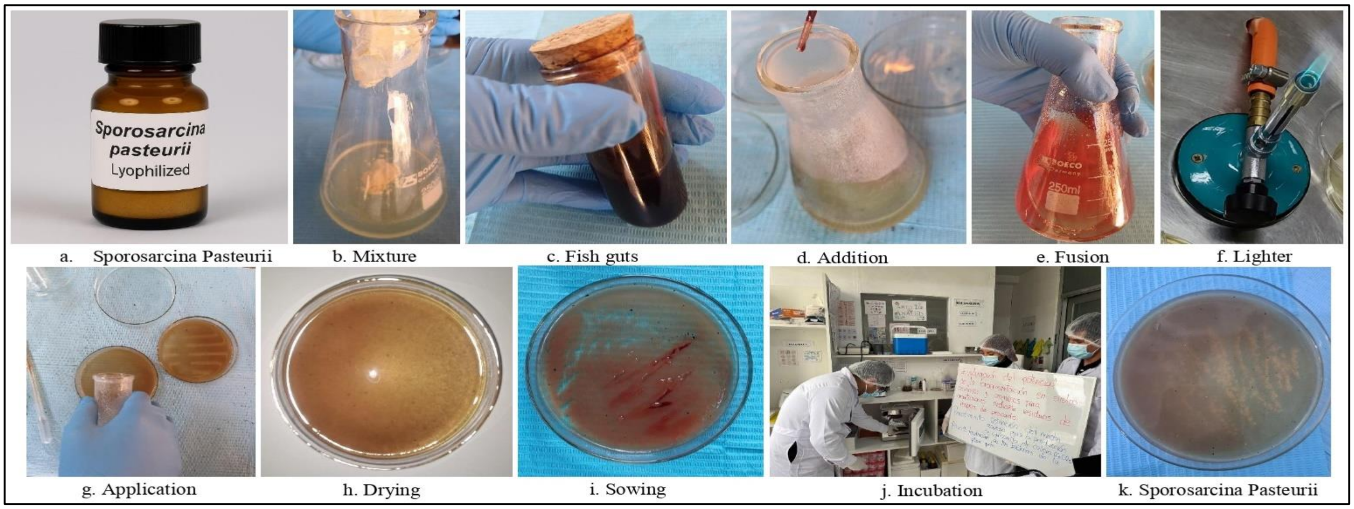

Fig. 3 Process for obtaining Sporosarcina pasteurii

2.4. Test Configuration

Sporosarcina pasteurii is a Gram-positive, aerobic bacterium that is motile due to peritrichous flagella and sporulates. Classified within the phylum Firmicutes, class Bacilli, and family Planococcaceae [32], it exhibits high ureolytic activity induced by urease, which is why it has gained relevance in biotechnology and civil engineering, particularly in biocementation.

To determine the impact of the initial concentration of the bacterium, the volume of fish viscera as an organic substrate, and the temperature on calcium carbonate precipitation, a series of 18 experiments was prepared, the variables of which are detailed in Table 1.

Table 1. Experimental conditions for tests with Sporosarcina pasteurii

Test	Bacterial concentration (cfu/ml)	Fish viscera volume (ml)	Temperature (°F)
1	1.10×10^7	3	25
2	1.10×10^7	6	25
3	1.10×10^7	9	25
4	1.10×10^7	3	35
5	1.10×10^7	6	35
6	1.10×10^7	9	35
7	1.20×10^7	3	25
8	1.20×10^7	6	25
9	1.20×10^7	9	25
10	1.20×10^7	3	35
11	1.20×10^7	6	35
12	1.20×10^7	9	35
13	1.30×10^7	3	25
14	1.30×10^7	6	25
15	1.30×10^7	9	25
16	1.30×10^7	3	35
17	1.30×10^7	6	35
18	1.30×10^7	9	35

Microorganism-induced Calcium Carbonate Precipitation (MICP) was used to promote the deposition of Calcium Carbonate ($CaCO_3$). Initially, single colonies of Sporosarcina pasteurii were collected with a sterile loop and inoculated into 10 mL test tubes containing fish viscera extract (prepared as described in section 3.1.3). The inoculated medium was supplemented with 10 ml of ammonium sulfate solution and 15.75 ml of Tris base buffer to establish a defined ionic equilibrium and a pH suitable for enzymatic activity and alkalinity-driven carbonate precipitation.. The mixture was kept at a controlled temperature between 25 and 30°C in an incubator with constant agitation. This agitation promoted both the homogenization of the reagents and the oxygenation of the medium, conditions that stimulate bacterial activity. As the urease produced by the bacteria hydrolyzed the urea into ammonium and carbonate ions, these reacted with the calcium ions present, generating the formation of the characteristic white precipitate of Calcium Carbonate ($CaCO_3$).

Figure 5 shows the evolution of calcium carbonate precipitation over time in the different tests carried out with Sporosarcina pasteurii under various conditions of bacterial concentration, amount of fish viscera, and temperature. At time 0, all tests showed constant values of 0.0001 g, indicating the absence of initial precipitation. From hour 1 onwards, a progressive increase in calcium carbonate formation was observed, especially in tests with higher concentrations of viscera and higher temperatures. In particular, tests 3, 6, 9, and 12, performed with 9 ml of viscera, recorded significantly higher values, suggesting that a greater volume of viscera favors biomineralization. Similarly, tests 6, 12, and 16, performed at 35°C, showed a notable increase in precipitation, highlighting the positive effect of high temperature on bacterial activity. Likewise, tests 13 to 17, corresponding to a bacterial concentration of 1.30×10^7 cfu/ml, showed a slight but consistent increase in calcium carbonate production, highlighting the influence of a higher concentration of S. pasteurii. Overall, the highest values were reached at 12 hours, being most notable in tests 3 (0.0190 g), 6 (0.0194 g), 9 (0.0160 g), and 12 (0.0178 g), confirming that conditions of greater viscera quantity, higher temperature, and higher bacterial concentration significantly favor the biomineralization of calcium carbonate.

Figure 6 compares calcium carbonate production in Tests 15 and 18, both performed with a bacterial concentration of 1.30 × 10^7 cfu/ml and 9 ml of fish viscera, but at different temperatures: 25°C and 35°C, respectively. From the first hour, there was a slight difference in precipitation, with values of 0.0150 g for Test 15 and 0.0170 g for Test 18. Over the 12 hours, both tests showed a progressive and constant increase, reaching 0.1600 g in Test 15 and 0.1700 g in Test 18. Although Test 18 showed a slight advance in calcium carbonate formation, the results suggest that temperature does not significantly influence the biomineralization process under the conditions evaluated, with similar behaviors being maintained between both tests.

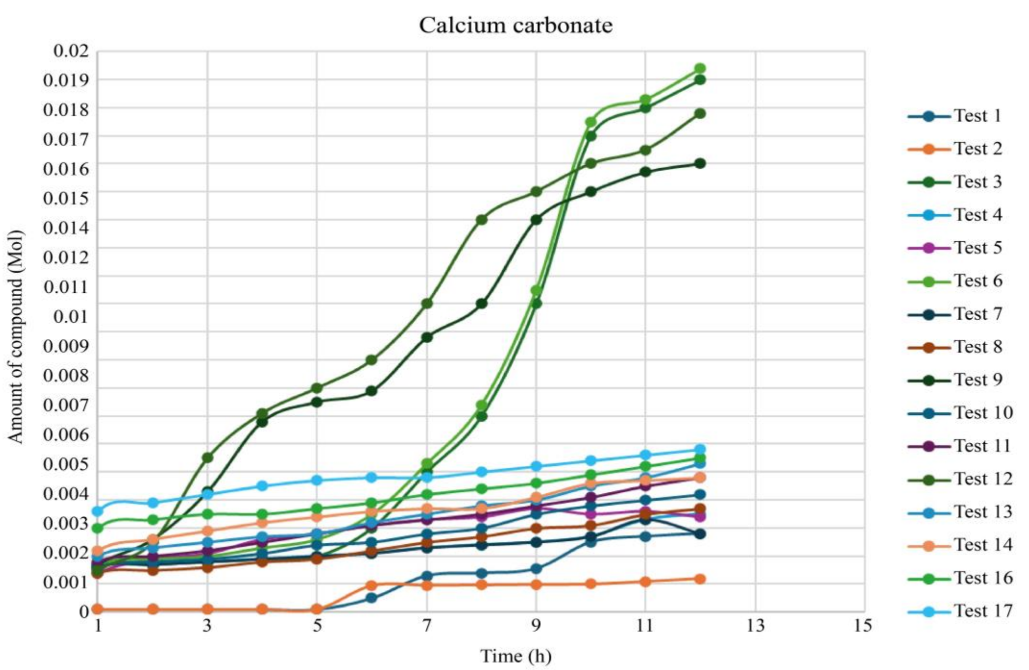

Fig. 4 Low calcium carbonate content

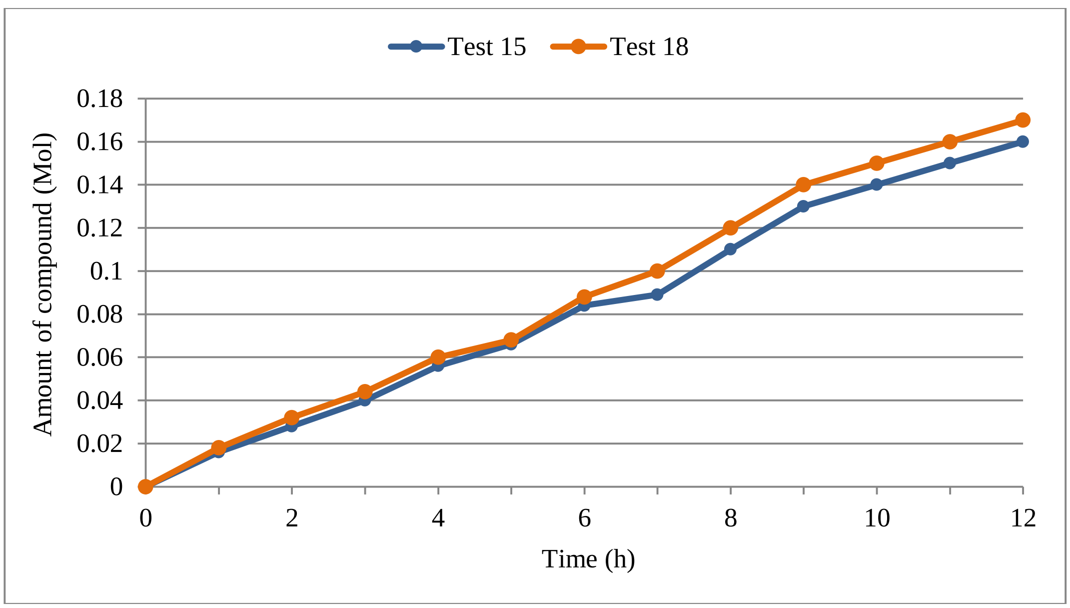

Fig. 5 High calcium carbonate content

According to the tests carried out and shown in Figure 6, the best result was obtained in test number 18. Based on this, the same configuration was used in subsequent tests. This result can be seen in Figure 7, which illustrates the generation of Calcium Carbonate ($CaCO_3$) as a direct outcome of the metabolic action of Sporosarcina pasteurii. Through the hydrolysis of urea, the microorganism releases carbonate ions (CO_3^{2-}), which subsequently interact with calcium species inherent in the fish-viscera extract, giving rise to the crystallization and deposition of $CaCO_3$, according to Equation (1):

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \downarrow \quad (1)$$

This crystal formation confirms the success of the bioprecipitation process induced by microorganisms under controlled conditions, using low-cost inputs of organic origin.

Fig. 6 Obtaining calcium carbonate

Therefore, in the following tests, these conditions will be taken as a reference for application in clay soil and mixtures of clay and sand, seeking to replicate or improve the observed efficiency [33].

2.5. Measuring Indicators for Soil Classification

2.5.1. Soil Sample

Soil samples were collected in the province of Huancayo. Clay soil was obtained from the Los Sauces quarry in Palián, while sandy soil was extracted from the 3 de Diciembre quarry. Both quarries supply the district of Huancayo, as they are located in strategic areas, approximately 15 minutes from the city center. In addition, these quarries have geotechnical characteristics representative of the region, making them suitable for soil studies in the field of civil engineering. The exact locations of both quarries are shown in Figure 8.

Fig. 7 Location of quarries

2.5.2. Granulometry of the Low Plasticity Clay Sample

The granulometric evaluation makes it possible to characterize the proportions of different soil fractions [34]. The test was performed in accordance with standard MTC E-107 [35], which establishes the use of a series of standardized sieves with progressive openings from the coarsest to the finest. The procedure was carried out manually, ensuring constant and uniform movement of the material over the sieves, as shown in Figure 9.

Fig. 8 Soil particle size distribution

2.5.3. Atterberg Limits

The liquid limit represents the water content at which the soil loses its shear strength and begins to behave like a fluid. To determine this, standard MTC E-110 [35] was applied, preparing a homogeneous soil sample mixed with water until it reached a suitable consistency. This was placed in the

Casagrande cup, maintaining a uniform depth of 10 mm. Subsequently, a groove was made with the standard grooving tool and impacts were made at a controlled frequency with a controlled frequency of approximately 2 impacts per second, noting the repetitions necessary for the groove to converge across a span of 13 mm.

The sample was collected after each series of impacts, as shown in Figure 10, and weighed to determine its moisture content. This procedure was repeated with different moisture contents until a series of data in the range of 15 to 35 blows was obtained, which allowed the liquid limit value to be interpolated.

Fig. 9 Liquid limit

The provisions of standard MTC E-111 [35] for the plastic limit were followed, using the cylinder formation method, as shown in Figure 11. The sample was kneaded manually on a smooth surface, forming small cylinders approximately 3 mm in diameter and 25 to 30 mm in length. The plastic limit was considered to have been reached when the cylinders began to disintegrate into fragments approximately 6 mm in length, indicating a loss of plasticity.

Fig. 10 Plastic limit

Peruvian Technical Standard NTP 339.135:1999 [36] guided the soil classification procedure and prescribes the adoption according to the Unified Soil Classification System (USCS). Under this framework, the material was categorized as SC, designating low plasticity clay, which is mainly composed of particles with diameters less than 0.002 mm [37] and is also distinguished by its low mechanical strength [38].

2.5.4. Classification of the Poorly Graded Sand Sample with Gravel

Particle size analysis is a test that determines the distribution of particle sizes in soil, which is essential for classifying it and understanding its mechanical behavior, especially with regard to compaction, permeability, and stability. Figure 12 shows the test performed on a sample of sandy soil using the manual sieving method. For this purpose, a series of sieves arranged in descending order of aperture was used, as established by technical standards ASTM D422 [39] and its national equivalent NTP 339. 128 [36], which recommends the use of sieves ranging from No. 4 (4.75 mm) to No. 200 (0.075 mm) for soils with a predominance of sandy fractions. Following these regulatory recommendations, this methodology was applied, which allowed us to obtain a representative granulometric curve of the analyzed material, thus facilitating its proper characterization.

Fig. 11 Sand soil grain size

According to Peruvian Technical Standard NTP 339.135: 1999 [36], the Unified Soil Classification System (USCS) was applied to the site material, resulting in an SP designation, which stands for poorly graded sand with gravel. This class comprises granular particles ranging from 0.06 mm to 2 mm [40] and is inherently characterized by inadequate cohesion to reliably support substantial vertical loads. Mitigation measures, such as compaction or the introduction of reinforcement layers, are recommended to improve its load-bearing capacity [41].

2.5.5. Direct Cut

The direct shear test is a test used to determine the shear strength of soils, evaluating their ability to resist horizontal forces that cause a sample to slide along a shear surface under controlled conditions. This test was performed using standard MTC E 123 [35], beginning with the preparation of a representative soil sample, which was sieved to obtain particles smaller than 19 mm. Figure 13, items a and b, shows the soil samples (clay and sand) that were placed in a direct shear box with an area of 50 cm² and a determined height of 3 cm, where they were compacted until the desired density was achieved. In item c, the shear box was adapted to place the sample; in item d, the box was positioned inside the shear box. In item e, the compaction process was carried out. In item f, the box was already correctly placed in the shear box, and in item g, the assembly was transferred to the machine, where a normal (vertical) load was applied to the sample, which was gradually increased to simulate real pressures. At the same time, a horizontal load was applied to cause the sample to slide along the cutting surface, while the horizontal and vertical displacements were measured with a high-precision displacement meter. In item h, the sample is seen after the test. The results obtained allowed the shear strength of the soil to be calculated, generating the shear stress versus displacement curve, which made it possible to determine the angle of internal friction (φ) and the cohesion (c) of the soil, fundamental parameters for the design of foundation works and structures.

Fig. 12 Direct shear test

2.5.6. Consolidation Test

The one-dimensional consolidation test was performed in an edometer to evaluate the compressibility of the soil and its behavior under load. Intact samples of clayey and sandy soils were extracted, with a diameter of 50-75 mm and a height of 20-25 mm. These samples were weighed, measured, and saturated with water for 24 hours. They were then placed in a metal ring Figure 14, items (b) and (c) inside the soil tablet Figure 14, item (a) and taken to the edometer machine Figure 14, item (d), where the measurement system was adjusted. Then, a minimum initial load was applied for settlement, followed by successive loads that doubled the pressure (0.1, 0.2, 0.4, 0.8, 1.6, 3.2, and 6.4 kg/cm²), maintaining them for 24 hours and recording the deformation at regular intervals. Finally, a progressive unloading was performed, and the recovery of the soil was measured. Parameters such as the consolidation coefficient (Cv), compression index (Cc), recompression index (Cr), and permeability (k) were calculated, obtaining the settlement vs. logarithm of time curve, which allowed for the prediction of settlements in structures and the design of adequate foundations.

Fig. 13 Consolidation test

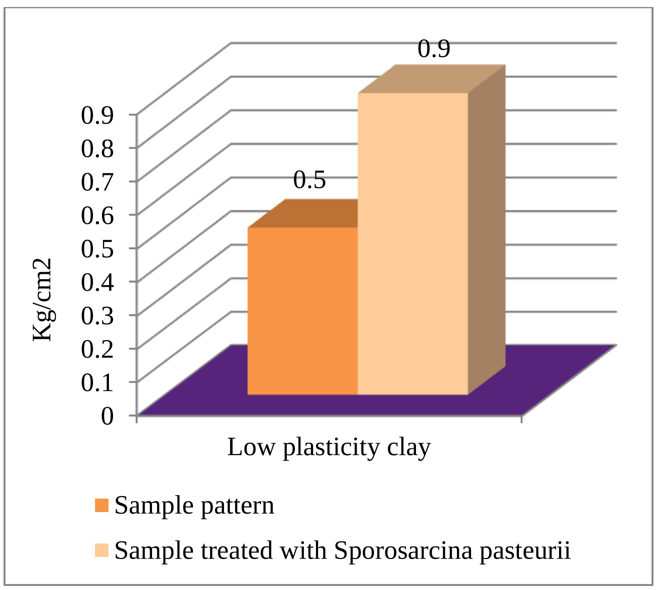

Fig. 14 Permissible capacity of low plasticity clay

3. Results

3.1. Permissible Capacity

3.1.1. Low Plasticity Clay

Figure 15 shows the results of the allowable capacity of low plasticity clay soil, comparing the control sample (without addition) with Sporosarcina pasteurii. This addition was made based on the previous tests described above, which allowed the optimal conditions for calcium carbonate production to be established. The control sample reached an admissible capacity of 0.50 kg/cm², while the treated sample increased this value to 0.90 kg/cm², representing an improvement of 80%. The observed increase in shear strength is related to the crystallization of calcium carbonate stimulated by microbial populations residing in the soil matrix, which effectively functioned as natural biocementing agents.

The resulting cementation bound individual soil particles together, leading to a measurable decrease in effective porosity and a concomitant increase in internal cohesion. Such alterations make the clay matrix more resistant to deformation and provide a reliable increase in load-bearing capacity, thus informing the engineering design of more robust foundations capable of accommodating higher service loads while minimizing the likelihood of instability or catastrophic failure.

3.1.2. Poorly Graded Sand with Gravel

Figure 16 illustrates the allowable bearing capacity of poorly graded sandy soil incorporating gravel, juxtaposing the behavior of the control specimen without any treatment with that of the specimen modified with the bacterium Sporosarcina pasteurii.

This addition was made per the previous tests, in which the necessary conditions were established to promote the formation of calcium carbonate. The allowable capacity of the soil in the control sample was 1.50 kg/cm², while in the treated sample it increased to 1.80 kg/cm², showing a 20% improvement.

This increase is due to the biocementing action of the microorganisms present, which facilitated $CaCO_3$ deposition between the sand particles, increasing internal friction and soil densification. As a result, the resistance of the soil is improved, which is favorable for the development of more stable foundations in granular soils.

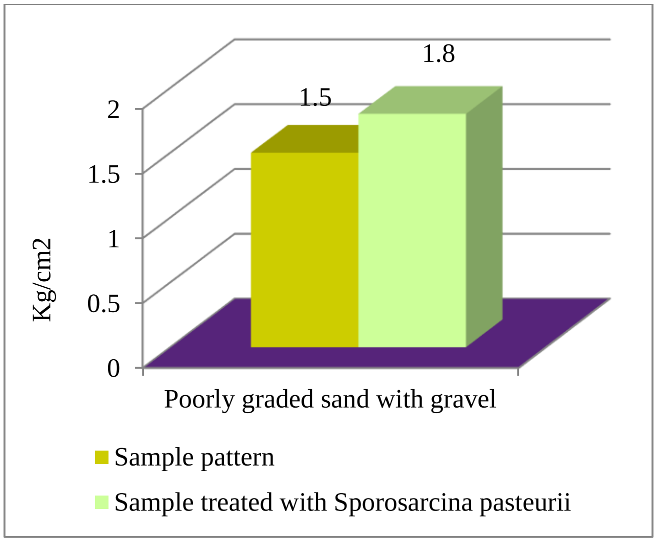

Fig. 15 Permissible capacity of poorly graded sand with gravel.

3.1.3. Low Plasticity Clay + 30% Poorly Graded Sand with Gravel

Figure 17 presents the outcomes of the bearing capacity of soil composed of low plasticity clay + 30% poorly graded sand with gravel, comparing the control sample (without addition) with the bacterium Soporosarcina pasteurii. This dosage was selected according to the previous tests described, which defined the optimal conditions for inducing calcium carbonate formation. The control sample achieved an allowable capacity of 0.98 kg/cm², while the sample with bacterial addition increased this value to 1.21 kg/cm², representing a 23% improvement. This increase is due to the biocementing effect of the microorganisms, which promoted $CaCO_3$ deposition between the soil particles, reducing their porosity and improving cohesion. This improvement in the strength of the mixed soil is favorable for applications in foundation projects, providing greater bearing capacity and structural stability.

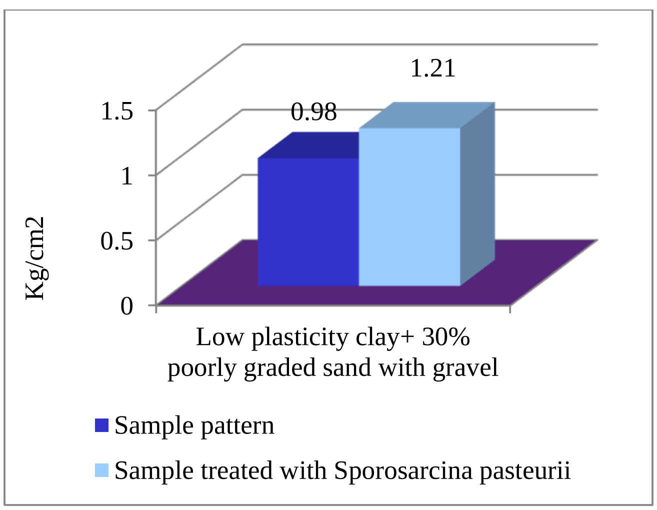

Fig. 16 Permissible capacity of low plasticity clay + 30% poorly graded sand with gravel

3.1.4. Low Plasticity Clay + 40% Poorly Graded Sand with Gravel

Figure 18 shows the results of the allowable capacity of soil composed of low plasticity clay + 30% poorly graded sand with gravel, comparing the control sample (without addition) with the bacterium Soporosarcina pasteurii. This addition was made following the conditions determined in previous tests to promote the efficient formation of calcium carbonate. The control sample reached an admissible capacity of 1.01 kg/cm², while the treated sample increased this value to 1.32 kg/cm², representing a 31% improvement. This improvement is attributed to the biocementing action of the microorganisms present in the viscera, which precipitated calcium carbonate between the soil particles, increasing strength and internal cohesion. This type of stabilization is beneficial for the design of foundations on mixed soils, allowing for better structural performance and greater load-bearing capacity.

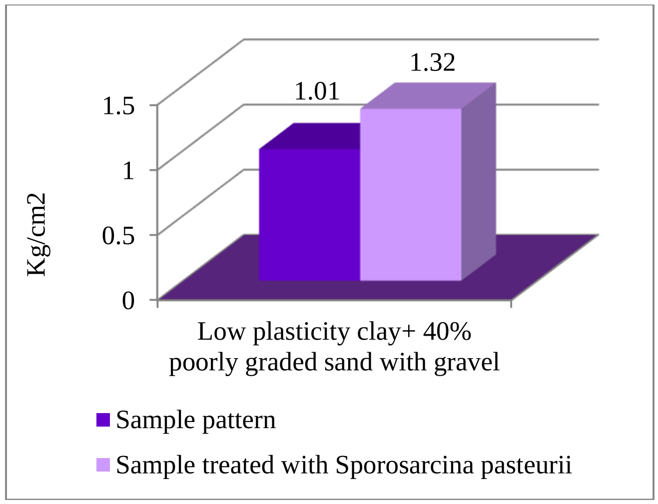

Fig. 17 Permissible capacity of low plasticity clay + 40% poorly graded sand with gravel

3.2. Consolidation

3.2.1. Low Plasticity Clay

The consolidation test was carried out with the aim of evaluating the behavior of low plasticity clay soil subjected to different load levels, both in its natural state and in treated condition. This test is essential for determining the soil's ability to resist compression and its behavior under progressive loads, which is essential in geotechnical engineering, especially for the design of foundations and structures that require a solid and stable base.

In Figure 19, corresponding to low plasticity clay soil (pattern), a progressive increase in deformation with load was observed, reaching a maximum value of 0.667 mm at 6.4 kg/cm². This response is typical of untreated clay soil, showing considerable susceptibility to compression. Soil consolidation under these conditions indicates a high capacity for deformation under moderate to high loads.

On the other hand, Figure 20 shows the results for low-plasticity clay soil treated with Sporosarcina pasteurii. In this case, the maximum deformation reached only 0.210 mm under the same load of 6.4 kg/cm², representing an improvement of 68.52% compared to untreated soil. This improvement is due to the action of the bacteria, which promote the formation of calcium carbonate and reinforce the soil structure, increasing its ability to resist compression and significantly reducing its deformability.

The bacterial treatment proved effective in improving the mechanical properties of clay soil, as evidenced by greater stiffness and less deformation under load. This suggests that the application of this bacterial treatment could be a viable option for improving the load-bearing capacity of soils in civil engineering projects, such as land stabilization for road construction, pavements, and building foundations, reducing the need for conventional stabilization methods such as lime or cement.

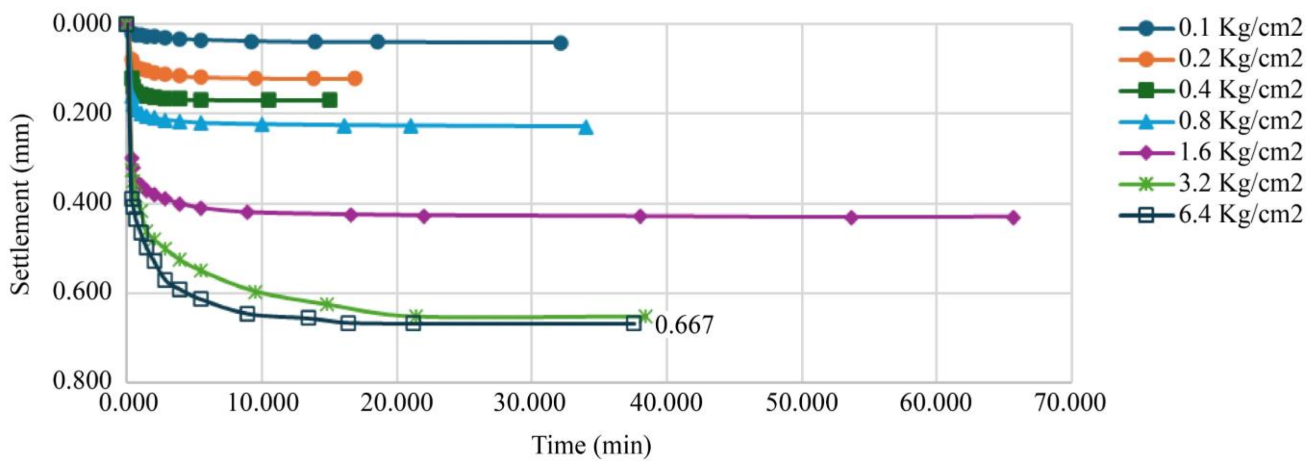

Fig. 18 Standard soil (low plasticity clay) in the settlement test

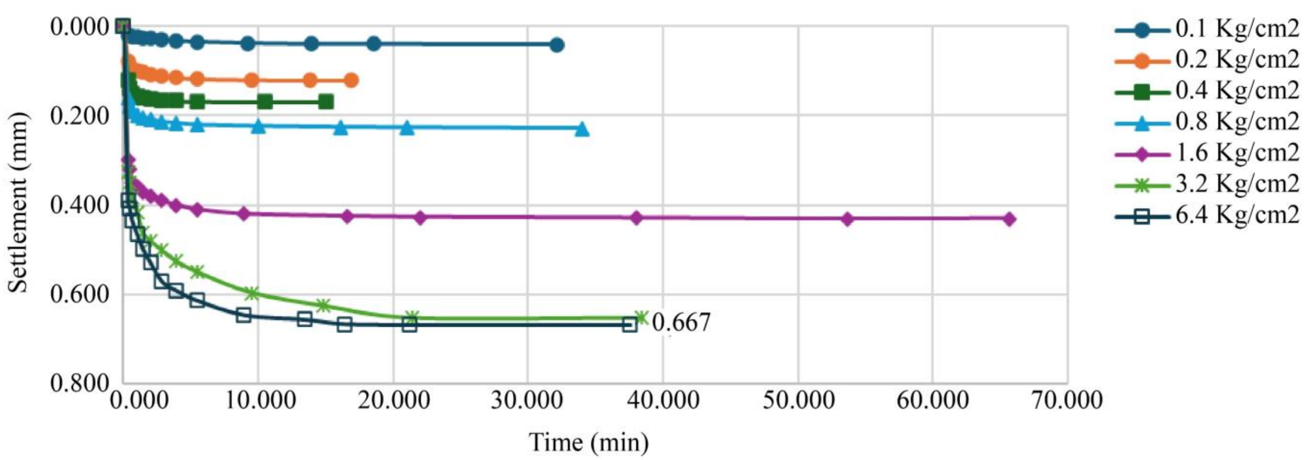

Fig. 19 Modified soil (low plasticity clay) in the settlement test

3.2.2. Poorly Graded Sand with Gravel

A one-dimensional consolidation analysis was conducted on gravelly sandy soil to assess its deformation characteristics under different stress levels in natural and bacterially enhanced conditions. The outcomes supply essential data for predicting settlement behavior, which is widely used in civil engineering to optimize the design of pavements and foundation systems.

In Figure 21, corresponding to poorly graded sandy soil with untreated gravel, a progressive increase in deformation was observed as the applied load increased. As the load increased, the soil showed high susceptibility to compression, reaching a maximum deformation of 2.29 mm at 6.4 kg/cm². This behavior is characteristic of unstabilized sandy soils, which tend to experience high deformation under moderate to high loads.

In comparison, Figure 22 shows the results obtained for poorly graded sandy soil with gravel treated with Sporosarcina pasteurii. The soil treated with bacteria showed a much more controlled response, reaching a maximum deformation of only 1.487 mm at 6.4 kg/cm². This reduction in deformation is significant, with a 35.07% improvement compared to untreated soil.

Bacterial treatment in sandy soil showed a clear improvement in the mechanical properties of the soil, reducing its deformability under load. This treatment, which promotes the precipitation of calcium carbonate, reinforces the soil structure, increasing its compressive strength. These results indicate that the application of bacteria in sandy soils could be an effective alternative for improving their performance in civil engineering projects, such as soil stabilization for the construction of structures, pavements, or foundations, reducing the need for more costly and complex conventional treatments.

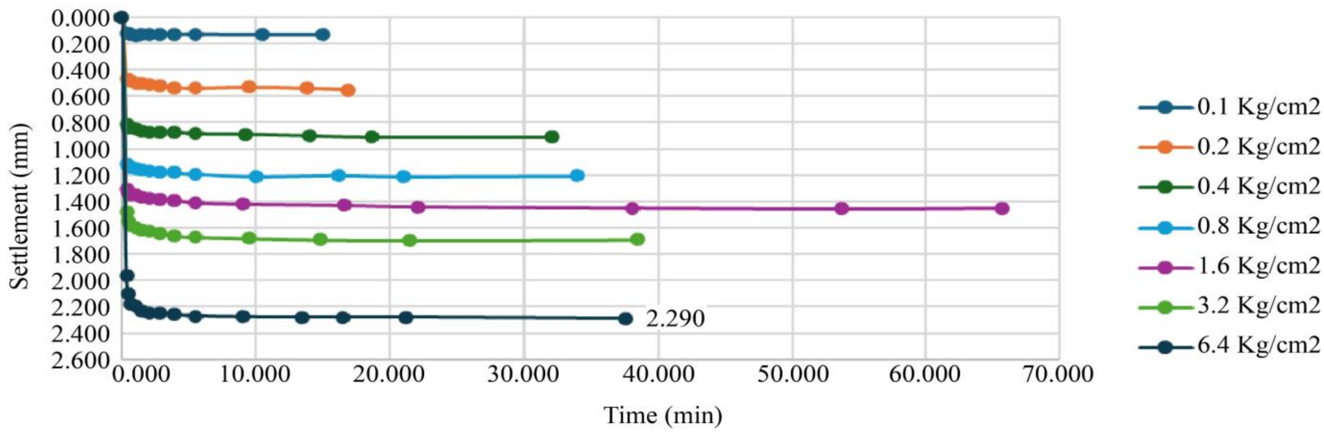

Fig. 20 Standard soil (poorly graded sand with gravel) in the settlement test

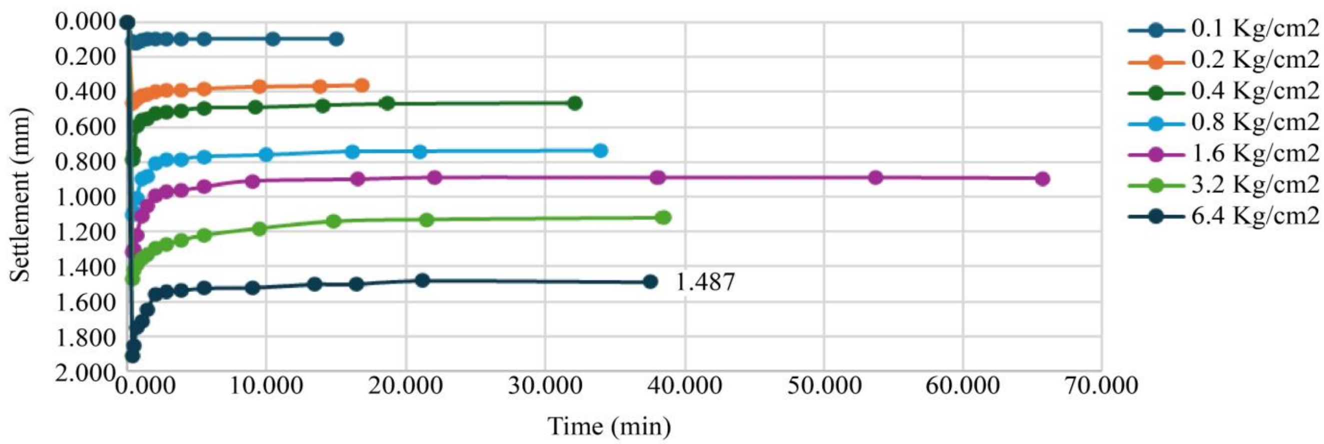

Fig. 21 Modified soil (poorly graded sand with gravel) in the settlement test

3.2.3. Low Plasticity Clay + 30% Poorly Graded Sand with Gravel

The consolidation test was carried out to evaluate the behavior of low plasticity clay soil with 30% poorly graded sand with gravel, both in its natural state and when treated with Sporosarcina pasteurii bacteria, which induce $CaCO_3$ deposition.

This test is fundamental for determining the soil's deformation capacity under different pressures, which is essential in geotechnical engineering applications, such as the construction of foundations and pavements. In Figure 23, corresponding to low plasticity clay soil with 30% poorly graded sand with gravel in its natural state, a progressive increase in deformation was observed as the applied load increased. As the load increased, the soil showed high susceptibility to compression, reaching a maximum deformation of 1,290 mm at 6.4 kg/cm². This behavior is characteristic of unstabilized soils, which tend to experience high deformations under moderate to high loads.

On the other hand, Figure 24 shows the results of low plasticity clay soil with 30% poorly graded sand with gravel treated with Sporosarcina pasteurii, reaching a maximum deformation of only 0.798 mm at 6.4 kg/cm², representing a significant reduction of 38.14% compared to untreated soil.

This bacterial treatment promoted $CaCO_3$ deposition, which reinforced the soil structure and increased its resistance to settlement, demonstrating that the use of Sporosarcina pasteurii is an effective and sustainable alternative for improving the mechanical properties of these soils in construction projects, reducing dependence on more costly and complex conventional treatments.

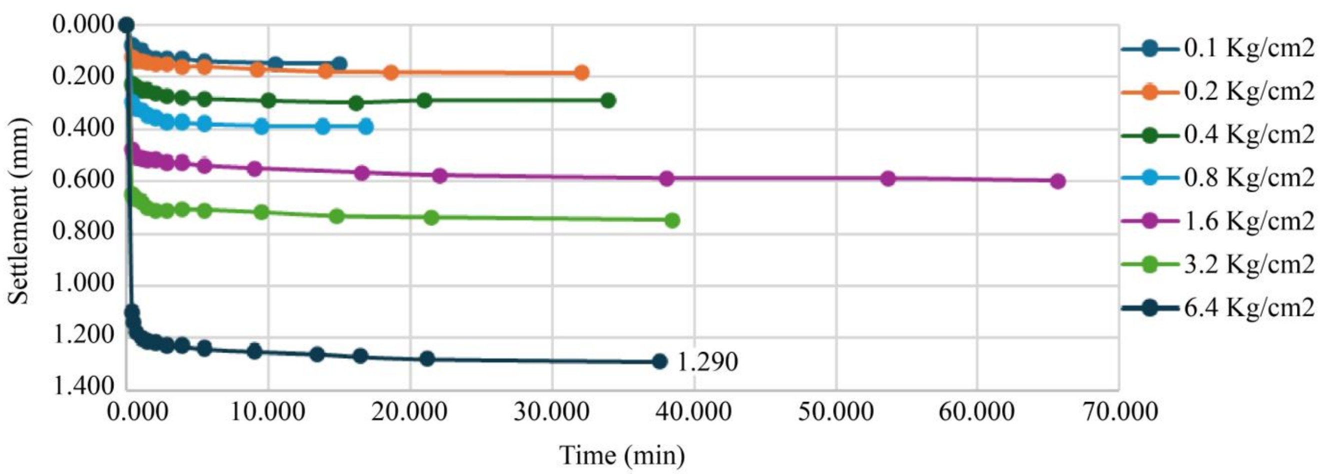

Fig. 22 Permissible capacity of low plasticity clay + 40% poorly graded sand with gravel

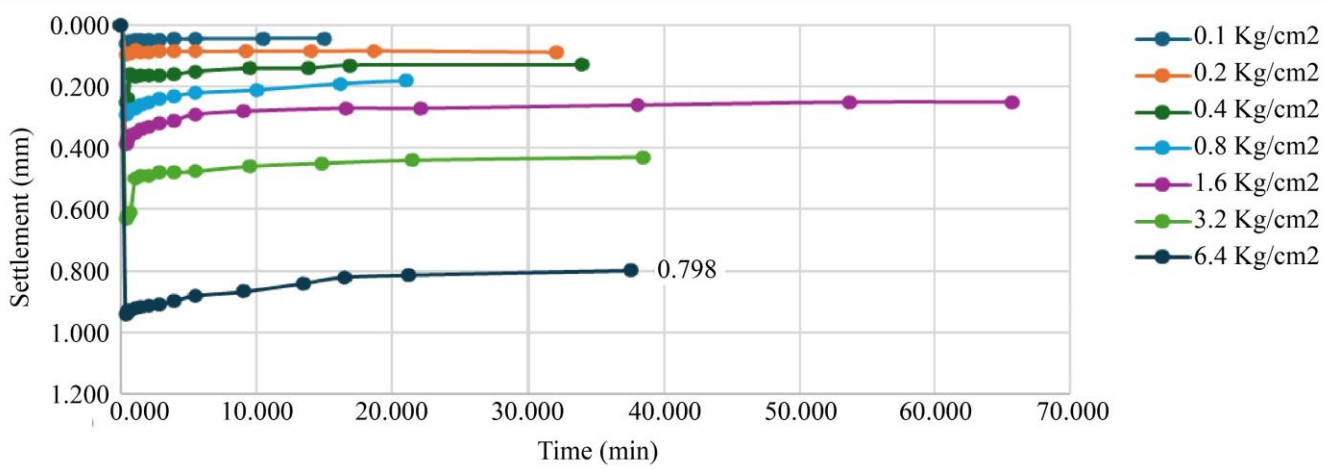

Fig. 23 Modified soil (low plasticity clay + 30% poorly graded sand with gravel) in the settlement test

3.2.4. Low Plasticity Clay + 40% Poorly Graded Sand with Gravel

The consolidation test was carried out to evaluate the behavior of a low-plasticity clay soil with 40% poorly graded sand with gravel, both in its natural state and treated with Sporosarcina pasteurii bacteria, which induce the precipitation of calcium carbonate. This test is essential for determining the soil's deformation capacity under different pressures, which is crucial in civil engineering applications such as foundation and pavement construction.

In Figure 25, corresponding to low plasticity clay soil with 40% poorly graded sand with gravel in its natural state, a progressive increase in deformation was observed as the applied load increased. As the load increased, the soil showed high susceptibility to compression, reaching a maximum deformation of 1.55 mm at 6.4 kg/cm². This behavior is characteristic of unstabilized soils, which tend to experience high deformations under moderate to high loads. On the other hand, Figure 26 shows the results obtained for low plasticity clay soil with 40% poorly graded sand with gravel, treated with Sporosarcina pasteurii, which induced $CaCO_3$ deposition. In this case, the soil showed a more controlled response, reaching a maximum deformation of only 0.686 mm at 6.4 kg/cm², representing a 55.74% reduction compared to the untreated soil. This would confirm that treatment with Sporosarcina pasteurii promotes $CaCO_3$ deposition, demonstrating the improvement in the structure of these soils and establishing it as an effective and sustainable alternative for construction projects, reducing the need for more costly and complex conventional methods.

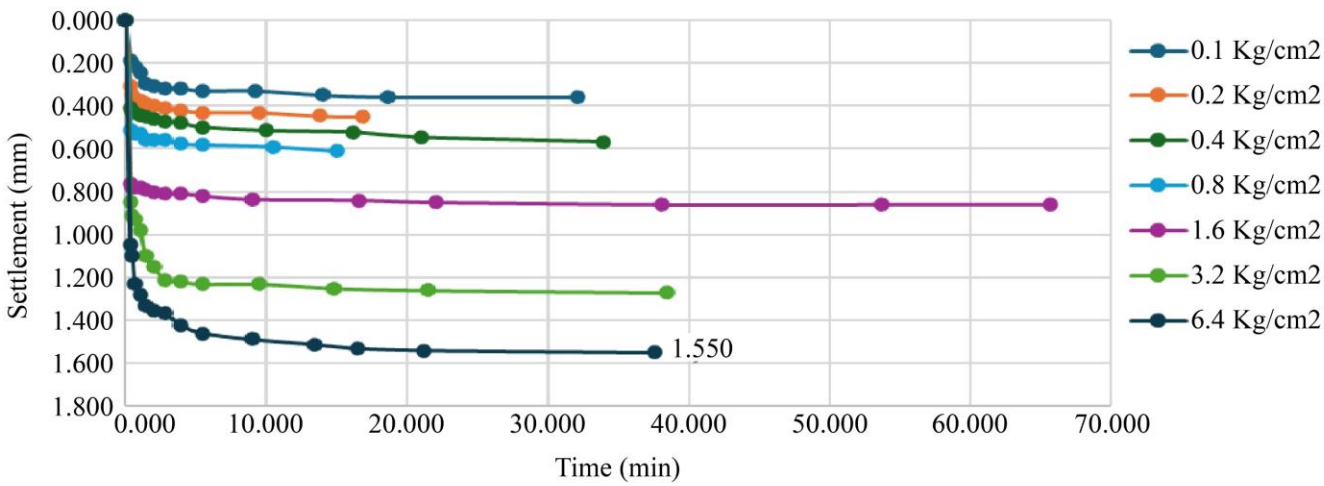

Fig. 24 Standard soil (low plasticity clay + 40% poorly graded sand with gravel) in the settlement test

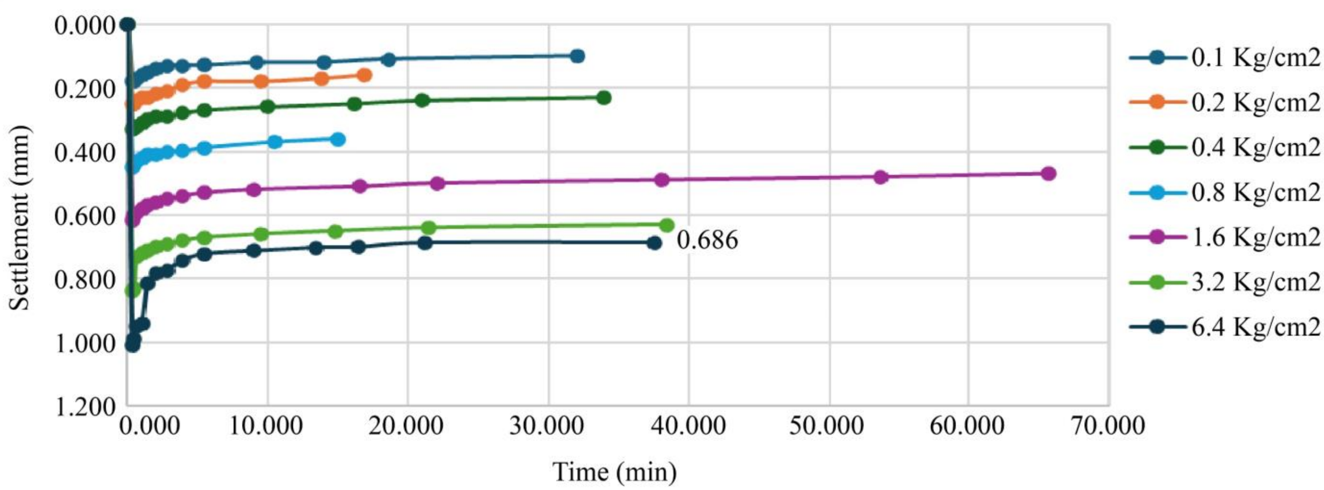

Fig. 25 Modified soil (low plasticity clay + 40% poorly graded sand with gravel) in the settlement test

4. Discussion

Extensive research has demonstrated that Microbially Induced Calcite Precipitation (MICP) using Sporosarcina pasteurii enhances soil performance by reducing porosity, strengthening interparticle bonding, and improving load transfer through the formation of $CaCO_3$ bridges [10, 11, 15-19, 22, 23, 42, 43]. The results of the present study validate these mechanisms under a novel nutrient pathway based on fish-viscera extract, confirming that such residual substrates can sustain ureolytic activity and produce measurable gains in bearing capacity and consolidation behavior of natural soils.

4.1. Low-Plasticity Clay (CL)

The 80% increase in allowable capacity and the 68.52% reduction in settlement observed in CL soils demonstrate that MICP substantially modifies the mechanical behavior of fine-grained matrices. The large surface-to-volume ratio of clay particles provides abundant nucleation sites for calcite precipitation, leading to cemented aggregates that limit platelet sliding and reorientation under stress. This results in higher apparent cohesion and lower compressibility. The phenomenon is reflected in a shift of the stress-strain curve, where treated clays exhibited significantly lower axial strains at equivalent vertical stresses, consistent with reductions in the Compression Index (Cc) reported for biocemented fine soils [19, 23].

Bernat-Masó et al. [42] showed that biocementation increased the apparent cohesion of compressed earth blocks, enabling stabilization without additional clay. The present results corroborate those findings, but under a nutrient regime derived from organic waste rather than synthetic media. This distinction is critical because it demonstrates that ureolytic pathways can be sustained by protein- and nitrogen-rich residues, which stimulate urease synthesis and metabolic activity. From an engineering design perspective, improvements in CL translate into higher mobilized cohesion (c′), allowing shallow foundations to operate under greater service loads with reduced settlement risks.

4.2. Poorly Graded Sand with Gravel (SP)

The 20% improvement in bearing capacity and the 35.07% reduction in settlement in SP soils are attributed to calcite precipitation primarily at grain-to-grain contacts. In granular frameworks, strength gains are driven less by cohesion and more by increases in the internal friction angle (φ′). Calcite deposits enlarge real contact areas, promote particle interlock, and reduce localized stress concentrations, producing stiffer and less deformable granular skeletons.

Yang et al. [15] documented similar results in sand columns, where in situ flocculation promoted uniform carbonate precipitation and significant UCS increases. Omoregie et al. [17] reported UCS values in the range of 11 MPa under percolation protocols, which exceed the outcomes here. This difference is attributable to treatment uniformity and reagent dosing: their percolation approach maximized ionic penetration, whereas the natural heterogeneity of the gravelly sand tested here limited homogeneous deposition.

Nevertheless, the settlement reduction confirms that even partial cementation is sufficient to densify granular frameworks and improve load transfer. This suggests that MICP can serve as a sustainable alternative to mechanical densification methods, particularly in contexts where compaction is constrained by accessibility or energy requirements.

4.3. Mixed Soils (CL+SP 30% and 40%)

The CL+SP mixtures exhibited synergistic behavior, achieving 23–31% increases in bearing capacity and 38–55% reductions in settlement. This synergy arises from the complementary roles of fines and coarse fractions: clays enhance nucleation due to their high specific surface, while sands provide permeable pathways that facilitate nutrient transport and bacterial mobility. Together, these mechanisms promote a more homogeneous distribution of $CaCO_3$, a well-recognized challenge in MICP applications highlighted by Omoregie et al. [19] and Dong et al. [18].

The superior performance of the 40% SP mixture compared with the 30% case indicates that permeability is a dominant factor in ensuring effective precipitation. Higher sand content improves ionic transport and prevents precipitation from being confined to isolated clusters, distributing cementation across the soil skeleton. This observation is consistent with Osinubi et al. [16], who emphasized that soil gradation and bacterial concentration jointly govern the magnitude of biocementation. This suggests that well-graded mixtures are optimal candidates for field-scale MICP, balancing transport efficiency with nucleation density.

4.4. Link between $CaCO_3$ Yield and Geotechnical Performance

Phase I confirmed that the maximum $CaCO_3$ yield was 0.0194 g at 12 hours under optimized conditions (1.30×10^7 CFU/ml, 9 ml extract, 35 °C). Although these values are lower than those reported in extended percolation or reactor-based protocols using synthetic nutrients [17, 30], they are remarkable given that they were achieved with a low-cost residual substrate. The observed trend of higher yields with larger extract volumes and elevated temperatures demonstrates that fish viscera's protein and amino acid-rich composition sustains ureolytic activity and promotes biomineralization.

The correlation between $CaCO_3$ production in Phase I and mechanical improvements in Phase II was direct: soils treated under optimized conditions displayed the highest gains in bearing capacity and the greatest reductions in settlement. This validates the mass of $CaCO_3$ as a quantitative predictor of geotechnical improvement, consistent with the culture optimization findings of Omoregie et al. [19] and the mineralization kinetics described by Carter et al. [30].

4.5. Sustainability and Novelty

Unlike prior studies that relied on synthetic substrates [17, 42, 43], the present research demonstrated that fish-viscera extract provides sufficient nutrients and micronutrients to sustain microbial viability and induce effective $CaCO_3$ precipitation. The lipid fraction additionally promoted hydrophobic microdomains, favoring nucleation at solid–liquid interfaces [30]. This dual biochemical contribution explains why the yields and soil improvements achieved here were competitive with benchmarks obtained using conventional nutrient media. Moreover, the valorization of fishery waste aligns with circular economy strategies in construction [6-8], reducing organic waste streams while simultaneously providing a sustainable soil stabilization method. While similar conclusions were reached using kitchen waste [31], this study extends the concept to protein-rich residues from the fishing industry, adapting it to Peru’s national waste-management priorities.

4.6. Boundary Conditions and Implications

4.6.1. Distribution of Precipitation

Achieving a uniform distribution of $CaCO_3$ remains one of the primary challenges in MICP. Yang et al. [15] emphasized that injection or percolation methods often generate preferential flow paths in granular soils, leading to zones of overcementation alongside untreated pockets. In this study, such effects were partially mitigated in CL+SP mixtures, where the fine fraction enhanced nucleation and the sandy fraction facilitated ionic transport. However, the heterogeneity observed in SP soils confirms that distribution uniformity remains a limiting factor for field-scale applications.

4.6.2. Durability under Aggressive Environments

International studies have shown that MICP-induced strength can degrade in chemically aggressive environments. Whitaker et al. [22] reported strength reductions of nearly 29% in treated sands exposed to acid rain, while Fu et al. [25] observed partial dissolution of calcite in marine conditions. These findings are particularly relevant to Peru, where urban soils may be exposed both to acid rainfall and saline coastal environments. Future applications should therefore incorporate protective measures or maintenance strategies to ensure long-term durability.

4.6.3. Management of Ureolysis By-Products

A further limitation is the release of ammonium (NH_4^+) as a by-product of urea hydrolysis. Without proper management, this can generate environmental impacts on groundwater and soil ecosystems [10-12]. While laboratory-scale testing in this study allowed control over ammonium accumulation due to small volumes, field-scale implementations must include effluent treatment systems, such as biofilters or nitrifying bacterial consortia, to mitigate secondary pollution.

4.6.4. Design Implications

These three factors, distribution, durability, and by-product management, define the boundary conditions for safe and effective MICP deployment. From a geotechnical perspective, the results translate into higher mobilized cohesion (c′) in CL, greater friction angle (φ′) in SP, and reduced compressibility indices (Cc) in mixed soils. These outcomes demonstrate that shallow foundations constructed on treated soils can safely sustain higher service loads with reduced settlements, offering a viable alternative to lime- or cement-based stabilization.

4.7. Overall Contribution

The findings provide robust experimental evidence that MICP using S. pasteurii with fish-viscera extract significantly enhances the geotechnical behavior of clays, sands, and mixed soils. The results validate $CaCO_3$ production as a reliable predictor of soil improvement, demonstrate that residual nutrient substrates sustain microbial activity at levels comparable to synthetic media, and highlight the environmental and economic benefits of adopting organic waste. This approach advances MICP research by integrating biochemical innovation with geotechnical validation, offering a sustainable, low-cost, and scientifically grounded alternative for soil stabilization in urban environments.

5. Conclusion

The findings support the proposition that biocementation mediated by Sporosarcina pasteurii… represents a pioneering and environmentally responsible means of soil improvement suitable for geotechnical work in sustainable civil engineering applications. Formation of Calcium Carbonate ($CaCO_3$) within the soil matrix was achieved through this technique, which had direct effects on its bearing capacity and behavior under sustained loads through microbial consolidation mechanisms.

In the case of low-plasticity clay soil, an 80% increase in allowable capacity was recorded, from 0.50 to 0.90 kg/cm². This improvement is highly significant, considering that cohesive soils such as clay tend to have low strength and high deformability. The action of calcium carbonate promoted the bonding between fine particles, reduced plasticity, and densified the soil structure, generating a more competent material for shallow foundations. This implies that, in contexts where the use of lime or cement is not viable for environmental or economic reasons, biocementation presents itself as a technical alternative with high substitution potential.

In poorly graded sandy soils with gravel, the allowable capacity increased from 1.50 to 1.80 kg/cm², representing a 20% improvement. Although the percentage is lower than in clay, this result is equally valuable, since granular soils do not have natural cohesion, and the reinforcement induced by the precipitation of $CaCO_3$ promoted the formation of mineral bridges between the grains, improving internal friction and reducing the risk of displacement. This makes them more suitable for light foundations or paving elements in areas with low natural compactness.

In the mixture of low plasticity clay + 30% poorly graded sand with gravel, the allowable capacity increased by 23%, while in the mixture with 40% poorly graded sand with gravel, the increase was 31%. These results reflect that hybrid soils, with characteristics intermediate between cohesive and granular, respond favorably to microbial treatment, combining improvements in both cohesion and friction. The inclusion of sand probably facilitated the permeability and penetration of the treatment, while the clay acted as a retention matrix. These treated soils are optimal candidates for stabilized subgrades or base layers in rural and urban roads.

From the point of view of consolidation behavior, the low-plasticity clay soil showed a notable reduction in its compressibility under sustained load after being treated. Vertical deformation was reduced from 0.667 mm to 0.210 mm at 6.4 kg/cm², representing an improvement of 68.52%. This decrease in deformability implies greater rigidity and long-term bearing capacity, reducing the risk of differential settlement in sensitive structures. Technically, this translates into greater structural safety and less need to oversize foundations, optimizing the use of materials and resources.

In poorly graded sandy soil with gravel, deformation decreased from 2.29 mm to 1.487 mm under the same load, representing an improvement of 35.07%. This difference is significant because granular soils are particularly prone to large initial settlements. Biocementation reduced this deformability, stabilizing the particle arrangement and allowing the material to work more like a compact mass than a loose mass.

This feature is particularly advantageous for projects with expected dynamic loads or repetitive vehicular traffic. Low-plasticity clay with 30% poorly graded sand with gravel showed a 38.14% reduction in deformation, from 1.29 mm to 0.798 mm. This indicates that the mixture responded favorably to the treatment, showing a balance between strength and ductility. In the case of the mixture with 40% poorly graded sand with gravel, the improvement was even greater, with a reduction of 55.74% (from 0.155 mm to 0.686 mm).

This result is particularly valuable, as it demonstrates that an appropriate dosage of components and biological conditions can generate a matrix with low compressibility and a high degree of rigidity, ideal for infrastructure elements that require high stability, such as foundation slabs, retaining walls, or industrial platforms. These improvements are directly associated with treatment efficiency based on three key variables: bacterial concentration, nutrient volume, and temperature. Optimal conditions were obtained with 1.30×10^7 cfu/ml, 9 ml of viscera extract, and 35°C incubation, achieving maximum $CaCO_3$ precipitation and, therefore, the greatest mechanical impact on soils. This finding allows replicable parameters to be established for future applications in the laboratory and the field. Although the results were highly positive, certain limitations must be considered. The study was conducted under controlled laboratory conditions, so it is necessary to evaluate the reproducibility of the treatment under real conditions, with more heterogeneous soils, climatic influences, and moisture cycles.

Likewise, long-term performance evaluation was not addressed; therefore, resistance to phenomena such as surface erosion, freeze-thaw cycles, or microbiological attacks remains an outstanding issue. Finally, although organic waste reduces costs compared to industrial nutrients, large-scale implementation will require economic and logistical feasibility studies for its production, conservation, and on-site application. Nevertheless, this research has proven that it is possible to develop geotechnical stabilization technologies from local biological inputs, promoting environmentally responsible and technically valid solutions for regions with low-bearing soils.

Acknowledgments

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