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Comparison of three different bio-based methodologies to improve sandy soils

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Article

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Abstract

This work compares the effectiveness of different biogrout agents to improve the mechanical properties (strength and stiffness) of two sandy soils. Based on the results of unconfined compressive strength tests for different curing times (3-180 days), the results obtained from microbial induced calcium carbonate precipitation using two bacteria (Sporosarcina pasteurii and Idiomarina insuliasalsae) are compared with employing urease to promote calcium carbonate precipitation and the use of the biopolymer xanthan gum (3-28 days). Additionally, the methodologies of calcite precipitation and the use of xanthan gum are also analyzed by examining scanning electron microscopy images. For a curing time of 28 days, the results show that the use of urease is the best of the three bio-based methodologies to improve sand, while the least efficient methodology is the use of the biopolymer xanthan gum. The use of both bacteria increases the unconfined compressive strength during the curing time (until 180 days); regarding the use of urease and xanthan gum, the unconfined compressive strength remains approximately constant for a curing time longer than 7-14 days. In terms of stiffness, the use of the bacteria Idiomarina insuliasalsae and urease result in a better improvement, while the use of the bacteria Sporosarcina pasteurii and xanthan gum has only a marginal effect on the stiffness.

1. Introduction

When the mechanical properties of foundation soils are not compatible with the actions induced by engineering structures (such as: embankments, dams, tunnels, airports, buildings among others), it is necessary to improve the characteristics of these soils. One of the methods most used in the world to enhance a soil's properties is the stabilization of the soil with cement-based chemical binders, for instance Portland cement, quicklime, fly ash, blast furnace granulated slag, etc.

Nowadays, cement is the chemical binder most used in geotechnical engineering, due to its excellent properties and low cost in some countries of the world. However, the impact of the cement industry on man's ecological footprint is far from negligible, since it contributes to approximately 2.6% of the global CO_2 emissions (IPCC, 2022). Further, the use of cement to stabilize soil induces significant and permanent impacts on the environment, associated with an increase in the *pH*, which has a negative effect on the vegetation growth and the microbiome development. Additionally, the composite material obtained from such stabilization is practically irreversible (Chang et al., 2016), therefore its use in environmentally sensitive areas is very questionable.

To mitigate the ecological footprint associated with cement-based stabilization techniques, some alternative methodologies have been emerging/investigated/tested in the last few years, namely the use of bio-based methodologies (Venda Oliveira & Rosa, 2020), the use of biodegradable materials (Nguyen et al., 2018; Mendonça et al., 2021a; Venda Oliveira & Reis, 2023) and recycling/reuse building materials (Gomes et al., 2014; Rios et al., 2024; Guimarães et al., 2024), among others.

The techniques based on the production of a bio-cement (biocementation), by using bacteria or enzymes, are some of the most studied and promising alternatives to replace the use of cement-based strategies under certain conditions. In general, biocementation techniques involve the precipitation of calcium carbonate and its deposition in the voids of the soil, which bonds the soil particles together and fills (at least partially) the pore spaces of the soil, that is, the material deposited behaves as a bio-cement. The production of this bio-cement can be achieved by the use

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of bacteria or enzymes, processes called microbial induced calcium carbonate precipitation (MICP) and enzyme induced calcium carbonate precipitation (EICP), respectively. In general, the soil improvement induced by both techniques, MICP and EICP, has some similarities; in fact, some bacteria used in MICP, such as Sporosarcina pasteuriii (S. pasteuriii), are able to produce the enzyme urease. However, the EICP technique uses urease, which has been previously extracted from plants (Nemati & Voordouw, 2003; Yasuhara et al., 2012; Neupane et al., 2013, 2015a, b; Putra et al., 2017; Carmona et al., 2018) or from bacteria (Hoang et al., 2020). When comparing both methodologies, the main advantage of MICP is its low cost, while EICP is more user-friendly since this technique does not imply the cultivation and storage of bacteria (Venda Oliveira & Neves, 2019).

Urease significantly increases the rate of urea ($CO(NH_2)_2$) hydrolysis (Blakeley & Zerner, 1984) into ammonium (NH_4^+) and carbonate (CO_3^{2-}) ions, described by the chemical Equations 1-3 (Hammes & Verstraete, 2002; Venda Oliveira & Rosa, 2020; Carmona et al., 2018):

$$\underbrace{CO(NH_2)_2(s)}_{Urea} + 2H_2 0(l) \xrightarrow{Urease} 2NH_3(aq) + H_2CO_3(aq) \quad (1)$$

$$NH_3(aq) + H_2O(l) \leftrightarrow NH_4^+(aq) + OH^-(aq) \quad (2)$$

$$H_2CO_3(aq) \leftrightarrow HCO_3^-(aq) + H^+(aq) \leftrightarrow 2H^+(aq) + CO_3^{2-}(aq) \quad 3)$$

The calcium carbonate ($CaCO_3$) precipitates spontaneously (Equation 4) (Chou et al., 2011; Burbank et al., 2013) in an environment with a high *pH* and in the presence of a source of calcium ions (Ca^{2+}), provided by the addition of calcium chloride ($CaCl_2$):

$$Ca^{2+}(aq) + CO_3^{2-}(aq) \leftrightarrow CaCO_3(s) \quad (4)$$

In spite of the differences between EICP and MICP, both methodologies promote a decrease in soil porosity (Whiffin et al., 2007), which naturally induces a decrease in the soil's coefficient of permeability (Chou et al., 2011; Al Qabany & Soga, 2013; Nemati & Voordouw 2003; Yasuhara et al., 2012; Neupane et al., 2013). In parallel, the deposition of the $CaCO_3$ in the soil's voids also creates a network of bonds between the soil particles that changes the characteristics of the stabilized material, namely: i) an increase in strength (Al Qabany & Soga, 2013; Venda Oliveira et al., 2015, 2016; Venda Oliveira & Neves, 2019; Lin et al., 2016; Liu et al., 2018; Xiao et al., 2019c; Venda Oliveira & Rosa, 2020; Yasuhara et al., 2012; Neupane et al., 2015a, 2015b; Carmona et al., 2018); ii) an increase in stiffness (van Paassen et al., 2010; Mortensen et al., 2011; Venda Oliveira & Rosa, 2020; Carmona et al., 2018; Venda Oliveira et al., 2016); (iii) an increase in the brittleness of the stress-strain behavior, which is directly associated with the level of stabilization reached (Venda Oliveira et al., 2015, 2016; Venda Oliveira & Neves, 2019); (iv) an increase in resistance to the liquefaction of the soil (Montoya et al., 2012; Burbank et al., 2013; Cheng et al., 2013); v) an increase in the resistance to erosion of gravel-sand mixtures (Jiang & Soga, 2016), sands (Zomorodian et al., 2023); silty clayey sands (Shafii et al., 2019), granite residual soil (Wang et al., 2023), silt and clays (Lajevardi & Shafiei, 2023); (vi) promotes the stabilization of dunes (Naeimi, et al., 2023); vii) and also changes the behavior of loose sands leading to volumetric expansion during shear (Chou et al., 2011).

The studies about the MICP methodology have revealed that this technique can be used successfully: (i) for different types of mineralogy (Mortensen et al., 2011); (ii) for different soil particle sizes and gradations (Mortensen et al., 2011), although the reduction in the regularity of the particles leads to a high level of calcification (Xiao et al., 2019b); (iii) with fresh or salt water (Lin et al., 2023); (iv) for various degrees of saturation, although lower saturation increases the method's efficiency (Cheng et al., 2013); (v) when temperature control (Xiao et al., 2019a) and a reserve injection of the biogrout solution is used (Xiao et al., 2019c); (vi) with other types of bacteria, besides S. pasteuriii, namely:

- Indigenous bacteria such as Bacillus spHaericus LMG22257 and Bacillus sp. (I.001) (Shirakawa et al., 2011) to mitigate the liquefaction potential of sands (Burbank et al., 2011, 2013);
- Idiomarina Isuliasalsae to enhance the strength and stiffness of sands (Venda Oliveira et al., 2015);
- Bacillus megaterium to increase the shear strength of a silty soil (Mukherjee et al., 2022);
- Bacillus sp. (UPB) VS1 to decrease the hydraulic conductivity and increase the stiffness (Chu et al., 2012);
- Bacíllus spHaericus (MCP-11) (DSM 23526) to simultaneously increase the strength and decrease permeability (Cheng et al., 2013).

Besides the common advantages of the previously mentioned MICP methodology, research about EICP has revealed some specific aspects, in particular: (i) EICP has better results in well graded sandy and silty soils (Venda Oliveira et al., 2016); (ii) the level of stabilization depends directly on the amount of $CaCO_3$ precipitated (Yasuhara et al., 2012; Neupane et al., 2015a; Carmona et al., 2018; Venda Oliveira & Neves, 2019); iii) a *pH* of about 8.0 maximizes the urease activity (Venda Oliveira et al., 2016); (iv) the EICP methodology is also viable for a high range of organic matter (*OM*) contents, 0-11% (Venda Oliveira & Neves, 2019), although an inhibition of the process for a conjugation of a *pH* lower than 4.5 and an *OM* content of about 11.0% has been observed (Venda Oliveira et al., 2016) ; (v) the effectiveness increases after repeated injections of the biogrout solution (Yasuhara et al., 2012; Neupane et al., 2013, 2015a; Putra et al., 2017; Hoang et al., 2020); (vi) the rate of enhancement of a soil's properties occurs fundamentally for a curing time of 7 days, a urease content of 4 kU/L and a content of urea-calcium chloride of 0.75-1.0 mol/L (Venda Oliveira et al., 2016; Carmona et al., 2018); (vii) the inclusion of magnesium sulfate induces the formation of crystals of aragonite and gypsum, in addition to calcite, which optimizes the process (Putra et al., 2017); (viii) particle packing influences the strength of the treated soil (Almajed et al., 2018).

An alternative bio-based methodology to the use of MICP or EICP consists in the use of biopolymers, which are biodegradable polysaccharides produced by living organisms. Although different biopolymers have been used to improve soil properties (guar, gellan, agar, chitosan, curdlan, β-Glucan and Zein biopolymer (Babatunde & Byun, 2023), among others), the biopolymer xanthan gum (XG) is the focus of this work, since it is one of the most successfully used in geotechnical engineering and due to the significant decrease in the market price over the last decade (Mendonça et al., 2021a). The main features of XG in soil improvement are summarized as follows: (i) it induces increases in the strength and stiffness of sands (Chang et al., 2015a; Cabalar et al., 2017), silty sands (Lee et al., 2019; Venda Oliveira & Cabral, 2023), clayed soils (Chang et al., 2015a; Kwon et al., 2019), bentonite, kaolinite (Latifi et al., 2017; Chang et al., 2015a, 2019, 2021) and residual soils (Chang et al., 2015c; Sulaiman et al., 2022); (ii) it induces an increase in the mechanical properties within a range of *OM* content between 1.5 to 5.5%, while a detrimental effect is seen for a higher *OM* content (7.7%) (Venda Oliveira & Reis, 2023); (iii) it decreases the undrained shear strength of a montmorillonite clay (Chang et al., 2021); (iv) it contributes to erosion mitigation due to two effects, a reduction in the soil's erosion and the growth of the vegetation (Chang et al., 2015b); (v) it reduces the soil's porosity and the stabilized soil's coefficient of permeability, due to the hydration of the hydrogels present in the biopolymer (Khachatoorian et al., 2003; Bouazza et al., 2009; Cabalar et al., 2017; Dehghan et al., 2019; Mendonça et al., 2021b); (vi) the curing in dry environments promotes a higher level of improvement than the curing in humid conditions and also a high stability over time is seen in dry conditions (Latifi et al., 2017; Chang et al., 2015a; Cabalar et al., 2017); (vii) the stabilization of a silty sand submitted to curing in humid conditions is potentiated for a curing time of 7-14 days and for an *XG* content of about 1% (Venda Oliveira & Cabral, 2023), while a curing time of less than 3 days results in a detrimental effect (Venda Oliveira & Cabral, 2023); (viii) a greater effectiveness of the XG to stabilize clays rather than coarse-grained soils is observed, since in clays there is a cumulative contribution of the deposition of the XG in the voids (also seen in coarse soils) and the hydrogen bonds between the XG and the surfaces of clays, which are electrically charged (Chang et al., 2015a, 2016).

2. Scope of the work

The potentialities and main features of three bio-based methodologies (MICP, EICP and the use of XG) to mitigate some weaknesses of several soils under different conditions, that is, grain size distribution, organic matter content, curing conditions (dry/humid), among others, are described in the previous section. Studies comparing the effectiveness of these three bio-based methodologies to strengthen soils are practically non-existent. Thus, the aim of this work is to contribute to filling this gap in geotechnical research.

This work is based on the laboratory results of unconfined compression strength (UCS) tests on two sandy soils stabilized with two different bacteria (Sporosarcina pasteurii and Idiomarina insuliasalsae), urease and with the biopolymer xanthan gum for different curing times. The samples are compared in terms of the brittleness index and in terms of gain of unconfined compressive strength and stiffness (Table 1). The curing time used for the three bio-based methodologies was selected in order to highlight the capability of each methodology; longer curing times (28-180 days) were used for MICP than the other two methodologies, EICP and XG (3-28 days). In order to allow a direct comparison of the results obtained from the three different bio-based methodologies and for both soils, a curing time of 28 days was considered in all the methodologies and the EICP technique was used for both soils (A and B).

Additionally, scanning electron microscopy (SEM) images obtained from some specimens are also compared. Table 1 shows the UCS tests considered in this work.

3. Materials

3.1 Characteristics of soils A and B

The main characteristics of the two soils (A and B) used in the experimental work are shown in Table 2 and the grain size distribution is illustrated in Figure 1. Soils A and B show some similarities, in fact both soils are inorganic, non-plastic, show a similar gradation (Figure 1) and present a *pH* in the range of 8.0-8.4, which corresponds to the range that potentiates urease activity and consequently maximizes the precipitation of calcium carbonate (Stocks-Fischer et al., 1999; Declet et al., 2016). Both soils are composed fundamentally of sand (77.4-94.2%), although soil B also shows a non-negligible content of silt (16.1) and clay (6.5%). Thus, soil A is classified as a poorly graded sand (SP) and soil B is classified as a silty sand (SM) (ASTM, 2000). Indeed, the major differences between the two soils are found in terms of the mechanical properties when prepared for the optimum condition stated by the standard Proctor test (ASTM, 2003). The better gradation of soil B induces a much higher maximum unconfined compressive strength (q_u) and secant Young's modulus (E_{u50}) than soil A.

3.2 MICP - Biogrout agents

The bacteria Sporosarcina pasteurii and Idiomarina insuliasalsae were used to implement the microbial induced calcium carbonate precipitation (MICP). Both bacteria produce

Table 1. UCS tests - Testing program.

Biogrout agent		Soil type	Curing time (days)	UCS Tests (N)
Type	Content			
Non-stabilized soil	---	A	---	6
	---	B	---	6
Bacteria: Sporosarcina pasteuri	9 g/l	A	28	3
			90	3
			150	3
Bacteria: Idiomarina insulisalsae	9 g/l	A	28	3
			60	2
			90	2
			180	3
Enzyme urease	4 kU/l	A	3	2
			7	2
			14	2
			28	2
		B	3	3
			7	3
			14	3
			28	3
Biopolymer xanthan gum	1%	B	3	3
			7	3
			14	3
			28	3

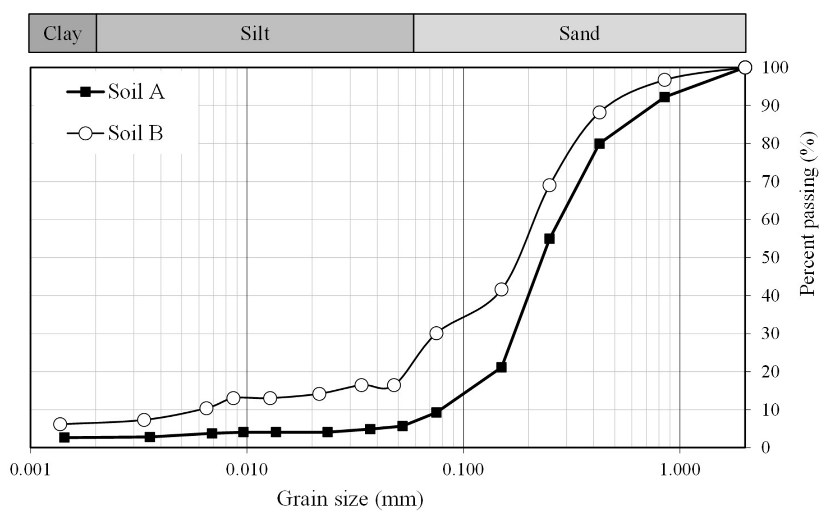

Figure 1. Grain size distribution of the two sands (A and B) used in the experiments.

urease, are aerobic, non-pathogenic and are thereby classified as risk group 1, that is, unlikely to cause human disease. The most favorable conditions for the S. pasteurii (DSM 33) to grow are: an appropriate ambient temperature (optimum value of 30 °C), a *pH* in the range of 6-9 and the presence of freshwater or sea water (Mortensen et al., 2011). Cultivation of this bacteria was performed in medium 220 (DSMZ, 2024) with a urea concentration of 20g/l at a temperature of 30 °C and an incubation time of 3 days (Yoon et al., 2001). The I. insulisalsae (CVS-6T) was isolated from the "Ilha do Sal" (the salt island) in the Cape Verde Archipelago and the most favorable conditions for growth (Taborda et al., 2009) are: a temperature between 10-45 °C (optimum 37- 40 °C), a content of *NaCl* in the range of 1-12% and a *pH* between 5.5-10.0 (optimum 7.5). The process of cultivation of I. insulisalsae is similar to S. pasteurii, but requires another growth medium (Marine Broth- Difco 2216), a temperature of 37 °C and an incubation time of 24 hours.

Table 2. Average properties of the soils used in the work (based on Venda Oliveira & Rosa, 2020; Carmona et al., 2018; Venda Oliveira & Cabral, 2023).

Property	Soil A	Soil B
Grain size distribution:		
Sand (%)	93.0/94.2	77.4
Silt (%)	3.7/7.0	16.1
Clay (%)	0.0/2.7	6.5
Coefficient of uniformity, Cu (D_{60}/D_{10})	3.7	32.3
Coefficient of curvature, Cc ($D^2_{30}/(D_{60}\times D_{10})$)	1.4	4.1
Specific gravity, G	2.63	2.65
Consistency limits	NP*	NP*
Standard Proctor test (ASTM D698):		
Maximum dry unit weight, γ_{dmax}	17.3/18.8	18.8/19.3
Optimum water content, w_{opt}	11.7/12.0	9.5/11.0
Organic matter content, OM (%)	0.0	0.0
pH	8.4	8.0
Soil classification, USCS[#] (ASTM D2487)	SP	SM
Maximum unconfined compressive strength, q_u (kPa)[&]	10.2/18.1	100.8/125.8
Secant Young's modulus for $0.5\times q_u$, E_{u50} (MPa)	0.95/1.05	5.7/8.5

*Non-plastic; [#]Unified Soil Classification System; [&]Obtained from the optimum conditions of Proctor test.

The grout solution used to prepare the specimens is composed of the bacterial concentration required, urea (purity level of 99.5%) and a nutrient medium (B4 and B4a) that feeds the microorganisms and provides the source of the Ca^{2+} ion required to promote the precipitation of $CaCO_3$. The B4 medium (yeast extract 4g, glucose 5g and calcium acetate ($Ca(CH_3COO)_2$) 15g per liter of solution) was adopted for S. pasteuriii, and provides the source of Ca^{2+} by Reaction 5.

$$Ca\left(CH_3COO\right)_{2(aq)} \rightarrow Ca^{2+}\left(aq\right) + 2CH_3COO^-\left(aq\right) \quad (5)$$

The B4a medium (yeast extract 4g, glucose 5g and calcium chloride ($CaCl_2$, purity level of 95.0%) 18g per liter of solution), promotes the activity of I. insulisalsae (Venda Oliveira et al., 2015) and provides the Ca^{2+} required by Reaction 6.

$$CaCl_2\left(aq\right) \rightarrow Ca^{2+}\left(aq\right) + 2Cl^-\left(aq\right) \quad (6)$$

3.3 EICP - Biogrout agents

The EICP methodology to promote biocalcification requires mixing the soil with a commercial biogrout agent composed of urease, urea (purity level of 99.5%) and calcium chloride (purity level of 95.0%). The urease used in the experiments was extracted from jack beans (Canavalia ensiformis), it is supplied commercially in powder form and has an activity of 34,310 U/g (i.e., 1U hydrolyses of 1.0 μ_{mol}/min of urea for pH 7.0 at 25 °C).

3.4 Biopolymer xanthan gum

Xanthan gum (XG) is a biopolymer produced by the Xanthomonas campestris bacterium, supplied commercially in powder form, and shows a great stability under a wide range of pH (1-13) and temperature (10º-80º). In contact with water, there is a hydration of the XG's viscous hydrogels inducing an increase in the viscosity (Bouazza et al., 2009; Kwon et al., 2019), which potentiates its use in several sectors as a thickening agent, such as food and pharmaceutical industries, cosmetics, lubrication of equipment and enhanced oil recovery, among others (García-Ochoa et al., 2000; Bouazza et al., 2009).

4. Preparation and testing of the specimens

Remolded samples were used in this work to reduce the intrinsic variability of the natural soils, without changing their mineralogical composition and the grain size distribution. Thus, previously to the preparation of the test specimens, the larger particles of both soils (A and B) were removed using a 2.0 mm-mesh sieve. The preparation of specimens used in the UCS tests included the following main stages: (i) preparation of the biogrout solution (which depends on the bio-based methodology used) according to Table 1 and the previous section; (ii) the specimens of a specific soil (A or B) and an amount of the biogrout solution corresponding to optimum moisture content (Table 2) were mixed in order to obtain a homogeneous paste; (iii) the paste was put inside the PVC molds (37 mm in diameter, 76 mm high) for the UCS tests in three layers; (iv) each layer was compacted with an energy equivalent to the standard Proctor tests to obtain the dry unit weight indicated in Table 2; (v) before placing the following layer, the surface of each layer was scarified to promote an effective bond between both layers; (vi) the specimens within the PVC molds were stored in a room with controlled humidity (95±5%) and temperature

(20±2 °C) during the curing time stated in Table 1; (vii) after the required curing time, the specimens were removed from the PVC molds and inspected visually to verify their quality; (viii) the approved specimens were placed in the testing equipment for the UCS tests; (ix) the automatic data acquisition system was turned on and the electronic measurement devices (displacement transducer and load cell) were checked and adjusted; (x) finally, the UCS tests were performed under a strain rate of 1%/min (ASTM, 2005).

The majority of the UCS tests were repeated three times (Table 1) to assure the reliability of the procedure used and the quality/reproducibility of the results obtained. Additionally, some representative specimens were subjected to SEM images to observe the microstructure and a local chemical analysis through energy dispersive x-ray (EDX) to identify the existence of $CaCO_3$ in a specimen subjected to EICP.

5. Results and discussion

Although soils A and B show significant similarities, they are not equal, mainly in terms of the gradation curve, as analyzed in section 3.1. These differences induce the significant discrepancies of the maximum unconfined compressive strength (q_u) shown in Table 3. It should be mentioned that the differences obtained for soils A and B, were obtained in distinct testing campaigns, which reflect some slight intrinsic differences (Table 2). In order to allow the comparison of the results obtained by the different bio-based methodologies, the analysis was performed in terms of the unconfined compressive strength ratio ($q_u/q_{u,unstab.}$) and secant modulus ratio at $0.5q_u$ ($E_{u50}/E_{u50,\ unstab.}$), which reproduce the gain or loss of strength/stiffness in relation to the value correspondent to the unstabilized soil. In this way, it is possible to compare, in relative terms, the effectiveness of the three bio-based methodologies used for both soils (A and B). However, it should be emphasized that the EICP methodology was used for the two soils, which also makes a direct comparison between them viable.

5.1 Stress-strain behavior

Figure 2 illustrates the stress-strain curves obtained from the UCS tests, for a curing time of 28 days, of the two soils for the unstabilized condition and stabilized specimens with the three bio-based methodologies (MICP, EICP and XG). In general, the results clearly show that the use of the three bio-based methodologies to stabilize the two soils (A and B) leads to an improvement of the mechanical properties, evidenced by an increase in the unconfined compressive strength ratio ($q_u/q_{u,unstab.}$) and secant modulus ratio at $0.5q_u$ ($E_{u50}/E_{u50,\ unstab.}$).

Table 3. Maximum unconfined compressive strength, q_u, obtained from the different specimens (based on Venda Oliveira & Rosa, 2020; Carmona et al., 2018; Venda Oliveira & Cabral, 2023).

Methodology	Soil	Curing time (days)	q_u (kPa)			
			Test -T_1	Test -T_2	Test-T_3	Average
MICP S. pasteurii	A	Unstabilized	9.20	12.04	9.22	10.2
		28	13.84	9.87	12.33	12.0
		90	13.49	14.79	13.36	13.7
		150	14.13	12.69	16.53	14.5
MICP I. insuliasalsae	A	Unstabilized	9.20	12.04	9.22	10.2
		28	16.97	12.33	16.93	15.4
		60	---	20.76	17.71	19.2
		90	24.50	22.22	---	46.7
		180	22.12	25.84	30.48	26.2
EICP Enzyme urease	A	Unstabilized	18.20	18.00	---	18.1
		3	33.44	23.28	---	28.4
		7	33.66	---	---	33.7
		14	---	31.04	33.55	32.3
		28	35.97	33.70	----	34.8
	B	Unstabilized	100.76	98.17	103.50	100.8
		3	141.15	143.65	151.52	145.4
		7	166.40	169.10	153.83	163.1
		14	180.20	181.65	182.29	181.4
		28	185.62	167.47	197.40	183.5
Biopolymer xanthan gum	B	Unstabilized	113.79	123.03	140.56	125.8
		3	96.98	104.68	115.78	105.8
		7	138.79	142.39	158.82	146.7
		14	130.94	160.13	171.28	154.1
		28	158.09	163.02	163.23	161.5

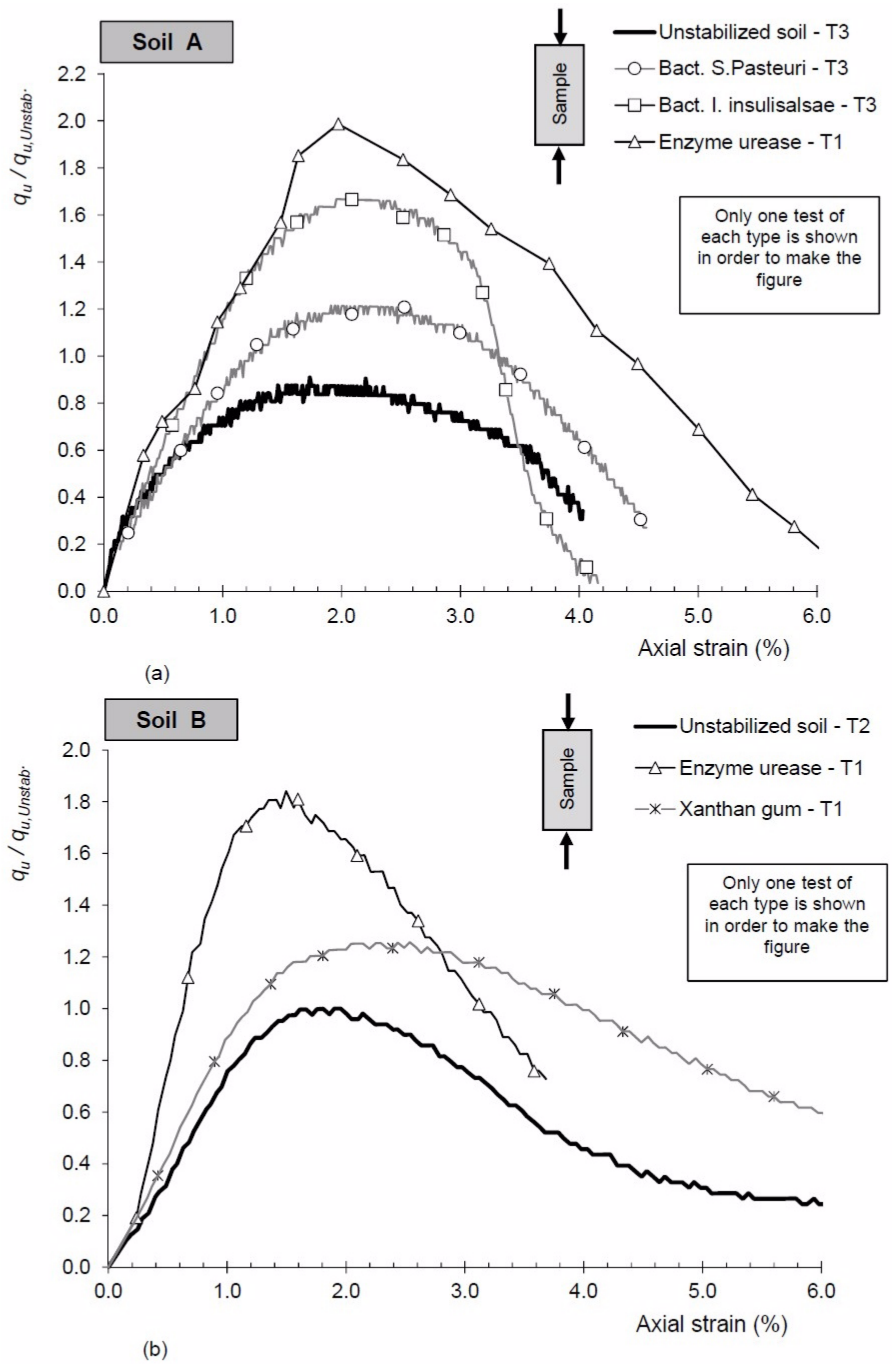

Figure 2. Results of UCS tests for a curing time of 28 days. Effect of biogrout agents on the stress-strain behavior: (a) sand A; (b) sand B.

Additionally, the results also show that, for a curing time of 28 days, the use of urease (EICP) induces the highest level of improvement, while the use of the biopolymer xanthan gum leads to the lowest level of improvement.

The results also seem to show that the two cases with higher levels of improvement, soil A stabilized with the bacteria I. Insulisalsae (MICP) and soil B stabilized with EICP, are associated with the highest loss of strength after the peak-failure, i.e., a significant increase in the brittleness of the stress-strain behavior. The effect of stabilization with MICP, EICP and XG on the stress-strain behavior (in terms of ductility or brittleness) is highlighted in Figure 3, through the quantification of the brittleness index (I_b), described by Equation 7 (Venda Oliveira & Neves, 2019; Venda Oliveira & Cabral, 2023):

$$I_b = 1 - \frac{q_{\left(\frac{f_{ax}}{f_{axf}}=2\right)}}{q_u} \tag{7}$$

where q ($\varepsilon_{ax}/\varepsilon_{ax,f}$=2) is the unconfined compressive strength corresponding to the double of the strain at the peak strength ($\varepsilon_{ax,f}$).

The increase of I_b from 0 to 1 indicates an increase in the brittleness of the stress-strain behavior, that is, I_b equal to 1 corresponds to a complete loss of strength after peak-failure, and I_b equal to 0 indicates an ideal ductile stress-strain behavior. The high range of standard deviations (from the mean value), shown by error bars (Figure 3), indicates some scattering of the results. However, it is observed that the use of MICP methodologies with both bacteria (Soil A) induces an increase in the I_b from 0.31 to 0.52 (i.e., an increase in the brittleness), while the results with EICP (with the exception of soil A for a curing time of 3 days) and XG seem to induce a slight increase in the ductility, i.e., a decrease of the I_b value in relation to the unstabilized soil.

5.2 Unconfined compressive strength ratio

The effect of the three different bio-based methodologies on the evolution of the $q_u/q_{u,stabil}$. ratio, for different curing times, is depicted in Figure 4. As observed in Figure 2, most of the results clearly show the positive effect of the three bio-based methodologies used to stabilize the two sands, as they describe gains in strength of up to 158%. The exception to this tendency is seen with the use the biopolymer xanthan gum for a curing time of 3 days, where a detrimental effect of about 16% was observed, which seems to be due to the hydration of the hydrogels of the *XG* (Venda Oliveira & Cabral, 2023).

Despite the high scattering of the results, Figure 4 also shows that the curing time has a significant effect on the MICP methodology, the greatest influence is observed

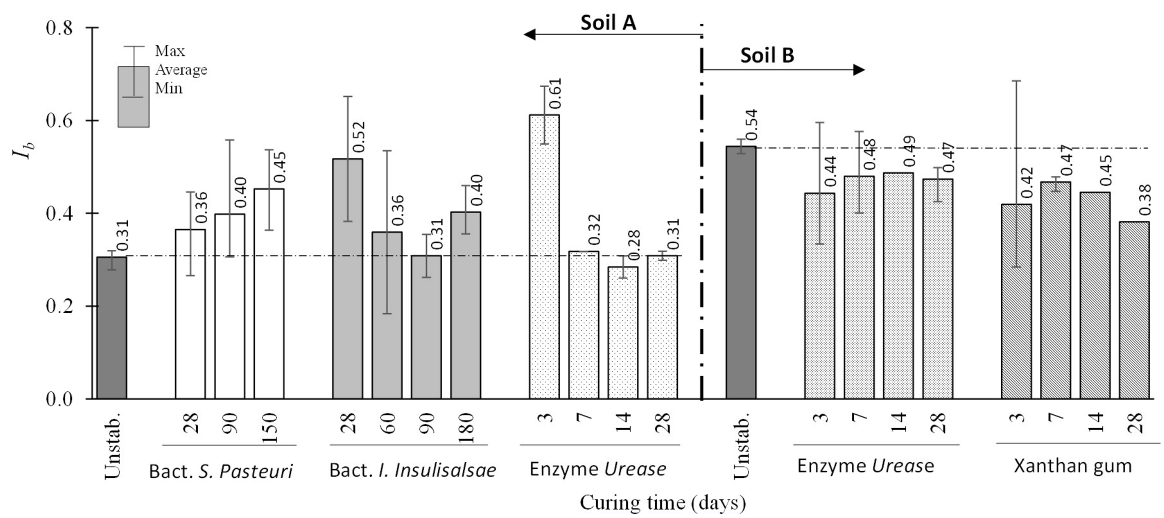

Figure 3. Results of UCS tests. Effect of the biogrout agent on the brittleness index.

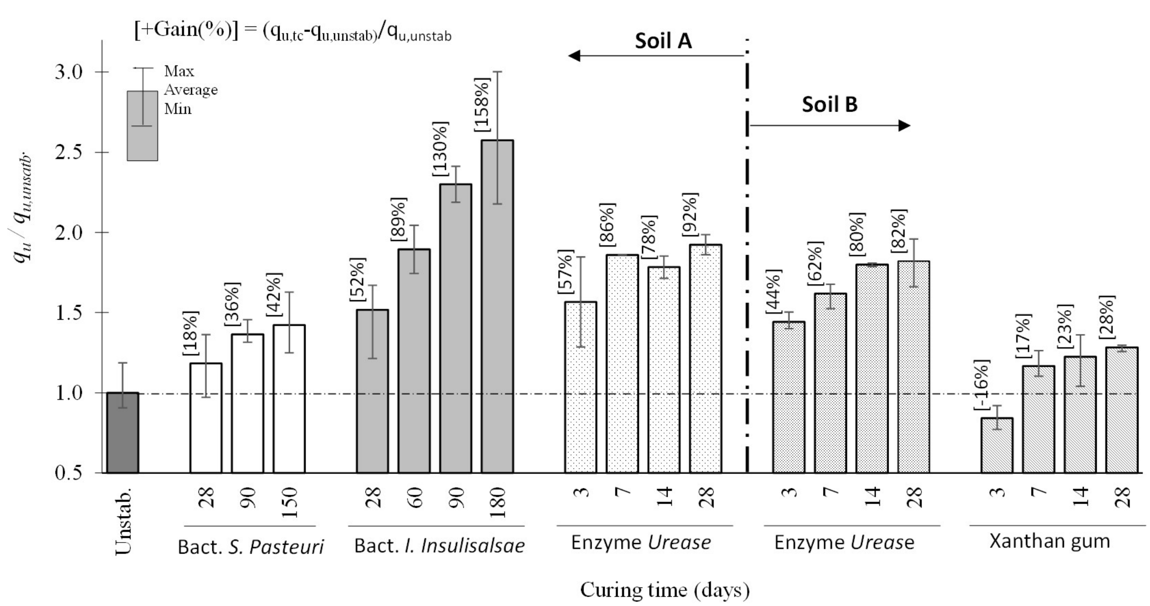

Figure 4. Results of the UCS tests. Effect of the biogrout agent on the increment of the unconfined compressive strength ratio, $q_u/q_{u,unstab}$.

using I. insuliasalsae with gains in strength from 52% (28 days) to 158% (180 days), while the gain in strength with S. pasteurii is more modest changing from 18% (28 days) to 42% (150 days). The increase in strength with time concerning urease (EICP) occurs fundamentally during the first 7-14 days, with a maximum gain in the range of 82-92%. This tendency is in line with the results of Nemati & Voordouw (2003), Carmona et al. (2018) and Venda Oliveira & Rosa (2020), which indicate that the majority of the chemical reactions (precipitation of $CaCO_3$) with EICP tend to occur in the short-term. The results also show that the use of the biopolymer xanthan gum induces only a moderate increase in strength, with gains lower than 30%, and these gains tend to occur essentially over the short-term (until 7-14 days), which is in line with the results obtained by Venda Oliveira & Cabral (2023) with specimens cured in humid conditions (95±5%). These results are much lower than those obtained from specimens of sandy soils subjected to air-dried curing (Chang et al., 2015a; Cabalar et al., 2017; Lee et al., 2019), which reflects the effect of some suction associated with the dehydration of the hydrogels of the XG.

5.3 Secant modulus ratio

An analysis of the effect of the curing time of the biostabilized specimens regarding the evolution of the secant modulus ratio ($E_{u50}/E_{u50,stabil.}$) (Figure 5) reveals results that are not totally coincident with the evolution of $q_u/q_{u,stabil.}$ (Figure 4). Indeed, the results show gains in the secant modulus higher than 110% with the use of the bacteria I. insuliasalsae and urease. On the other hand, the gains obtained from the bacteria S. pasteuriii and the biopolymer xanthan gum are marginal (maximum of 28%), or may even be detrimental as seen with the use of XG for 3 and 7 days of curing time. In terms of the secant modulus, the effect of the curing time is more significant for soil B, with a significant increase in the secant modulus ratio from 7 to 28 days. For instance, the results obtained from EICP for soil B show a gradual increase in the ratio $E_{u50}/E_{u50,stabil}$ from 3 (+37%) to 28 days (+158%), while for soil A, EICP induces an abrupt increase in the ratio $E_{u50}/E_{u50,stabil}$ from 3 (+47%) to 7 days (+118%), followed by approximately constant values after that.

5.4 SEM images

Figure 6a shows the SEM images with energy EDX analysis for a specimen of soil A stabilized with urease at a concentration of 4 kU/l. The SEM image shows not well-defined soil particles, which is a characteristic of sand specimens subjected to a biocementation process (Venda Oliveira & Rosa, 2020). When evaluating the local chemical composition, the EDX analysis confirms the existence of vestiges of the precipitation of $CaCO_3$, described by a non-negligible concentration of oxygen (40.2%), carbon (14.2%) and calcium (3.9%). It should be em*pH*asized that the MICP and EICP methodologies are similar in terms of improvement, since they both promote the precipitation of $CaCO_3$ within the porous medium.

The SEM image of the specimen of soil B stabilized with XG (Figure 6b) shows the existence of a network of microfibers, which links the soil particles together, inducing a phenomenon similar to a micro reinforcement of the solid skeleton and consequently a strengthening of the soil. This image matches the results observed by other authors for coarse soils stabilized with XG (Chang et al., 2015a; Ayeldeen et al., 2017; Venda Oliveira & Cabral, 2023). The EDX analysis

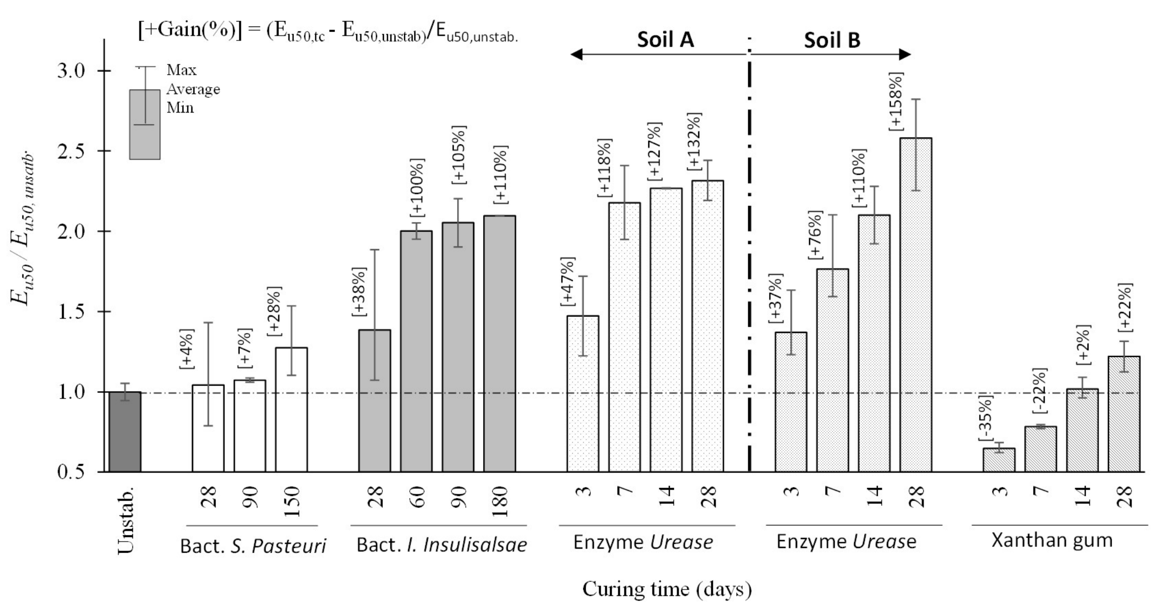

Figure 5. Results of the UCS tests. Effect of the biogrout agent on the increment of the secant Young's modulus, $E_{u50}/E_{u50,unstab}$.

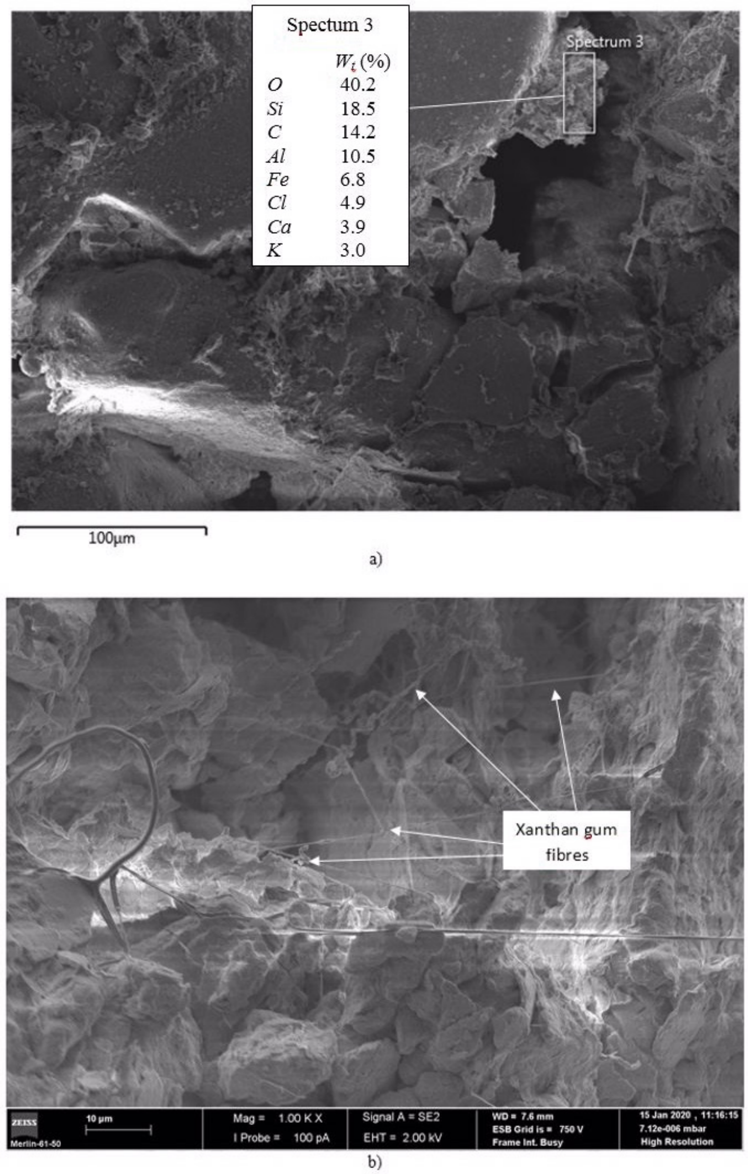

Figure 6. SEM images: (a) soil A stabilized with the enzyme urease (4 kU/l); (b) soil B stabilized with 1% of xanthan gum.

shows a significant concentration of the carbon (*C*) component, which constitutes evidence of the microstructure promoted by the XG-stabilized soil (García-Ochoa et al., 2000; Venda Oliveira & Cabral, 2023).

6. Conclusions

Based on the results of the UCS tests, the effectiveness of three bio-based methodologies (MICP, EICP and biopolymer) was compared in terms of the improvement of the mechanical properties of two sandy soils. Two bacteria (Sporosarcina pasteuriii and Idiomarina insuliasalsae) were used in the MICP process, the EICP was based on the performance of the enzyme urease to promote biocalcification, and the biopolymer selected in this work was xanthan gum. Following the experimental program, the following can be stated:

i. In terms of the stress-strain behavior, stabilization with MICP methodologies (for both bacteria) induces a slight increase in the brittleness (i.e., an increase in the brittleness index), while most of the results with EICP and the use of XG seem to induce a slight increase in the ductility (i.e., a decrease in the brittleness index) when compared with the behavior of an unstabilized soil;
ii. For a curing time of 28 days, the EICP methodology is the best to improve the mechanical properties of both the sands tested, with strength gains of 57%, while the gains obtained from S. pasteurii, I. insuliasalsae and XG are 18%, 52% and 28%, respectively;
iii. The results of the MICP methodology show that the use of the bacteria I. insuliasalsae is more effective than S. pasteuriii, as it induces a greater increase in

the unconfined compressive strength and stiffness, with gains of 52% and 38% for 28 days of curing time, respectively. Additionally, the use of I. insuliasalsae shows a significant increase in the $q_u/q_{u,stabil}$ ratio with the curing time, with gains from 52% (28 days) to 158% (180 days);

iv. When employing S. pasteuriii, the secant modulus ratio $E_{u50}/E_{u50,stabil}$ only increases from 90 to 150 days of curing time, while with the use of I. insuliasalsae the gain of E_{u50} occurs fundamentally during the first 60 days of curing time, without a significant change after that;

v. The use of EICP has similar results for both soils characterized by maximum strength gains in the range of 82-92% for 28 days of curing time and the increase in $q_u/q_{u,stalib}$ occurs fundamentally during the first 7-14 days of this time;

vi. The maximum E_{u50} gain with EICP is not very different for both soils (+ 132% for soil A; +158% for soil B). The evolution of $E_{u50}/E_{u50,stabil}$ with the curing time seems to depend on the type of soil; thus for soil A (SP soil) the gain of $E_{u50}/E_{u50,stabil}$ occurs fundamentally during the first 7-14 days, as seen for the strength gain. However, for soil B (SM soil) there is a gradual increase in the ratio $E_{u50}/E_{u50,stabil}$ from 3 to 28 days of curing time;

vii. The use of the biopolymer xanthan gum induces only a moderate increase in q_u and E_{u50}, with gains of less than 28% and 22%, respectively. The gains in strength occur fundamentally in the short-term (until 7-14 days). For a curing time of less than 3 days for q_u and less than 7 days for E_{u50}, a detrimental effect of the use of XG is observed, which seems to be due to the instantaneous hydration of the biopolymer's hydrogels.

viii. An analysis of the SEM images from the EICP methodology that promotes the biocalcification (similar to MICP), and the use of the XG biopolymer clearly shows that these methodologies have a distinct effect on the microstructure of the stabilized soil. Thus, the methodologies inherent to the biocalcification process originated the deposition of calcite crystals in the voids, which create bonds between the soil particles, inducing the strengthening of the stabilized soil. Conversely, the stabilization of a sandy soil with the biopolymer XG induces a network of microfibers that bonds the soil particles together, inducing a process similar to the micro-reinforcement of the soil.

The main advantage of the bio-based techniques for soil improvement addressed in this work is their potential to reduce the ecological footprint associated with the use of cement-based methods, and simultaneously promote a low to medium level of enhancement. Although MICP and EICP can be employed for long-term ground improvement, the biopolymer xanthan gum, due to its biodegradability, is particularly suitable for temporary applications, especially when there is a need to minimize the long-term impact of the work for sustainability reasons.

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Declaration of interest

The author has no conflicts of interest to declare. The author affirms the contents of the paper and there is no financial interest to report.

Data availability

All data produced or examined in the course of the current study are included in this article.

Declaration of use of generative artificial intelligence

This work was not prepared with the assistance of Generative Artificial Intelligence (GenAI).

List of symbols and abbreviations

pH	Potential of hydrogen
q_u	Maximum unconfined compressive strength (kPa)
$q_{u,stalib}$	Maximum unconfined compressive strength for the stabilized soil (kPa)
$CaCl_2$	Calcium chloride
$CaCO_3$	Calcium carbonate
Ca^{2+}	Calcium ion
$Ca(CH_3COO)_2$	Calcium acetate
CO_2	Carbon dioxide
$CO(NH_2)_2)$	Urea
$Ca\left(CH_3COO\right)_2$	Calcium acetate
CH_3COO^-	Acetate ion
CO_3^{2-}	Carbonate ion
NH_4^+	Ammonium ion
H_2O	Water
H_2CO_3	Carbonic acid
NH_3	Ammonia
OH^-	Hydroxide ion
SP	Poorly graded sand

SM	Silty sand
ASTM	American Society for Testing and Materials
DSM	German Collection of Microorganisms
DSMZ	German Collection of Microorganisms and Cell Cultures GmbH
CVS	Consumer Value Stores
B4	Medium with yeast extract 4g, glucose 5g and calcium acetate ($Ca(CH_3COO)_2$) 15g per liter of solution
B4a	Medium with yeast extract 4g, glucose 5g and calcium chloride ($CaCl_2$, purity level of 95.0%) 18g per liter of solution),
PVC	Polyvinyl chloride
C	Carbon
O	Oxygen
Si	Silicon
Al	Aluminum
Fe	Iron
Cl	Chlorine
Ca	Calcium
K	Potassium
Cc	Coefficient of curvature
Cu	Coefficient of uniformity
FCT/MCTES	Portuguese Foundation for Science and Technology
EDX	Energy dispersive x-ray analysis
EICP	Enzyme induced calcium carbonate precipitation
E_{u50}	Secant Young's modulus for $0.5q_u$ (kPa)
$E_{u50,stabil}$	Secant Young's modulus for $0.5q_u$ for the stabilized soil (kPa)
G	Specific gravity
I_b	Brittleness index
II	*Idiomarina insuliasalsae* (bacteria)
I.	*Idiomarina*
ISISE	Institute for Sustainability and Innovation in Structural Engineering
MICP	Microbial induced calcium carbonate precipitation
OM	Organic matter content
PIDDAC	Central Government Investment and Development Expenditure Programme
SEM	Scanning electron microscopy
SP	*Sporosarcina pasteurii* (bacteria)
S	*Sporosarcina*
UCS	Unconfined compressive strength
XG	Xanthan gum (biopolymer)
w_{opt}	Optimum water content (%)
γ_{dmax}	Maximum dry unit weight (kN/m^3)
ε_{ax}	Axial strain (%)
$\varepsilon_{ax,f}$	Axial strain at failure (%)

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