Physical and geotechnical properties of a silty sand soil treated with calcium carbonate fixing bacteria

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Abstract. The objective of the present study is to develop a biotechnological tool for a new application of silty sand soil as stabilized materials in linear works replacing chemical stabilizer (e.g. cement and lime) by natural cement, formed by precipitated calcium carbonate generated by microorganisms of the Sporosarcina family. For this purpose, it is conducted a chemical and mineralogical characterization and an examination of physical and geotechnical properties, being very important from the engineering standpoint. The results of different tests are presented here. The data show that the effects of bacteria are reducing the soil specific surface and increasing its plasticity. The reason for this result could be the addition of a plastic component to the natural soil, or the result of the more aggregated structure promoted during the treatment. The pore size distribution of the soil changes in an approximate range 3 - 30 µm, where the pore mode tends to disappear. The change in the pore density function is reflected in the mechanical behaviour of the treated soil, which presents typical features of a less dense soil with respect to the natural untreated one. The friction angle of the treated soil is slightly higher, and its compressibility is consistently lower than that of the natural soil. As the bacteria do not seem to produce any cementation effect on the soil skeleton, collapse upon wetting does not seem to be significantly affected by the treatment. On the contrary, comparison of collapse data shows that occurrence and amount of collapse are ruled by the as-compacted dry density. The tests performed seem to suggest that the microbiological technique may be effective to improve the mechanical characteristics of the compacted soil. For that, it is necessary to provide more energy in compacting the treated soil that it will be stabilized, so as to achieve a high initial dry density. From this viewpoint, it seems that higher compaction effort is even more effective than increasing the amount of bacteria introduced to stabilize the soil.

1 Introduction

Microbiological calcite ($CaCO_3$) precipitation (MCP) is a biocalcification or biocementation process as a soil strengthening method for ground cementation [1-9]. Thus, there is an increasing in mechanical strength by bounding soil grains together with the precipitation of calcium carbonate. The process involves the enzymatic hydrolysis of urea into ammonium and carbonate using bacteria, leading to the formation and precipitation of calcium carbonate in the medium by addition of calcium soluble salts. Improvements of the mechanical properties of treated soils have been described by several authors [3, 4, 7-15].

Whiffin et al. [4] evaluated MCP as a soil strengthening process by biocementation using a sand column treated with bacteria and chemical reagents. The analysis was conducted on triaxial equipment to determine strength and stiffness. Al-Thawadi et al. [7] enriched and isolated highly urease active bacteria, which also allow a biocementation process. This was the first study to use biological cementation to produce high strength comparable to that of traditional cemented construction materials, such as sandstone and concrete with high penetration depth.

Other studies have focused on the reduction of soil permeability, for instance those carried out by Stabnikov et al. [6] and Chu et al. [12], used the microbially induced carbonate precipitation to treat the soil surface. Van Paassen et al. [10, 11] developed the so-called "BioGrout" technique as a soil improvement method through sand column experiments. After this achievement, the next challenge was to establish homogeneous strengthening throughout larger soil volumes, evaluating the new technology with a step-wise scale-up approach.

Other authors [16] prepared cementing solutions using *Sporosarcina pasteurii* (DSM 33), urea and calcium chloride, with samples of sand, silt and calcarenite. These investigations were of interest to improve soil stability, to build roads and paths, and to restore monuments, among other applications.

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The influence of chemical parameters, such as calcium and nutrients concentration, is an interesting subject of study on the efficiency of the bio-deposition process. Previous research indicated that the concentrations of these components influence the amount and type of precipitates that are formed [2, 5, 17, 18]. Cardoso et al. [18] studied the effects of clay's chemical interactions on biocementation analyzing samples of a uniformly graded sand and samples of the same sand combined with white kaolin clay to reduce porosity to half. The bacteria *Sporosarcina pasteurii* was used along with urea and calcium chloride solutions. Henze and Randall [19] investigated the microbial induced calcium carbonate precipitation (MICP) at elevated pH values (> 11) using *Sporosarcina pasteurii* to create more sustainable building products. Morales et al. [20] studied the MICP using clay phyllites to replace chemical stabilizers (cement or lime). The effect of these raw materials on MICP was discussed providing physical and hydromechanical results.

The present study describes the development of a biotechnological tool to be used for stabilizing soils in linear works, i.e., it is intended to replace chemical stabilizers (e.g. cement and lime) using natural carbonate binders generated by microorganisms.

2 Material and methods

2.1. Sample preparation and experimental procedures

The soil sample (designed B-3) were prepared following established initial conditions to carry out the hydro-mechanical tests, i. e., the tests are made on a compacted sample at maximum dry density and with a water content between the optimal (w_{opt}) and the optimal humidity w_{opt} < 2% from standard Proctor test (SP). The soil is previously passed through a sieve #10 (2 mm) (except for granulometry and Proctor tests, which have followed the normative). For soil-bacteria inoculum preparation, the natural soils (untreated soil) is passed through a sieve #10 (2 mm) before treatment. After treatment, the soil is compacted at standard Proctor energy with water content between w_{opt} and w_{opt} < 2% corresponding to its respective untreated soil. The microbiological treatment is summarized in Figure 1.

Treated soil (BT-3) is prepared by inoculating the natural soil with microorganisms. A microorganism of the Bacillaceae family is used in the precipitation of carbonate minerals in the investigated soils. The microorganisms (*Bacillus pasteurii*), introduced in a particular chemical composition in solution (designed as complex-Na), are added to the natural sample together with compaction water. This chemical composition is a proprietary and patented composition [21] containing urea and several salts in aqueous solution (sodium bicarbonate, ammonium sulphate or chloride). The mixture was added to the compaction water content without sterilising the soil prior to inoculation. The treated soil sample is left ageing in a humid chamber for

at least 7 days, after which the treatment is interrupted by increasing the temperature
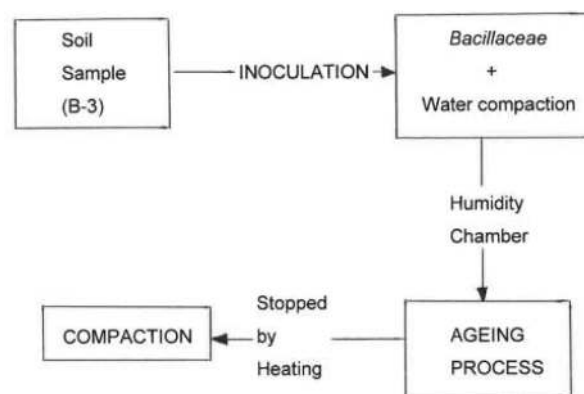

Fig. 1. Microbiological treatment.

The microorganism dose is described in this paper as: a) zero concentration or untreated sample (designed B); b) simple dose or treated sample: (designed BT) and c) simple dose, re-compacted sample.

2.2. Chemical and mineralogical characterization

The pH was determined potentiometrically following the UNE-EN 13037 standard [22] and the soluble salts according UNE103205 standard [23] Gypsum was estimated according to UNE 103206 standard [24] and the sulfates were estimated using turbidimetry (UNE 103201) [25]. The content of organic matter was estimated according to UNE 103204 standard [26] and the cation exchange capacity (CEC) by the USDA method [27]. Carbonate proportion (as calcite) was estimated using the UNE 103200 standard [28]. This test was completed with Dietrich-Frühling calcimeter conforming to the Italian UNI 11140 standard [29]. The conductivity was determined following the UNE77308 standard [30].

X-ray fluorescence (XRF) for chemical analysis was performed on cylindrical compacted specimens. The quality of the analytical results was checked by measurements using standard certified materials [31].

The mineralogical characterization of the samples was carried out by X-ray powder diffraction (XRD). The raw sample was gently ground in an agate mortar. The mineralogical composition after crystalline phase analysis was determined using the classical methods [32] applied in previous research by several authors [33-35].

2.3. Physical and hydro-mechanical characterization

Humidity was determined by thermogravimetry and drying in oven (110 ºC). Particle size distribution was determined by sieving (ASTM D6913-04) [36] and hydrometer analyses (ASTM D422) [36]. Specific gravity was determined using ASTM D854-10 [36]; consistence limits of the fine fraction (Liquid Limit, Plastic Limit, and Plasticity Index of Soils) following

ASTM D4318-10 [36]. Normal Proctor (Standard Proctor ASTM D698) [36] and Modified proctor (ASTM D1557, 12) [36].

The tests are performed on an automatic triaxial equipment. The lower end of the sample is connected to an automatic piston at a constant back pressure of 30 kPa for soil B-3. The upper end is maintained at atmospheric pressure. Confining pressure is maintained at 50 kPa, for samples B-3 and BT-3. The permeability coefficient of saturated water, kw, under stationary conditions is determined by the equation:

$$K_w = [(\Delta V/\Delta t)/A]/i \qquad (1)$$

being $i = [\Delta(z + u_w/\gamma_w)]/L_0$ where L_0 is the initial length of the sample and $\Delta V/\Delta t$ the fluid flow readings recorded between periods of time, A is the cross sectional area of the sample, z is the reference height, u_w is the water pressure and γ_w is the unit weight of water.

Direct Shear test (ASTM D3080) [36] was conducted on a cylindrical specimen, confined laterally by the walls of a ring 25 mm high and 60 mm in internal diameter, and cut on a horizontal plane. Tests were carried out on three specimens to describe the failure envelope.

Compressibility parameters were determined under conditions of lateral confinement of a sample according to different net/effective vertical stress applied in successive steps of loading (compression/consolidation) and unloading.

To perform the Mercury intrusion porosimetry (MIP) test, the samples were air-dried (air drying conditions).

Finally, adsorption and desorption of nitrogen gas was carried out until relative pressures near to the unity are achieved. The surface area is calculated by the models of Brunauer, Emmett and Teller (BET isotherm) and Lagmuir [37, 38]. The pore size distribution was perfomed following the BJH method [39].

3 Results and discussion

3.1. Chemical characterization of the soil and precipitation of calcium carbonate

A quantitative evaluation by XRF of the content of elements, in weight percent, was carried out. The results are presented in Table 1. The principal oxides are CaO and SiO_2, representing about 83% of the total of the sample.

Table 2 shows the results obtained of various chemical parameters, both for natural (untreated) B-3 and treated BT-3 soil samples.

From these results it can generally be said that the pH of treated soil increases compared to that of natural soil. It is because the urea degrading bacteria produces ammonia from the hydrolysis of urea, causing alkaline conditions, which are required for the precipitation of calcium carbonate [1-15]. Urease enzyme is responsible to hydrolyse intracellularly 1 mol of urea to 1 mol of ammonia and 1 mol of carbamate. Carbamate (carbamic acid) is decomposed by spontaneous hydrolysis to produce another molecule of ammonia and carbonic acid. Two ammonia molecules and carbonic acid subsequently equilibrate in water with their deprotonated and protonated forms. Then, these products are equilibrated in water to form bicarbonate and 2 moles of ammonium and hydroxide ions. The last two reactions give rise to a pH increase, which in turn shifts the bicarbonate equilibrium, resulting in the formation of carbonate ions. In the presence of soluble calcium ions, calcium carbonate is precipitated [1-15].

**Table 1.** Chemical composition of sample B-3 determined by XRF.

SiO_2 (%)	41.16
Al_2O_3 (%)	6.71
Fe_2O_3 (%)	2.32
K_2O (%)	1.77
CaO (%)	41.38
MgO (%)	1.73
Na_2O (%)	0.265
TiO_2 (%)	0.35
P_2O_5 (%)	0.07
SUM (%)	95.81

Table 2. Chemical characterization of untreated (B-3) and treated (BT-3) samples.

Soil	B-3	BT-3
pH	6.59	8.84
Electrical conductivity (dS/m)	1.1	1.41
Soluble salts (%)	0.55	0.09
Gypsum (%)	2.11	0.29
$CaCO_3$ eq (%)	32.30	14.66
Sulfates (mg/L)	0.09	293.70
Organic matter (%)	0.12	0.14
Cation exchange capacity (meq/100 g Soil)	7.5	10.2

The content of soluble salts decreases and electrical conductivity (EC) slightly increases after the treatment. There is also a relationship between gypsum/sulphates that is inversely proportional. There is a decrease in the percentage of gypsum after treatment, while the sulphates content increases in all cases. This is justified as follows. Gypsum and/or anhydrite are dissolved at alkaline pH and in carbonate-rich environments, which are unsaturated with respect to gypsum, and are in contact with any of these mineral phases. At the dissolution start, calcium and sulphate ions pass to the fluid phase supersaturated with respect to the more insoluble polymorph, $CaCO_3$. Furthermore, the solubility

is not very high and it slows the solution and its effect is prolonged. The solubilized gypsum is dissociated as follows:

$$CaSO_4 \cdot 2H_2O \leftrightarrow SO_4^{2-} + Ca^{2+} \qquad (2)$$
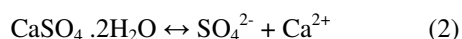
The released calcium can precipitate when it is combined with the carbonate generated by microorganisms or, it may be exchanged with the sodium of the change complex (Complex-Na) and that is combined with the excess of sulphate to produce sodium sulphate:

$$Complex\text{-}Na + Ca^{2+} \leftrightarrow Complex\text{-}Ca + Na^+ \qquad (3)$$
$$SO_4^{2-} + 2Na^+ \leftrightarrow NaSO_4\, H_2O \qquad (4)$$
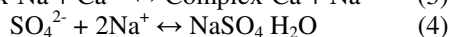
This arrangement of calcium cations, in the soil exchange Complex-Na, can be justified by the increase in CEC, which is related to these two chemical parameters, sulphates and gypsum. Calcium carbonate precipitation reduces the concentration of calcium in the solution and, consequently, favours the dissolution of more gypsum/anhydrite. In turn, this addition of calcium to the fluid phase leads to the continuation of crystallization of $CaCO_3$. The dissolution-crystallization processes define a feedback loop that continues to operate while the carbonate concentration in the solution is sufficiently high and there is still gypsum/anhydrite to dissolve. The reactions that occur in the soil take place due to the incorporation of calcium into the adsorbent particle and release of sodium to the solution [40].

3.2. Mineralogical characterization

The results estimated for the untreated soil B-3 by mineralogical analysis by XRD confirmed the presence of illite, chlorite, anorthite, albite, calcite and quartz, as main components. In this particular case, calcite ($CaCO_3$) seems to be predominant with respect to quartz. The semiquantitative mineralogical estimation from XRD is presented in Table 3. These results indicate a high content of calcite (the most stable polymorph of calcium carbonate). That is to say, the natural soil (untreated soil) has an important content of carbonates. It is also important the quartz content, about 40 wt. %.

Table 3. Mineralogical composition of sample B-3.

	Quartz	**Calcite**	**Illite + Chlorite**	**Albite + Anorthite**
wt. (%)	40	50	<5	05-Oct

3.3. Textural analysis by determination of particle size, specific gravity, plasticity and surface area

The granulometric analysis shows a particle size distribution before and after microbiological treatment. For natural soil, the samples are crushed with a mallet to break up the aggregations and the soils are left in water for posterior drying and sieving (according to the ASTM D6913-04 standard procedure) [36]. The problem in this test is that the particle aggregations can be broken as consequence of procedure described in the standard. For that, two comparatives process are used. The first one follows the standard and it is called "crushed aggregations". In the second one, the treated soils are not crushed with the mallet to avoid the breaking of aggregations ("with aggregations"). Figure 2, shows the granulometric curves of tested samples B-3/BT-3. It can be observed that when the treated soils are crushed the amount of fines is the same or slightly greater than untreated soil, probably as a consequence of the calcium carbonate breaking. However, when the granulometry is carried out with aggregations the percentage of passing grain is lower than in untreated soils. Therefore, it can be concluded that the microbiological treatment tends to aggregate the soil, so that eventually an apparent coarser distribution results. If the bacteria dose or ageing time is doubled, the result is greater aggregates for BT-3.

The results obtained by standard specific gravity test, Atterberg limits and specific surface area test are summarised in Table 4. The solid density (ρ_s) and the classification of the material are presented. The specific surface area of the material obtained by nitrogen adsorption gas test is also included. As mentioned before, the comparison shows that the microbiological treatment tends to aggregate the soil, so that eventually an apparent coarser distribution results. In accordance with Unified Soil Classification System (USC), the sample B-3 is a silty sand (SM) and after the treatment is classified as a clayey-sandy gravel (GC).
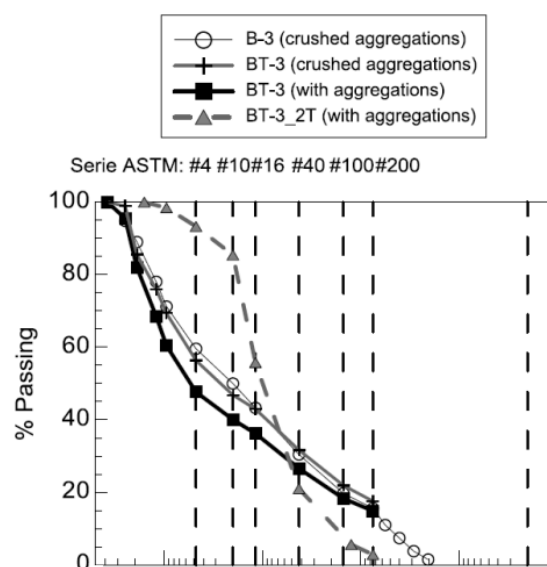

Fig. 2. Granulometry of untreated soil B-3 (crushed aggregations) and treated soil BT-3 (with aggregations and with crushed aggregations) and BT-3_2T.

With regard to consistency limits of the soil, the plasticity index increases as a consequence of a liquid limit increase and the plastic limit decreases for samples B-3 and BT-3. The specific surface area is related to the liquid limit of the material, so that as the specific surface increases the liquid limit remaining almost constant for samples B-3/BT-3.

Table 4. Properties of untreated (B-3) and treated (BT-3) samples. USC Clasif.= Classification; LL= Liquid Limit; PL= Plastic Limit; PI= Plasticity Index; S= Specific Surface Area; ρ_s= density of the solid.

Sample		B-3	BT-3
USC Clasif.		SM	GC
% pass	100 (mm)	60	48
	0.4 (mm)	30	27
	0.08 (mm)	16	15
LL (%)		34	36
PL (%)		27	22
PI (%)		7	14
ρ_s		2.7	2.69
S (m²/g)	BET	24.4	23.6
	Langmuir	33.7	32.7

3.4. Compaction test

Figure 3 presents compaction curves obtained for different energy levels of the Proctor tests. Standard Proctor compaction performed on the natural soil B-3 gives an optimum dry density (2.00 Mg/m³), which can be achieved with a water content of w_{opt} = 0.10. For the treated soil BT-3 only a point is calculated. This point is obtained from the initial conditions of sample preparation, i.e., at SP energy and optimum humidity w_{opt} < 2 % corresponding to their respective natural soils.
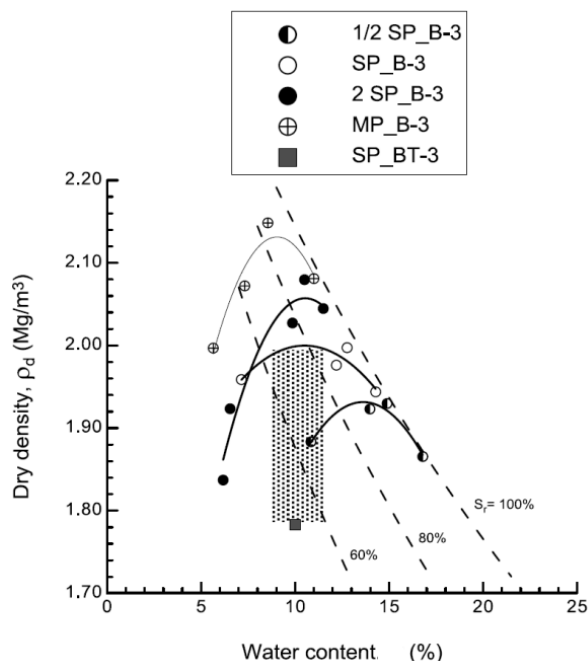

Fig. 3. Plane compaction for natural soil B-3 at different energy levels. Point obtained for treated soil BT-3 at SP energy keeping constant dry density and humidity.

In general, it can be said that some energy is dissipated during compaction as a consequence of the breaking soil aggregates formed by the microbiological treatment. The final compaction energy (for treated soils) becomes similar or minor to half of the standard proctor energy (1/2SP) applied in natural soils. Humidity content (w), void ratio (e), porosity (n) and density (ρ_d= dry density and ρ_{sat}=saturated density) of the analysed materials are indicated in Table 5. These data represent the range of values in which tests are performed. In all cases, the treated soils show e values higher than natural soils. This is because treated soils are compacted with water content between optimum (w_{opt}) and optimum minus 2% (w_{opt} < 2%) corresponding to the natural soil. Therefore, the treated sample does not achieve the optimum humidity value.

The treated soils present a higher content of hard aggregates. As a consequence of these aggregates it is not possible to achieve the same value of ρ_d and w_{opt} as that of the natural soils. So that, the compacted conditions of the soils tend much more towards the dry side of the plane of compaction. This also causes that treated soils present lower degrees of saturation (about 20-30% lower). On the other hand, the studied soils come from areas of south-central Spain [41], places characterized by suffering periods of drought. This fact justifies the soils preparation in the dry side of the compaction plane (degrees of saturation below 80%) to carry out the different hydro-mechanicals tests. The soils compacted in this side have higher soil stiffness and saturated permeability and tend to develop collapse during wetting at high stresses.

Table 5. Data of humidity (w), void ratio (e), porosity (n), degree of saturation (Sr) and density (ρ_d = dry density and ρ_{sat} = saturated density) of untreated (B-3) and treated (BT-3) samples.

Sample	w (%)	e	n (%)	Sr (%)	ρ_d (Mg/m³)	ρ_{sat} (Mg/m³)
B-3	8.12 to 10.1	0.35	25.9	62.6 to 77.9	2	2.26
BT-3	9.34 to 11.5	0.63	38.7	39.9 to 49.5	1.65	2.04

3.5. Direct shear

Figure 4 shows the evolution of the shear stress (τ) for different applied vertical effective stress, σ_v (50, 100 and 150 kPa), corresponding to the direct shear tests on saturated conditions, performed on the samples B-3/BT-3 respectively. The comparison shows that the treated soils are more contractile than the natural one, due to its higher initial e value, except BT-3 that is more dilatant than its natural soil. Nonetheless, the shear strength which can be mobilized is slightly higher at the stress levels investigated [42].
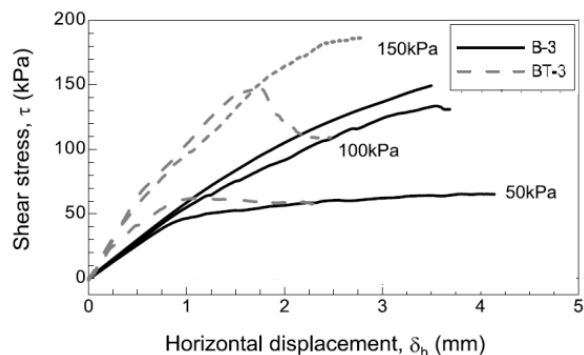
Fig. 4. Evolution of shear stress (τ) and vertical displacement (δ_h with respect to horizontal displacement for different applied vertical effective stress. σv (50, 100 and 150 kPa), on sample B-3 and BT-3.

Calcite precipitation from bacteria does not give an apparent cohesion to the soil. Therefore, it should not be interpreted as a cementation effect. This fact might be caused by the breakage of a great part of the aggregates formed during the microbiological treatment, i.e., part of the natural cement material may break during the compaction process, eliminating potential $CaCO_3$ bridges formed between particles and becoming part of a filling material of the pores. Cardoso et al. [18] have discussed this process in a previous investigation on MCP using sand and kaolin clay as model samples.

3.6. Pore size distribution and pore volume. Results by mercury intrusion porosimetry (MIP) and nitrogen adsorption/desorption gas.

The results of pore system characterization of natural and treated compacted samples B-3/BT-3, are depicted in Figure 5. The pore size density function relates the log differential intrusion curve versus the pore size, which aids in the visual detection of the dominant pore modes. The results obtained by mercury intrusion porosimetry are compared with those obtained by nitrogen desorption gas porosimetry test following the BJH method on the desorption path [39]. The comparison between the data for natural soil and those for the treated soil shows that the appreciable effect of microbiological treatment is to reduce the porosity an intermediate range between 3 µm – 30 µm, approximately, and the porosity from 1µm of equivalent entrance diameter. Besides this, compaction at the same energy is less effective in reducing the pores of larger dimensions. BT-3 samples present a reduction of the pore size density in pores with equivalent entrance diameter to 10 µm and 7 µm, respectively.
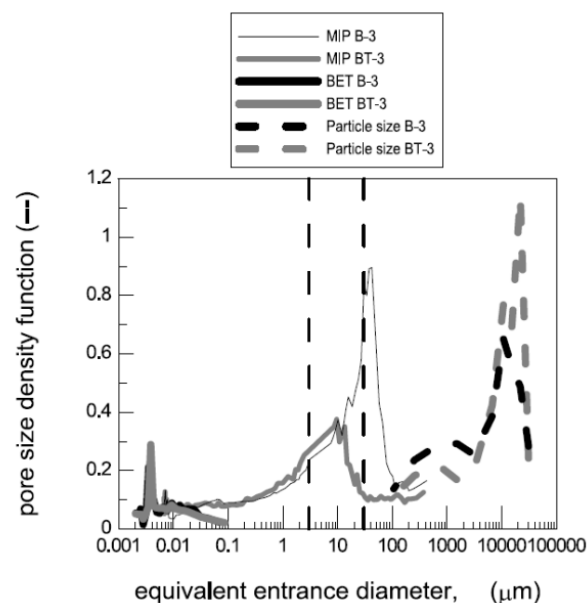

Fig. 5. Pore density function versus pore size conducted for sample B-3 and BT-3.

4 Conclusions

The data show that the effects of bacteria are reducing the soil specific surface and increasing its plasticity. The reason for this result could be the addition of a plastic component to the natural soil, or the result of the more aggregated structure promoted during the treatment.

The results of different tests are presented here The data presented display a consistent behaviour pattern, suggesting that precipitation of calcium carbonate from bacteria takes place in the pores of the soil. As a result, the pore size distribution of the soil changes in an approximate range 3µm - 50 µm, where the pore mode tends to disappear. The change in the pore density function is reflected in the mechanical behaviour of the treated soil, which presents typical features of a denser soil with respect to the natural untreated one. The friction angle of the treated soil is slightly higher, and its compressibility is consistently lower, than that of the natural soil. As the bacteria do not seem to produce any cementation effect on the soil skeleton, collapse upon wetting does not seem to be significantly affected by the treatment.

As a whole, the tests performed in this investigation seem to suggest that the microbiological technique may be effective to improve the mechanical characteristics of the compacted soil. For that, it is necessary to provide more energy in compacting the treated soil that it will be stabilized, so as to achieve a high initial dry density. From this viewpoint, it seems that higher compaction effort is even more effective than increasing the amount of bacteria introduced to stabilize the soil.

The results obtained show that the post-ageing compaction destroys the initial binding/cementation effect. Calcium carbonate acts as a filler, filling pore sizes between 3 and 50 µm, approximately, as detected by MIP. This fact results in a filling of the macroporosity that affects the water retention properties inducing a

higher air-entry value. On the contrary, a lower adsorption capacity is detected in the high-suction range on wetting (consistent with the lower specific surface of the treated material).

Acknowledgements

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