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ORIGINAL ARTICLE

Investigation of the Consolidability of Different Limestones Used in Historical Buildings by Using *Myxococcus xanthus* and *Bacillus subtilis*

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Abstract
Natural stones have been used as basic building materials in many of the historical buildings and monuments that are cultural heritage from past to present. Limestones are the most preferred material group in many historical buildings and monuments in our country due to their aesthetic appearance and ease of workability. However, these stones are more easily affected by atmospheric conditions compared to other natural stones and gradually degrade over time due to physical, chemical, and mechanical effects. In order to make these Stones more resistant to degradation, there is a need to improve or reinforce their in-situ properties. Microbial consolidation is a new approach developed to recreate a structure similar to the original microstructure of a stone in order to achieve maximum compatibility in the consolidation of the stone. In this study, the effects of the microbial consolidation method on stones were investigated by using *Myxococcus xanthus* and *Bacillus subtilis* strains before and after the application of the mercury porosimetry test, mineralogical and petrographic analysis, color measurements, and scanning electron microscope (SEM) analysis. At the end of the study, total porosity values of Kufeki stone, Sazlıbosna stone, and Suloglu stone samples showed percentage decreases between 14–83%. Mineralogical, petrographic, and scanning electron microscopy (SEM) analyses revealed that calcium carbonate precipitation occurred in all stone samples. Bacterial cells completely covered the pore walls of the stones after the calcium carbonate precipitation. Bacterial cells are attached to the calcite grains without blocking the pores. The promising results obtained on the stone samples after the application of the microbial consolidation method showed that the use of bacteria offers potential as a new intervention method for the conservation of porous limestones sensitive to atmospheric conditions.

Keywords Bacterial biomineralization · Stone consolidation · *Myxococcus xanthus* · *Bacillus subtilis* · Calcium carbonate precipitation

Introduction

Turkey, home to various civilizations, has numerous structures and artifacts bearing traces of its history. In order to preserve architectural and cultural history, it is crucial that these buildings and artifacts that have survived to the present day be preserved, repaired, and passed on to future generations. Many cultural, artistic and historical buildings and objects are made of natural stones. Carbonated stones, such as limestone, are the most widely used material in heritage buildings. These stones were frequently used in arches, columns, body walls, dome construction, flooring and exterior garden walls of historical monuments and buildings. Factors such as air pollution, temperature changes, freeze–thaw and crystallization-dissolution of water-soluble salts (sulfate, nitrate, etc.) cause limestones to degrade over time. Pore size and pore distribution play an important role in

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the ability of the material to resist moisture-induced degradation. Carbonate stones such as limestone are especially more easily affected by atmospheric conditions than other natural stones due to their porous structure, microvoids, and interrelated microcracks. In addition to these, earthquakes, other natural disasters, and rapid urbanization in our country and the fact that historical buildings and the stones used in the facades of these buildings are under great threat against these adverse conditions have led to the need to protect the stones by strengthening them in place. In this context, Küfeki stone is an important building material frequently used in many historical structures in Istanbul, especially in the works of Mimar Sinan. Küfeki stone is used in both load-bearing and decorative elements in the fundamental structures of Ottoman architecture, such as the Sultanahmet Mosque, the Süleymaniye Mosque, the Rüstem Paşa Mosque, and the Topkapı Palace. However, the lack of access to the original quarries where this stone was extracted today necessitates the in-situ preservation and consolidation of existing stones. Additionally, with limited use in historical structures, the Sazlıbosna and Süloğlu quarries are currently preferred over Küfeki stone in restoration applications. Scientific studies on the consolidation potential of these stones are quite limited; in particular, their evaluation within the scope of conservation strategies developed using microbial methods remains a significant gap in the literatureConsolidation should be such that it can repair the binder cement of the natural stone that has weathered or been weakened by surface erosion, improve the cohesion in the mineral composition of the stone, and connect the weathered surface to the intact part of the stone (Torraca 1988; Ginell et al. 2001, Henry 2006; Ersen 2011; İpekoğlu 1998).

The performance criteria expected from the reinforcements used for the consolidation of natural stones are that they penetrate the stone well, protect the stone from degradation caused by atmospheric conditions, do not clog the pores of the stone, do not increase the microvoids of the stone, don't cause a negative change in the color and texture of the stone, don't react chemically, don't form water-soluble salts as by-products, do not adversely affect water vapor permeability, and are economical and environmentally friendly. Considering the principles specified in the Venice Regulation, such as minimum intervention, recyclability, and re-applicability of conservation practices, it is extremely important to reinforce the carbonated stones, which are frequently used in historical buildings and monuments, with a repair material with new technology compatible with itself, in order to preserve the original and aesthetic values of historical buildings and monuments that are cultural heritage and to ensure their sustainability at the same time. In recent years, the increasing interest in innovative green technologies in the field of cultural heritage has led to the development of new methods, such as bioconsolidation.

Microbial calcium carbonate ($CaCO_3$) formation is commonly referred to as microbiologically induced carbonate precipitation (MICP), which occurs in soil, water, and marine sediments. But in the process, the concentration of dissolved inorganic carbon in the medium and the pH of the medium increase (De Muynck et al. 2010).

Calcium carbonate precipitation occurs with the following relation (1).

$$Ca^{2-} + CO_3^{2-} \leftrightarrow CaCO_3 \tag{1}$$

Carbonate ions are produced in water by the following equilibrium reactions (2,3) (Stumm and Morgan 1981).

$$HCO_{3-} \leftrightarrow CO_3^{2-} + H^+ \tag{2}$$

$$HCO_{3-} + OH^- \leftrightarrow CO_3^{2-} + H_2O \tag{3}$$

For microbial calcium carbonate formation to take place, microbial processes such as urease enzyme, ammonification, nitrate, and sulfate reduction are involved. In the literature, urease enzyme and ammonification are the processes commonly used in the biological consolidation of limestones. The *M. xanthus* bacteria used in the study perform ammonification, and *B. subtilis* bacteria hydrolyze urea to form calcium carbonate.

Ammonification is the formation of NH_3 by the breakdown of proteins in various organic wastes, dead plants, and animal remains. While NH_3 formed in this process increases the pH of the environment, CO_3 (carbonate) ions and Ca^{2+} ions combine and precipitate as crystals. *M. xanthus,* which provides crystal formation by this mechanism, breaks down amino acids in the environment and forms NH_3 and CO_3 ions. The *M. xanthus* bacteria penetrates deeper and carries the newly produced calcium carbonate to the pore system, healing and strengthening the stones for a long time. A large number of bacteria, if in an environment with calcium and urea, perform significant mineralization of calcium carbonate ($CaCO_3$: calcite, aragonite, vaterite) (Spanos and Koutsoukos 1998; Al-Thawadi 2011; Pıñar et al. 2010; Yildirim et al. 2016).

Urea Hydrolysis: Urea undergoes a biochemical reaction with the urease enzyme produced by the alkalophilic microorganism. As a result of this reaction, 1 mol of ammonia (NH_3) and 1 mol carbamic acid (NH_2COOH) are formed (4). These substances are spontaneously transformed into 1 mol of ammonium $(NH_4)^+$ and 1 mol of carbonic acid (H_2CO_3) (5). The products can be easily decomposed and transformed into $H2CO_3$, HCO_3^- and H^+ ions (6). Ammonia (NH_3) combines with water (H_2O) and decomposes into

2 mol of ammonium $(NH_4)^+$ ions and 2 mol of hydroxide $(OH)^-$ ions (F.7). At the end of these processes, the pH of the medium increases, and Ca^{2+} ions in the medium combine with the negatively charged bacterial cell (F.8) In alkaline environments, dissociated $(HCO_3)^-$ ions also combine with the formed structure and precipitate (9). (Hall-Stoodley et al. 2004; Patro et al. 2015; Yıldırım et al. 2016).

B. subtilis bacterial strain provides crystal formation by this mechanism.

$$CO(NH_2)_2 + H_2O \longrightarrow NH_2COOH + NH_3 \quad (4)$$

$$NH_2COOH + H_2O \longrightarrow NH_3 + H_2CO_3 \quad (5)$$

$$H_2CO_3 \longrightarrow HCO_3^- + H^+ \quad (6)$$

$$2NH_3 + 2H_2O \longrightarrow 2NH_4^+ + 2OH^- \quad (7)$$

$$Ca^{2+} + Cell \longrightarrow Cell - Ca^{2+} \quad (8)$$

$$Cell - Ca^{2+} + CO_3^{2-} \longrightarrow Cell - CaCO_3 \quad (9)$$

In recent years, bacterial-induced carbonate mineralization has been proposed as an environmentally friendly method for preserving weathered carbonate rocks. (Adolphe et al. 1990; Castanier et al. 2000; Orial et al. 1993; Tiano et al. 1999). This new preservation method mimics a natural process that has been occurring in nature for a very long time, as many carbonate rocks are cemented by calcium carbonate precipitation triggered by microorganisms (Buczynski and Chafetz 1991; Folk 1993; Rodriguez-Navarro et al. 2003). In this study, the term "environmentally friendly" refers to a consolidation approach based on natural biological processes. Unlike traditional chemical consolidators, microbial consolidation promotes calcium carbonate precipitation through bacterial metabolism, minimizing the use of chemicals that could cause environmental pollution or be incompatible with historical materials. Furthermore, because the resulting calcium carbonate has a composition similar to the original mineralogical structure of the stone, the applied method demonstrates high compatibility with the stone material. Therefore, this method is considered a sustainable, environmentally friendly, and structurally compatible conservation technique for the preservation of historical stone materials.

This study investigates the microbial consolidation capability of *M. xanthus* and *B. subtilis* bacterial strains in limestones of varying characteristics commonly used in historical buildings and monuments in Istanbul, utilizing their naturally occurring carbonate precipitation properties. Simultaneously, this research aims to validate the biomineralization process as a consolidation method for porous limestones. Several studies in the literature have investigated the bacterial consolidation capability of historical limestones. However, this study examines the microbial consolidation potential of Küfeki stone, commonly used in historical buildings in Istanbul, and Sazlıbosna and Süloğlu limestones, which are sometimes preferred as alternatives in restoration applications. The limited access to the original quarries from which the Küfeki stone is extracted today increases the importance of scientific studies on the in-situ preservation of existing building stones. In addition, the consolidation effectiveness of two different bacterial strains (*M. xanthus* and *B. subtilis*) was evaluated comparatively in this study. Furthermore, the effects of microbial treatment on the pore structure, mineralogical composition, and microstructure of the rocks were investigated using a holistic approach that employed multiple analytical methods, including mercury porosimetry, petrographic examinations, XRD, SEM, and color measurements. This multifaceted evaluation provided a more comprehensive understanding of the changes in rock microstructure induced by the microbial consolidation process.

Material and Methods

Material

Stone Materials and Their Geological Characteristics

This study examines three different types of limestone with varying characteristics. The first sample group consists of weathered stone specimens from the construction of the Karadeniz Madrasa, built by Sultan Mehmed II between 1463 and 1470 in the Fatih district of Istanbul. One of the main building stones commonly used in historical structures in Istanbul is the Küfeki stone, also known as "Bakırköy stone." This stone, which is fossiliferous, lumachelite, and mactra limestone, is also known as Bakırköy limestone, mactraite limestone, and lumachelite limestone. These stones are extracted from Neogene (Sarmatian) aged units extending westward from the center of Istanbul to Küçükçekmece. These units: The ground consists of sand, clay, gravel, marl, coarse limestone, and layered limestones, and fossils such as Mactra, Unio, and Melanopsis are commonly found, especially in sandy and carbonate levels (Sayar and Erguvanlı 1954). According to its macroscopic characteristics, the hand sample of Küfeki stone is described as an off-white-colored, thin-medium-thick-layered, hollow-porous, heterogeneous limestone with abundant macrofossil clasts (Fig. 1a,b).



Fig. 1 Limestone samples used in the research; **a**: Karadeniz Madrasah and the place where the Kufeki stone was taken, **b**: Küfeki stone, **c**: Hadimkoy quarry and Sazlıbosna stone was taken, **d**: Sazlıbosna stone, **e**: Süloğlu quarry and Süloğlu stone was taken, **f**: Süloğlu stone

The second sample consists of rock specimens belonging to the Sazlıbosna Formation, known as "Sazlıbosna limestone." These samples were obtained from a quarry located northeast of the Sazlıdere Dam. The Sazlıbosna limestone used in this study was taken from its original quarry and belongs to the Eocene-aged Soğucak Formation. In line with previous studies, the age of the unit has been determined as Middle-Late Eocene. Lithologically, the dominant rock type is reefal limestone. These limestones are generally off-white, light cream, or gray in color and mostly exhibit massive or thickly layered structures. They also contain abundant macrofossils and benthic foraminifera. (İBB DEZİM 2011). The hand sample of Sazlıbosna stone was described as yellowish beige, fine-grained limestone according to its macroscopic characteristics (Fig. 1c,d). The Süloğlu limestones, which constitute the third sample group, were obtained from a quarry located south of the Süloğlu district of Edirne province, on the Süloğlu–Havsa road. The Süloğlu limestones obtained from different quarries within the scope of this study belong to the Oligocene–Miocene-aged Pınarhisar Formation. According to İBB DEZİM (2011), the formation lies on top of the Eocene-aged Soğucak Formation and begins with a 5–6 m-thick limestone level at the base, containing abundant congeria. In this level, lamellibranches, gastropods, and fish teeth are commonly found, with congeria being the main component, while fish molds are observed in the upper levels. The upper sections of the formation contain loosely cemented, lime-cemented sandstones and conglomerates, along with limestones containing oolites, oncolites, and abundant macrofossil shells, as well as claystone and marl units. The depositional environment is defined as a shallow marine coastal and lagoon environment.. The stone samples forming this group are very similar to Bakırköy Küfeki stone in terms of abundant fossils and appearance. It is known as the Bademli Küfeki or Süloğlu stone (Fig. 1e,f).

Bacterial Strains Used in This Study In the study, *M. xanthus* and *B. subtilis* bacterial strains were used to consolidate the stone samples. The *M. xanthus* bacterial strain is a gram-negative, non-pathogenic, common soil bacterium belonging to the decomposition of proteobacteria. Depending on the chemistry of the culture medium, the *M. xanthus* bacterial strain can induce the formation of phosphates (struvite, schertelite, newberite), carbonates (calcite, Mg-calcite, vaterite), and sulfates (Jimenez-Lopez et al. 2008). *M. xanthus* bacterial strain ATCC 25232 (American Type Culture Collection) was commercially available.

Bacillus genus bacteria are gram-positive, aerobic or facultatively aerobic, spore-forming, rod-shaped bacteria belonging to the family "Bacillaceae", although some species are variable. They are found in soil, water, and various foods, and although they are mostly mesophilic, psychrotrophic and thermophilic species are also known (Ayhan 2000). The diameters of vegetative cells vary between 0.5×1.2 m and 2.5×10 m in diameter. The colony morphology of the Bacillus genus varies. They generally have white or cream-colored colonies. In some species, pigmented colonies in yellow, pink, orange, and black colors are also found (Kuşaklı 2011). *B. subtilis* is a highly diverse bacterial species that can grow in many environments. *B. subtilis* can

be isolated from many terrestrial and aquatic environments, indicating that this species is ubiquitous and widely adapted to grow in different environments in the biosphere (Sonenshein et al. 2002; Ricca et al. 2004; Earl et al. 2008). The *B. subtilis* bacterial strain was obtained from the culture collection maintained in the Aquatic Microbial Ecology Laboratory of Istanbul University, Faculty of Aquatic Sciences.

Since *M. xanthus* and *B. subtilis* bacterial strains have a high ability to produce calcium carbonate, these microorganisms are also called carbonatogenic microorganisms (Cameotra et al. 2012).

Preparation of Media: Solid (nutrient agar) and liquid (nutrient broth) media were prepared to grow the bacterial strains. For the liquid (nutrient broth) medium, 1.6 g of nutrient broth was added to 200 ml of distilled pure water and then mixed with a magnetic stirrer to homogenize the medium. After the medium was prepared, it was distributed into glass closed tubes in a volume of 9 ml and then sterilized in an autoclave. While preparing the solid medium, 5 g of nutrient agar was added to 250 ml of pure water and mixed with a magnetic stirrer. After the nutrient agar was sterilized in an autoclave, it was poured into Petri dishes under aseptic conditions and kept at room temperature until solidified. The solidified media were stored at+4 °C for later use.

Preparation of Bacterial Culture: After the preparation of *M. xanthus* and *B. subtilis* bacterial strains, nutrient agars stored at+4 °C were kept at room temperature before inoculation. The commercially purchased *M. xanthus* bacterial strain was first enriched by inoculation into liquid medium since it was lyophilized and then inoculated into the petri dish under aseptic conditions using the spread plate method. It was then incubated at 37 °C for 24 h. At the end of 24 h, both bacterial strains were found to grow as dense colonies. The *B. subtilis* bacterial strain was placed in a ball containing *B. subtilis* (BioBall Biomérieux, France) medium under aseptic conditions. 200 µl of sterile distilled water was added and waited for 30 s. The medium was spread on a petri dish using a Drigalski spatula and incubated at 37 °C for 24 h. Bacterial suspensions of 1 L at a density of 1×10^8 CFU/mL (colony-forming units per milliliter) were obtained from 24 h cultures of bacterial strains. This was done separately for *M. xanthus* and *B. subtilis* bacterial strains. The prepared flasks were placed in a shaking oven at 100 rpm (rotation speed/min) and 25 °C.

Methods

For the microbial method to be applied using *M. xanthus* and *B. subtilis* bacterial strains in the study, a total of 108 stone samples were prepared in 2*1*1 cm sizes to be used in experiments and analyses. Half of the 108 stone samples were separated to be incubated with the *M. xanthus* bacterial strain and the other half with the *B. subtilis* bacterial strain. Half of the samples used in the study were sterilized, and the other half were used in experimental studies without sterilization. The sterilization process was carried out in order to destroy the bacterial and fungal formations present in the stones. Sterilization of the stones is important in terms of comparing the effects of the microbial method to be applied on the stones with the presence of microorganisms naturally present in the stones and the effects of the microorganisms eliminated on the stones. The sterilization process was carried out with an autoclave device at 120 °C at 1 atm pressure. Two samples each for three different sample groups were set aside as a control group. The stone samples were immersed in culture media prepared separately with *M. xanthus* and *B. subtilis* bacterial strains for incubation for the 7th, 17th, and 27th days without any mechanical stirring. The stone samples were removed from the sterile media in which they were immersed on the 7th, 17th and 27th days and the open porosity (effective) porosity values of the samples were determined with a mercury porosity meter. As a result of the experiment, the best results were obtained in some stone samples at 7 days and in some stone samples at 27 days. In this study, petrographic analyses, X-ray diffractometry analysis (XRD), scanning electron microscopy (SEM) analyses, and color measurements were performed on the stone samples on the days with the best results during the 27-day incubation period, and the results were shared.

The flowchart of the experimental studies conducted within the scope of this research is presented in Fig. 2.

The following section describes the experimental procedures and analytical methods applied to the stone samples.

Petrographic Analyses: Within the scope of petrographic analyses, the mineralogical composition, texture, pore distribution, pore structure, and microcrack characteristics of the samples were identified based on thin-section observations. The TS EN-12407 2002 standard was used as the basis for petrographic analyses. Petrographic analyses were conducted using a Nikon Eclipse 50i POL polarizing microscope.

X-Ray Diffractometry Analysis: The mineralogical composition of the samples was determined by X-ray diffractometry (XRD) analysis, and changes in their mineralogical properties after the application of the microbial method were detected. X-ray diffractometry analysis was performed using a Bruker D8 Discover model, with a Ni filter, at 40 kV-30 microamperes, and a Vantec 500 detector. The measurement range used was 3° b 2θ b 80°.

Mercury Intrusion Porosimetry (MIP) Test: The effective (open) porosity of the stone samples was determined using a mercury intrusion porosimeter. The measurements were conducted in accordance with TS 699 2009 standard.

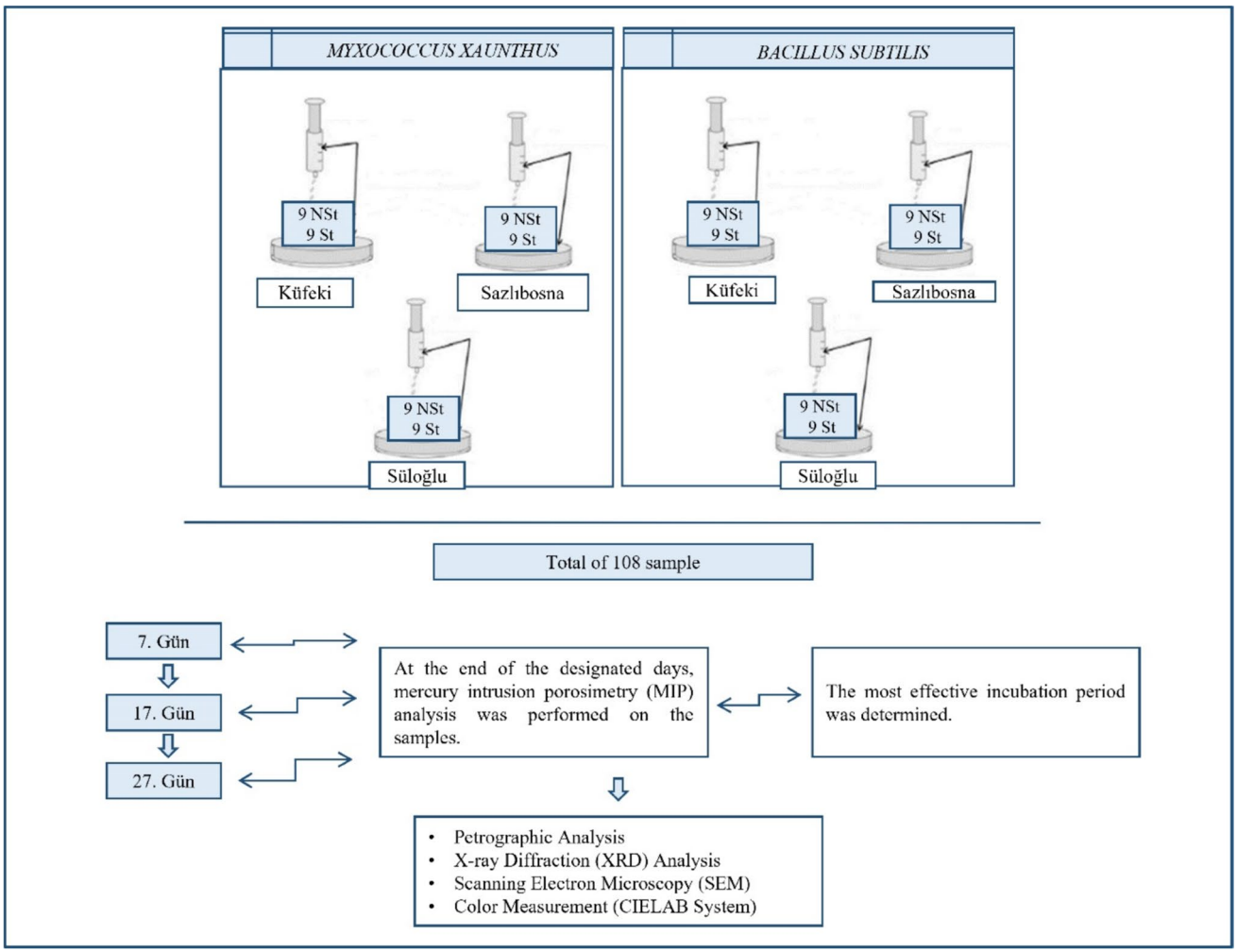


Fig. 2 Flowchart of the analytical procedures performed in this study

All analyses were performed at the Central Research Laboratory of Yıldız Technical University using a Quantachrome Poremaster 60 instrument.

Color Measurement with Spectrophotometer: Color measurements of the samples were performed using a color spectrophotometer based on the CIE L*a*b* color space. The CIE L*a*b* system is an internationally accepted measurement method that aims to numerically represent color. In this analysis, the color changes on the stone sample surfaces after the application of the microbial method were quantitatively evaluated. First, the L*, a*, and b* color values were measured, and based on these values, ΔL (lightness–darkness change), Δa (change along the red–green axis), and Δb (change along the yellow–blue axis) were calculated. These three parameters were then used to determine the total color difference (ΔE), providing a single numerical value that summarizes the visual change induced on the stone surface by the treatment. Measurements were taken from three different regions of each sample surface, and the average of the obtained values was given. Color measurement was performed using a Konica Minolta CM-2600d spectrophotometer.

Scanning Electron Microscopy (SEM) Analysis: Following the application of the microbial method with Scanning Electron Microscopy (SEM) analyses, the pore structure, surface morphological characteristics, and microstructural texture of newly formed calcium carbonate ($CaCO_3$) in the stone samples were examined. Before starting the analyses, a gold plating process was applied to the samples to ensure surface conductivity. SEM analyses were performed using an FEI Quanta 650 FEG scanning electron microscope.

The experiments and analyses on the samples were carried out at the Mimar Sinan Fine Arts University, Cultural Heritage and Artworks Materials Application and Research Center.

The stone samples used in the study were coded according to the regions from which they were taken in order to make the experimental studies more understandable (Table 1).

Table 1 Limestone samples codes used in the study

Sample Code	Explanation
KM-NSt-C	Non-sterile Küfeki Stone Control Sample
KM-St-C	Sterile Küfeki Stone Control Sample
KM-*Mx*	Küfeki Stone Incubated with *M.xanthus*
KM-*Bs*	Küfeki Stone Incubated with *B.subtilis*
H-NSt-C	Non-sterile Sazlıbosna Stone Control Sample
H-St-C	Sterile Sazlıbosna Stone Control Sample
H-*Mx*	Sazlıbosna Stone Incubated with *M.xanthus*
H-*Bs*	Sazlıbosna Stone Incubated with *B.subtilis*
E-NSt-C	Non-sterile Süloğlu Stone Control Sample
E-St-C	Sterile Süloğlu Stone Control Sample
E-*Mx*	Süloglu Stone Incubated with *M.xanthus*
E-*Bs*	Süloglu Stone Incubated with *B.subtilis*

Results

Mercury Porosimetry Analysis

According to the results of the mercury porosimetry experiment on unsterilized stone samples, the open porosity value of the control sample of Küfeki stone was obtained as 2.37%. After the application of the microbial method, the lowest open porosity values were obtained as 1.21% among the samples immersed in the culture medium inoculated with the *M. xanthus* bacterial strain after 17 days of incubation and 2.38% among the samples immersed in the culture medium inoculated with the *B. subtilis* bacterial strain after 7 days of incubation. The open porosity value of the control sample of Sazlıbosna stone was obtained as 6.13%. After the application of the microbial method, the lowest open porosity values were obtained as 5.14% after 7 days of incubation among the samples immersed in the culture medium inoculated with the *M. xanthus* bacterial strain and 5.23% after 27 days of incubation among the samples immersed in the culture medium inoculated with the *B. subtilis* bacterial strain. The open porosity value of the control sample of Suloğlu stone was obtained as 3.28%. After the application of the microbial method, the lowest open porosity values were obtained as 1.51% among the samples immersed in the culture medium inoculated with the *M. xanthus* bacterial strain after 7 days of incubation and 2.47% among the samples immersed in the culture medium inoculated with the *B. subtilis* bacterial strain after 7 days of incubation (Fig. 3).

According to the results of the mercury porosimetry test performed on the sterilized stone samples, the open porosity value of the control sample of Küfeki stone was obtained as 3.34%. After the application of the microbial method, the lowest open porosity values were obtained as 1.49% among the samples immersed in the culture medium inoculated with the *M. xanthus* bacterial strain after 17 days of incubation and 1.70% among the samples immersed in the culture medium inoculated with the *B. subtilis* bacterial strain after 17 days of incubation. The open porosity value of the control sample of Sazlıbosna stone was obtained as 5.00%. After the application of the microbial method, the lowest open porosity values were obtained as 2.06% among the samples immersed in the culture medium inoculated with the *M. xanthus* bacterial strain after 27 days of incubation and 2.46% among the samples immersed in the culture medium inoculated with the *B. subtilis* bacterial strain after 7 days of incubation The open porosity value of the control sample of Süloğlu stone was obtained as 3.64%. After the application of the microbial method, the lowest open porosity values were obtained as 0.63% among the samples immersed in the culture medium inoculated with the *M. xanthus* bacterial strain after 27 days of incubation and 2.28% among the samples immersed in the culture medium inoculated with the *B. subtilis* bacterial strain after 7 days of incubation (Fig. 4).

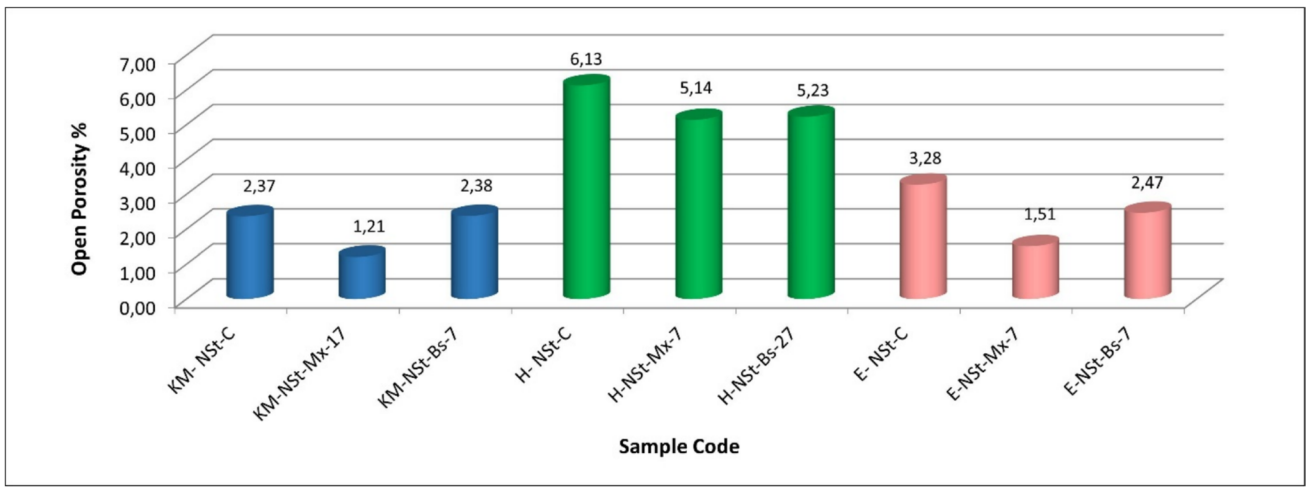


Fig. 3 Open porosity values of unsterilized limestone samples with *M. xanthus* and *B. subtilis* bacterial strains

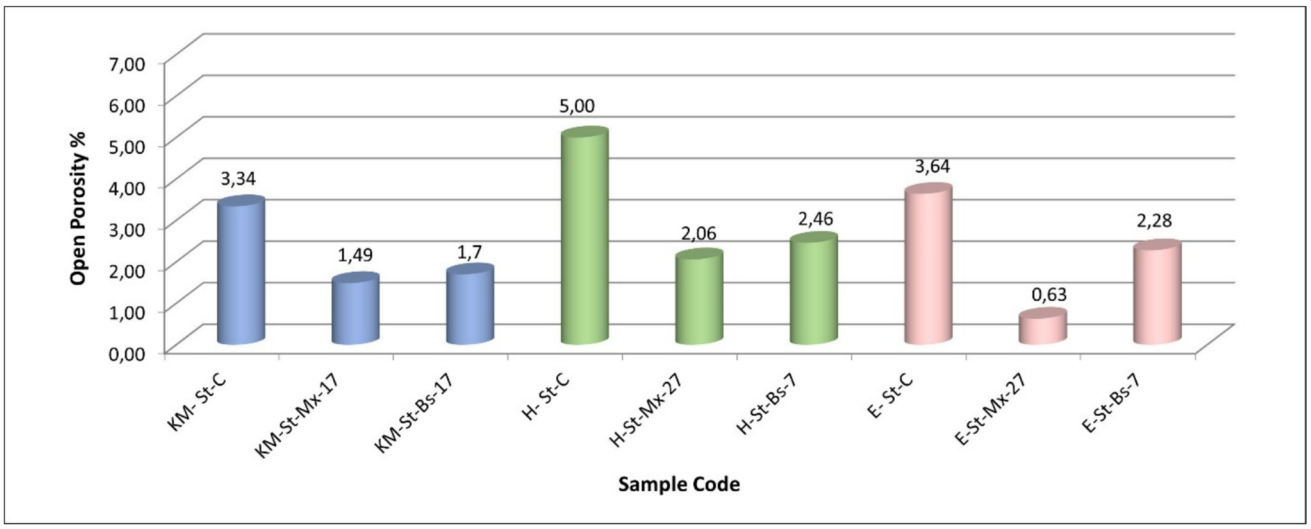


Fig. 4 Open porosity values of sterilized limestone samples with *M. xanthus* and *B. subtilis* bacterial strains

Table 2 Open porosity values obtained before and after the application of the microbial method

Sample Code	Open Porosity (Effective Porosity) Value %
	Percentaged Change %
KM-NSt-*Mx*	−48,95
KM-NSt-*Bs*	0,42
H-NSt-*Mx*	−16,15
H-NSt-*Bs*	−14,68
E-NSt-*Mx*	−53,96
E-NSt-*Bs*	−24,70
KM-St-*Mx*	−55,39
KM-St-*Bs*	−49,10
H-St-*Mx*	−58,80
H-St-*Bs*	−50,80
E-St-*Mx*	−82,69
E-St-*Bs*	−37,36

The percentage changes in open porosity values obtained from stone samples after microbial treatment are presented in Table 2. According to these results, when non-sterilized stone samples are evaluated within themselves, in Küfeki stone, open porosity decreased by 48.95% in samples immersed in culture medium containing *M. xanthus*, while it increased by 0.42% in samples immersed in medium containing *B. subtilis*. This increase is negligible. In the Sazlıbosna stone sample, a decrease of 16.15% occurred with *M. xanthus* and 14.68% with *B. subtilis*. In the Süloğlu stone sample, a decrease of 53.96% was detected as a result of incubation with *M. xanthus* and 24.70% with *B. subtilis*. More significant decreases were obtained in sterilized stone samples. In the Küfeki stone, a decrease of 55.39% was observed with *M. xanthus* and 49.10% with *B. subtilis;* In the Sazlıbosna stone sample, a reduction of 58.80% was observed with *M. xanthus* and 50.80% with *B. subtilis.* In the Süloğlu stone sample, a significantly higher reduction of 82.69% was obtained after incubation with *M. xanthus*, whereas the reduction with *B. subtilis* was 37.36%. According to the results, the fact that good results were obtained in 7 days in some stone samples and in 17 days in others suggests that the process can vary across bacterial applications.

Mineralogical and Petrographic Analyses

As a result of the petrographic analyses, the mineralogical composition of the Küfeki stone sample consists mainly of carbonate minerals (calcite and dolomite), with a small amount of detrital material such as quartz or feldspar and clayey levels. It is generally a sparitic calcite limestone, crystalline, hollow in places, and called ““Biosparite”” according to Folk (1962). The parts, which also contain micritic texture in places, consist of clay-sized calcite mud (Fig. 5a, b). Sterilization of the Küfeki stone resulted in a more porous structure compared to the unsterilized sample. This was due to partial dissolution of the limestones, resulting in a more void-like structure (Fig. 5b). Before the application of the microbial method, the edges of the voids in the carbonate texture of both the sterilized and unsterilized Kufeki stone samples were found to be in the form of a sharp line (Fig. 5a, b). After the application of the microbial method, bacterial calcite formations in the form of thin lines compatible with the bedrock were observed at the gap edges of both sterilized and unsterilized Küfeki stone samples. It was determined that *M. xanthus* and *B. subtilis* bacterial strains formed small crystals on the cavity edges of the samples. These formations are observed under the microscope as lighter colored, brighter, and finer crystalline (Fig. 5c, d, e, f).

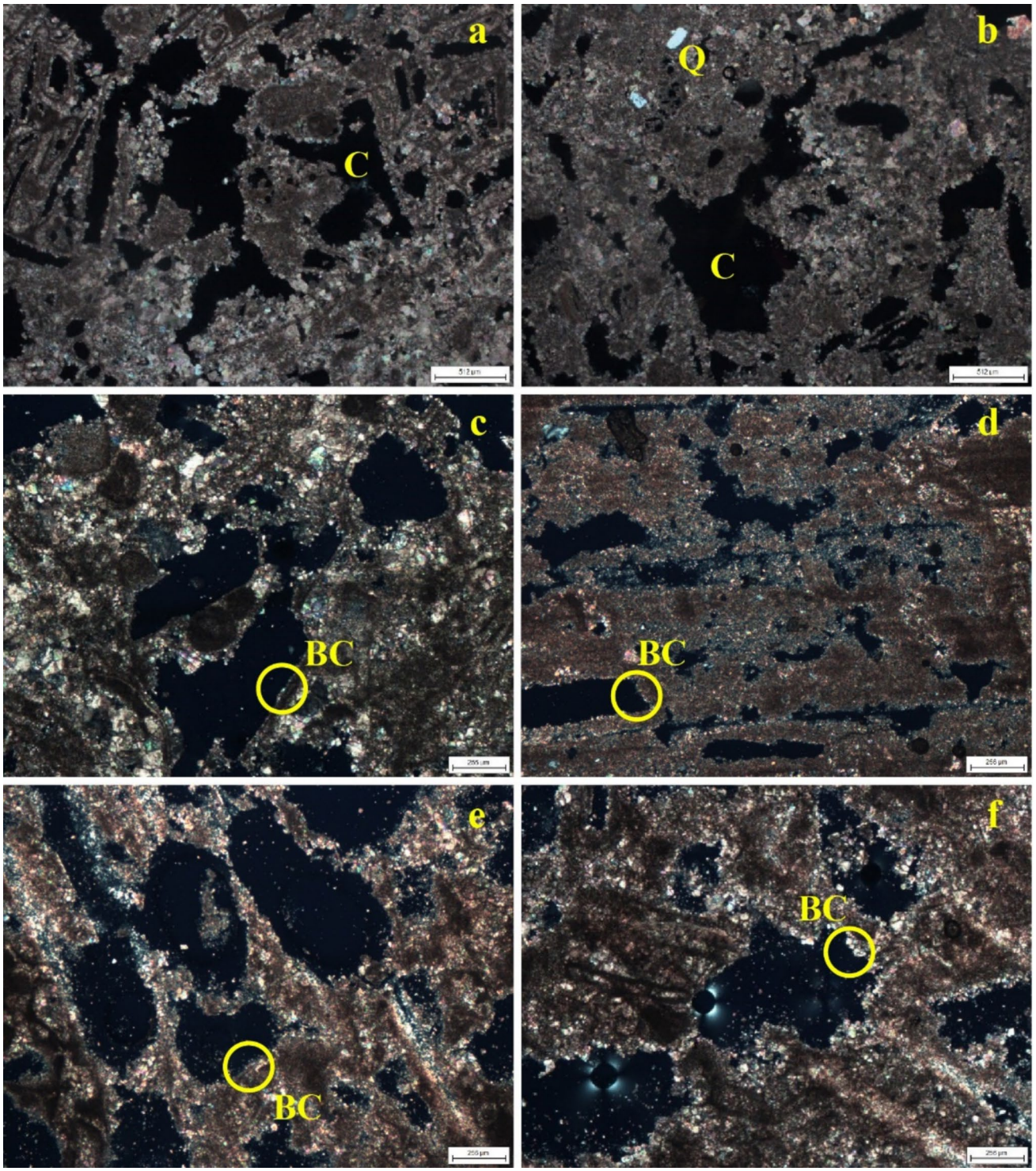


Fig. 5 Polarizing microscope images of Küfeki stone samples in double niches; **a:** KM-NSt-C, **b**: Km-St-C, **c:** KM-Nst-*Mx*, **d:** KM-St-*Mx*, **e:** KM-Nst-*Bs*, **f:** KM-St-*Bs* (Q: Quartz, C: Voids, BC: Bacterial Calcite)

As a result of the petrographic analysis, the Sazlıbosna stone sample was defined as ""Intrabiosparite"" in the crystalline structure, containing very little quartz. There is very little clay in the micritic part. It contains coral and Nummulite fossils (Fig. 6a, b). The sterilization process resulted in a more porous structure in the Sazlıbosna stone sample(Fig. 6b). Before the application of the microbial method, it was determined that the void boundary lines in the carbonate tissue of both sterilized and unsterilized Sazlıbosna stone samples were sharp (Fig. 6a, b). After the

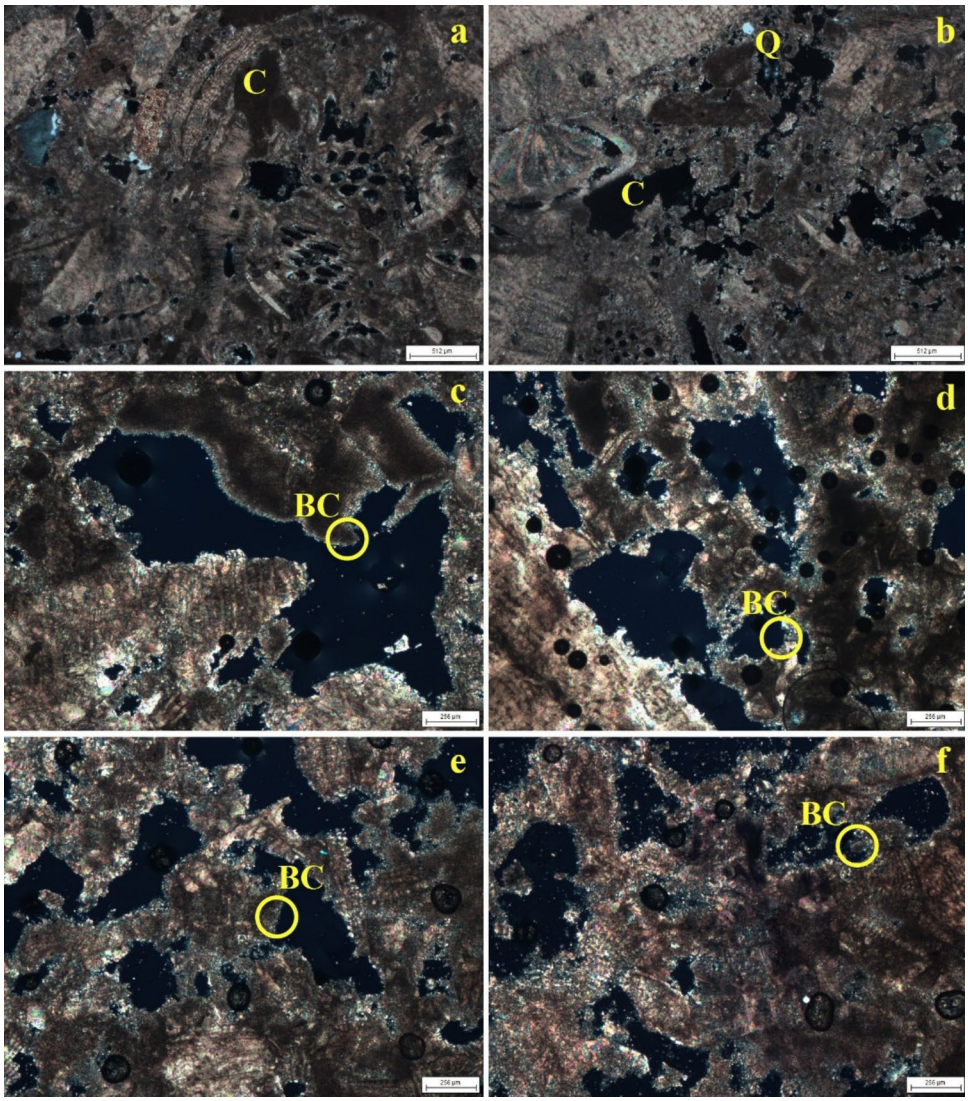


Fig. 6 Polarizing microscope images of Sazlıbosna stone samples in double niche; **a:** H-NSt-C, **b**: H-St-C, **c**: H-Nst-*Mx*, **d:** H-St-*Mx*, **e:** H-Nst-*Bs*, **f:** H-St-*Bs* (Q: Quartz, C: Voids, B.C: Bacterial Calcite)

application of the microbial method, bacterial calcite formations in the form of thin lines compatible with the bedrock were observed at the edges of these voids. In particular, bacterial calcite formations form an indented and protruding structure in these voids. It was determined that *M. xanthus* and *B. subtilis* bacterial strains formed recrystallization by using small $CaCO_3$ at the edges of the voids (Fig. 6c, d, e, f). *B. subtilis* and *M. xanthus* bacterial strains tried to bridge not only the gap edges but also between the gaps. It was found that calcite production was more intense in the fine-grained parts.

The Süloğlu stone sample is a crystalline limestone defined as “”litho-sparite”” containing very small traces of quartz and micritic inclusions, with quartz and very small detrital material (Fig. 7a, b). With the sterilization of the Süloglu stone sample, the sample gained a more porous structure and presented a more hollow structure compared to the unsterilized sample (Fig. 7b). Prior to the application of the microbial method, both sterilized and unsterilized Süloglu stone samples were found to have sharp void boundary lines in the tissue (Fig. 7a, b). It was determined that *M. xanthus* and *B. subtilis* bacterial strains formed recrystallization by using calcium carbonate ($CaCO_3$) at the gap edges. After the application of the microbial method, bacterial calcite formation was observed in the fine-grained parts compatible with the bedrock at the edges of these voids, while these formations were not observed at the edges of the voids with coarse crystals (Fig. 7c, d, e, f).

X-Ray Diffractometry Analysis

X-ray diffractometer analysis of unsterilized Kufeki stone samples immersed in culture medium inoculated with *M. xanthus* and *B. subtilis* bacterial strains, both before the microbial consolidation method was applied, revealed that the dominant mineral phase was calcite.

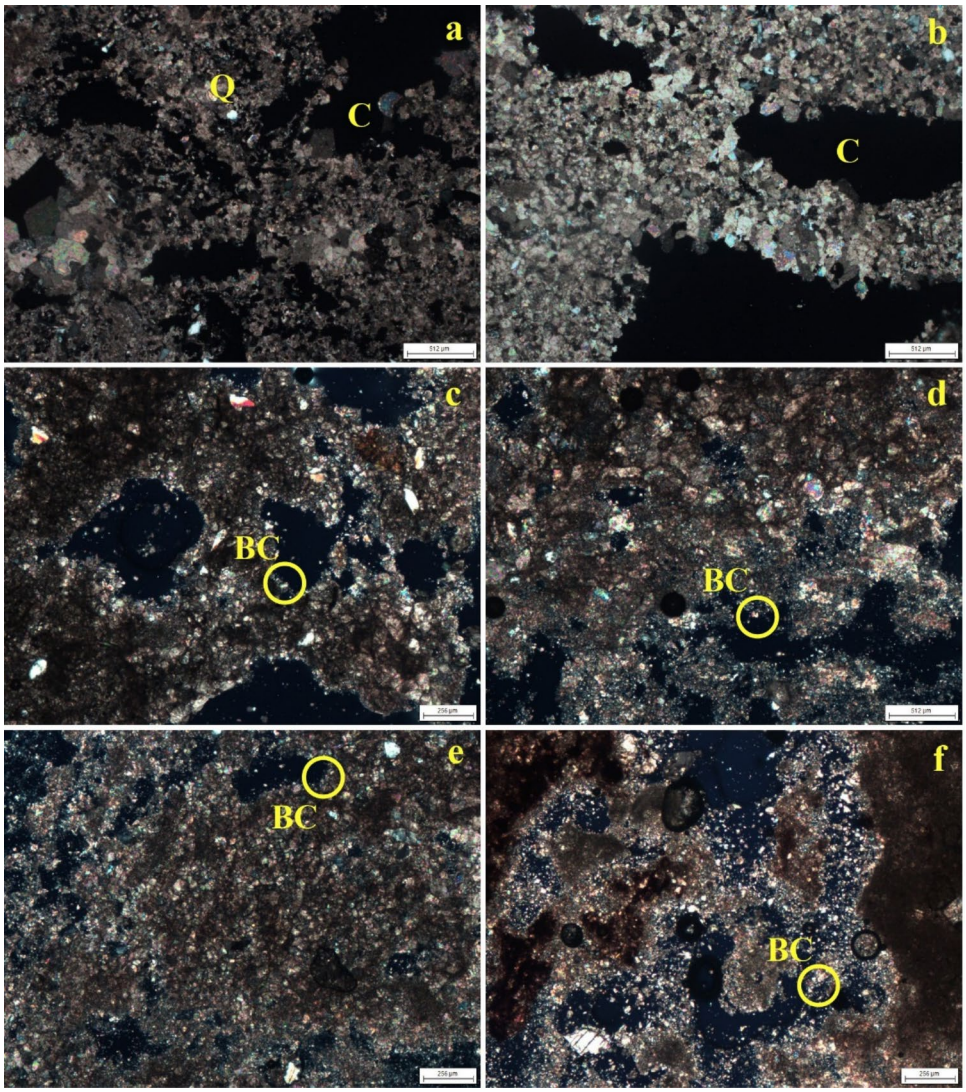


Fig. 7 Polarizing microscope images of Süloğlu stone samples in double niche; **a:** E-NSt-C, **b**: E-St-C, **c:** E-Nst-Mx, **d:** E-St-*Mx*, **e:** E-Nst-*Bs*, **f:** E-St-*Bs* (Q: Quartz, C: Voids, BC: Bacterial Calcite)

Unsterilized Sazlıbosna stone samples revealed that the dominant mineral phase was calcite. A small amount of quartz mineral was determined in the sample. After the application of the microbial method, the main phase was determined as calcite in the samples in culture medium inoculated with the *M. xanthus* and *B. subtilis* bacterial strain. A small amount of quartz mineral was determined in the samples. Unsterilized Süloğlu stone samples were analyzed using an X-ray diffractometer (XRD), which showed that calcite was the predominant mineral phase with a trace quantity of quartz. Following the use of the microbial approach, calcite was identified as the primary phase in the samples that had been inoculated with bacterial strains of *M. xanthus* and *B. subtilis* (Fig. 8).

Scanning Electron Microscopy (SEM) Analysis

Figures 9a and b show the microsparite texture of the Küfeki stone sample, which was sterilized and unsterilized before the application of the microbial method. Calcite crystals on the stone surfaces are observed as self-shaped rhombohedral crystals. Intercrystal spaces are evident.

Calcium carbonate precipitation occurred in unsterilized and sterilized samples immersed in the culture medium inoculated with the *M. xanthus* bacterial strain, and new bacterial calcite formations were observed on the surfaces of the stone samples and between self-shaped rhombohedral calcite crystals (Fig. 9c, d).

Calcium carbonate precipitation occurred in the sterilized and non-sterilized samples immersed in the culture medium inoculated with the *B. subtilis* bacterial strain, and newly formed, abundant calcified bacterial cells were detected (Fig. 9e, f). Epitaxially growing calcite crystals developed a crystallographic orientation on pre-existing calcite crystals (Fig. 9f). Within the scope of the formation mechanisms of biofilm formations in the Küfeki stone sample, extracellular polymeric substances (EPS) formations and fimbria, which allow bacteria to adhere, were observed between calcite

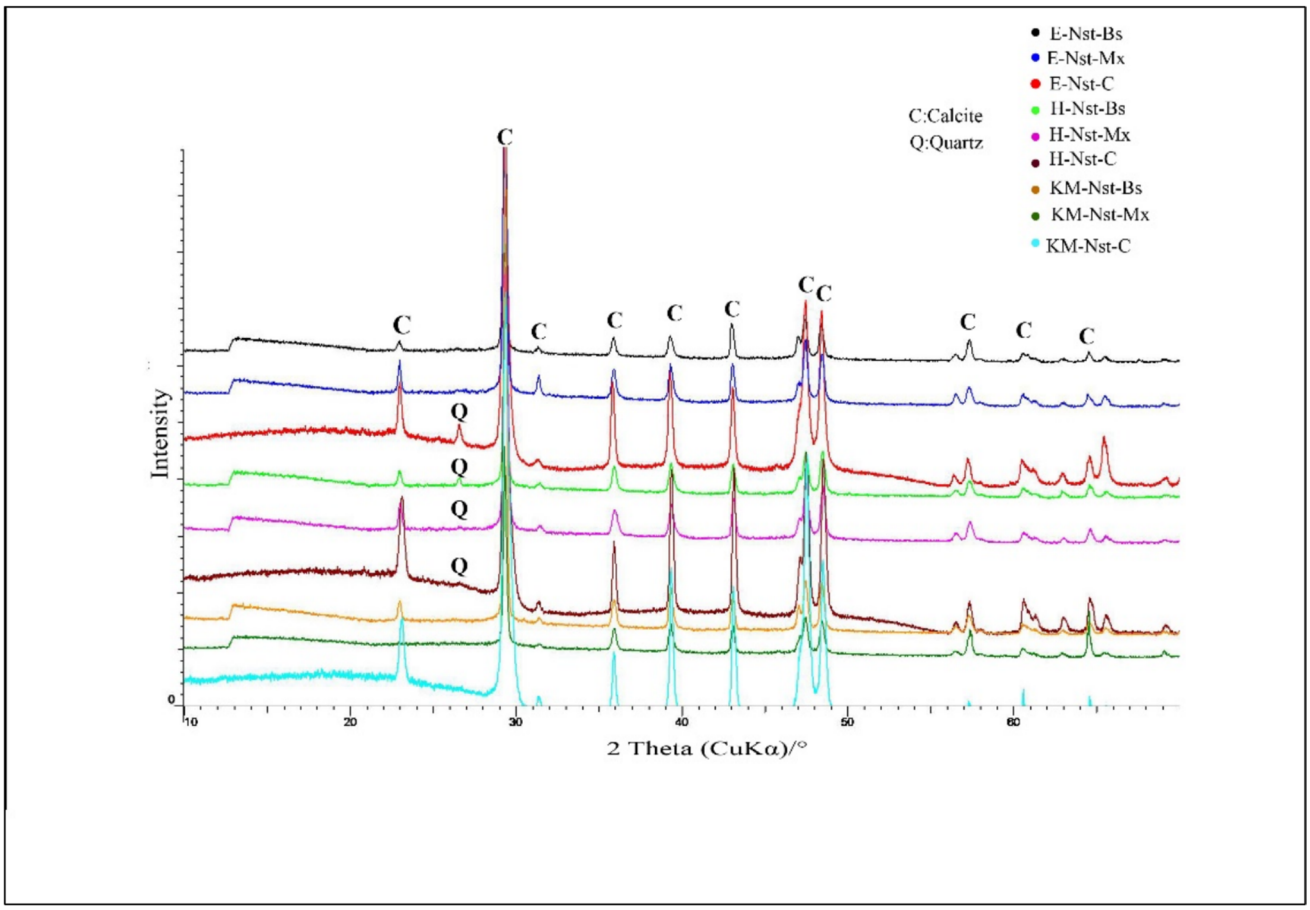


Fig. 8 XRD diffractometer of unsterilized limestone samples

crystals (Fig. 9c,d,e).. Extracellular polymeric substances (EPS) formations were more prominent in unsterilized samples (Fig. 9c, e).

Both the sterilized and unsterilized Sazlıbosna stone samples had self-shaped rhombohedral calcite crystals and pores between the particles, according to a scanning electron microscopy (SEM) analysis of the sample. Additionally, the appearance of microsparite texture was clearly visible (Fig. 10a, b). Both sterilized and non-sterilized stone samples incubated in the culture medium inoculated with the bacterial strains *M. xanthus* and *B. subtilis* experienced calcium carbonate precipitation following the application of the microbial method. Additionally, new bacterial calcite formations were seen on the surfaces of the stone samples and in between self-shaped rhombohedral calcite crystals (Fig. 10c, d, e, f). The bacterial cells of *M. xanthus* were found between and on the surface of calcite crystals, and they stuck to the stone surface (Fig. 10c, d). Epitaxially developing calcite crystals formed a crystallographic orientation on pre-existing calcite crystals in the sample submerged in culture media injected with the *B. subtilis* bacterial strain. On the surface of the stone sample, new calcite formations (bacterial calcite) developed as a result of the bacterial activity seen there (Fig. 10e, f).

The Süloğlu stone sample was analyzed using scanning electron microscopy (SEM), which showed a sparite texture and self-shaped rhombohedral calcite crystals with pores between the particles in both the sterilized and unsterilized samples (Fig. 11a, b). Both sterilized and non-sterilized samples incubated in the culture medium inoculated with *M. xanthus* and *B. subtilis* bacterial strains experienced calcium carbonate precipitation following the application of the microbial method. Additionally, new bacterial calcite formations were seen on the stone surfaces and in between self-shaped rhombohedral calcite crystals (Fig. 11c, d, e, f).

Significant extracellular polymeric substances (EPS) formation was observed in non-sterilized samples incubated with *M. xanthus* and *B. subtilis* (Fig. 11c,e). In contrast, EPS formation was found to be quite limited in sterilized samples incubated with *B. subtilis* (Fig. 11f). Numerous calcified bacterial cells were observed in samples submerged in culture media inoculated with both bacterial strains (Fig. 11e,f).

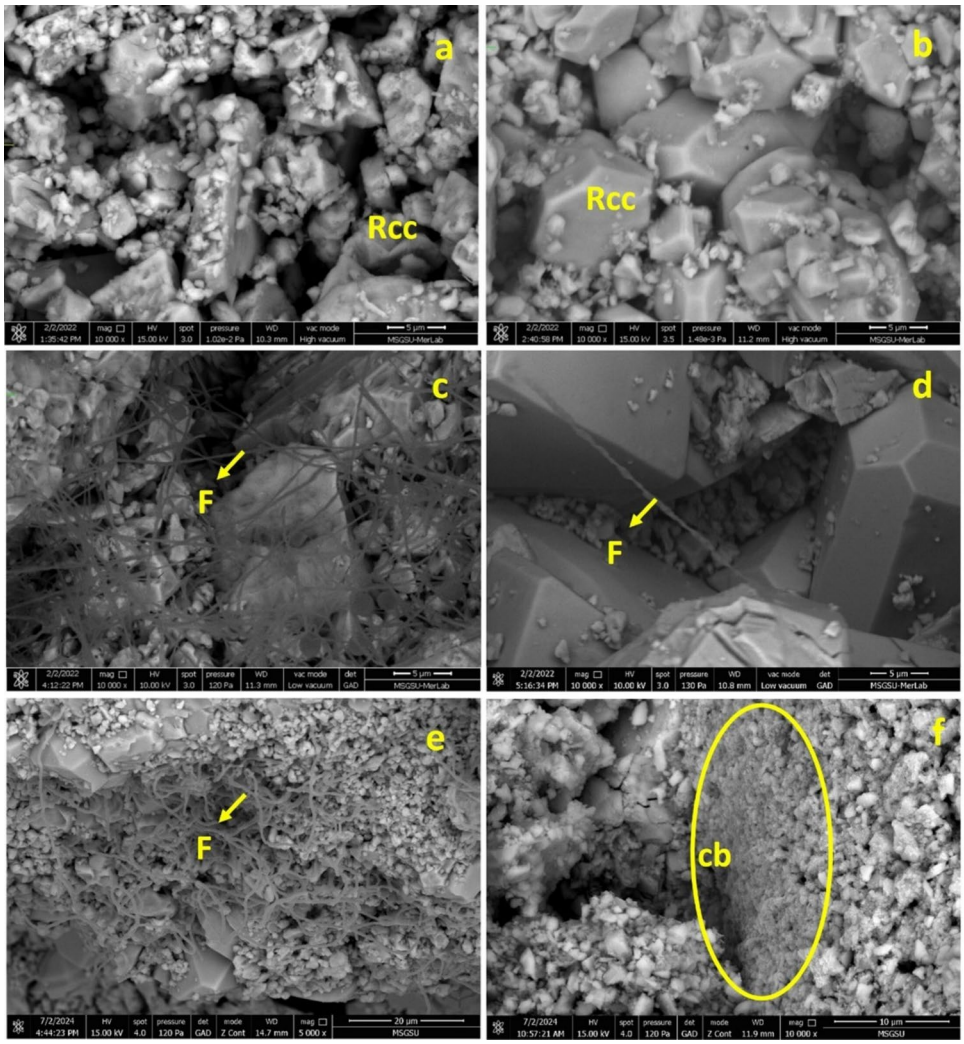


Fig. 9 SEM images of Küfeki stone sample; **a:** KM-NSt-C **b:** KM-St-C, **c**: KM-Nst-*Mx,* d: KM-St-*Mx,***e:** KM-Nst-*Bs*, **f**: KM-St-*Bs* (Rcc: Rhombohedral calcium carbonate crystals, cb: Calcified bacteria F: Fimbriae)

Colour Measurement with Spectrophotometer

The ΔL, Δa, Δb, and ΔE values determined in the samples after applying the microbial method are shown in Table 3. Color measurements (ΔL, Δa, Δb) taken on sterile and non-sterile stones revealed differences based on the stone type. The Küfeki (KM) stones generally exhibited a more stable color behavior compared to the other stones. In Sazlıbosna (H) stones, ΔE* values were low, indicating limited color change, with the only exception being the sterile samples incubated with *M. xanthus*. On the other hand, the Süloğlu (E) stones showed the highest color changes under both sterile and non-sterile conditions. Generally, positive ΔL* values suggest a tendency for surface lightening (whitening) after treatment. Conversely, no significant change was seen in Δa* values. When considering the effect of microorganisms, it was found that applying *M. xanthus* in non-sterile samples generally resulted in higher ΔE values (7.42 for Süloğlu stone), while *B. subtilis* produced higher ΔE values in some sterile samples (6.84 for Süloğlu stone). Analyzing the standard deviations of the ΔE values showed high variability (above±2) in certain samples, likely due to the heterogeneous structure of the stone surfaces.

Discussion

The bacterial strains *M. xanthus* and *B. subtilis* are microorganisms with a high ability to precipitate calcium carbonate. CO_2 concentration occurs under alkaline conditions, and calcite precipitation occurs. CO_2 is produced by bacteria. CO_2 solubilizes both HCO_3^- and CO_3^{-2} at high pH. Carbonate ions are supplied by K_2CO_3 in the culture medium. Heterogeneous nucleation in the bacterial cell walls produces calcium carbonate in calcite crystals once adequate saturation has been reached. Carbonate precipitation causes a decrease in pH. The pH decrease occurs when the calcium carbonate precipitation rate (weight gain) is close to maximum. The newly formed carbonate of Ca^{+2} and CO_3^{-2} causes a pH decrease (Rodriguez-Navarro et al. 2003). The

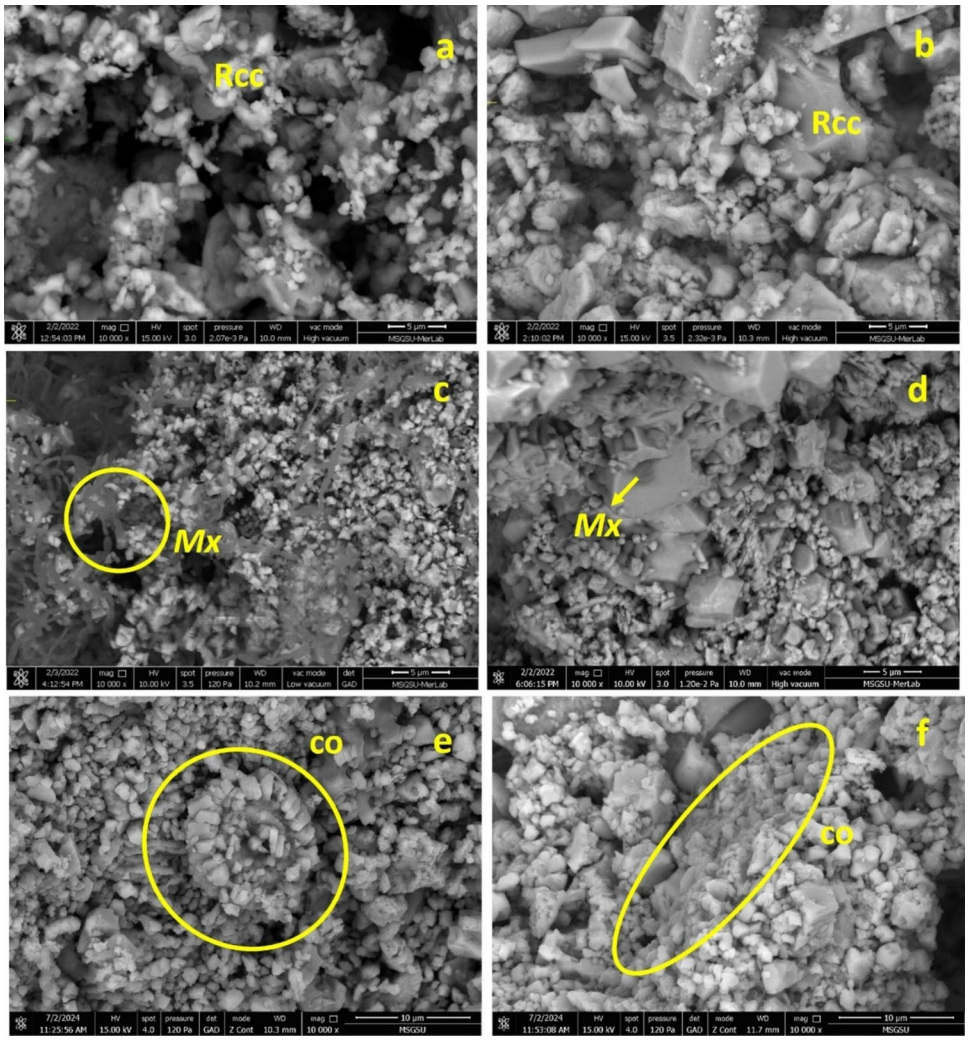


Fig. 10 SEM images of Sazlıbosna stone sample; **a**: H-NSt-C, **b**: H-St-C, **c**: H-Nst-*Mx*, **d:** H-St-*Mx*, **e:** H-Nst-*Bs*, **f:** H-St-*Bs* (Rcc: Rhombohedral calcium carbonate crystals, *co:crystallographic orientation, Mx*:*M.xanthus*)

findings of this study revealed that calcium carbonate precipitation is related to bacterial activity. The new calcite formed as a result of bacterial activity was determined as a result of mineralogical, petrographic, X-ray diffractometry (XRD), and scanning electron microscopy (SEM) analyses performed on the stone samples after the application of the microbial method, and the findings obtained supported the results of the mercury porosimetry experiment.

According to the results of the mercury porosimeter experiment, in unsterilized stone samples inoculated with the *M. xanthus* bacterial strain, open porosity decreased by an average of 16–54%, whereas in sterilized stone samples, open porosity decreased by 55–83%. In unsterilized stone samples inoculated with the *B. subtilis* bacterial strain, the open porosity values decreased by an average of 14–25%, while in sterilized stone samples, the open porosity values decreased by 37–51% (Table 2). According to the analysis results, the effect of microbial treatment on open porosity varies depending on both the type of bacteria used and the sterilization status. The study shows that sterilization enhances the effectiveness of microbial consolidation applications and that the *M. xanthus* strain outperforms *B. subtilis* across all stone types. Furthermore, the initial pore structure of the stones appears to have a significant impact on microbial sedimentation efficiency..The fact that the highest percentage reductions in open porosity values occurred in sterilized samples indicates that bacterial strains are more effective alone, without any cooperation with other microorganisms. On the other hand, the significant reductions in open porosity values in unsterilized stone samples suggest that the bacterial strains *M. xanthus* and *B. subtilis* also cooperated with other microorganisms naturally present in the stones. However, antagonistic and synergistic relationships between bacterial strains and other microorganisms naturally present in the stone samples were not investigated in this study. In their 2025 study, Benedetti et al. evaluated the effectiveness of a carbonatogenic bacterial strain (Lysinibacillus fusiformis) on three natural carbonate stones with different porosities: Calcarenite, Travertine, and Marble.. The results showed that biomineralization

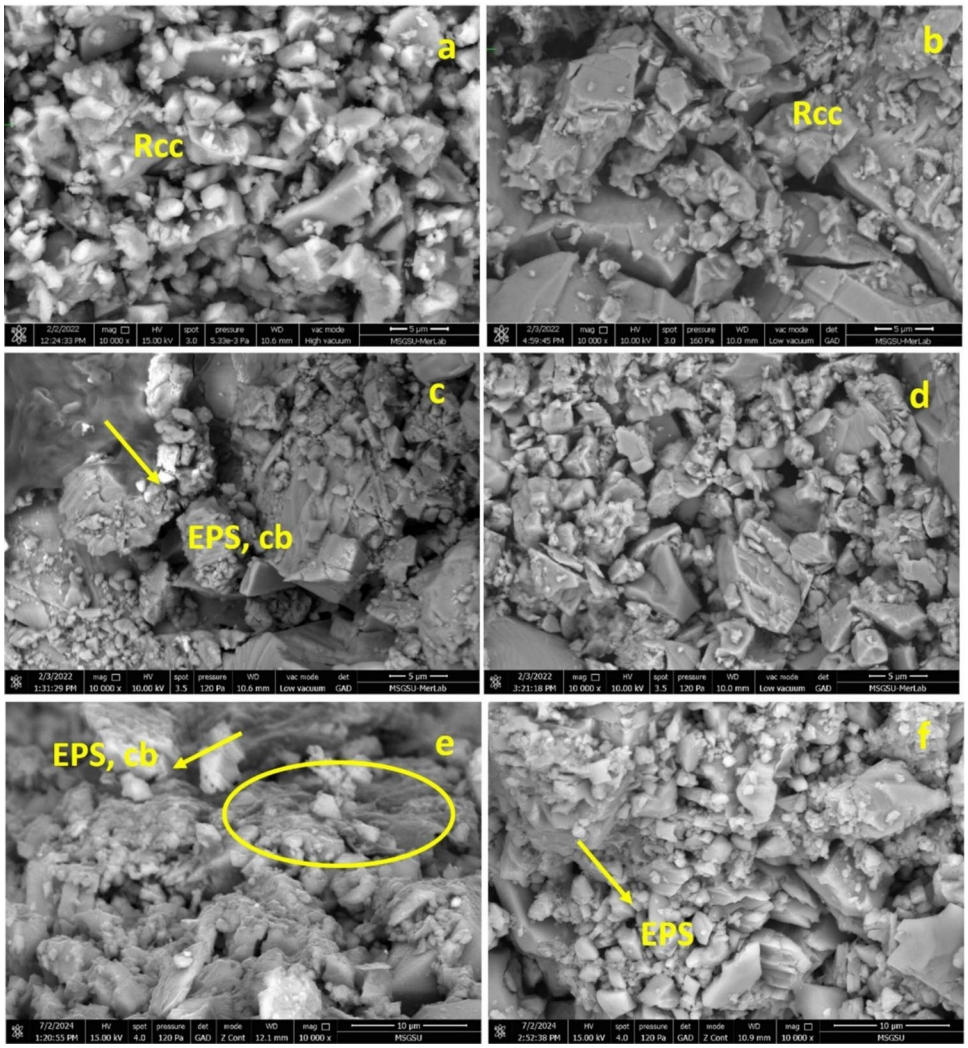


Fig. 11 SEM images of Süloğlu stone sample; **a:** E-NSt-C, **b:** E-St-C, c: E-Nst-*Mx*,**d**: E-St-*Mx*, **e**: E-Nst-*Bs*, **f:** E-St-*Bs* (Rcc: Rhombohedral calcium carbonate crystals, cb: calcified bacteria, EPS: Extracellular polymeric substances)

Table 3 Colour measurement results of samples

Sample Code	Δl*	Δa*	Δb*	Δe*	Standart Deviation Δe
KM-NSt-*Mx*	3,24	−0,03	−1,73	3,84	2,21
KM-NSt-*Bs*	3,01	0,25	0,67	3,18	1,52
H-NSt-*Mx*	0,90	−0,24	−1,04	2,82	1,73
H-NSt-*Bs*	0,39	−0,21	−0,24	1,59	1,01
E-NSt-*Mx*	5,29	−0,02	1,34	7,42	2,67
E-NSt-*Bs*	3,11	0,06	1,28	3,65	1,29
KM-St-*Mx*	3,33	−0,01	1,17	3,68	1,37
KM-St-*Bs*	3,61	0,57	0,94	3,83	0,85
H-St-*Mx*	0,01	0,07	−0,84	4,09	2,12
H-St-*Bs*	0,74	0,13	−0,26	2,86	2,17
E-St-*Mx*	2,65	−1,04	−3,84	5,46	3,07
E-St-Bs	3,18	−0,15	−1,77	6,84	2,47

treatment was ineffective on marble samples, but the physical and mechanical properties of calcarenite and travertine samples improved. This difference was attributed to the lithologies' porosity characteristics. Specifically, pore size, shape, and degree of interconnectedness directly affect bacterial penetration into the stone and calcium carbonate precipitation. The better performance of calcarenite samples with high interconnected porosity and suitable pore geometry supports the idea that bioconsolidation largely occurs in the connection zones, defined as "pore throats". (Agrawal et al. 2021; Nelson 2009). This situation reveals that microstructural characteristics play a critical role in the success of bioconsolidation. The results obtained in this study are consistent with these findings. When the unsterilized stones were evaluated within themselves, it was observed that the most significant decrease in porosity occurred in Küfeki and Süloğlu stones in both *M. xanthus* and *B. subtilis*-treated samples. A significant decrease in porosity was detected in unsterilized samples in all three stone types. While the decrease was more balanced between sterilized and unsterilized samples in the Küfeki stone, the decrease in porosity in sterilized Sazlıbosna stone samples differed from that in unsterilized samples. Similarly, it was determined that the decrease in sterilized samples of Süloğlu stone differed from that in unsterilized samples. Küfeki stone, thanks to its regular void geometry and highly connected pore structure,

allowed biomineral precipitation to occur more effectively along the pores. The Süloğlu stone sample, although having relatively weaker pore connections, is characterized by a larger void volume and larger calcite crystals, findings confirmed by petrographic analysis.In contrast, Sazlıbosna stone has a more limited void volume and exhibits a fine-grained texture. However, in this sample, pore interconnectivity is relatively well developed, but due to the finer grain size, microbial precipitation develops under a different microstructural control. De Muynck (2010) investigated the effectiveness of bioconsolidation in five types of limestone with varying properties using mercury porosimetry. According to the results, pore size emerged as the main factor determining bacterial adhesion to the surface and, consequently, the location and amount of precipitated carbonate. The study showed that biogenic carbonate formation occurred in macroporous stones in greater quantities and at deeper levels than in microporous stones. As a result, the biodeposition application exhibited the highest protective performance in macroporous stones. Our findings also support these results. In Küfeki and Süloğlu stones, which have larger, more interconnected pores, the reduction in porosity and in carbonate precipitation resulting from bioconsolidation is more pronounced. This situation once again highlights the crucial role of pore structure in the bioconsolidation process.

Petrographic examinations revealed that all three different stone samples had the same carbonate structure. The samples from the three different groups are limestone. The Küfeki and Sazlıbosna stone samples have a crystalline structure and exhibit microsparite texture (Figs. 5 and 6a, b). The Süloğlu stone sample is a sparitic limestone. It has larger crystals compared to the Küfeki and Sazlıbosna stone samples (Fig. 7a, b). In the study by Benedetti et al. (2025), petrographic examinations of three carbonate rocks revealed significant microstructural changes in calcarinite and travertine samples after microbial treatment, whereas no significant changes were observed in marble samples. After the application of the microbial method, new micrometric crystal formations were identified along the outer edges of the samples; this indicates that the microbial calcium carbonate precipitation (MICP) process primarily begins in areas close to the surface. Towards the interior, crystal accumulations continued along the pore walls, forming aggregates over time. The petrographic findings obtained in this study similarly revealed bacterial calcite formations in the form of thin lines, consistent with the bedrock, at the pore edges within the carbonate texture in both sterilized and unsterilized stone samples after microbial consolidation treatment. These formations were observed to be concentrated particularly at the pore edges, with crystallized edges, and the calcite precipitates, due to bacterial activity, developed with a bright, light-colored, and fine-crystalline character (Figs. 5, 6, and 7c, d, e, f). These findings are consistent with the literature and support the idea that the MICP process primarily affects the surface and pore edges.

As a result of X ray diffractometer (XRD) analysis, it was determined that the mineralogical structure of the stone samples in three different groups was the same. As a result of X ray diffractometer (XRD) analysis to determine the mineral phase of the culture medium and bacterial activity in the solution, calcite was determined as the main phase in the control group and in all stone samples after the microbial method (Fig. 8). This finding is consistent with bioconsolidation studies on *M. xanthus* and *B. subtilis* that demonstrate calcite formation (Benedetti et al. 2025; Marín-Ortegaa et al. 2026).

Scanning electron microscopy (SEM) analyses clearly showed self-shaped rhombohedral calcite crystals forming the carbonate cement of the sterilized and non-sterilized control group samples (Figs. 9, 10, and 11a, b). After the application of the microbial consolidation method, calcium carbonate ($CaCO_3$) precipitation occurred in all stone samples, both sterilized and non-sterilized, and abundant calcified bacterial cells grew in association with the newly formed carbonate aggregates. In both sterilized and non-sterilized stone samples, new calcite forms formed as a result of the metabolic activity of *M. xanthus* and *B. subtilis* bacterial strains were detected, especially in the cavity walls of the stones and on the stone surface. These observations demonstrate that microbial calcium carbonate precipitation (MICP) is not limited to a surface coating but actively progresses within the pore network of the stone. Biocement, formed through biomineralization, is defined as the accumulation of $CaCO_3$ that develops through microorganism activity in calcium-rich environments (Marjadi 2016) and plays a critical role in consolidation performance due to its interaction with the stone matrix. Indeed, Rodriguez-Navarro et al. (2003) stated that bacterial adhesion to carbonate grains forms a strong cement phase between the newly formed carbonate and the substrate. Similarly, Jimenez-Lopez (2008) showed that a new cement phase that integrates with the stone matrix forms in systems inoculated with *M. xanthus*, thereby increasing mechanical strength. In this study, the new cement phases detected by SEM analysis provide results consistent with the literature.

Bio-cement formed as a result of bacterial calcite deposits after the application of the microbial consolidation method was determined in sterilized and non-sterilized stone samples (Figs. 9, 10, and 11c, d, e, f). This indicates that bacterial inoculation, applied independently of the naturally occurring microbial population in the environment, results in effective carbonate precipitation and creates a void-filling and binding effect within the stone's microstructure. However, SEM observations detected biofilm formations and

the associated development of extracellular polymeric substances (EPS) in all stone samples.

Bacterial biofilms and their extracellular polymeric substances (EPS) are especially good at binding ions from solutions and creating mineral deposition nucleation surfaces (Decho 2000). Since biofilms retain moisture and provide nutrients, they have been suggested to be the optimal environment for further bacterial colonization (Rodriguez-Navarro et al. 2003).

The formation of biofilms and their extracellular polymeric substances (EPS) was observed in both sterilized and unsterilized samples of all stone samples. It was determined that the formation of extracellular polymeric substances (EPS) was higher especially in unsterilized stone samples (Fig. 9c, e, 10c, 11c, e, f). Within the scope of the formation mechanisms of biofilm forms, filaments that enable the attachment of bacterial strains are seen. Among the stone samples immersed in the culture medium inoculated with the *M xanthus* bacterial strain, filaments were detected in both sterilized and unsterilized samples of Küfeki stone and only in the unsterilized sample of Sazlıbosna stone. Similarly, filaments were detected only in the unsterilized sample of Küfeki stone among the stone samples immersed in the culture medium in which the *B subtilis* bacterial strain was inoculated (Fig. 9e). However, these formations were more prominent and more abundant in the unsterilized stone samples (Fig. 9c, e and 10c). Bacterial biofilms are formed when bacteria produce extracellular polymeric substances (EPS) and bind irreversibly to a surface. The short and flat fimbriae allow the bacteria to attach to various surfaces and twitch in movement. Based on these observations, the interaction of the formed biofilm and EPS structures with the stone surfaces was evaluated. The fact that EPS formations remained limited on the stone surfaces and did not completely block the pores indicates that the applied microbial consolidation method provided consolidation without negatively affecting the stone's permeability. Therefore, when both biofilm formation and calcite precipitation are considered together, it becomes clear that microbial processes create a controlled, balanced healing mechanism within the stone's internal structure.

Color measurement analyses performed before and after the application of the microbial method are of great importance for quantitatively evaluating the visual changes on the stone surface. Especially in stone materials of cultural heritage value, it is a fundamental requirement that the applied consolidation or improvement methods maintain the aesthetic integrity of the material. Compared to previous studies, the color change after biogenic applications varies depending on the application conditions and stone type. Benedetti et al. (2025) reported that bioconsolidation application led to significant changes in stone surfaces, with ΔE values above the generally accepted threshold values (ΔE < 5–6) for stone surface applications. Similarly, De Muynck et al. (2010) observed relatively high ΔE values in limestones after microbial application and attributed this largely to the colored components in the culture medium. On the other hand, Marin-Ortego (2026) found that color changes after bioconsolidation with *B. subtilis* were mostly imperceptible (ΔE* < 5). In the reproduction industry, a typical customer's tolerance generally ranges from 1 to 6 ΔE. Most observers cannot distinguish color differences of 2–4 ΔE units from the standard color (Sharma Abhay 2004; Köse and Şahinbaşkan 2008). In this study, when evaluated in terms of total color difference (ΔE), the highest ΔE value was observed in non-sterile Süloğlu stone samples with *M. xanthus* application (7.42 ± 2.67), while the lowest value was obtained in Sazlıbosna stone samples with *B. subtilis* application (1.59 ± 1.01). In sterile samples, the highest ΔE value was determined in the Süloğlu stone–*B. subtilis* sample (6.84 ± 2.47), and the lowest value was determined in the Sazlıbosna stone–*B. subtilis* sample (2.86 ± 2.17). After incubation with two different bacteria, both sterile and non-sterile samples of Küfeki stone had ΔE values of approximately 3, making it the only stone group that remained color-stable. In general, in both conditions, Süloğlu stone samples showed a significant color change with higher ΔE values, while Sazlıbosna stone samples exhibited lower ΔE values. In all samples except the Süloğlu stone samples (E–NSt–*Mx* and E–St–*Bs*), the ΔE values, representing the total color change, being below 6 indicates that the color change is minimal, acceptable, and generally stable..

While research into the consolidation of carbonate stones used in historical structures with Microbial Carbonate Precipitation (MICP) has yielded promising short-term results, the long-term durability of MICP applications remains unclear. Environmental factors such as freeze–thaw cycles, acid rain, and biodegradation can affect the longevity of the method. Although challenges remain, including long-term durability, optimizing reaction rate, controlling biofilm formation, compatibility with different historical materials, and regulatory requirements, MICP stands out as a sustainable and potentially cost-effective solution aligned with environmental sustainability and cultural heritage preservation goals (Wang et al. 2014; Wang and Wang 2025).

Conclusions

Strong evidence that *M. xanthus* and *B. subtilis* bacterial strains have the ability to cause stone consolidation has been presented based on the results. The positive results obtained in the trials where the microbial communities in the stone samples were not eliminated indicate that *M. xanthus* and

B. subtilis bacterial strains do not form an antagonistic relationship with other microorganisms in the stone that would negatively affect the stone properties. In order to solidify the stones and shield them from the impacts of the atmosphere, future research should use parts or variations of the bacterial strains *M. xanthus* and *B. subtilis*, or experiment with other microorganisms. The newly created calcite brought about by bacterial activity is consistent with the stone's native structure following the use of the microbial consolidation process, establishing a crucial preservation strategy for the original material. Since limestones are far more sensitive to atmospheric conditions than other natural stones and have been used extensively in ancient buildings, it is believed that the novel method created in this study will significantly help with the existing repair issues. These recently created methods of affordability and environmental friendliness are additional benefits.

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Author Contributions Özge Boso Hanyalı: Methodology, Stone analysis and investigation, Original draft preparation, Writing, Atiye Tuğrul: Methodology, Stone analysis, Review and editing, Gülşen Altuğ: Methodology, Bacteriological analysis, Review and editing.

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Data Availability All relevant data used in the study is included in the published article in the form of figures and tables.

Declarations

Ethical Approval Not applicable.

Conflict of ınterest Authors declare that they have no conflicts of interest.

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References

Abhay S (2004) Understanding Color Management. Thomson Delmar Learning, USA

Agrawal P, Mascini A, Bultreys T, Aslannejad H, Wolthers M, Cnudde V, Butler IB, Raoof A (2021) The impact of pore-throat shape evolution during dissolution on carbonate rock permeability: pore network modeling and experiments. Adv Water Resour 155:103991. https://doi.org/10.1016/j.advwatres.2021.103991

Al-Thawadi SMJ (2011) Adv Sci Eng Res 1(1):98–114

Ayhan K (2000) Gıdalarda Bulunan Mikroorganizmalar Gıda Mikrobiyolojisi ve Uygulamaları Ankara Üniversitesi Ziraat Fakültesi Gıda Mühendisliği Bölüm Yayını. Sim Matbaacılık Ltd., Ankara, pp 43–44

Benedetti F, Atanasio P, Buccini L, Kratter M, Mura F, Passeri D, Rossi M, Trippetta F, Rinald T (2025) Bioconsolidation strategies for carbonate lithologies: effectiveness and mechanisms in calcarenite, travertine, and marble. Sci Total Environ 971(179092):1–11. https://doi.org/10.1016/j.scitotenv.2025.179092

Buczynski C, Chafetz HS (1991) Habit of bacterially induced precipitates of calcium carbonate and the influence of medium viscosity on mineralogy. J Sediment Res 61:226–233

Cameotra SS, Tikam C, Dakal CT (2012) Carbonatogenesıs: mıcrobial contribution to the conservatıon of monuments and stone artwork**,** Institute of Microbial Technology, Sector-39A, Chandigarh-160036, India Conservation Science and Culturel Heritage, 79–101

Castanier S, Le Me´tayer-Levrel G, Orial G, Loubie`re JF, Perthuisot JP (2000) Bacterial carbonatogenesis and applications to preservation and restoration of historic property. In: Ciferri O, Tiano P, Mastromei G (eds) Of microbes and art: the role of microbial communities in the degradation and protection of cultural heritage. Plenum, New York, N.Y., pp 201–216

Decho AW (2000) Microbial biofilms in intertidal systems: and overview. Continent Shelf Res 20:1257–1273. https://doi.org/10.1016/S0278-4343(00)00022-4

TS 699 (2009) Doğal Yapı Taşları- İnceleme ve Laboratuvar Deney Yöntemleri

Earl AM, Losick R, Kolter R (2008) Ecology and genomics of *Bacillus subtilis*. Trends Microbiol. https://doi.org/10.1016/j.tim.2008.03.004

Patro Sanjaya K, Chandra KS, Sugandha S, Chand S, Sahu SK, Manimaran S (2015) Effect of bacteria on the properties of concrete using Portland Slag Cement, Proceedings of the National Conference on Recent Advances and Future Prospects in Civil Engineering (RAFPCE-15), pp 89–98

Folk R (1993) SEM imaging of bacteria and nannobacteria in carbonate sediments and rocks. J Sediment Res 63:990–999

Hall-Stoodley L, Costerton JW, Stoodley P (2004) Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2(2):95–108. https://doi.org/10.1038/nrmicro821

Henry A (2006) Stone Conservation. In: Henry A (ed). Donhead, London

İBB DEZİM (2011) İstanbul İl Alanının Jeolojisi. İstanbul Büyükşehir Belediyesi, İstanbul

İpekoğlu B (1998) Theoretical and technical principles of stone conservation in historic monuments. Pamukkale Univ Muhendislik Bilim Derg 4(3):787–795

Jimenez-Lopez C, Jroundi F, Chiara Pascolini C, Rodriguez-Navarro C, Pinar-Larrubia G, Rodriguez-Gallego M, Gonzalez-Munoz MT (2008) Consolidation of quarry calcarenite by calcium

carbonate precipitation induced by bacteria activated among the microbiota inhabiting the stone. Int Biodeterior Biodegrad 62:352–363. https://doi.org/10.1016/j.ibiod.2008.03.002

Köse E, Türkün Şahinbaşkan T (2008) Renk yönetiminde kullanilan standart ICC profillerinin Türkiye'de Oluşturulmuş Bazı profiller ile karşilaştirilmasi. Politeknik Derg 11(4):365–371

Kuşaklı S (2011) Bacıllus Subtilis'in Peyniraltı Suyu İçersinde Fermantasyonu ile Polisakkarit ve Surfaktin Üretiminin Araştırılması, Sakarya Üniversitesi, Fen Bilimleri Enstitüsü, Gıda Mühendisli Bölümü, Yüksek Lisans Tezi

Marjadi DS (2016) Conservation and restoration of cultural heritage: a biotechnological approach Pelagia Research Library. Adv Appl Sci Res 7(4):159–167

De Muynck W, De Belie N, Verstraete W (2010) Microbial carbonate precipitation in construction materials: a review. Ecol Eng 36:118–136. https://doi.org/10.1016/j.ecoleng.2009.02.006

Nelson PH (2009) Pore-throat sizes in sandstones, tight sandstones, and shales. AAPG Bull 93:329–340. https://doi.org/10.1306/10240808059

Orial G, Castanier S, Le Me´tayer-Levrel G, Loubiere JF (1993) The biomineralization: a new process to protect calcareous stone applied to historic monuments. In: Ktoishi H, Arai T, Yamano K (eds) Proceedings of the 2nd International Conference on Biodeterioration of Cultural Property. International Communications Specialists, Tokyo, Japan, pp 98–116

Pıñar G, Jımenez-Lopez C, Sterflınger K, Ettenauer J, Jroundı F, Fernandez-Vıvas A, Gonzalez-Moñoz MT (2010) Bacterial community dynamics during the application of a *Myxococcus xanthus* inoculated culture medium used for consolidation of ornamental limestone. Microb Ecol 60:15–28

Adolphe JM, Loubie`re, JF, Paradas J, Soleilhavoup F (1990) Proce´de´ de traitement biologique d'une surface artificielle. European patent 90400G97.0 (after French patent 8903517, 1989)

Ricca E (ed) (2004) Bacterial Spore Formers: Probiotics and Emerging Applications. Horizon Scientific Press

Rodriguez-Navarro C, Rodriguez-Gallego M, Chekroun KB, Gonzalez-Munoz MT (2003) Conservation of ornamental stone by *Myxococcus xanthus* induced carbonate biomineralization. Appl Environ Microbiol. https://doi.org/10.1128/AEM.69.4.2182-2193.2003

Sayar M, Erguvanlı K (1954) Türkiye Mermerleri ve İnşaat Taşları. İstanbul Teknik Üniversitesi, İstanbul

Sonenshein AL (ed) (2002) Bacillus subtilis and Its Closest Relatives: From Genes to Cells. ASM Press

Spanos N, Koutsoukos GP (1998) The transformation of vaterite to calcite: effect of the conditions of the solutions in contact with the mineral phase. J Cryst Growth 191:783–790. https://doi.org/10.1016/S0022-0248(98)00385-6

Folk RL (1962) Spectral subdivision of limestone types. In: Ham WE (ed.), Classification of carbonat rocks. Amer Assoc Petroleum Geologist Mem 1:62–84

Stumm W, Morgan JJ (1981) Aquatic chemistry. Wiley-Interscience, New York

Ersen A (2011) Taş Korumada Son 20 Yıldaki Çalışmalar ve Yenilikler, Restorasyon ve Konservasyon Dergisi

Tiano P, Biagiotti L, Mastromei G (1999) Bacterial bio-mediated calcite precipitation for monumental stones conservation: methods of evaluation. J Microbiol Methods 36:139–145

Torraca G (1988) Porous Building Materials. ICCROM Publication, Rome, Materials Science for Architectural Conservation

TS EN-12407 (2002) Doğal Taşlar, Deney Metotları, Petrografik İnceleme, Türk Standartları Enstitüsü, Ankara

Wang H, Wang S (2025) Applications of microbial induced calcium carbonate precipitation in historical architecture restoration – a mini review. J Infrastruct Preserv Resil 6(12):2–10. https://doi.org/10.1186/s43065-025-00125-3

Wang JY, Snoeck D, Van Vlierberghe S, Verstraete W, De Belie B (2014) Application of hydrogel encapsulated carbonate precipitating bacteria for approaching a realistic self-healing in concrete. Constr Build Mater 68:110–119

Yıldırım N, Gürtuğ Y, Sesalı C (2016) Mikrobiyal Kalsiyum Karbonat Oluşum Mekanizmaları ve Uygulama Alanları. Marmara Fen Bil Derg 2:70–80. https://doi.org/10.7240/mufbed.73209

Marín-Ortegaa S, Iglesias-Campos MA, Calvo i Torras MA (2026) Comparative evaluation of fossil bone bioconsolidation via two endogenous bacterial strains: Bacillus subtilis and Sporosarcina pasteurii, Journal of Cultural Heritage 77 (2026): 51–66. http://creativecommons.org/licenses/by-nc-nd/4.0/

Ginell WS, Wessel D, & Searles C (2001) ASTM E2167-01: Standard Guide for Selection and Use of Stone Consolidants. West Conshohocken, PA: ASTM International.

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