Sigma J Eng & Nat Sci 37 (3), 2019, 953-965

Sigma Journal of Engineering and Natural Sciences
Sigma Mühendislik ve Fen Bilimleri Dergisi

Research Article

THE EFFECTS OF DIFFERENT SOURCES OF CALCIUM IN IMPROVEMENT OF SOILS BY MICROBIALLY INDUCED CALCITE PRECIPITATION (MICP)

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Received: 23.06.2019 Revised: 16.08.2019 Accepted: 26.08.2019

ABSTRACT

Several soil improvement techniques are successfully implemented today. Studies are increasingly becoming popular on microbially induced calcite precipitation (MICP) as an environment-friendly and sustainable method that is an alternative to other soil improvement techniques. Our study examined the effects of different sources of calcium on an improvement that was carried out on a sand soil with a 35% relative density by using the MICP method. The results were analyzed by using unconfined compression, permeability, calcite formation percentage, pH, SEM (scanning electron microscopy) and XRD (x-ray diffraction) experiments. As a result of the unconfined compression test, the strength values were obtained as 2406 kPa in the specimens where calcium chloride was used as a calcium source, 2435 kPa in the specimens where calcium nitrate was used and 64 kPa in the specimens where calcium acetate was used. The permeability experiment revealed a decrease in permeability of 80.8% for calcium chloride, 23% for calcium nitrate and 90.4% for calcium acetate. According to the results of the SEM analyses, the structures that formed in all specimens bound the grains to each other and coated the surfaces of the grains. In the XRD analyses, calcite formation was observed in the specimens where calcium chloride and calcium nitrate were used as the source, while, in contrast, vaterite formations were also observed in the specimens where calcium acetate was used as the source. It was determined that different sources of calcium had different effects on improvement of sand soils by microbially induced calcite precipitation (MICP).
Keywords: MICP, microbially induced calcite precipitation, bio-cementation, bio-clogging, calcium source, soil improvement.

Abbreviations

$ dollar
rDNA Ribosomal DNA
cfu Colony forming unit
ml Milliliter
g Gram
M Molarity

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mg Milligram
µm Micrometer
mmMillimeter
e Void ratio
s Second
kPaKilopascal
Cu Uniformity coefficient
Cc Coefficient of curvature
D_{10} 10% of the particles are finer than this size
D_{30} 30% of the particles are finer than this size
D_{60} 60 % of the soil particles are finer than this size
pH Potential of hydrogen
rpm Revolution per minute
SP Poorly graded sands
USCS Unified soil classification system
HCL Hydrochloric acid
UCS Unconfined compressive strength
SEM Scanning electron microscopy
XRD X-ray diffraction

1. INTRODUCTION

Cements and chemicals have been frequently used as binding agents in improvement of soils, and increases in shear strength and decreases in water permeability have been achieved. However, usage of cements and chemicals in soil improvement is costly and time-consuming. Additionally, there is also a need for more inexpensive soil improvement methods to be able to apply disaster mitigation precautions in larger areas with a limited budget. For example, as seen in the earthquake disasters in New Zealand and Japan, liquification has been defined as a great threat for a long time. However, soil work that is carried out to reduce the risk of liquification is conducted in prioritized structures such as seaports and airports. The reason for this is that soil work that is conducted to prevent liquification has high costs. If a more inexpensive method that will reduce liquification can be developed, larger-scale anti-liquification soil work may be carried out, and thus, the harmful effects related to liquification may be reduced. The need for new and sustainable construction materials is undeniable in terms of reducing the usage of chemicals and cement in soil improvement and minimizing costs [1].

A biological technique for soil improvement, MICP (Microbially Induce Calcite Precipitation), emerged as a new approach to soil improvement techniques. Although studies where the MICP technique is used are increasing in numbers today, there are also earlier studies that were carried out on this topic [2-5].

With these studies, it was aimed to improve soils as a result of some chemical and biological processes involving bacteria. While these processes may occur spontaneously in nature, they may also be achieved artificially by using biological and chemical mediators. This technique also has several advantages over existing soil improvement techniques. The cost of the microbial soil improvement technique ($\$0.5\text{-}9/m^3$) was reported to be much lower than that of chemical injection ($\$2\text{-}72/m^3$) [6]. As microorganisms are abundant in nature and can be inexpensively reproduced, this is a sustainable technique. With microbial biotechnology, it will be possible to apply some existing construction processes in a simpler way. For example, bio-cement may be in a liquid or solid form. In the liquid form, the bio-mortar has lower viscosity and can flow like water. For this reason, in comparison to cement and chemicals, distributing it into soil is easier. Furthermore, while it may take weeks to reach complete strength when cement is used, the reaction time may be shortened if needed when bio-cement is used [1].

MICP is achieved as a result of a set of reactions. This technique involves formation of ammonium and carbonate ions as a result of hydrolysis of urea by the urease enzyme after addition of aerobic bacteria with high urease production capacity into the soil. The chemical reaction in this process is shown in equation 1.

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

In the presence of a calcium source (usually calcium chloride, $CaCl_2$), as seen in the chemical reaction presented in equation 2, calcium carbonate (CaCO3, calcite) is formed along the soil matrix [7].

$$Ca^{2+} + CO_3^{2-} \Rightarrow CaCO_{3\ (s)} \quad (2)$$

The species of bacteria that are used are usually those that have high urease production capacity [8]. In the literature, most studies on calcium carbonate formation utilized the bacterium *Bacillus pasteurii* (*Sporosarcina pasteurii*) [9-16]. There are also studies which were conducted by using different microorganisms other than this species [17-26].

In the calcium carbonate formation reaction, the quantity of the mass where urea activity will take place is also an important factor. Urea activity may differ in the same environmental conditions with different species of bacteria [15]. In contrast to the literature, our study used the bacterium *Viridibacillus arenosi*.

Different sources of calcium are also a parameter that is effective on improvement. Choi et al. [27] aimed to reduce costs by using a different source of calcium in their study. Zhang et al. [28] used different calcium sources in their study and investigated how these affected the process of soil improvement and the structures that formed as a result. In our study, in difference to the literature, using a different species of bacteria and a different implementation method, the effects of different sources of calcium on improvement were determined on a sand soil.

2. MATERIAL AND METHOD

2.1. Material

2.1.1. Bacteria

For the calcium carbonate formation reaction to be possible, we used an isolate of Viridibacillus arenosi K64 (GenBank ID:KR873397) which is known to have a high urease activity, take a role in precipitation and is a Gram-positive species that can precipitate calcium carbonate under in vitro conditions. The isolate was previously obtained from caves and found to create calcium carbonate precipitation. The bacterium was identified by using 16S rDNA sequencing analyses. This was achieved at the Molecular Biology and Bacteriology Laboratory at the Department of Biology at Atatürk University Faculty of Science. Bacterium density was found by spectrophotometry analysis as $1x10^9$ cfu/ml.

2.1.2. Broth Medium and Treatment Solution

In the study, we used the broth medium that was used in the study by Li [29] and contains Tryptic Soy Broth: 30 g/L, urea: 20 g/L, $MnSO_4 \bullet H_2O$: 12 mg/L and $NiCl_2 \bullet 6H_2O$: 24 mg/L. As the treatment solution, 1.5 M of urea and 0.75 M of calcium source (calcium acetate, calcium nitrate, calcium chloride) were used. While preparing the broth medium and treatment solution, all chemicals except urea were sterilized in an autoclave. Urea was added to the mixture by passing through a 0.45-µm filter. The materials were measured so that the mixture would have a total volume of 1 L and combined in an aseptic environment.

2.1.3. Soil

As the soil, the silica sand whose granulometry is shown in Figure 1 was preferred. Table 1 shows the index characteristics of the soil. The soils were placed into unconfined compression experiment molds at 35% relative density. According to the Unified Soil Classification System (USCS), the soil that was used in the experiments was found to be poorly graded (SP) soil.

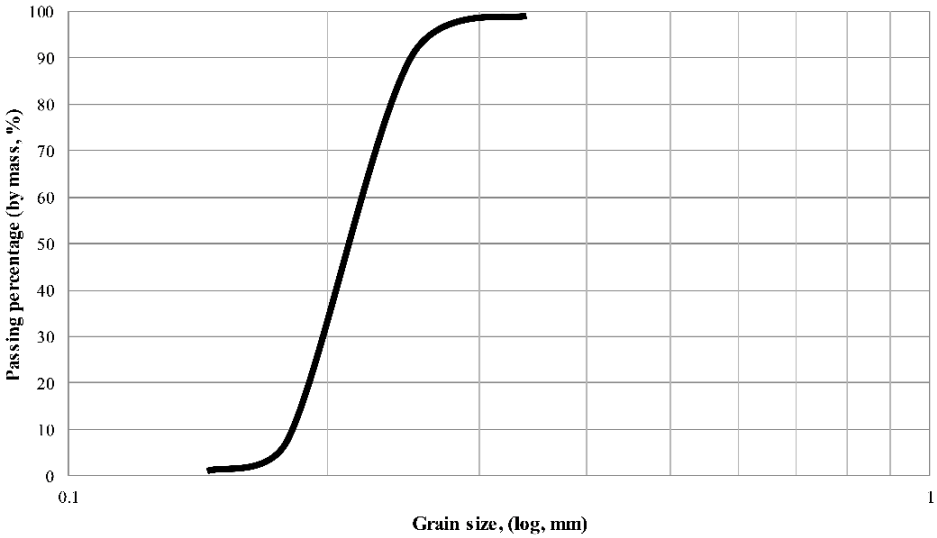

Figure 1. The granulometry curve of the sand soil that was used in the experiments

Table 1. The index characteristics of the SP soil that was used in the experiments

	Sand Soil
Specific gravity [a]	2.65
e_{max}	0.86
e_{min}	0.61
Relative density (%)	35
D_{10} [b]	0.18
D_{30} [b]	0.2
D_{60} [b]	0.22
Uniformity coefficient, Cu [b]	1.22
Coefficient of curvature, Cc [b]	1.01
Soil classification (USCS)	SP

[a]ASTM D854-14, [b]ASTM D2487-11

2.2. Method

2.2.1. Treatment Method

The broth medium that was prepared was firstly sterilized, and the bacteria were inoculated into the medium under aseptic environmental conditions. The medium inoculated with bacteria was incubated in a shaker for 48 hours at 120 rpm, and it was made ready for soil treatment. The soils were placed into PVC molds with a diameter of 38 mm and height of 150 mm at a relative

density of 35%. Filters were placed to the bottom and top of the specimens. While treating the soil, firstly the culture medium, and after 24 hours, the stock solution were introduced from above with silicone hoses using peristaltic pumps at a rate of 0.40 ml/s for 10 days, and the fluid was filtered from below by the effect of gravity. When clogging started, the overflowing part of the fluid was transferred to the container kept below via silicone hoses. This why, through circulation, it was aimed for the culture medium and/or stock solution (calcium source + urea) to reach every part of the soil. The experimental setup is shown in Figure 2.

Figure 2. Experimental setup

2.2.2. Unconfined Compression Test

Unconfined compression tests were carried out on the soils that were subjected to improvement. These experiments were achieved based on ASTM D2166.

2.2.3. Measurement of the Seepage Rate

Chu et al. [30] used seepage rate to analyze the reduction in permeability as a result of biological improvement. The permeability test was carried out based on the principle of constant level of permeability in the PVC molds that were prepared for the treatments instead of the standard molds that were reported previously [31]. Additionally, the permeability coefficient of the untreated sand soil was determined by a constant-head permeability experiment.

2.2.4. Measurement of Calcium Carbonate Contents and pH Analysis

Calcite content was determined by using the method of weight loss after acid treatment. There are several different methods for determining calcium carbonate rates. Selection of this method was due to its easiness and having the apparatuses at hand [32]. In this method, 5 g of the specimen is mixed with 20 ml 1 M HCL acid. Then, the dissolved particles are washed with

distilled water for 10 minutes with filter paper at mesh no 200. All soluble calcium sources are washed out with the washing process. The remaining soil particles are sieved again and weighed after drying. The weight difference between the first specimen (A) and the specimen that is washed and dried (B) provides the weight of the calcium carbonate. The calcium carbonate ratio is then calculated with the following formula [33].

Calcium carbonate percentage (%) = 100 - (B/A) x 100 (3)

The calcium carbonate ratio in the untreated specimen was found as 0.36. During the treatment, pH was measured at intervals of 24 hours, and the results were analyzed.

2.2.5. SEM (Scanning Electron Microscopy) Analysis

The structures that formed as a result of soil improvement were analyzed by using the SEM (Scanning Electron Microscopy) Quanta 450 FEG device at the laboratory of Erzincan Binali Yıldırım University.

2.2.6. XRD (X-Ray Diffraction) Analysis

To determine the structures before and after improvement, the Empyrean Series 2 X-ray Diffraction System device at the laboratory of Erzincan Binali Yıldırım University was utilized. The diffraction angle was selected as 2theta.

3. RESULTS AND DISCUSSION

3.1. Unconfined Compression Test Results

The images of the improved sand soil and the specimens obtained after the unconfined compression experiment are shown in Figure 3, while Figure 4 shows the plot of the axial stress – axial strain obtained as a result of the unconfined compression test. As a result of the unconfined compression test, the strength values were obtained as 2406 kPa in the specimens where calcium chloride was used as a calcium source, 2435 kPa in the specimens where calcium nitrate was used and 64 kPa in the specimens where calcium acetate was used. The strength results that were obtained showed that different calcium sources affected the treatment process differently. While calcium nitrate and calcium chloride provided similar results, a much lower strength value was obtained by applying calcium acetate. Zhang et al. [28] investigated the effects of different calcium sources on strength by using the *Sporosarcina pasteurii* bacteria. According to the results of the UCS (unconfined compressive strength) test, as the source of calcium, calcium acetate provided better results than the other sources. However, the same chemical provided the lowest strength value in our study. It is believed that these contradicting results were caused by different treatment methods and usage of different bacteria.

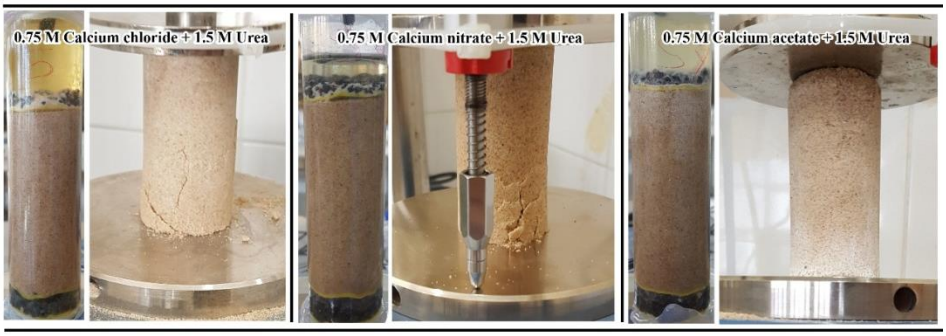

Figure 3. The specimens that were treated and their images that were obtained at the end of the unconfined compression test

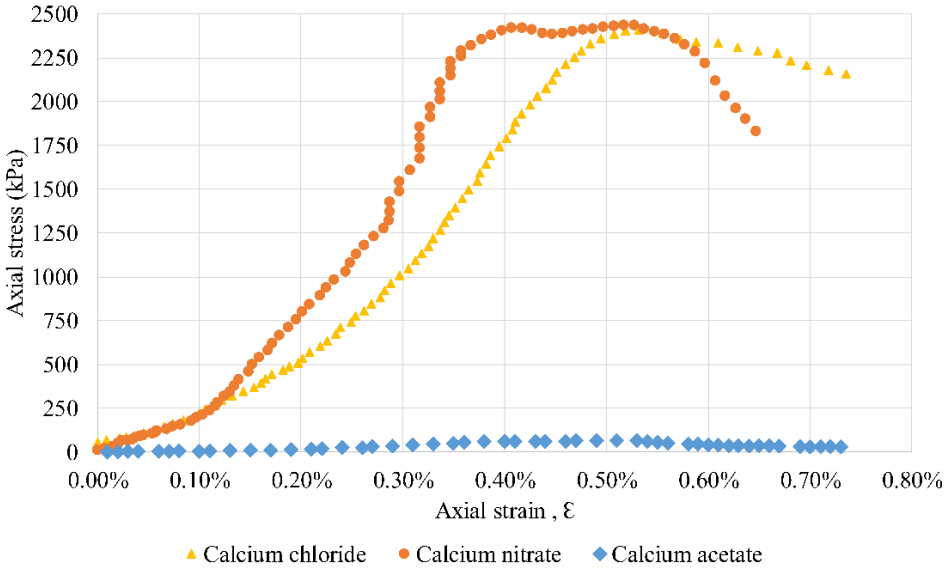

Figure 4. The axial stress – axial strain plot obtained as a result of the unconfined compression test carried out on the specimens that were improved

3.2. Permeability Test Results

According to the fixed level permeability experiment results, the permeability coefficient of the untreated sand soil was determined as 0.01375 cm/s. Figure 5 shows the percentage decreases in the permeability values of the improved specimens. The permeability experiment revealed a decrease in permeability of 80.8% for calcium chloride, 23% for calcium nitrate and 90.4% for calcium acetate. While similar values were obtained for the specimens where calcium chloride and calcium acetate were used as the sources, calcium nitrate provided a much lower reduction value. Consequently, it is believed that, in cases where it is aimed to reduce permeability, 1.5 M of urea and 0.75 M of calcium acetate should be used as the treatment solution.

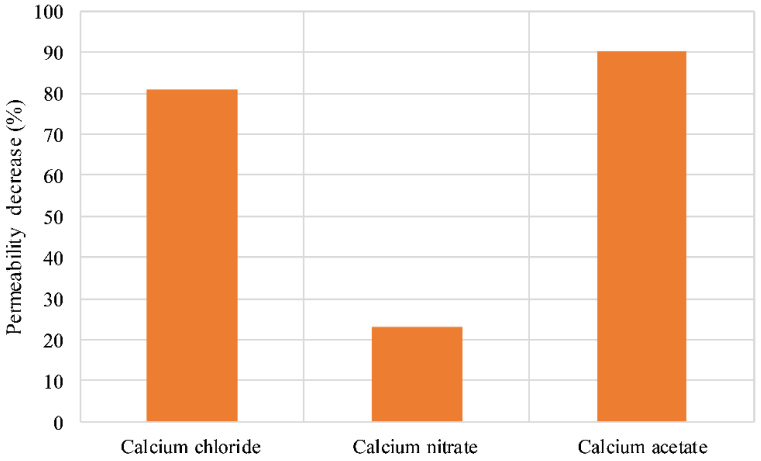

Figure 5. Percentage permeability decreases in the sand soils that were improved

3.3. Measurement of Calcium Carbonate Contents and pH Analysis Results

The sand soils that were improved were divided into three sections, and their calcite ratios were determined. These ratios are shown in Figure 6. The mean percentage of calcite that formed was the highest in calcium nitrate, followed respectively by calcium chloride and calcium acetate. Similarly, the results of the unconfined compression test revealed higher strength values in the specimens treated with calcium nitrate and calcium chloride, whereas much lower strength values were obtained with calcium acetate. However, the decrease in permeability in the specimens that were treated with calcium acetate was greater than those achieved with the other calcium sources. In their study, Cui et al. [34] examined calcite crystals that formed and divided them into two categories as effective and ineffective calcite crystals. They described the calcite crystals that bound grains of sand to each other as effective and those that clung onto the surface of the grains as ineffective. They emphasized that effective calcite crystals have a significant effect on the expected increase in the strength of a specimen. In the specimens where calcium nitrate and calcium chloride were used, calcite crystals connected the grains to each other and increased the strength values. On the other hand, as ineffective calcite crystals, the crystals obtained with calcium acetate were effective on permeability rather than strength.

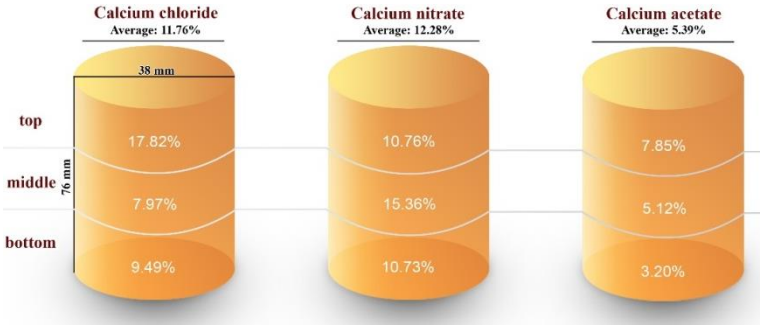

Figure 6. Calcite percentage analysis results

During the treatment process, pH measurements were made, and the results are shown in Figure 7. Moravej et al. [35] determined that pH values continuously decreased as a result of bacterial calcite precipitation and biochemical reactions. They reported that the rate of decrease in pH was fast due to formation of calcite particles, but it slowed down in time. Considering that the pH value in our study usually dropped along the treatment process, it may be understood that calcite formation continued throughout the process.

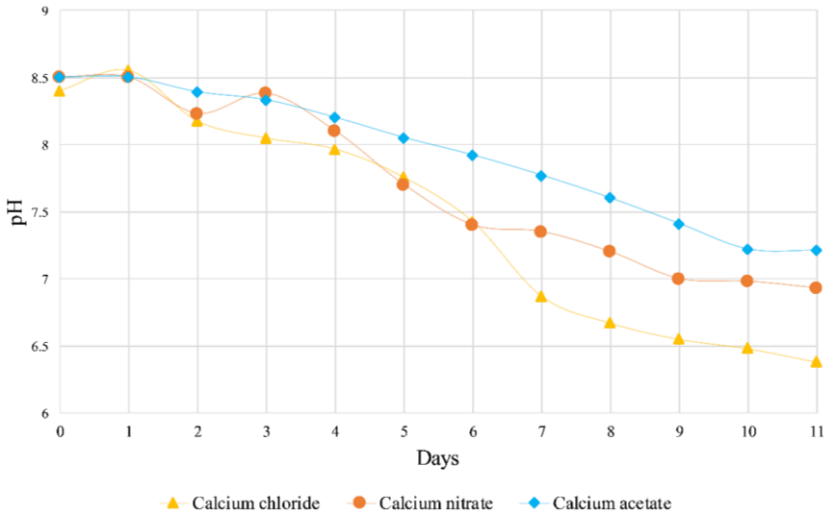

Figure 7. pH analysis results

3.4. SEM (Scanning Electron Microscopy) Analysis Results

Figure 8 shows images of the SEM analysis that was carried out on the soil specimens obtained as a result of improvement. The calcites that formed in all soil specimens treated with the calcium sources usually filled the gaps in the grain and coated their surfaces. Information on the composition of the structures that formed between grains and on surfaces is given in the XRD analysis results.

3.5. XRD (X-Ray Diffraction) Analysis Results

Figure 9 shows the results of the XRD analysis that was carried out on the treated and untreated specimens. In the comparison of the XRD results of the untreated soil and the soil that was treated with calcium chloride, an increase in the peaks on the plot may be observed. As a result of the analysis, these peaks were determined to show calcite. In the XRD analysis results on the specimen where calcium acetate was applied, calcite formation was observed in similarity to the other sources, but differently, vaterite formation was also observed. Zhang et al. [28] observed calcite and aragonite formation in their XRD analyses as a result of calcium acetate treatment. In contrast, in our study, vaterite formation was determined. It is believed that these different results were caused by the method of treatment and usage of different bacteria. In the specimen that was treated with calcium nitrate, in similarity to the specimen where calcium chloride was used, calcite formations were observed. Kalantary and Kahani [36] conducted a study on biological improvement of soils, and as a result of their XRD analyses, they determined calcite, vaterite and aragonite formations. Our XRD analysis results revealed similar structures.

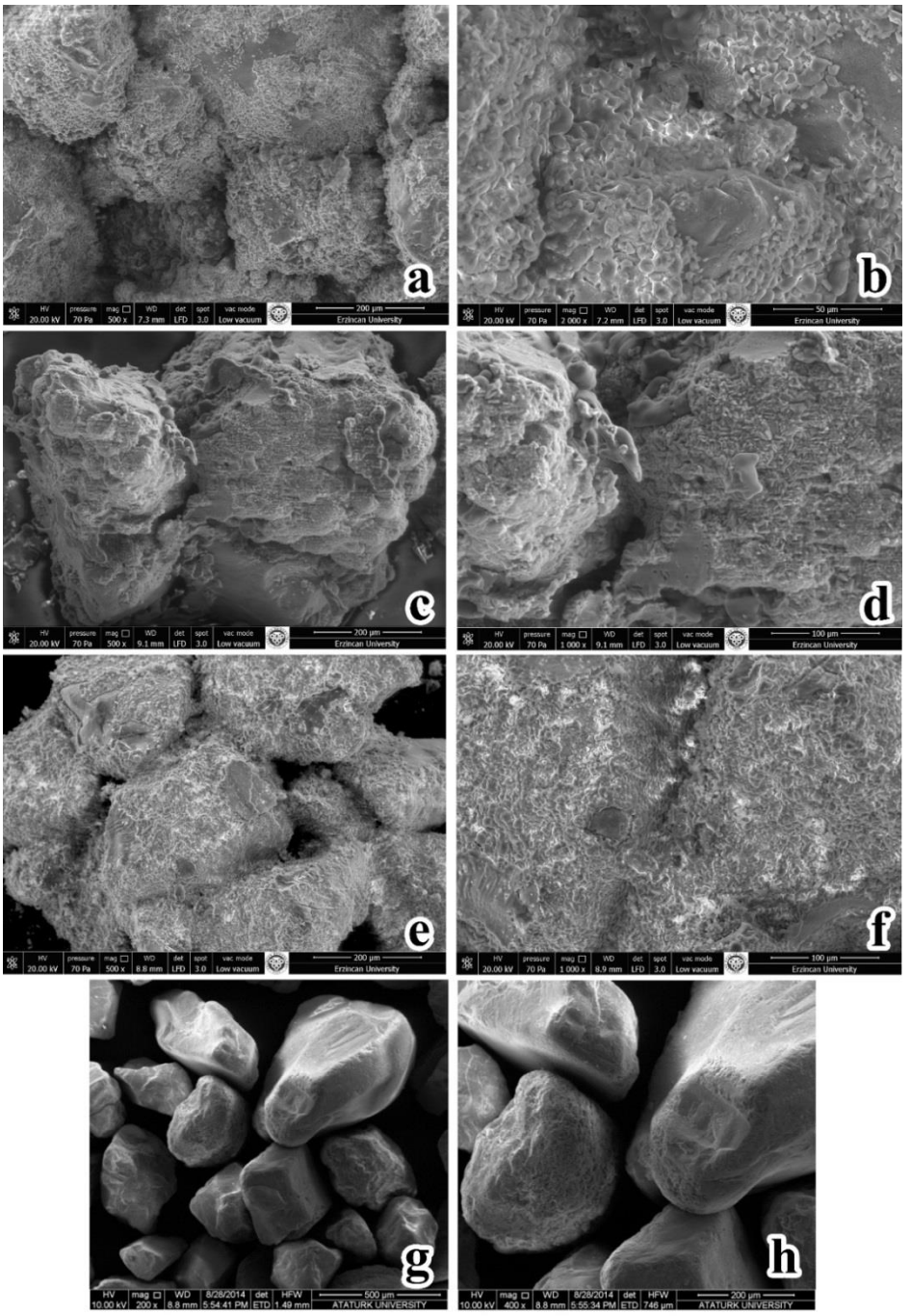

Figure 8. SEM Analysis Results (a, b) Calcium chloride, (c, d) Calcium nitrate, (e, f) Calcium acetate, (g, h) Untreated soil

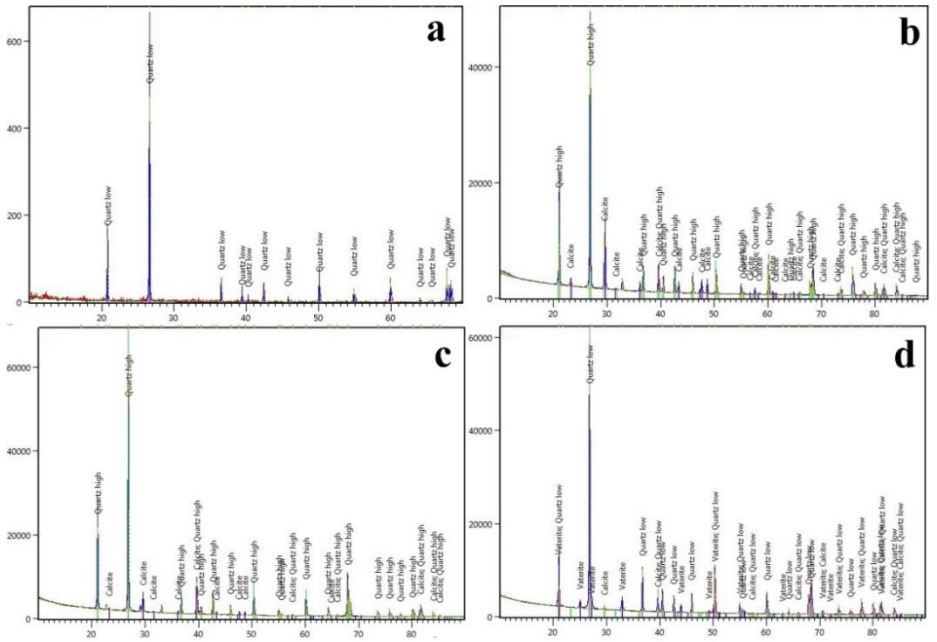

Figure 9. XRD analysis results (a) untreated soil, (b) soil treated with calcium chloride, (c) soil treated with calcium nitrate, (d) soil treated with calcium acetate

4. CONCLUSION

This study investigated the effects of different calcium sources in biological improvement of soils on strength and permeability. As a result of the experiments and analyses that were carried out, it was observed that different sources of calcium provided different results in terms of both strength and permeability. While better results were obtained in the specimens treated with calcium chloride and calcium nitrate in terms of strength, better results were obtained in terms of permeability in the specimens treated with calcium acetate. In the SEM analyses, it was seen that the structures that formed as a result of biological improvement filled the gaps between the grains and connected these grains to each other. In the XRD analysis, it was seen that calcite formation occurred in treatments with all calcium sources in the study, while additional vaterite formation was observed for calcium acetate. The highest mean calcite percentages were found in the specimens treated with calcium chloride and calcium nitrate, which was similar to the results on strength values. In calcium acetate, the mean calcite percentage was lower, as in the case of the lower strength values it provided. Decreases in pH were observed in all specimens throughout the treatment process, and this indicated that the reaction continues through the improvement procedure. Considering that the cost of calcium nitrate is higher, and calcium acetate and calcium chloride had similar percentage permeability decrease results, using calcium chloride may provide better results in the process of reducing permeability and obtaining better strength values. Better results may be obtained in studies to be conducted with different treatment methods, molarities and bacteria species.

Acknowledgements

The study was produced based on the doctoral dissertation study carried out at the School of Sciences at Atatürk University titled Investigation of the Effects of Some Variables in Biological

Improvement of Soils by Using Microbially Induced Calcite Precipitation. The dissertation study was carried out with the support provided by the Scientific Research Project coded 2019/6880 at Atatürk University. We would like to thank Atatürk University for the support and their contributions.

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