

Microbiological induced carbonate (CaCO₃) precipitation using clay phyllites to replace chemical stabilizers (cement or lime)

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ABSTRACT

Clay phyllites as raw materials for several technological applications is a subject of interest in previous investigations, such as impermeabilization of roofs, waste landfills, concrete, filler and composites with cements or lime. However, the microbiological induced carbonate precipitation using these raw materials is not yet studied. The objective of the present study is to develop a biotechnological tool for a new application of clay phyllites 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 *Bacillaceae* family. For this purpose, it is conducted a chemical and mineralogical characterization and an examination of physical and geotechnical properties. The results of the present paper show that the effect of bacteria using clay phyllites as raw material is an increase of the calcium carbonate content, with a reduction of the specific surface area and plasticity values. It is a consequence of an addition of a non-plastic component to the clay phyllite producing a more aggregated structure by precipitation of calcium carbonate from the bacteria, which is filling the pores of this material. The microbiological treatment of clay phyllites tends to aggregate the original particles, being in part these aggregates associated to the formation of calcium carbonate. This process is influenced by the curing and compaction procedures on the samples, with a breakage of the carbonated structures formed during the treatment. As a consequence of the breaking process of the aggregates, some of the compaction energy is lost and the treated samples do not reach the maximum dry density as the natural state for the same level of compaction energy applied. The change in the pores density function is reflected in the mechanical behaviour. Treated samples display a slightly larger friction angle with no cohesion, being consistent with the filling properties and denser condition. The compressibility is consistently lower than that of the natural one. Comparison of collapse data shows that occurrence and amount of collapse are ruled by the as-compacted dry density. The results of physical and geotechnical tests suggest that the microbiological technique may be effective to improve the mechanical characteristics of the compacted sample. It is found that higher compaction effort is even more effective than increasing the amount of bacteria introduced to stabilize the sample, with a filling of the pores (size ranges 3-50 μm) with calcium carbonate. However, the post-ageing compaction destroys the initial binding/cementation effect.

Keywords: calcium carbonate, *Bacillaceae*, clay phyllites, physical, geotechnical

1. Introduction

The traditional use of clay phyllites found in southeast Spain has been for very specific purposes: to cover an impermeabilize roofs, to construct the central area of the ponds, for heterogeneous dams and reservoirs, and for urban waste landfills due to their compaction properties and low permeability levels (Garzón et al., 2009a, b; Garzón et al., 2010; Garzón et al., 2016b). A few papers reported on different technological applications of phyllite clays, for instance as a filler in plastisols (Valera et al., 2002) and concrete (Adom-Asamoah and Afrifa, 2010). In recent reports, new studies on composites phyllite clay-cement and clay-lime, having improved engineering properties and material applications have been investigated (Garzón et al., 2015; Oliveira et al., 2015; Garzón et al., 2016a). The application of clay phyllites in biotechnological solutions using microorganisms is a subject of interest. In an early work, Stocks-Fischer et al. (1999) showed that microorganisms directly participated in the calcite precipitation by providing a nucleation site and by creating an alkaline environment, which favoured the precipitation of calcite. That is to say, these authors examined the physical and biochemical properties of CaCO_3 precipitation induced by *Bacillus pasteurii*, a microorganism of alkaline soils. The analysis by X-ray diffraction quantified the composition of mineral deposited in a sand column and the analysis classified the CaCO_3 crystals as calcite. The assessment carried out by scanning electron microscopy (SEM), identified the bacteria in the middle of the calcite crystals, which act as nucleation centres. The improvement of the mechanical properties of soils highlight the works of Whiffin et al. (2007); Van Paassen et al. (2007); Al-Thawadi (2008); DeJong et al. (2006) and (2010); Ozdogan (2010) and Chou et al. (2011).

It is interesting to remark that Whiffin (2004), presented the first study to achieve effective consolidation of loose sand via the application of bacterial cultures. Whiffin et al. (2007) evaluated microbial calcite precipitation –MCP- (biocementation process) as a soil strengthening process. A five meter sand column was treated with bacteria and reagents. After dismantling of the column, it was conducted to determine strength and stiffness on triaxial equipment. Porosity was determined from the wet and dry densities of the samples after strength testing, indirectly and the permeability (k) was measured by a constant head test. The main problem these authors found was the non-homogeneous distribution of carbonates along the sand column. The authors observed that a minimum amount of carbonate precipitation per m^3 of sand was required in order to obtain a significant consolidating effect. The latter is also known to play a role in the speed and, hence, the type of crystals that are formed, affecting the global effectiveness of a treatment. Others studies focused on the reduction of soil permeability, for instance those carried out by Stabnikov et al. (2011) and Chu et al. (2012), who use the microbially induced calcium carbonate precipitation on surface of soil.

When stabilization of a soil mass is required at depth, Van Paassen et al. (2007, 2009a and 2009b) considered that these superficial techniques are insufficient and strengthening techniques, like deep mixing, cement or chemical grouting (Karol, 2003). However, as the zone of influence of these methods is limited to the proximity of the mixing equipment or due to the high viscosity or short hardening time of the injected grouts, these traditional methods are not suitable for treating large volumes. The potential of biological techniques for ground reinforcement -biogrout- has being investigated (Whiffin et al., 2007; DeJong et al., 2006; Ivanov and Chu 2008). In this sense, Ismail et al. (2002), developed a technique of cementing porous materials artificially using calcite, based on flushing a mixture of chemical solutions through a porous medium. The different soils response to the cementation process was analysed.

These researchers discuss several factors to determine the response of geomaterials to calcite treatment. It has been found that the strength of a calcite-treated material increases with: (a) intrinsic strength of the individual grains, (b) density, (c) decreasing particle size of the host grains, (d) pre-coating of grains with calcite, and (e) roundness and non-angularity of soil grains. The cementation technique had proved successful in reproducing the mechanical behaviour of natural calcarenite from the North West Shelf of Australia.

Van Paassen et al. (2009a and 2009b), as described above, developed “BioGrout” as a soil improvement method with sand column experiments. The next challenge was established homogeneous strengthening over larger soil volumes, evaluating the new technology in a step-wise scale-up approach. Biogrouting was applied in sand filled boxes in which the sand was treated over a distance of 5 m using screens of injection and extraction wells. The tests showed that it was possible biologically to turn sand into sandstone. Further investigations by Van Paassen et al. (2010a) checked the feasibility of their method, in a field scale experiment. It had been shown that biogrouting-ground improvement by microbial-induced carbonate precipitation can be used to significantly improve the stiffness of granular soils. Van Paassen et al. (2010b) proposed that the occurrence of inhibitive intermediates (nitrite and nitrous oxide) at high concentrations and the heterogeneous distribution of calcium carbonate in the sand column still negatively affect the potential of denitrification as a soil reinforcement method and require further studies. As the rate of calcium carbonate formation by denitrification is still far lower than for the urease process, it requires further optimization for practical applications.

In a next advance on these investigations, Harkes et al. (2010) described a methodology to distribute and fix bacteria relatively homogeneously in a sand bed, before supplying cementation reagents, in order to improve the potential of MCP as a ground reinforcement technique in fine-grained sand. The methodology is based on a two-phase injection procedure: a bacterial suspension is injected into the sand column, immediately followed by a fixation fluid. It is proposed that bacteria are retarded by adsorption and filtration processes and are permanently adsorbed to the sand grains when overtaken by the fixation fluid. The authors could observe that the low ionic strength would promote bacterial transport over large distances and improve the homogeneity of the distribution of bacteria throughout a large sand body and *vice versa*. Al-Thawadi (2008) and Al-Thawadi et al. (2011) enriched and isolated highly urease active bacteria, which allow a biocementation process. This was the first study to use biological cementation to produce high strength comparable to that of the traditional cemented construction materials such as sandstone and concrete with high penetration depth. These studies developed a method of producing high strength cemented sandy materials (up to 30 MPa, equivalent to construction cement) through bacterial hydrolysis. This high strength production was achieved without a significant decrease in the permeability. Moreover, high penetration depth up to 1 m was achieved. These studies suggested that the mechanical strength enhancement of cemented sandy materials was caused by the point-to-point contacts of rhombohedral CaCO_3 crystals and adjacent sand grains.

On the other hand, the research of DeJong et al. (2006) was conducted in MCP and gypsum-cemented specimens, which were assessed non-destructively by measuring the shear wave velocity with bender elements. A series of isotropically consolidated undrained compression (CUC) triaxial tests indicate that the MCP-treated specimens exhibit non-collapse strain softening shear behaviour, with a higher initial shear stiffness and ultimate shear capacity than untreated loose specimens. This behaviour was similar to that of the gypsum-cemented specimens, which represent typical cemented sand behaviour. By means of the SEM technique, it was observed that the cementing effect occurred as a result of the precipitated calcite forming bonds at the particle-particle contacts of sand grains. It is concluded, with increasing concentrations of precipitated carbonate, increasing bond formation and hence, consolidation can

be obtained. Furthermore, DeJong et al. (2010) use several non-destructive geophysical methods for the analysis of the effects this treatment has on the soil. Shear wave velocity, compression wave velocity, and resistivity are the three primary geophysical methods used by these authors.

Ozdogan (2010) conducted a research on biologically treated sand in a triaxial equipment. The test specimens were prepared using air pluviation and moist tamping (these approaches simulate natural deposition methods), which achieve a relatively loose initial void ratio that is more prone to collapse during soil shear. Microorganisms that were suspended in solution were introduced to the soil and the microorganisms were supplied with necessary nutrients *via* cycling with a peristaltic pump. The author concluded that the bio-treated specimens generally did not exhibit stress-strain behaviour that was significantly different from the control specimens.

It is important to note the work of Qabany et al. (2011), in which the shear (S) wave velocity measurements have often been used to obtain valuable soil information. The authors proposed as a possible technique the use of S-wave velocity as an indicator of the effectiveness of the MCP due to its non-destructive nature. The objective of that study was find a correlation between the amount of calcite precipitation and S-wave velocity. It is used as an indicator for cementation as it requires a medium with shear stiffness to propagate, and thus the change in their velocity would reflect the change in specimens shear stiffness. Chou et al. (2011) performed research with *Sporosarcina pasteurii* to improve the mechanical properties of a soil. The authors observed that the growing cells improved the sand properties due to the microbial induced precipitation and related pore volume changes, while dead and resting cells generally caused smaller increases in friction angle and bearing strength. Analysis of the sand treated with growing cells demonstrated that the microbial and chemical processes both contributed to the clogging of the porous medium. Direct shear and California bearing ratio (CBR) tests were conducted on sand specimens subjected to treatment by growing, resting, and dead *Sporosarcina pasteurii* cells in completely stirred tank reactors and completely mixed biofilm reactors, respectively. The results of the study show that the bacterial cells effectively improved the geo-mechanical properties of the sand. Mayoral González and González Díez (2015) prepared cementing solutions using *Sporosarcina pasteurii* (DSM 33), urea and calcium chloride, with samples of sand, silt and calcarenite. Their laboratory work obtained successful results as demonstrated by a SEM study, although further research was necessary. These investigations were of interest to improve soil stability, to build roads and paths, to restore monuments and more applications.

In contrast, less attention has been paid to the influence of chemical parameters, such as calcium and nutrients concentration, on the efficiency of the bio-deposition process. Previous research indicate that the concentrations of these components influence the amount and type of precipitates that are formed (Jiménez-Lopez et al. 2007; Al-Thawadi 2008; De Muynck et al. 2010a; Qabany 2013). In a recent paper, Cardoso et al. (2018) have studied the effects of clay's chemical interactions on biocementation considering samples of a uniformly graded sand and samples of the same sand to which kaolin clay (with predominant kaolinite with minor quartz and muscovite) was added to reduce the porosity to the half. The bacteria *Sporosarcina pasteurii* was used besides urea and calcium chloride solution. The results of tests allow concluding that chemical interactions between the feeding solution and the clay minerals must be considered when using microbially induced calcite precipitation in soils containing them. A physical interpretation of the samples has been proposed by these authors considering the coating introduced by the clay soil and calcium carbonate bonds (Cardoso et al., 2018).

The main objective of the present research is to develop a biotechnological tool for application in clay phyllites stabilization in linear work. i.e., it is intended to replace chemical stabilizer (e.g. cement and lime) by natural cement generated by microorganisms. In this paper

the inclusion of biotechnological solutions from the bacterial treatment will allow the advance in the knowledge of different branches of the engineering. This use is not only focuses on the soil stabilization, but also includes other uses in repairing or enforcing construction materials as consolidation and protection of ornamental stones, selective plugging for enhanced oil recovery, reinforcement in concrete structures, remediation of cracks in concrete and is also focused in others matters related with biological wastewater treatment and in situ bioremediation of polluted soils (DeJong et al., 2013). Physical and geotechnical properties are determined, showing interesting results concerning the microbiological induced precipitation of calcium carbonate in clay phyllites.

2. Material and methods

In this paper, the inclusion of the experimental programme to achieve the main objective as described above, includes several physical and geotechnical tests.

2.1. Selection of bacterial species to induce the cementing property

Data related to the different microbiological doses and bacterial species are under patent and so are not detailed. Microorganism pertains to the *Bacillaceae* family. The microorganism dose is described in this paper as: a) zero concentration (natural sample): untreated (B), b) simple dose (treated sample): treated (BT) and c) simple dose, re-compacted sample: treated_RC (BT_RC)

The procedure of sample preparation is described, in summary, in Figure 1. After Standard Proctor compaction, some of the treated samples are statically re-compacted to match the void ratio of the untreated material: a) simple dose, double time of ageing (treated sample): BT_2T and b) double dose: BT_2D

2.2. Sample preparation

The clay phyllite sample (B-4) is 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 (wopt) and the optimal humidity minus 2 wt% (wopt – 2 wt%) from standard Proctor test (SP). The soils are previously passed through a sieve of 2 mm (except for granulometry and Proctor tests, which have followed the standards ASTM D6913-04, ASTM D698 and ASTM D1557).

Treated clay phyllites (BT-4) are prepared by inoculating the sample with microorganisms. As mentioned above, a microorganism of the Bacillaceae family is used. The microorganisms are added to the raw samples together with compaction water. It was allowed ageing in a humidity chamber under a relative humidity higher than 97 % for a minimum of seven days, after which the treatment (and bacterial activity) is interrupted by increasing the temperature.

For soil-bacteria inoculum preparation, the raw clay phyllites are also passed through a sieve of 2 mm before treatment, except those intended for the granulometry, which are not sieved. After treatment, the sample is compacted at standard Proctor energy with water content between wopt and wopt < 2 wt% corresponding to its respective untreated sample.

2.3. Chemical and mineralogical characterization

The pH is determined potentiometrically following the standard UNE-EN 13037 (2001) and the electrical conductivity (EC) of saturation extract with a conductimeter according to UNE 77308 (2001). The soluble salts have been calculated by the Richards's formula (UNE103205, 2006) and the sulfates are estimated by turbidimetry (UNE 103201, 1996). The content of organic matter has been estimated according to the UNE 103204, 1993 and the cation exchange capacity (CEC) is carried out using the methodology recommended by the Soil Conservation Service of the Department of Agriculture (acetate method, USDA, 1972). The method of Richards is used to estimate gypsum content (UNE 103206, 2006) and carbonate concentration is estimated by the method of Barahona in accordance with the UNE 103200 (1993). This test has been completed with Dietrich-Frühling calcimeter conforming to the Italian standard UNI 11140 (2004).

Two pellets are prepared by compression using aliquots of the sample and a boric acid medium. This test is conducted in usual conditions to obtain a chemical analysis by X-Ray Fluorescence (XRF) analysis using a sequential spectrometer (Model S4 PIONEER WDXRF). Once registered signals from the different elements, with more suitable patterns for these natural samples, it is carried out a quantitative evaluation of the content of the identified elements (major and minor) in weight percent.

X-Ray powder diffraction diagrams (XRD) are obtained in random preparations using a Bruker diffractometer, model D-501, with Ni-filtered $\text{CuK}\alpha$ -radiation, graphite monochromator, at 36 kV and 26 mA, and a scanning speed of 1° in 2θ min^{-1} .

The microstructure features of the untreated and treated samples are studied using Scanning Electron Microscopy (SEM) at different magnifications together with Energy-Dispersive X-Ray spectroscopy (EDS) in an equipment Hitachi S-3500 N X-ray microprobe, model INCA X-Sight, Oxford and through metallization using a sputtering system Bal-Tec SCD 005.

2.4. Physical and hydro-mechanical characterization

The characterization consists of physical description (Unified Soil Classification System). The test is performed in untreated and treated samples.

The humidity is determined by thermogravimetric balance (using infrared heating at 110°C) and drying in oven. The loss of weight by calcination due to a heat treatment is performed after heating for one hour at 1000°C using a furnace.

Particle size distribution is determined by sieving (ASTM D6913-04 (2009) and hydrometer analyses (ASTM D422). Standard specific gravity using ASTM D854 - 10. Consistency limits of the fine fraction (Liquid Limit, Plastic Limit, and Plasticity Index of Soils following ASTM D4318-10).

Normal Proctor (Standard Proctor ASTM D698) and Modified Proctor (ASTM D1557, 12), California Bearing Ratio (CBR) and swelling test (ASTM D1883) on compacted samples at Standard Proctor.

The tests are performed on automatic triaxial equipment (GDS Instruments Ltd). The dimensions of the specimens are 76 mm high and 38 mm in diameter. The lower end of the sample is connected to an automatic piston at a constant back pressure of 80 kPa for B-4 and for

treated sample BT-4. The upper end is maintained at atmospheric pressure. Confining pressure is maintained at 100 kPa for B-4 and for BT-4. The permeability coefficient of saturated water (k_w) under stationary conditions is determined by the equation [1]:

$$K_w = \frac{(\frac{\Delta V}{\Delta t})/A}{i}; \quad i = \frac{\Delta(z + \frac{u_w}{\gamma_w})}{L_0}$$

[1]

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) is performed on a cylindrical specimen, confined laterally by the walls of a ring 25 mm high and 60 mm in internal diameter, and cut in a horizontal plane. For each sample, which are cut at different vertical loads (50, 100 and 150 kPa), tests are carried out on three specimens to describe the failure envelope. The equipment is a WF 25300 house Wykeham Farrance Engineering Limited, and oedometric compression in oedometer Bishop and oedometric cell with load application by the lever arm type Bishop (ASTM D4186).

Determination of the compressibility parameters is performed 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.

Oedometer cells with rings of 50 mm diameter and 20 mm height are used. The samples are extracted from the central zone of the Proctor sample. For each sample two specimens are prepared. The specimens are charged at 20 kPa (vertical strain) and the first sample is saturated at 50 kPa and at 100 kPa, the second. The tests follow a phased procedure charging up to 800 kPa of vertical effective stress and the unload is performed in five steps (400, 200, 100, 50 and 20 kPa, in the most cases). The time interval is 24 hours between application of each step and the next (except for loads applied before saturation, when the time range is lower). To perform this test, the sample is air-dried (air drying conditions) and the equipment Autopore IV (Micromeritics) is used. In some samples the drying of the material is carried out by the "freeze drying" technique.

Adsorption and desorption isotherms of nitrogen gas are carried out until relative pressures near to the unity are achieved. The specific surface areas are calculated by the adjustment process to the mathematical models of Brunauer, Emmett and Teller (BET) and Lagmuir. To perform this test, the samples are treated at 120°C for 2 hours and degassed before measurements (Micromeritics ASAP 2010 V4.00 G). The test is carried out on compacted samples. The pore sizes are calculated using the BJH method (Barret et al., 1951) using the desorption branch.

The mercury intrusion porosimetry (MIP) tests were performed on a Micromeritics-AutoPore IV equipment, attaining maximum intrusion pressures of 220 MPa. The pore sizes were calculated using the Washburn equation following a previous study (Romero and Simms, 2008).

Water retention curve is conducted with the equipment WP4, Decagon Devices (chilled-mirror dew-point Psychrometer). This equipment can determine the relative humidity in equilibrium with the sample and involves a wide range of readings in short times, based on the difference between sample temperature and air temperature when reaching the dew point at equal vapour pressure. The psychrometer used can determine total suction from 1 to 300 MPa, with an accuracy of ± 0.1 MPa from 1 to 10 MPa and $\pm 1\%$ from 10 to 300 MPa. The purpose of this test is the determination of the water retention properties that a sample has during air-drying processes and gradual wetting, i.e. it measures the water potential through vapour transfer (total suction). The estimation of the soil total suction is based on the psychrometric law (Fredlund and Rahardjo, 1993) following equation [2]:

$$\psi = - \frac{RT\rho_w}{M_w} \ln(RH)$$

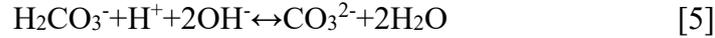
[2]

where ψ is the soil total suction, R the gas constant (8.314 J/mol·K), T the absolute temperature, M_w the molecular mass of water (18.016 kg/kmol), ρ_w the pure water density (998 kg/m³ at 293 K), and $RH=p_v/p_{v0}$ the relative humidity value of the vapour pressure, p_v , relative to the saturation vapour pressure, p_{v0} according to Cardoso et al. 2007 and Decagon Devices, 2003.

3. Results and discussion

3.1. Chemical and mineralogical characterization

Table 1 shows the results obtained of several chemical parameters, both for the samples B-4 (clay phyllite) and BT-4 (treated clay phyllite). From these results it can generally be said that the pH of treated samples increases slightly as compared to that of natural sample. This is because the urea degrading bacteria produces ammonia by hydrolysis, causing alkaline conditions, which are required for the precipitation of calcium carbonate. In sample B-4 there is an increase of the pH. Urease enzyme is responsible to hydrolyse intracellularly 1 mol of urea to 1 mol of ammonia and 1 mol of carbamate (Reaction 1). Carbamate is decomposed by spontaneous hydrolysis to produce another molecule of ammonia and carbonic acid (Reaction 2). The two ammonia molecules and carbonic acid subsequently equilibrate in water with their deprotonated and protonated forms (Reactions 3 and 4). 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 (Reaction 5). In presence of soluble calcium ions, calcium carbonate is precipitated (Reaction 6), in accordance with Sarda et al. 2009. The reactions, in summary, are as follows:

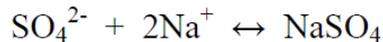
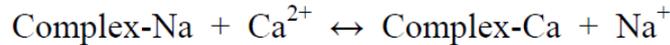


Electrical conductivity (EC) and soluble salts are directly proportional in all samples (Table 1). The electrical conductivity decreases for treated sample BT-4, with a clearly remarkable decline. The content of soluble salts decreases in all cases.

There is also a relationship between gypsum/sulfates that is inversely proportional. There is a decrease in the percentage of gypsum after treatment, while the sulfates content decrease in BT-4. The gypsum dissolves at alkaline pH. Gypsum and/or anhydrite are dissolved in 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 starts, calcium and sulfate ions pass to the fluid phase supersaturated with respect to the more insoluble polymorphs, CaCO_3 . As in a recent work (Cardoso et al., 2018), polymorphs calcite and vaterite were not determined in this investigation. Furthermore, the solubility is not very high and slows the solution, being a prolonged effect. The gypsum is dissociated in aqueous medium as follows:



The released calcium can precipitate when it is combined with the carbonate generated by microorganisms or it may be exchanged with sodium of the complex, being combined with the excess of sulfate to form sodium sulfate:



This arrangement of calcium cations, in the sample exchange complex, can be justified by the increase in CEC, which is related to these two chemical parameters, sulfates and gypsum.

Calcite crystallization 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 sample take place due to the incorporation of calcium adsorbed into the particle and release of sodium to the solution (Fernández-Díaz et al., 2010).

The estimation of calcium carbonate (Reaction [6]) presents some difficulties, because of the gas volumetric variability as a consequence of the influence of certain factors, such as pressure and temperature. To avoid this, the Dietrich-Frühling's method is used, instead of Barahona's method. In treated sample BT-4 there was a considerable increase in the carbonates concentration. This fact, together with the other chemical parameters, reveals the influence of microorganisms on the samples.

According to Table 1, the cation exchange capacity (CEC) increases in the treated sample, since the divalent cations Ca^{2+} present in the gypsum possibly displace the sodium into the soil solution. However, the organic matter undergoes no significant changes in any of the cases after the microbiological treatment.

The average of elemental chemical composition determined by XRF for the sample B-4 is presented in Table 2, together with the main oxides. The results confirm the existence of SiO_2 (49 wt. %) and Al_2O_3 (26 wt. %), from the present silicates, besides the alkali elements mainly K_2O in a content of 4.2 wt. % which is associated with the mineral mica. The content of Fe_2O_3 equal to 10.1 wt. %, CaO and MgO (both about 3 wt. %) are also relevant. The others components are irrelevant, complementing the constituents remaining 4.49 wt. %.

XRD analysis of the crystalline phases present in the untreated clay phyllite (sample B-4), reveals the presence of mica (illite), chlorite and dolomite, as well as quartz and iron oxide as the major components. While the feldspar and a type of interstratified mineral, illite-smectite or illite-chlorite, are observed besides other minor components (Figure 2). A semi-quantitative estimation is performed through the diffractogram of a random powdered sample giving the results presented in Table 3.

The SEM study shows the existence of large masses of irregular particles with other smaller ones, with a wide size distribution (Figure 3). Sometimes, the particles are bonded with the outer part that contains some sheets that are associated to phyllosilicates identified by XRD (Figure 2). However, these sheets also appear isolated in the mass. Chemical analysis by EDS shows the predominance of silicon (Si) and aluminium (Al), which is associated with the free quartz identified by XRD, together with those come from the silicates. Iron (Fe) and potassium (K) traces are also features of the content in mineral phases of mica (illite) and chlorite.

The image of treated sample, BT-4, obtained by SEM shows a homogeneous surface of particles bonded by the natural cementing material (Figure 4). Figure 5 shows a selected SEM at micrograph at higher magnification, where it can be observed the bacteria used in the microbiological treatment.

3.2 Physical and hydro-mechanical characterization

Microbiological treatment has a noticeable effect on the samples that consists in the formation of aggregates. This initial assessment will allow understanding certain results later. Aggregate formation is studied by SEM. A selected micrograph is presented in Figure 6.

The results obtained for sample B-4 are: the received sample had apparently some degree of division. Humidity of the as-received sample (determined by thermogravimetric balance using infrared heating at 110°C): 1-2 wt. %. The loss on heating at 1000°C for 1 hour: 7.02 wt.%. This weight loss is attributed to the presence of hydroxylated phyllosilicates identified by XRD (Figure 2).

The granulometry shows a particle size distribution before and after microbiological treatment. For the clay phyllite all are slightly crushed to break up the aggregates and the samples are left in water for posterior drying and sieving (according to the standard ASTM D6913 – 04 (2009)). The problem that presented in this test is that the particle aggregations, generated during treatment, can be broken as a consequence of the procedure described in the standard. For that, two comparatives process are used. The first one follows the standard and it is called "crushed must be aggregations". In the second one, the treated samples are not crushed to avoid the breaking of aggregations ("with aggregations").

Figure 7 show the granulometric curves of tested samples B-4 and BT-4. The percentage of passing grains and particle size density are plotted in function of particle size. It can be observed that when the treated samples are crushed the amount of fines is the same or slightly greater than untreated samples, probably as a consequence of the calcium carbonate breaking. However, when the granulometry analysis is carried out with aggregations the percentage of passing grains is lower than in untreated samples. Therefore, it can be deduced that the microbiological treatment tends to aggregate the clay phyllite, so that eventually an apparent coarser distribution results. If the bacteria dose or ageing time is doubled, the result is greater aggregates for BT-4. According to the granulometry, the sample B-4 has about 30 wt% of fines associated to the clay minerals (Table 3).

The results obtained by standard specific gravity test, Atterberg limits and surface area tests are summarised in Table 4. On the one hand, the results of the fractions which pass through the sieves 100 mm, 0.4 mm and 0.08 mm, liquid limit values (LL), plastic limit (PL) and plasticity index (PI) are presented. On the other hand the classification of the material and solid density (ρ_s), are presented. The specific surface areas of the samples obtained by nitrogen gas adsorption (BET and Langmuir), are also included. As mentioned before, the comparison shows that the microbiological treatment tends to aggregate the clay phyllites, so that eventually an apparent coarser distribution results. In accordance with Unified Soil Classification System the sample B-4 is a clayey sand with some aggregates (SC), maintaining the same classification after treatment.

With regard to consistency limits, the PI increases as a consequence of a LL increase and PL decrease for samples B-4 and BT-4. The specific surface area seems to be related to the liquid limit of the material, so that as the specific surface increases the liquid limit and the PI also increases for samples B-4 and BT-4.

Following the tests experiments, Figure 8 present compaction curves obtained for different energy levels of the Proctor test (B-4). The plotted curves are obtained at standard Proctor compaction energy, as well as the corresponding curves at half of the standard proctor energy (1/2SP), twice the standard Proctor energy (2xSP) and modified Proctor (MP) for the original sample. For treated samples only a point is calculated. This plotted point in treated samples is obtained from the initial conditions of preparation, i.e., at SP energy and optimum humidity minus 2% ($w_{opt} - 2 \text{ wt. } \%$), corresponding to their respective natural samples. The values obtained for sample B-4 describe an optimum dry density of $\rho_d = 2.05 \text{ Mg/m}^3$, which can be achieved with a water content of $w_{opt} = 0.12$. In general, it can be said that some energy is dissipated during compaction as a consequence of the breaking sample aggregates formed by the microbiological treatment. The final compaction energy (for treated soils) becomes similar or minor to 1/2SP energy applied in natural samples.

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 the cases, the treated sample show a void ratio (e) higher than natural sample. This is because treated sample is compacted with water content between optimum (w_{opt}) and optimum minus 2% ($w_{opt}-2\%$) corresponding to the natural sample. Therefore, the treated sample does not achieve the optimum humidity value. The treated sample presents a higher content of hard aggregates. As a consequence, it is not possible to achieve the same value of ρ_d and w_{opt} as that of the natural sample. So that, the compacted conditions of the sample tends much more towards the dry side of the plane of compaction. This also causes that treated samples present lower degrees of saturation (about 20-30 % lower).

On the other hand, the studied clay phyllites come from areas of south-central of Spain, which are characterized by suffering periods of drought. This fact justifies the sample preparation in the dry side of the compaction plane (degrees of saturation below 80%) to carry out the different hydro-mechanical tests. The samples compacted in this side have higher stiffness and saturated permeability and tend to develop collapse during wetting at high stresses.

From the results of the Proctor tests, two processes are proposed for the study of the microorganism effects on the sample. The different choices that could be pursued to compare the performance of the treated sample to that of the natural one are: 1) working at the same humidity (w) and compaction energy, so that the void ratio (e) and dry density (ρ_d) are different for natural and treated samples as a result of aggregate formation, and 2) working at different compaction energy maintaining constant void ratio (e) humidity (w) and dry density (ρ_d). As mentioned before, tests conducted in the preliminary phase are carried out at the same compaction energy (0.573 MJ/m^3), for subsequent comparative analysis.

The results obtained for CBR and the swelling index for B-4/BT-4 are presented in Table 6. In order to compare the results of the treated and untreated samples that undergo this test, it is decided to keep one of the properties that define the state of the sample, i.e. the degree of saturation, since neither w_{opt} nor ρ_d remain constant after microbiological treatment. This fact has led to worse outcomes of CBR index in the treated samples than in the natural ones. The values obtained indicate that the percentage of the force exerted by the piston on the cylinder for a given penetration, with relation to the same force corresponding to the same penetration of a probe type, is lower for treated sample. Besides this, the swelling is lower for treated sample.

Hydro-mechanical tests are performed on untreated and treated samples to analyse the effects of the treatment on the mechanical behaviour. The tests are performed at the as-compacted conditions and at the saturation water contents, simulating possible saturation in the field due to water infiltration. As a result of the formed aggregations by the effect of microorganisms, the values obtained indicate that treated sample present more open structures at the same compaction energy. Table 7 summarises the water permeability data as a function of the initial void ratio. As it can be observed, these more open structures generate higher permeability values in treated sample. To establish a comparative analysis, the original sample should commence from the same initial void ratio.

Figure 9 show the evolution of the shear stress (τ) and vertical displacement (δ) with respect to horizontal displacement 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-4/BT-4. Figure 10 plotted the break points and failure envelope of the material under drained conditions for the samples B-4 / BT-4, respectively. The comparison shows that the treated sample is more contractile than the natural one, due to its higher initial void ratio. Nonetheless, the shear strength which can be mobilized is slightly higher at the stress levels investigated. The result is confirmed by the shear strength envelopes, depicted in Figure 10 which gives higher friction angles. It is remarkable that calcite production from bacteria does not give an apparent cohesion to the sample. Therefore, it should not be interpreted as a cementation effect. Instead, it increases the friction angle, in spite of higher void ratio. 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.

Oedometer tests are run on the untreated and treated samples with the reference bacteria dose to study the bacterial stabilization effect on the volumetric stiffness. Figure 11 shows the

data of the tests in which the samples are flooded at $\sigma_v = 50$ kPa. The compression curves for the samples flooded at $\sigma_v = 100$ kPa are shown in Figure 12. The initial void ratios of the different samples are slightly different, due to microbiological treatment and the dissipated compaction energy. All samples show volumetric collapse upon wetting, but the amount of collapse does not seem to be influenced by the treatment. The data suggest that the initial void ratio at the time of wetting is the dominant parameter on the amount of collapse. The treatment apparently stiffens the sample, as the comparison of the compression curves after collapse suggests. This fact is confirmed by the direct shear test, which verifies the stiffening of the treated clay phyllites. In compression curves for the samples flooded at $\sigma_v = 100$ kPa, the samples showing the most important collapse are the naturals. Although in most cases, the untreated samples have the highest void ratio upon wetting, due to poor compaction operations.

Following the physical characterization of the materials, it is interesting to study the pore system. The results of the characterization of the pore system of natural and treated compacted samples B-4/BT-4 are depicted in Figure 13. The obtained graphs from the density function and intruded void ratio versus pore size distribution are plotted. Thus, 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 (MIP) are compared with those obtained by nitrogen desorption gas porosimetry test using the BJH method (Barret et al., 1951), as presented in Figure 14. The density distribution of particle size is also included. The comparison between the data for natural sample and those for the treated one shows that the appreciable effect of bacteria is to reduce the porosity from $1\ \mu\text{m}$ of equivalent entrance diameter. This value coincides with the bacterium size ($1\text{--}3\ \mu\text{m}$). It can be generally said that the effect of microbiological treatment is to reduce the porosity an intermediate range between $3\ \mu\text{m} - 30\ \mu\text{m}$, approximately. Besides, compaction at the same energy is less effective in reducing the pores of larger dimensions. BT-4 sample presents a reduction of the pore size density in pores with equivalent entrance diameter to $10\ \mu\text{m}$ and $7\ \mu\text{m}$, respectively.

The results obtained to assess the water retention properties (hygroscopicity and water storage capacity) are presented below. Water retention is essential for understanding the swelling capacity of the tested clay materials and soils (Musso et al., 2008). Figure 15 show the water retention curves (WRC) obtained, for the different materials during drying branch in function of the sample water content. Relative humidity and suction capacity are determined for the samples B-4/BT-4 as a function of the degree of saturation, in Figure 16. MIP data are also elaborated to provide an estimate for the water retention curve and the elaborations are compared with direct measurements obtained by the dew point probe (WP4) on compacted samples. First of all, the water storage capacity for the low-suction (about 1 MPa) is slightly lower for the treated sample. For this sample, the linear relationship Log suction-water content for a higher range of sample suction shows a minor slope flattening, indicating a minor specific surface in carbonated sample. The highest specific surface area (almost twice), which corresponds to the data presented in Table 4, is associated with an increase of hygroscopicity (specific surface of the material allows to absorb water) and a greater ability to undergo expansion volume changes in the wetting process (Romero and Vaunat, 2000).

Finally, the compressibility results ($-\delta e/\delta \ln \sigma_v$) obtained from oedometer test, shear strength (drained) and water permeability of the tested materials are given in Table 8. As a general tend, the compressibility of treated materials varies considerably, thus confirming the calcium carbonate precipitation due to the bacteria activity during the curing process. Sample BT-4 is more rigid with respect to the raw clay phyllites, but exhibits a similar tendency in stiffness. According to the results, the permeability values remaining almost constant for sample BT-4.

4. Summary and conclusions

The results presented in this investigation on microbial induced carbonate precipitation in clay phyllites show that the effects of bacteria are increasing calcite content of the sample, and reducing the soil specific surface and its plasticity. The reasons for the latter result are assumed the addition of a non-plastic component to the natural soil, or the result of the more aggregated structure promoted during the treatment. These results display a consistent behaviour pattern, suggesting that precipitation of calcium carbonate from bacteria takes place in the pores of the phyllite clay sample which are slightly bigger than the characteristic dimension of the bacteria themselves, which is around 1-2 μm .

As a consequence, the pore size distribution of the sample changes in an approximate range 3 μm - 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 sample, which presents typical features of a more dense solid with respect to the natural untreated one. The friction angle of the treated sample is slightly higher, and its compressibility is consistently lower, than that of the natural untreated sample.

As the bacteria do not seem to produce any cementation effect on the phyllites clay 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. As a whole, the physical and geotechnical tests performed in this investigation suggest that the microbiological technique may be effective to improve the mechanical characteristics of the compacted clay phyllites. For that, it is necessary to provide more energy in compacting the treated sample 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 sample. However, the results obtained in this phase show three main concluding features, as follows:

1) 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 mercury intrusion porosimetry;

2) 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, which is consistent with the lower specific surface of the treated material, and finally,

3) Treated sample displays a slightly larger friction angle with no cohesion, being consistent with the filling properties and its denser condition.

In the investigation it was found that the microbiological treatment tends to aggregate the studied clay phyllite samples, thus generating aggregates in part due to the formation of calcium carbonate. This process is heavily influenced by the curing and compaction protocols followed on the sample. That is to say, the compaction occurs after seven days of curing, resulting in a breakage of the carbonated structures formed during the treatment. This process was common to all the samples, but despite that, it allowed us to establish a number of differences between them. As a consequence of the breaking process of the aggregates, some of the compaction energy is lost. Therefore, the treated samples do not reach the same maximum dry density as the natural state, for the same level of compaction energy applied. These results are promiser to perform new studies on microbiological induced carbonate precipitation using other raw clay materials and soils

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Table 1

Chemical parameters of the tested samples, untreated (B-4) and treated (BT-4).

| Soil | pH | Electrical conductivity (dS/m) | Soluble salts (%) | Gypsum (%) | CaCO ₃ eq (%) | Sulfates (mg/l) | Organic matter (%) | Cation exchange capacity (meq/100 g. Soil) |
|------|------|--------------------------------|-------------------|------------|--------------------------|-----------------|--------------------|--|
| B-4 | 8.43 | 38.3 | 2.45 | 1.25 | 8.81 | 1192.18 | 0.39 | 8.73 |
| BT-4 | 8.81 | 13.23 | 0.85 | 0.44 | 9.08 | 1055.8 | 0.16 | 10.80 |

Table 2

Chemical composition of the sample B-4 determined by XRF (quantitative assessment).

| Sample | SUM (%) | SiO ₂ (%) | Al ₂ O ₃ (%) | Fe ₂ O ₃ (%) | MnO (%) | MgO (%) | CaO (%) | Na ₂ O (%) | K ₂ O (%) | TiO ₂ (%) | P ₂ O ₅ (%) | As (ppm) | Ba (ppm) |
|--------|----------|----------------------|------------------------------------|------------------------------------|----------|----------|----------|-----------------------|----------------------|----------------------|-----------------------------------|----------|----------|
| B-4 | 100 | 49 | 26 | 10.1 | 4.2 | 3.29 | 3.02 | 2.5 | 1.4 | 0.119 | 0.081 | 829.3 | 779.5 |
| | Dy (ppm) | Er (ppm) | Eu (ppm) | F (ppm) | Ga (ppm) | Gd (ppm) | Hf (ppm) | La (ppm) | Lu (ppm) | Mo (ppm) | Nb (ppm) | Ni (ppm) | Pb (ppm) |
| | 7.3 | 418.9 | 378.6 | 201.6 | 140.9 | 113.8 | 112.9 | 48.7 | 38.3 | 31.7 | 27.9 | 26.2 | 13.7 |

Table 3

Mineralogical composition of sample B-4.

| Mineral | Quartz | Illite | Chlorite | Feldspar | Dolomite | Iron oxide | I/S |
|---------|--------|--------|----------|----------|----------|------------|-----|
| wt (%) | 35 | 20 | 15 | 10 | 5 | 5 | 10 |

Note: I/S=Interstratified illite-smectite**Table 4**

Properties of the untreated (B-4) and treated (BT-4) samples.

| Sample | Clasif. | % pass | | | LL (%) | PL (%) | PI (%) | ρ _s | S (m ² /g) | |
|--------|---------|----------|----------|-----------|--------|--------|--------|----------------------|-----------------------|----------|
| | | 100 (mm) | 0.4 (mm) | 0.08 (mm) | | | | (Mg/m ³) | BET | Langmuir |
| B-4 | SC | 82 | 60 | 30 | 26 | 17 | 8 | 2.82 | 7.6 | 10.6 |
| BT-4 | SC | 64 | 14 | 5 | 32 | 15 | 17 | 2.89 | 16.5 | 22.9 |

Note: LL= Liquid Limit; PL= Plastic Limit; PI= Plasticity Index; S= Specific Surface Area; ρ_s= density of the solid; Clasif.= Classification.

Table 5

Humidity (w), void ratio (e), porosity (n), degree of saturation (Sr) and density (ρ_d : dry density and ρ_{sat} : saturated density) of the natural and treated materials.

| Sample | w (%) | e | n (%) | Sr (%) | ρ_d | ρ_{sat} |
|--------|--------------|-------|-------|--------------|----------------------|----------------------|
| | | | | | (Mg/m ³) | (Mg/m ³) |
| B-4 | 9.4 | 0.355 | 26.2 | 74.8 | 2.084 | 2.346 |
| BT-4 | 6.18 to 8.07 | 0.551 | 35.5 | 32.4 to 42.2 | 1.86 | 2.22 |

Table 6

Results obtained for CBR index and swelling index in untreated (B-4) and treated (BT-4) samples.

| Sample | CBR | | Swelling |
|--------|-----------------|----------------|----------|
| | 100% Compaction | 95% Compaction | |
| B-4 | 2.5 | 1.7 | 3.56 |
| BT-4 | 0.6 | 0.2 | 0.06 |

Table 7

Water permeability (k) for natural (B-4) and treated samples (BT-4) as a function of the initial void ratio (e_0).

| Sample | B-4 | BT-4 |
|-----------|--------------------|--------------------|
| e_0 | 0.355 | 0.418 |
| k (m/s) | $8 \cdot 10^{-10}$ | $2 \cdot 10^{-09}$ |

Table 8

Results obtained from the tests carried out in the natural (B-4) and treated (BT-4) samples.

| Material | Dry density (SP) ρ_d (Mg/m ³) | Saturated compressibility ($-\delta e / \delta \ln \sigma_v$) | | Direct shear (drained) | | Water permeability (m/s) | |
|----------|--|---|-------------|------------------------|------|--------------------------|-------|
| | | Load | Unload | θ' (°) | c' | k | e_0 |
| B-4 | 2.05 | 0.049-0.062 | 0.019-0.017 | 26 | 0 | $8 \cdot 10^{-10}$ | 0.355 |
| BT-4 | 1.86 | 0.068-0.063 | 0.016-0.017 | 30 | 0 | $2 \cdot 10^{-09}$ | 0.418 |

Note: θ' (°)= Internal friction angle, c' = Cohesion, k= Water permeability, e_0 = Void ratio

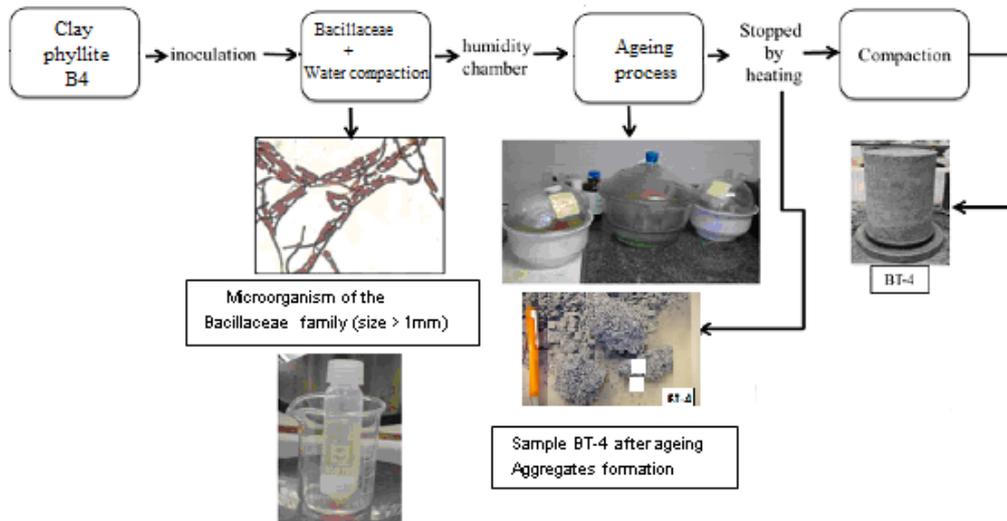
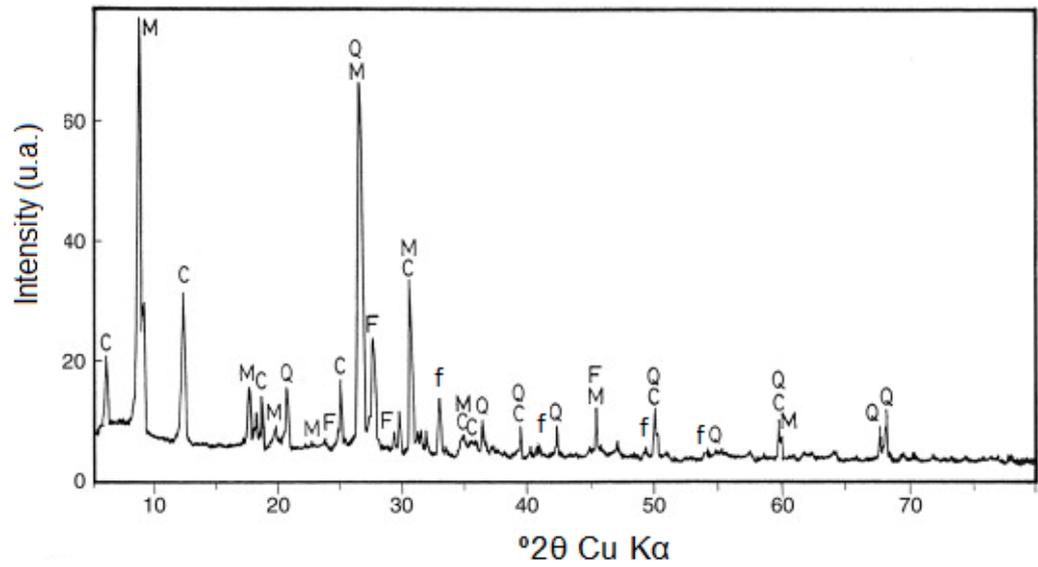
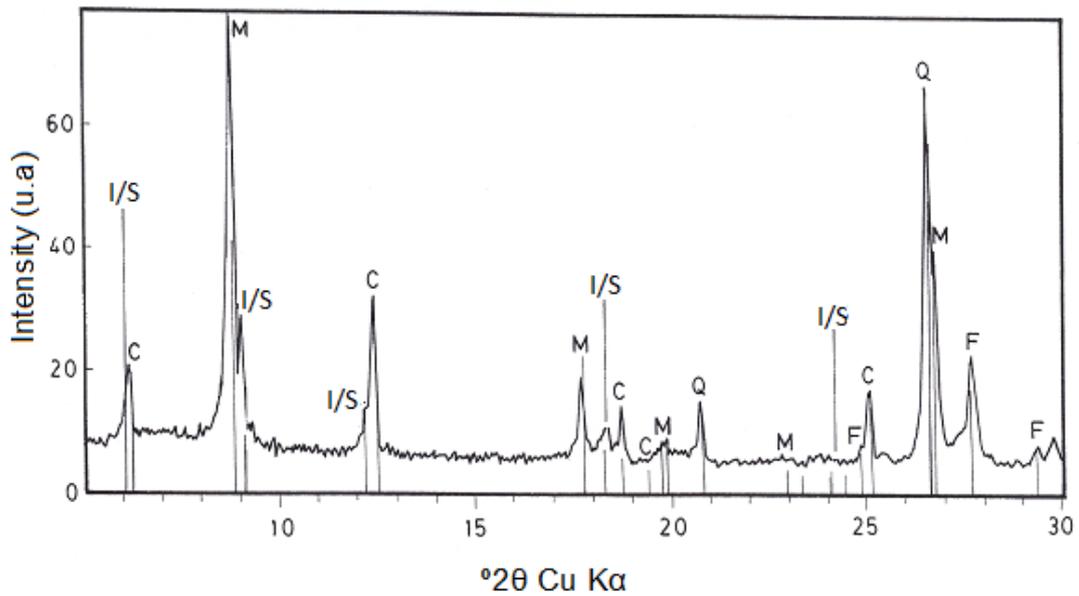


Fig. 1. Microbiological treatment.



a)



b)

Fig. 2. XRD powder diagram in random preparation (a) of the clay phyllite sample used in this work with the main crystalline minerals identified. They are mica (muscovite, illite), designed as M, chlorite (possibly clinochlore), designed as C, quartz (designed as Q), potassium feldspar (designed as F), and iron oxide (designed as f), and the same XRD powder diagram (b) in the zone 5-30 ° 2θ to show the presence of a mixed-layer or interstratified phase, possibly illite-smectite or illite-chlorite, denoted by I/S, with the relative intensities of each peak.

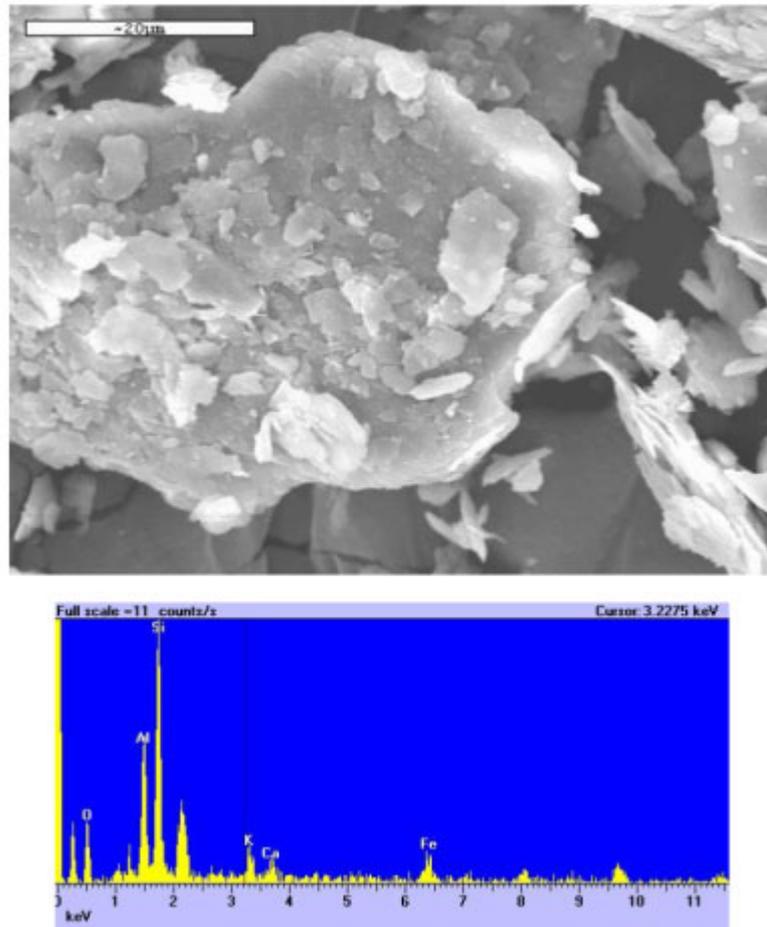


Fig. 3. SEM image and EDS analysis of untreated sample, B4.

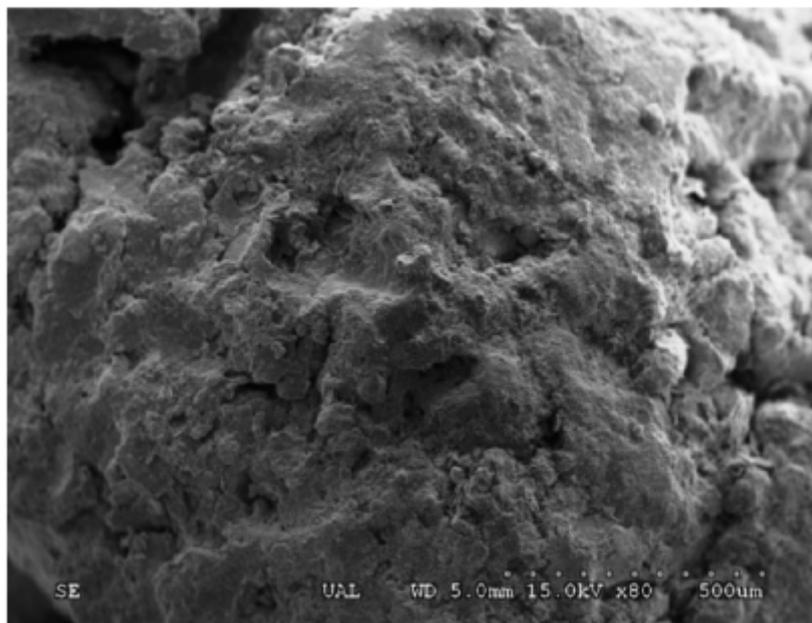


Fig. 4. SEM photomicrograph of the treated sample, BT-4 (obtained at 80x magnification).

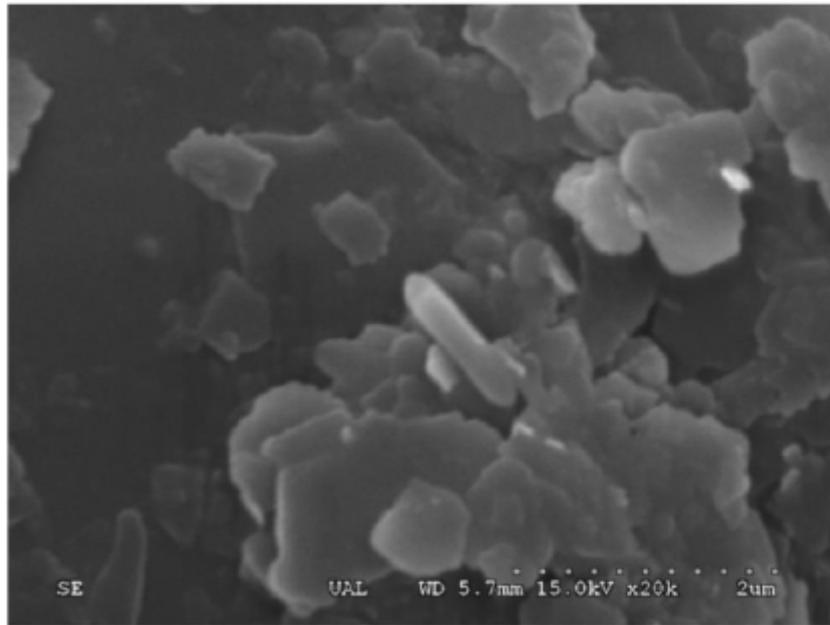


Fig. 5. SEM photomicrograph of the alive bacterium before calcification process.



Fig. 6. Detail of the aggregate formation of the treated sample BT-4 and detail of isolated compacted agglomerates.

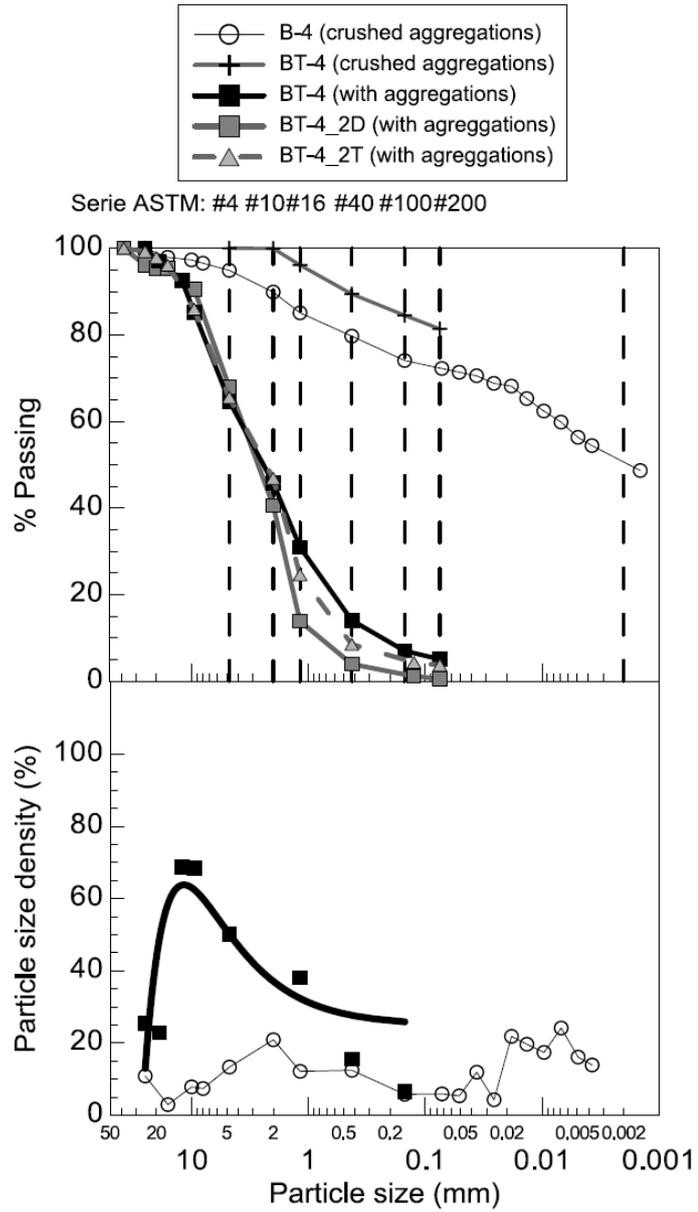
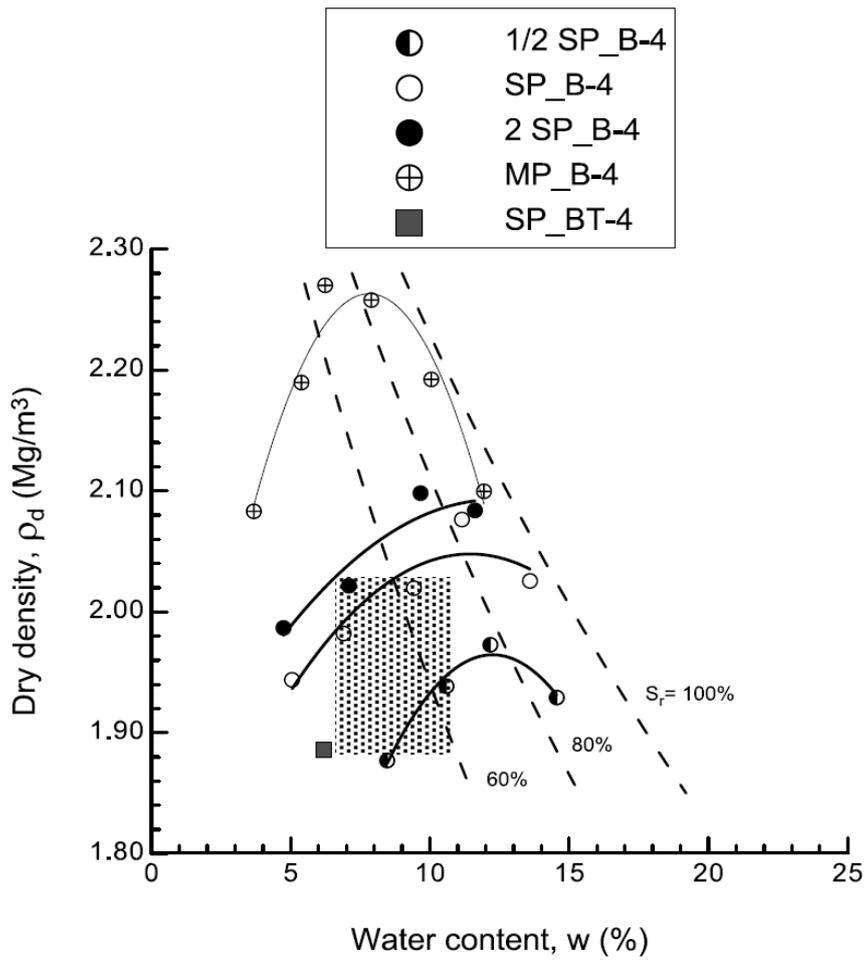


Fig. 7. Grain size distribution of untreated sample B-4 (crushed aggregations) and treated samples BT-4, BT-4_2D and BT-4_2T.



Note: S_r = degree of saturation

Fig. 8. Plane compaction for natural sample B-4 at different energy levels. Point obtained for treated sample BT-4 at SP energy keeping constant dry density and humidity.

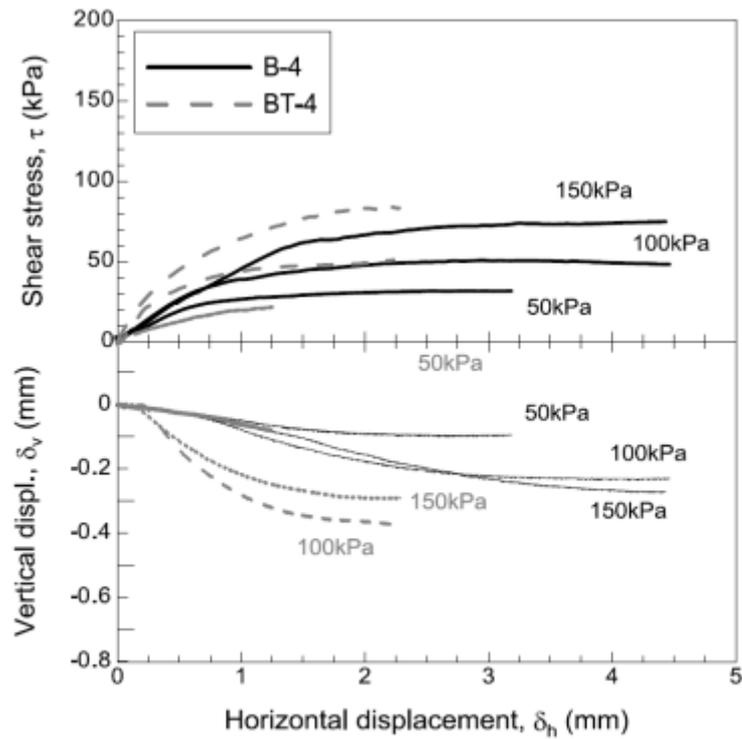


Fig. 9. Evolution of the shear stress (τ) and vertical displacement (δ_v) with respect to horizontal displacement for different applied vertical effective stress, σ_v (50, 100 and 150 kPa), on sample B-4 and BT-4.

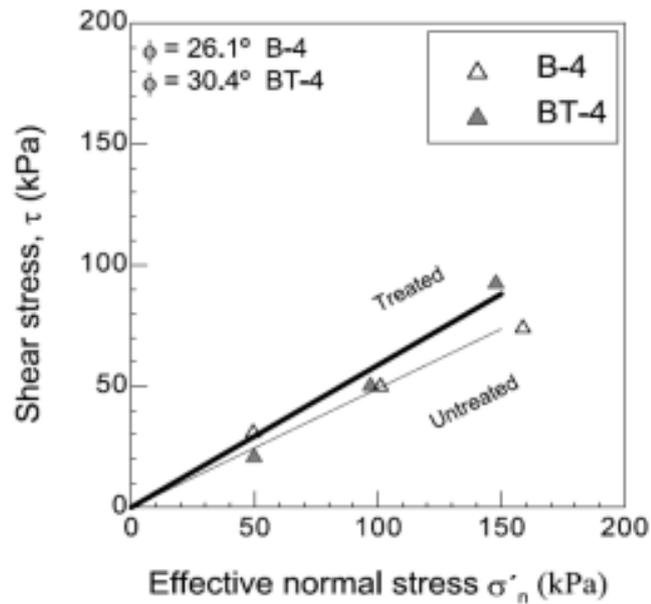


Fig 10. Results of break points and failure envelope of the material under drained conditions for B-4 and BT-4.

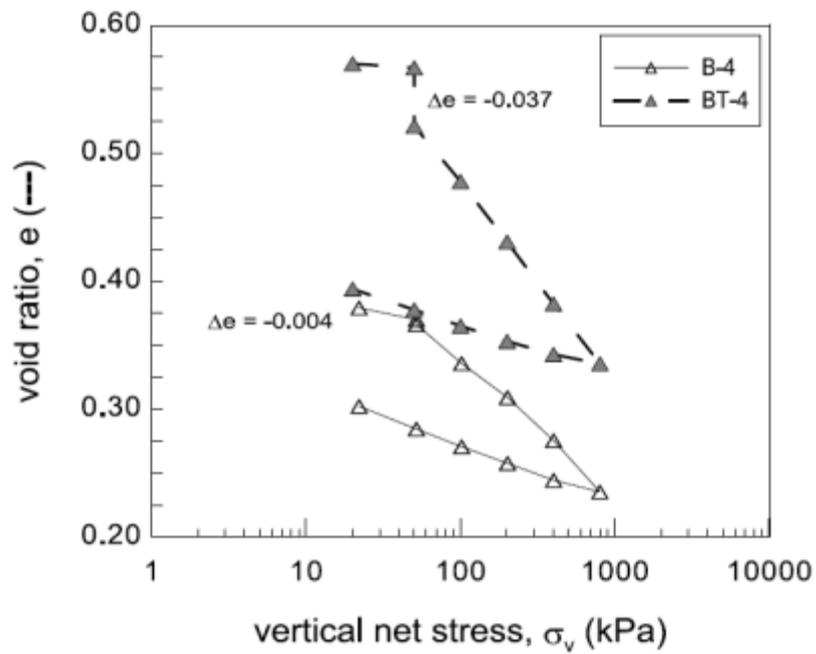


Fig. 11. Compressibility curves of the natural (B-4) and treated sample (BT-4), flooded at $\sigma_v = 50$ kPa.

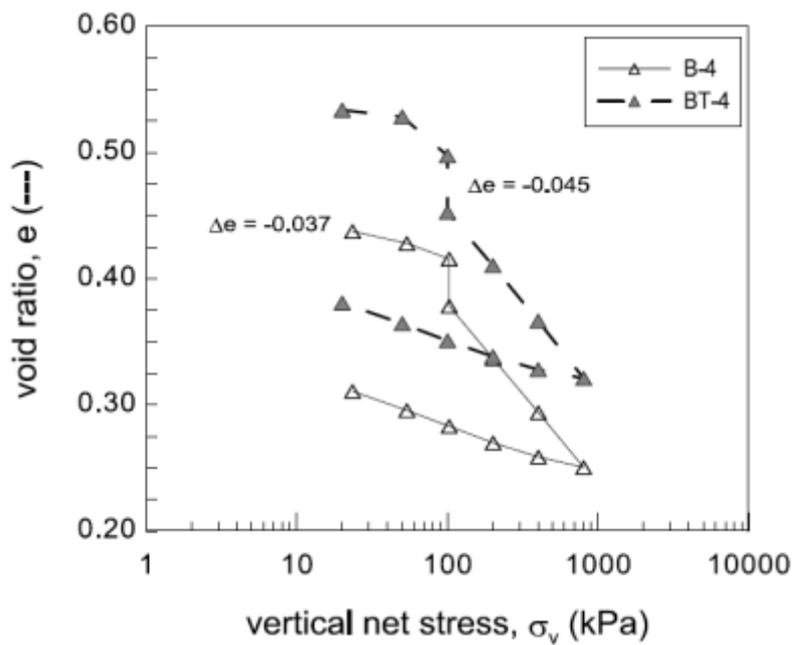


Fig. 12. Compression curves for natural B-4 and treated sample BT-4, flooded at $\sigma_v = 100$ kPa.

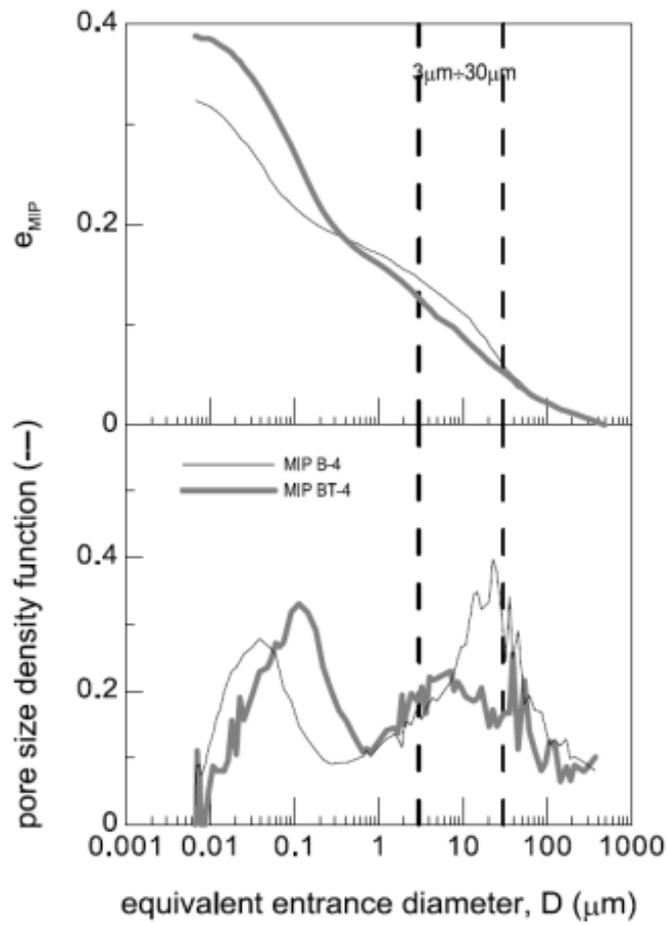


Fig. 13. Intruded porosity and pore density function depending on pore diameter. Mercury intrusion porosimetry carried out for the samples natural B-4 and treated BT-4, by freeze drying.

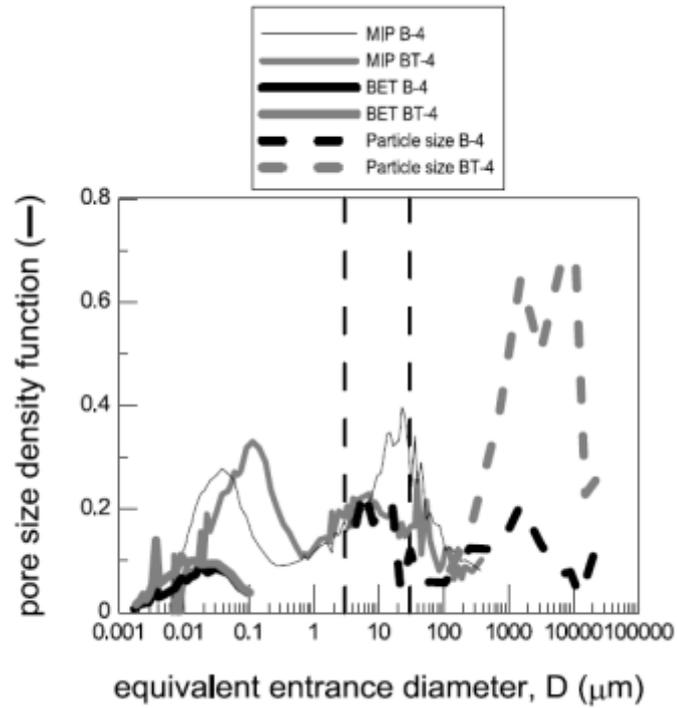


Fig. 14. Pore size density function versus pore size conducted for samples B-4 and BT-4. The particle density is included

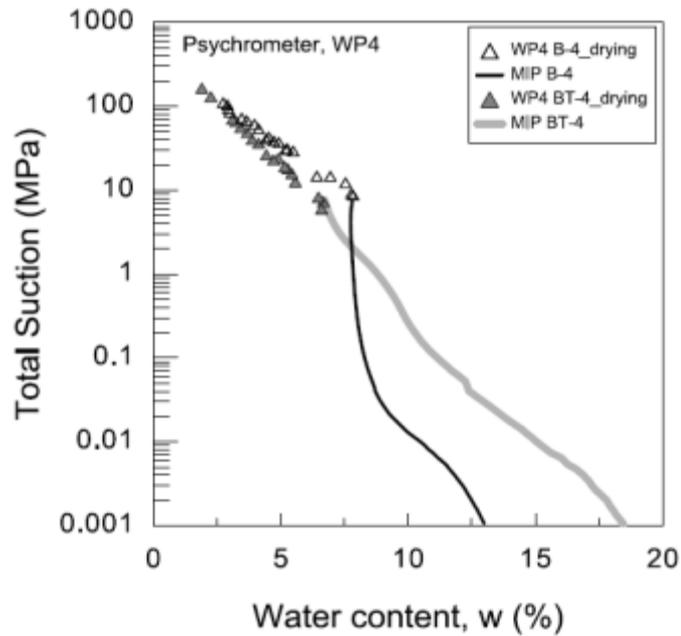


Fig. 15. Test results from water retention curves and MIP corresponding to samples B4 and BT-4.

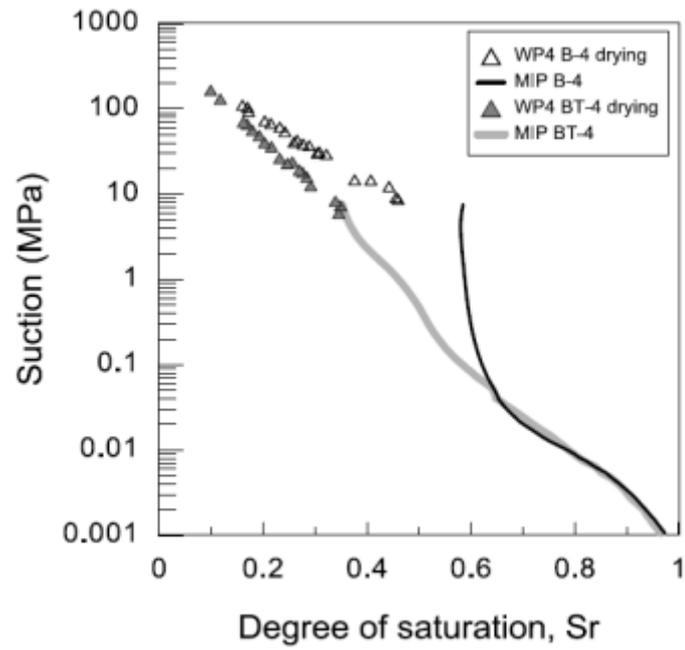


Fig. 16. Total suction as a function of saturation degree from water retention curves and MIP corresponding to samples B-4 and BT-4.