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EFFECT OF UREOLYTIC BACTERIA ON COMPRESSIBILITY OF THE SOILS WITH VARIABLE GRADATION

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Abstract
The aim of this study is to present the effect of treatment with ureolytic bacteria (*Sporosarcina pasteurii*) on the compressibility parameters of mineral and anthropogenic soils. In the presence of the urease enzyme, secreted by a strain of *Sporosarcina pasteurii* bacteria, urea hydrolysis occurs, allowing $CaCO_3$ to be precipitated. The literature suggests applying the Microbially Induced Calcite Precipitation (MICP) method to non-cohesive soils. In order to determine whether the biomineralization process occurs in other soil types, cohesive and anthropogenic soils were tested in the laboratory. Compressibility tests were carried out in the laboratory on MICP-treated and untreated soils as reference samples. The process of biocementation in the soil is made possible by the introduction of bacteria into the soil and subsequent activation by a cementation solution (consisting of urea and calcium ions Ca^{2+}). This paper presents the methodology for introducing bacteria into the soil, as well as the effect of the biomineralization process on the deformation parameters of the tested materials.

Keywords: **Biomineralization; MICP; Soil improvement; Soil parameters; Ureolytic bacteria.**

1. INTRODUCTION

Unfavourable soil and groundwater conditions have contributed to the development of ground improvement methods. Some of them use the effect of stabilising additives such as cement, lime, bentonite or fly ash to improve soil parameters. Although chemical additives, like lime or cement, bring ecological concerns. Additionally, cement is a material whose production generates CO_2 emissions and consumes a large amount of industrial energy [1]. That is why sustainable and environmentally friendly techniques of soil improvement are sought. In recent years, much attention has been paid to enhancing soil parameters through microbiological methods based on the living microorganisms. The biocementation/biomineralization method is connected to the calcium carbonate ($CaCO_3$) precipitation induced microbially. The phenomenon occurs in nature, for example in soil, geological sediments and waters [2, 3]. Microbially induced precipitation of calcium carbonate, known as MICP (Microbially Induced Calcite Precipitation). In the MICP method, precipitated calcium carbonate ($CaCO_3$) acts as a binder in the soil [4, 5]. MICP method is considered to be an eco-friendly alternative to conventional remediation techniques [1]. An additional advantage is that MICP have the removal behaviour of heavy metals from contaminated environments [6–8], and research have been conducted on

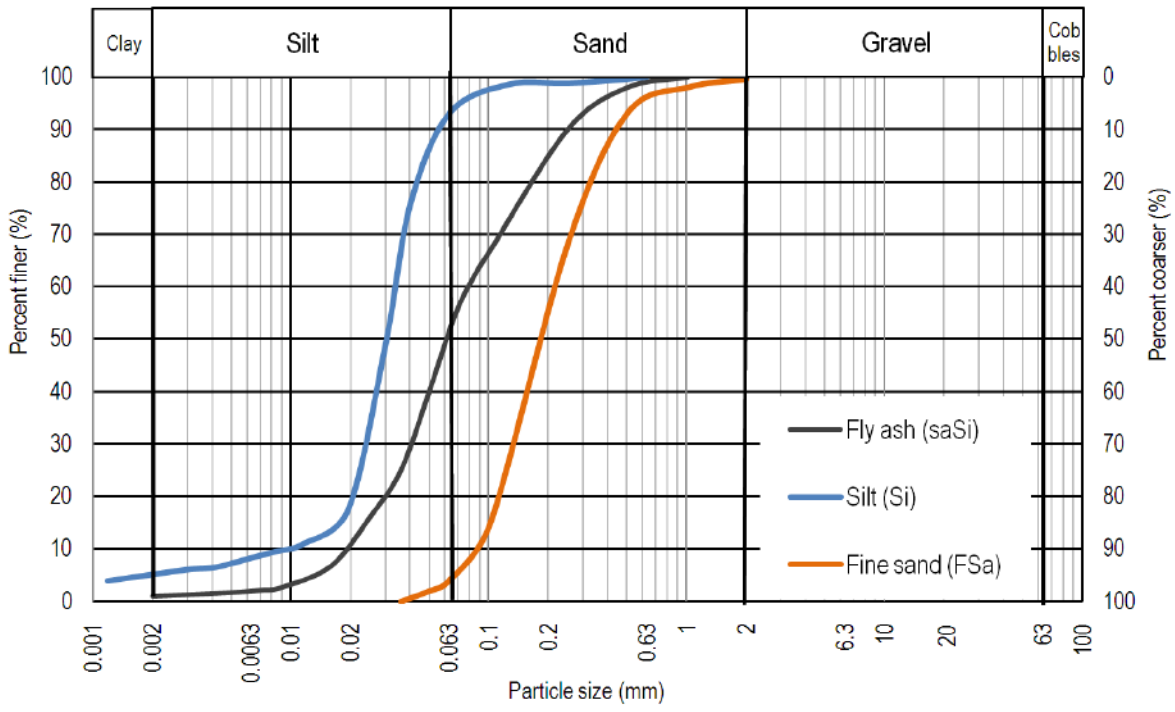


Figure 1.
Grain size distribution curves of tested materials

the ability to remediation of groundwater [9, 10].

Microorganisms characterised by the production of a large amount of urease, resulting in the creation of an alkaline environment for the precipitation of calcium carbonate precipitation, are ureolytic bacteria. In biomineralization, biominerals are formed by the reaction of metabolic products of microorganisms with the surrounding environment. Mineral precipitation occurs through the attachment of positively charged calcium ions to the negatively charged cell walls of microorganisms [11]. Microbially induced calcium carbonate precipitation (MICP) is the process where ureolytic bacteria (producing urease) hydrolyse urea to produce dissolved ammonium, inorganic calcium and CO_2. Ammonia increases the pH of the surrounding material and leads to the accumulation of $CaCO_3$ [12]. Chemical reactions of urea hydrolysis are presented through the equations [13, 14]:

$$CO(NH_2)_2 + 2H_2O \xrightarrow{Urease} 2NH_4^+ + CO_3^{2-} \quad (1)$$

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

The method has been used in different ways in civil engineering [15], such as concrete crack repair [12, 16], bioconcrete [11, 17], sand consolidation [18, 19], and soil improvement [20–28]. Referring to soil reinforcement, the following properties have been improved: shear strength [29, 30], permeability [31, 32], unconfined compressive strength [13, 28, 33, 34], compressibility [35–37], liquefaction resistance [38, 39].

Krajewska [1] after [21], compared the cost-effectiveness of biogrouting to chemical grouting in the soil as an improvement method. Assuming the same method of injection of the solution to the ground, the MICP method seems to be more economical (if cheap waste materials were used as nutrients for bacteria), which, apart from being environmentally friendly, argues in its favour compared to well-known, traditional methods.

The paper aims to present preliminary test results of the soil amended with the MICP method. For the biocementation process, non-cohesive, cohesive and anthropogenic soils were taken to assess if the process will occur and how it impacts the compressibility of treated soils. The experience throughout the sample preparation is also described.

2. MATERIALS AND METHODS

2.1. Soils

To conduct the preliminary research on the influence of bacteria on the compressibility parameter of the soil, three types of soils were used: non-cohesive soil, cohesive soil, and anthropogenic soil which was Class F [ASTM C618-19] fly ash [40], the coal combustion by-product. The chemical and phase composition of tested fly ash is presented in Table 1. In Fig. 1 the grain size distribution curves of tested materials are presented, according to the EN 933-1 standard. The physical parameters of presented materials, like the density of solid particles (ρ_s) and median particle diameter (D_{50}), are given in Table 2.

Based on Fig. 1, in accordance with EN ISO 14688-1 standard, the tested non-cohesive soil is fine sand (FSa), and the tested cohesive soil is silt (Si). Class F fly ash (FA) has a grain size corresponding to that of sandy silt (saSi), and in the literature, fly ash is called transitional soil [41].

Table 1.
Chemical and phase composition of tested fly ash (FA)

Phase-mineral composition (%)		Chemical composition (%)	
SiO_2	43.28	Si	22.37
Al_2O_3	31.81	Al	15.80
Fe_2O_3	1.82	C	3.49
CaO	1.10	K	2.46
MgO	2.16	Fe	1.87
K_2O	3.42	Mg	0.97
TiO_2	0.63	Ca	0.81
LOI	12.45	Ti	0.56

Table 2.
The parameters of tested soils

Material	D_{50} (mm)	ρ_s (g/cm^3)	I_p (%)
Silt (Si)	0.031	2.67	7.7
Fine sand (FSa)	1.90	2.65	–
Fly ash (FA)	0.060	2.18	–

2.2. Bacterial and cementation solution

In this paper, strain *Sporosarcina pasteurii* (DSM 33) was selected as a commonly used ureolytic bacteria in the MICP process [18, 20, 23, 42]. *Sporosarcina pasteurii* is a soil bacterium known as a strain which produces high levels of urease and tolerates extreme conditions [10]. The bacteria from the freeze-dried form was rehydrated and moved to solid medium nutrient agar with urea. After incubation at 25°C for 48 h, the bacterial colonies were formed. Activated bacterial colonies were moved to the flask which contained 50 mL of urea medium. The urea medium for bacteria, urea medium and cementation solution were prepared according to the procedure described in [33]. Finally, two flasks were prepared, one containing urea medium with bacteria, and the second one, cementation treatment consisting of urea medium and $CaCl_2$ stock solution (with a concentration of 0.5 M of Ca). Urea [$CO(NH_2)_2$] was used as ammonium and an energy source for the hydrolysis process [43]. The bacterial suspension was analysed for optical density measured at a wavelength of 600 nm (OD_{600}) using an ultraviolet-visible spectrophotometer. OD_{600} corresponds to bacterial concentration, which might be used to describe the activity of bacteria. The bacterial concentration was calculated according to [14]:

$$\textit{Bacterial density (cells/ml)} = OD_{600} \cdot 4 \cdot 10^8 \quad (3)$$

The bacteria concentration used in the presented research was $OD_{600} = 0.3$, which corresponds to the bacterial density of 1.2×10^8 cells/mL. In Fig. 2, the bacteria-urea solution is presented, the same bacteria-urea solution directly after the cementation solution is added and after a few days of storing at room temperature (21°C), where the precipitated particles are visible.

As reported in the literature [44], when the value of OD_{600} is higher than 0.15, the bacterial suspension is suitable for the biocementation process. In the research of Wang et al. [14], the bacterial solution had an optical density from 0.2 to 3.0. Other factors of the experiment were constant. The higher bacterial concentrations lead to higher activity, so faster and more crystals should precipitate (depending on the cementation solution concentration). As [14] observed in the microscale, OD_{600} had an impact on the sizes of the precipitated crystals. In the case of the lowest OD_{600} value, crystals formed slower and there was a lower amount of them, but the largest among the other cases. When the bacterial density was high, $CaCO_3$ crystals were small and unstable, but over time might transform into larger and more stable forms. The worst results were achieved for the very high bacterial density (OD_{600}=3.0), where the rate of $CaCO_3$ precipitation increased, but formed large amounts of unstable forms of $CaCO_3$. It might cause problems in the case of soils with small pores, where unstable forms might be trapped in pores and cause clogging and inhomogeneity of improved soil.

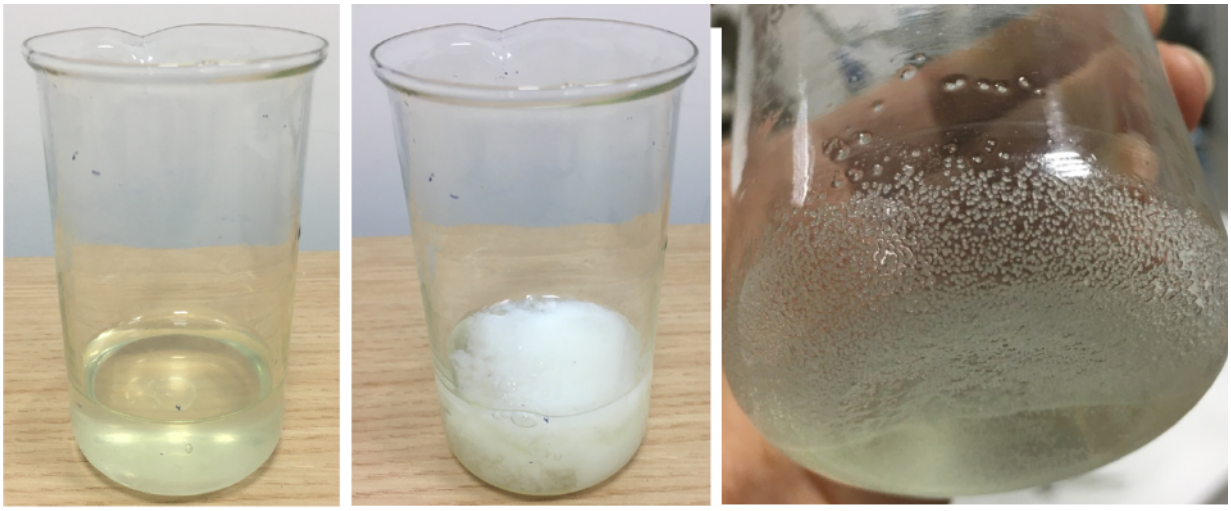


Figure 2.
The process of biomineralization in a flask: a) bacterial solution, b) bacterial solution right after $CaCl_2$-urea solution added, c) calcite crystals precipitated at the bottom of the flask, where bacterial solution and cementation solution were mixed (after a few days of storing at room temperature)

2.3. Sample preparation

Premixing was chosen as a method of introducing the solutions to the soil because the cohesive soil (Si) was used and the fly ash had the granulation of saSi. As the literature shows [5], percolation is a mainly used injection method. However, there might be a problem with the homogeneity of the biocementation process through the sample. Premixing resulted in a homogeneous sample [45]. Although the homogeneity of the samples was achieved, the results of the tests of unconfined compressive strength (UCS) revealed lower values of UCS that the one for the biocemented samples where the injection method was used. The uniformity of biocemented samples is important, because of the repeatability of the test results, and as Konstantinou et al. [46] claimed, the literature shows contradictory results.

In the literature [12, 13, 19, 46, 47], in most cases, sandy soils are used to conduct the MICP process, where the bacterial and cementation solution with the use of a peristaltic pump or other equipment, can easily flow through the soil particles. Mitchell & Santamarina [41] prepared a graph showing the bacteria size and types of soils, where the process of biomineralization may be a misguided soil improvement method. However, since then, few studies [48, 49], have tested the effect of MICP on fine-grained materials. In this study, sandy soil, silt, and fly ash were tested.

Before sample preparation, each soil was put into a laboratory dryer at a temperature of 100°C to get dry material and to be sure, that no other microorganisms are present. In order to prepare samples, the urea-bacterial solution was mixed with urea-cementation solution in the proportion 1:1 and directly added to the soil, followed by thorough mixing. The volume of the mixture that was added to the material was calculated according to the initial void ratio. The samples were to be placed in a mould (oedometer ring), so the mass of the soil was calculated and weighted before moulding. A different amount of mixture was added to each material as different moisture contents were desired.

Samples were prepared in a rigid ring for an oedometric test of 65 mm in diameter and 19.5 mm in height. On the bottom and top of the samples filter papers and porous stones were placed. Then, the samples were left at room temperature (protected from drying) to enable the biocementation process, which is connected to the retention of ureolytic bacteria in the soil. The simultaneous introduction of bacteria and calcium into the soil was the procedure followed by [35]. It was a good solution in a method where no peristaltic pump was used.

In the initial process of biocementation, microbes are attaching to the soil particles. Samples were kept in conditions of constant temperature (21°C) and protected from drying. Curing time, from the preparation of the sample to the tests lasted a total of 14 days, after which the compressibility tests were conducted in an oedometer. The curing time was based on the literature [50], where the researchers found

that bacterial activity decreased after 16 days of treatment.

The loading path of the oedometer test was 8, 15, 30, 60, 120, 240, 540 and 725 kPa. The unloading was 240, 90 and 30 kPa. Each load increment was applied to the sample for 24 hours. Before placing samples in the oedometer, the weight of each was checked. The samples were flooded with water. The vertical deformations during the test were collected by the computer program.

3. RESULTS AND DISCUSSION

The compressibility curves of the oedometric tests are presented in Figures 3–5 for the samples treated with the MICP method and reference samples (treated with a urea-$CaCl_2$ solution to be sure that bacteria contributed to the MICP process). The results are presented as a relation of relative vertical deformation ε_v to vertical stress σ_v'. In Table 2, compression indices of tested materials are presented.

Based on the presented compressibility curves, it can be seen, the different characteristics of the vertical deformation of each material. In all of the samples, the MICP process caused a change in the final value of vertical deformation ε_v. The biggest difference between the referenced sample and the treated sample in the ε_v value was observed in the FA sample (1.82%). As indicated in the literature, the compressibility of fly ashes is influenced by improving additives [51], but also by the method of sample preparation [52].

The smallest difference in the value of final ε_v in comparison of treated and untreated material was

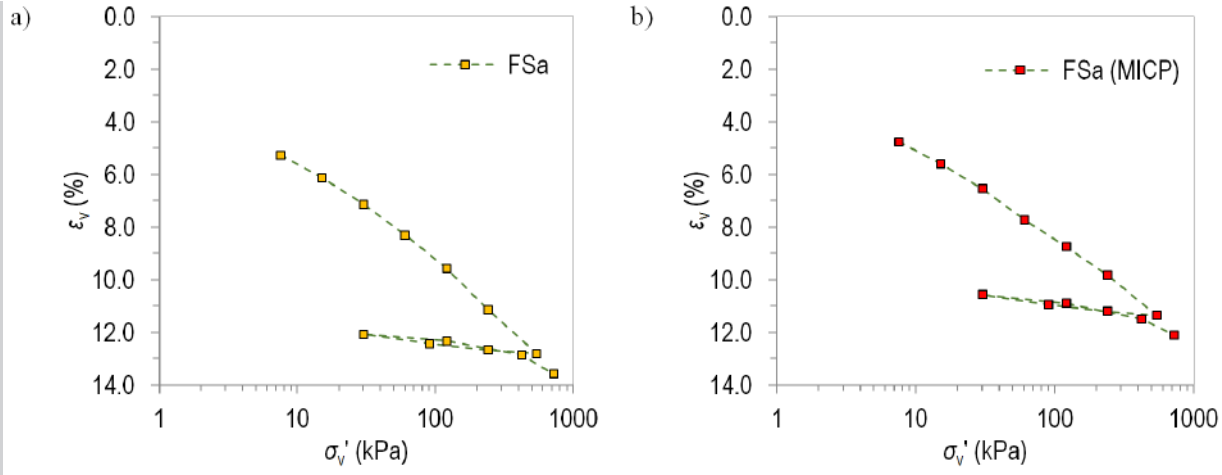


Figure 3.
Results of oedometric tests of: a) untreated fine sand (FSa), b) fine sand (FSa) improved with the MICP method

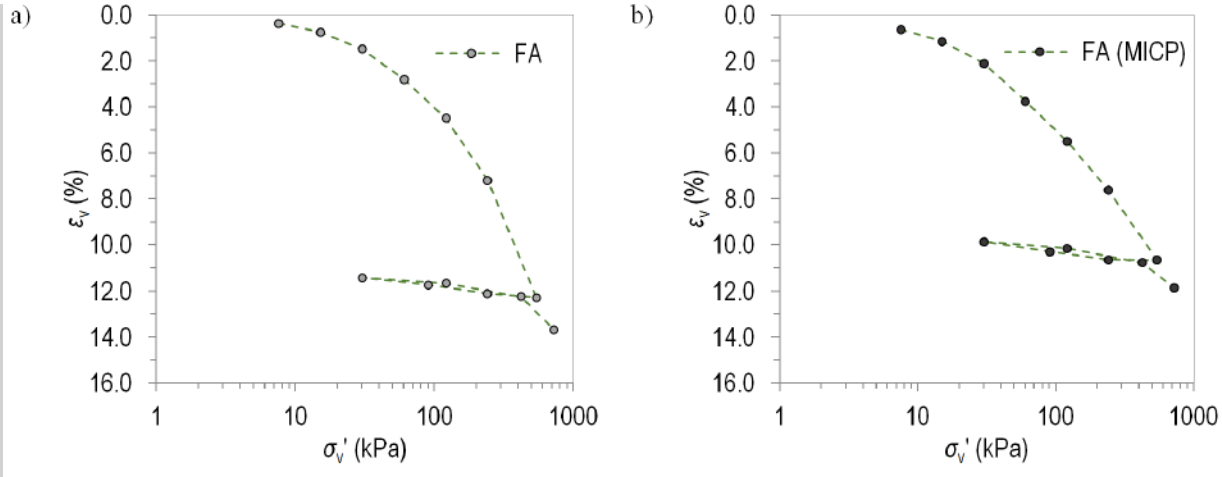


Figure 4.
Results of oedometric tests of: a) untreated fly ash (FA), b) fly ash (FA) improved with the MICP method

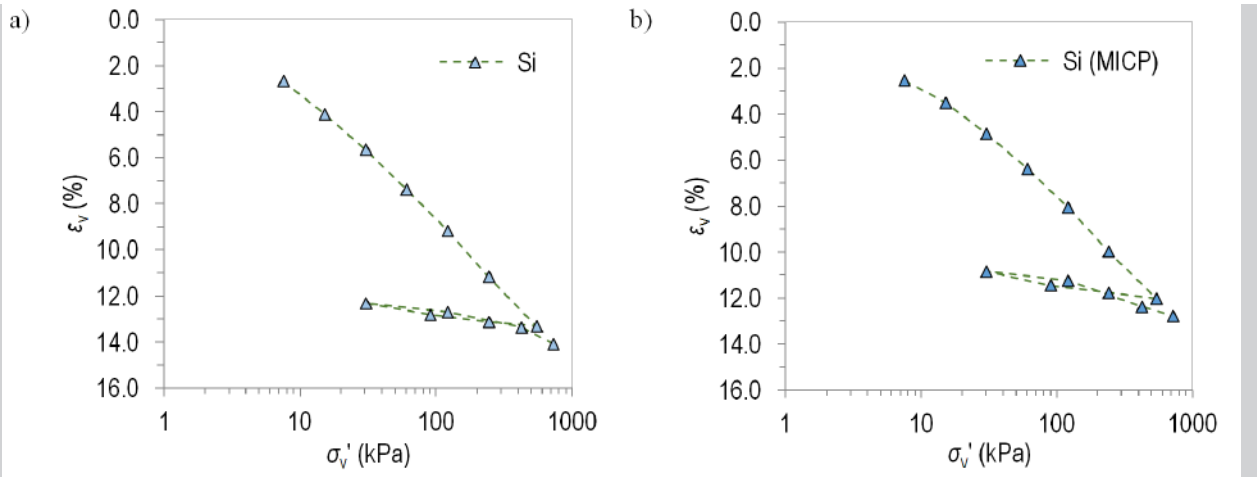


Figure 5.
Results of oedometric tests of: a) untreated silt (Si), b) silt (Si) improved with the MICP method

observed in the Si sample (1.30%). For the FSa sample, the difference in vertical deformation at the end of the test was equal to 1.47%.

Presented results of preliminary tests of samples prepared using premixing as a method of sample preparation, showed that the MICP method improved tested soils in terms of compressibility. The method is based on calcite precipitation and forms bonds between particles. That is how other researchers have obtained biocemented soils. In most cases, these soils were sandy soils, and the injection method was used as an introduction of bacteria and cementation solution into the soil. Additionally, a few injections of cementation medium were applied in time intervals, to achieve a higher level of biocementation. This resulted in the formation of rigid samples, which, before the treatment, were loose soils. According to that, in most of the research, the unconfined compressive strength is tested [13, 33]. However, the MICP method can also improve soils by filling pore voids with precipitated $CaCO_3$ [35]. During the oedometric test, the application of the load on the sample might cause the breakage of the bonds. Additionally, samples were tested with the presence of water, and, as [53] observed, the biocement might be dissoluble.

4. CONCLUSIONS

Based on the presented test results and experience collected during the preparation of the treatment and samples, the following conclusions might be drawn:

1. The mixing method, as a method of introducing bacterial and cementation solution to the soil, is effective, although the biocementation level might be lower than for the injection method. It is caused by the fact, that during the injection method, more cementation solution is added to the sample, so the biocementation level is higher. Further research on the mixing method is planned to achieve a higher level of biocemenation, which is dependent on a few factors, such as OD_{600} of the bacteria solution, the concentration of the cementation solution and retention time.
2. Compressibility of fine sand, silt and fly ash was reduced by biocementation. The highest decrease in the relative vertical deformation was observed for FA, where the final value of vertical deformation decreased by 1.82% compared to the value in untreated material. In treated FSa and Si samples, the decrease in ε_v value was respectively 1.47% and 1.30%.
3. Preliminary research showed that the biomineralization method, as a method for soil improvement, may be applied not only to coarse-grained soils such as sand, but also to fine-grained materials, like silt and fly ash.

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