






DOI 10.22363/1815-5235-2022-18-1-54-63  
UDC 691.3

RESEARCH ARTICLE / НАУЧНАЯ СТАТЬЯ


## Use of natural compounds as a nutrition for bacteria in self-healing mortar

Vera V. Galishnikova<sup>1</sup> , Sherif M. Elroba<sup>2,3</sup>  , Nbras Dayoub<sup>1</sup> , Ahmad Sakna<sup>3</sup> 

<sup>1</sup>Moscow State University of Civil Engineering (National Research University), Moscow, Russian Federation

<sup>2</sup>Peoples' Friendship University of Russia (RUDN University), Moscow, Russian Federation

<sup>3</sup>Egyptian Russian University (ERU University), Badr City, Arab Republic of Egypt

 eru.sherif@yahoo.com

### Article history

Received: November 22, 2021

Revised: January 20, 2022

Accepted: January 28, 2022

### For citation

Galishnikova V.V., Elroba S.M., Dayoub N., Sakna A. Use of natural compounds as a nutrition for bacteria in self-healing mortar. *Structural Mechanics of Engineering Constructions and Buildings*. 2022;18(1):54–63. <http://doi.org/10.22363/1815-5235-2022-18-1-54-63>

**Abstract.** Microbiologically induced calcite precipitation, or calcium carbonate  $\text{CaCO}_3$ , is used in remediating cracks and fissures in concrete. Since the microbial activity is pollution-free, natural, that process is extremely desired and may solve concrete cracking without sacrificing mechanical properties. The effects of different nutrient on the self-healing process are elucidated. Nutrients provide the required sources of energy for the bacterial growth and metabolic activities. A species of bacteria *Bacillus sphaericus* was added to the cement mix at a ratio of 0.6% of cement weight with three organic compounds for nutrients (calcium lactate, yeast extract and peptone) at 0.30% of cement weight. Effects on setting time, rate of water absorption, compressive strength and flexural strength were studied. It was found that bacteria nutrition acts as an accelerator for cement pastes for initial setting time mortar, while acts as a retarder of cement pastes for final setting time for all bacterial compared to control mortar. Finally, bacterial mortars with different types of nutrients showed an increase in compressive and flexural strengths with yeast extract showing the most promising enhancements, resulting in 26.5 and 60% increase in compressive and flexural strength respectively.

**Keywords:** crack, organic compounds, water absorption, microbial calcium carbonate, self-healing, compressive strength, *Bacillus sphaericus*

**Vera V. Galishnikova**, Doctor of Technical Sciences, Professor, Director of the International Department, Moscow State University of Civil Engineering (National Research University), 26 Yaroslavskoye Shosse, Moscow, 129337, Russian Federation; ORCID: 0000-0003-2493-7255, Scopus Author ID: 55303553900, eLIBRARY SPIN-code: 2765-7069; galishni@gmail.com

**Sherif M. Elroba**, PhD Student, Department of Civil Engineering, Academy of Engineering, Peoples' Friendship University of Russia (RUDN University), 6 Miklukho-Maklaya St, Moscow, 117198, Russian Federation; ORCID: 0000-0001-6002-3827, Scopus Author ID: 57220745777; ERU.SHERIF@yahoo.com

**Nbras Dayoub**, PhD Student, Department of Technology and Organization of Construction Production, Moscow State University of Civil Engineering (National Research University), 26, Yaroslavskoye Shosse, Moscow, 129337, Russia; ORCID: 0000-0003-3107-5796, Scopus Author ID: 57220744165, WoS Researcher ID: ABF-4840-2020, eLIBRARY SPIN-code: 5604-5652; nbrasdayoub@hotmail.com

**Ahmad Sakna**, Teaching Assistant, Faculty of Engineering, Construction Engineering Department, Egyptian Russian University, Cairo – Suez Road, Badr City, 11829, Arab Republic of Egypt; ORCID: 0000-0001-7712-5942; ahmadsakna96@gmail.com

© Galishnikova V.V., Elroba S.M., Dayoub N., Sakna A., 2022



This work is licensed under a Creative Commons Attribution 4.0 International License  
<https://creativecommons.org/licenses/by/4.0/>

## Использование природных соединений в качестве питания бактерий в самовосстанавливающихся рамах

В.В. Галишникова<sup>1</sup> , Ш.М. Элроба<sup>2,3</sup>  , Н. Даюб<sup>1</sup> , А. Сакна<sup>3</sup> 

<sup>1</sup>Московский государственный строительный университет, Москва, Российская Федерация

<sup>2</sup>Российский университет дружбы народов, Москва, Российская Федерация

<sup>3</sup>Египетско-Российский университет, Бадр, Арабская Республика Египет

✉ eru.sherif@yahoo.com

### История статьи

Поступила в редакцию: 22 ноября 2021 г.

Доработана: 20 января 2021 г.

Принята к публикации: 28 января 2021 г.

**Аннотация.** Микробиологически индуцированное осаждение кальцита, или карбоната кальция  $\text{CaCO}_3$ , используется для устранения трещин в бетоне. Микробная активность не загрязняет окружающую среду, поэтому данный процесс очень важен, а также он помогает решить проблему растрескивания бетона без ущерба для механических свойств. Выясняется влияние различных питательных веществ на процесс самовосстановления. Питательные вещества обеспечивают необходимые источники энергии для роста бактерий и метаболической деятельности. Вид бактерий *Bacillus sphaericus* был добавлен в цементную смесь в соотношении 0,6 % от массы цемента с тремя органическими соединениями для питательных веществ (лактат кальция, дрожжевой экстракт и пептон) в количестве 0,30 % от массы цемента. Изучено влияние на время схватывания, скорость водопоглощения, прочность на сжатие и прочность при изгибе. Обнаружено, что бактериальное питание действует как ускоритель цементных паст для начального времени схватывания раствора и одновременно как замедлитель цементных паст для окончательного времени схватывания для всех бактерий по сравнению с контрольным раствором. Наконец, бактериальные растворы с различными типами питательных веществ показали увеличение прочности при сжатии и изгибе, при этом дрожжевой экстракт продемонстрировал наиболее многообещающие результаты, что привело к увеличению прочности при сжатии и изгибе на 26,5 и 60 % соответственно.

**Ключевые слова:** трещина, органические соединения, водопоглощение, микробный карбонат кальция, самовосстановление, прочность при сжатии, *Bacillus sphaericus*

### Для цитирования

Galishnikova V.V., Elroba S.M., Dayoub N., Sakna A. Use of natural compounds as a nutrition for bacteria in self-healing mortar // Строительная механика инженерных конструкций и сооружений. 2022. Т. 18. № 1. С. 54–63. <http://doi.org/10.22363/1815-5235-2022-18-1-54-63>

## Introduction

Microbiologically induced calcite precipitation (MICP), or calcium carbonate  $\text{CaCO}_3$ , is used in re-mediating cracks and fissures in concrete. MICP is a method that may be seen in the production of calcite in various geological settings, such as soils, limestone caves, oceans, and soda lakes. It is part of a larger category of research known as bio-mineralization [1]. When a fracture forms, the implanted bacteria are activated, and the resulting calcium carbonate minerals fill the crack, as seen in Figure 1. MICP is extremely desired since the calcite precipitation caused by microbial activity is pollution-free, natural, and may solve concrete cracking without sacrificing mechanical properties [2]. In comparison to traditional chemical self-healing concrete, the bio self-healing method using MICP provides a permanent and ecologically benign solution to the cracking issue. The method

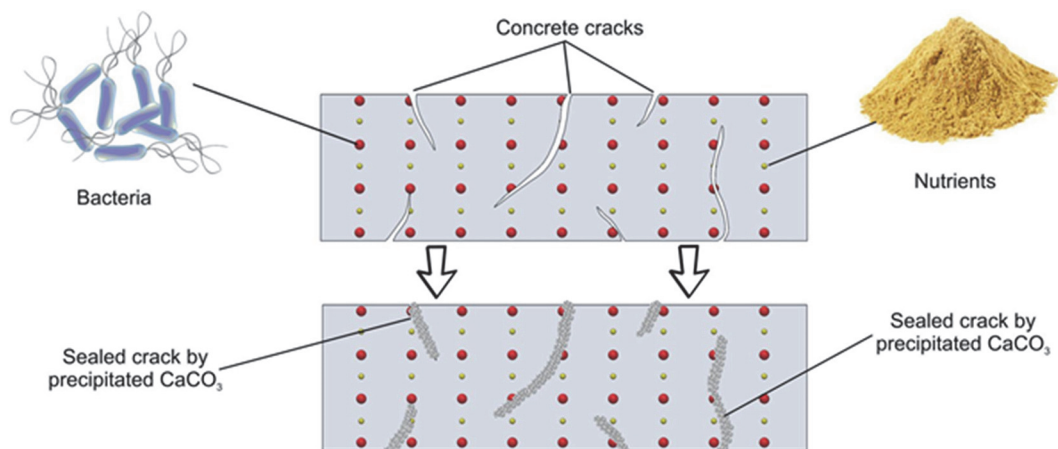
Галишникова Вера Владимировна, доктор технических наук, профессор, директор международного департамента, Московский государственный строительный университет (Национальный исследовательский университет), Российская Федерация, 129337, Москва, Ярославское шоссе, д. 26; ORCID: 0000-0003-2493-7255, Scopus Author ID: 55303553900, eLIBRARY SPIN-код: 2765-7069; galishni@gmail.com

Элроба Шериф М., аспирант, кафедра гражданского строительства, Инженерная академия, Российский университет дружбы народов, Российская Федерация, 117198, Москва, ул. Миклухо-Маклая, д. 6; ORCID: 0000-0001-6002-3827, Scopus Author ID: 57220745777; ERU.SHERIF@yahoo.com

Даюб Нбрас, аспирант, кафедра технологии и организации строительного производства, Московский государственный строительный университет (Национальный исследовательский университет), Российская Федерация, 129337, Москва, Ярославское шоссе, д. 26; ORCID: 0000-0003-3107-5796, Scopus Author ID: 57220744165, WoS Researcher ID: ABF-4840-2020, eLIBRARY SPIN-код: 5604-5652; nbrasdayoub@hotmail.com

Сакна Ахмад, ассистент, кафедра строительной инженерии, инженерный факультет, Египетско-Российский университет, Арабская Республика Египет, 11829, Бадр, дорога Каир – Суэц; ORCID: 0000-0001-7712-5942; ahmadsakna96@gmail.com

may be utilized to boost the compressive strength and stiffness of fractured concrete samples [3]. The efficiency of the MICP process is heavily dependent on the concentration of bacteria as well as the amount and quality of induced minerals.



**Figure 1.** Overview of the self-healing process in concrete matrix

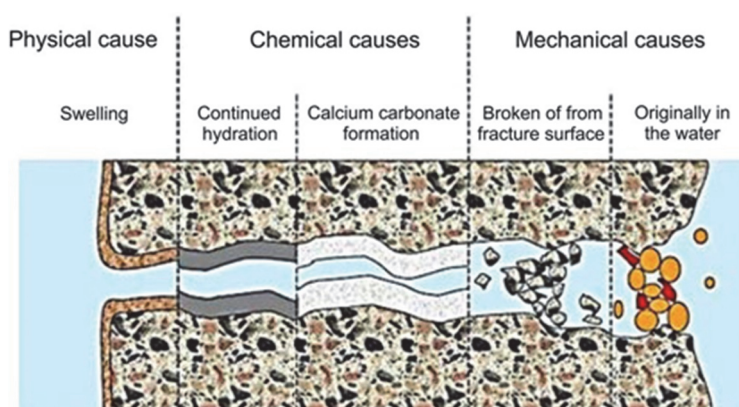
Bacillus species exhibit greater activity under certain environmental conditions. According to research, *B. sphaericus* is the most commonly used bacterium for MICP procedures that use a urea-enriched medium [4]. The concrete cubes are exposed to bacterial precipitation by several bacterial strains (*Bacillus pasturii* and *Bacillus sphaericus*) for 7, 14, 28, 90, and 120 days. MICP has been shown to enhance compressive strength, fracture self-healing, and porosity. General concrete cubes were exposed to a compressive strength test with and without microorganisms in this comparative study. Microbes are shown to be effective in improving concrete characteristics by achieving higher compressive strength than ordinary concrete at the same curing time. Bacterial precipitation of calcium carbonate has filled certain holes and gaps, making the texture denser and more compact. It enhances the compressive strength of concrete by making the structure resistant to seepage/water permeability [5]. The water/cement ratio of the matrix is reduced to increase the self-healing ability of cementitious materials. A large increase in the relative amount of cement or binder in the mixture leads to the creation of a self-healing buffer, i.e. the existence of non-or only partly reacted binder particles in the material matrix. High strength or high-performance concrete are typical examples of low water to binder ratio concrete [2]. Continuous healing of surface fractures reduces the material's permeability and greatly reduces the danger of early matrix deterioration and corrosion of the embedded steel reinforcement owing to intrusion of water and harsh chemicals. Because of the significant energy consumption and associated atmospheric CO<sub>2</sub> emissions, reducing the quantity of cement required in a concrete mixture is ecologically favorable [6]. Chemical infiltration and rebar corrosion are predicted to be reduced by an active and quick crack-healing process, resulting in a considerable improvement in the lifetime of concrete buildings. Because manual inspection and repair of big buildings is expensive, an autonomous repair or self-healing system is advantageous. However, the self-healing mechanism and/or self-healing agent in concrete should not have a detrimental impact on the mechanical properties of the original structure. Non-reacted or partially hydrated cement particles may be used as a repair agent. Water infiltration through fractures would cause these particles to undergo additional hydration processes. This might result in crack sealing [7]. The permeability of the concrete controls the rate at which water penetrates it. Recent advances in the characteristics of high-performance concrete with low water permeability have grown more evident in order to alleviate issues and increase resistance to water and other solution permeation, such as freeze-thaw degradation, sulfate/other chemical assault, and chloride-ion penetration corrosion of embedded reinforcing bars [8]. A bio-based agent composed of alkali-resistant bacteria and a food supply for the bacteria is proposed to increase the durability of the concrete repair system and its connection with the concrete substrate. When used in concrete, this bio-based chemical has the potential to generate calcite-based minerals inside fractures, decreasing concrete permeability [9]. The incorporation of microorganisms into mortar/concrete, resulting in the bio-mineralization process, is currently a promising area of research in concrete technology. The plan was to include microorganisms, which aid in the precipitation of calcium carbonate from dissolved inorganic carbon. The use of mineral-producing bacteria

for sand consolidation and monument repair was investigated. Concrete crack and fissure filling has been explored. Ureolytic bacteria are anaerobic and water-grown bacteria that thrive inside the concrete or mortar matrix in the absence of oxygen or food. In addition, the technique would significantly cut atmospheric carbon dioxide emissions since less cement will be required for self-healing fractures in mortar and concrete [10; 11]. *Bacillus sphaericus* increases the compressive strength of fissures. This shows the fully formed calcite crystal with defined and sharp edges all over the fracture surface, which acts as a plugging and repair agent. Scanning Electron Microscopy's imaging and microanalysis capabilities reveal the existence of calcite precipitation inside fractures. The development of microbial concrete will give a non-chemical sealing option. As a result, it will be both economical and ecologically friendly [12].

As a result, a realistic strategy is necessary. Biotechnological techniques for the production of a new generation of self-healing concrete have been proposed, inspired by microorganisms' inherent capacity to cause calcium carbonate precipitation [6].

Among the most essential dietary requirements are carbon and nitrogen supplies, which serve as energy sources and heterotroph survival respectively [13]. Different nutrients must be supplied in the reaction medium depending on the metabolic route. For example, bacteria in the non-methylotrophic methanogenesis pathway employ CO<sub>2</sub> for energy production and carbonate biosynthesis [14].

It's also worth noting that, in order to get the most calcium carbonate, the reagent concentrations must be kept within safe limits to prevent inhibiting microbial development.



**Figure 2.** Possible causes of self-healing concrete: swelling of the cement matrix, continued hydration, formation of calcium carbonate or calcium hydroxide and sedimentation of particles [14]

An overview was presented in [15] of natural sources of self-healing processes in concrete (physical, chemical, and mechanical). The physical reason, especially expansion of hydrated cement particles, results in a small crack blockage, as illustrated in Figure 2. Chemical treatments can also help to partially repair the fracture. The chemical process of hydrating unhydrated cement particles aids in the closure of tiny fractures. Its success, however, is greatly dependent on the availability of unhydrated cement, and it can be useful for new concrete with modest crack widths. The production of calcium carbonate on the fracture face is another chemical reaction that happens. This process is the most effective method for autogenous concrete healing [16; 17].

### Materials and methods

Distilled water (1000.0 ml), peptone, yeast extract, and agar [18] were used to grow bacteria with the pH set to 7.0. The addition of 10.0 mg MnSO<sub>4</sub> H<sub>2</sub>O to *Bacillus* strains is advised for sporulation. The conical flask is filled with media. The flask is then sealed with paper and a rubber band to make it airtight. The solution is then sterilized for 10–20 minutes using a flame burner. Before adding the bacteria, the solution should be devoid of impurities and a clear orange color [18]. Later, the flasks are opened, and 1 ml of the bacteria is added to each sterilized flask, which is then shaken at 150–200 rpm overnight at 30 °C. The bacterial solution was discovered to be a pale-yellow turbid solution after 24 hours.

*Materials selection and cultivation of calcite-producing bacteria:* here microbiologically the efficiency of the MICP process is heavily dependent on the concentration of bacteria as well as the amount and quality of induced minerals.

*Fine aggregates:* medium well-graded sand of fineness modulus 2.2 was used for mortar.

*Cement:* ordinary portland (type I) cement with grade 42.5 N.

*Water:* fresh tap water was used with water/cement ratio 0.45.

*Calcium lactate:* calcium lactate powder is produced by reacting lactic acid with calcium-based water-soluble compounds such as calcium carbonate or calcium hydroxide. The chemical formula is  $C_6H_{10}CaO_6$  and it is a white powder and it possesses an efflorescent odor. It is also known as calcium salt pent hydrate and the chemical formula of the powder is  $CaO_2$ . Jonkers et al. [9; 19] proposed the utilization of calcium lactate as the only source of carbon and energy for microbial productivity and mineral precipitation and concluded that the only calcium mineral compound that can be added to concrete without causing any loss in strength is calcium lactate and may increase the compression strength when adding up to 2% of cement mass [20].

*Yeast extract:* yeast extract is the primary carbon source for the urea hydrolysis process [21] as well as a nitrogen supply for the metabolic process. Other important minerals can also be found in it. The addition of yeast extract to the concrete mixture prevents the cement from setting and the concrete from hardening [21]. The inclusion of yeast extract, at 1% by mass of cement, reduces the strength of the concrete [19]. Furthermore, Jonkers et al. [19] discovered that adding yeast extract (1% by mass of cement) to concrete reduces the strength of the concrete. Paine's study [22] contradicts this. It has no influence on the strength of the mortar when it is less than 0.5% by mass of cement.

*Proteins (peptone):* peptone, tryptone, tryptone peptone, trypticase, and trypticase peptone are partly digested proteins that are widely utilized as a source of amino acids, peptides, proteins, and nitrogen in growth medium. Complex proteins are broken down either enzymatically or chemically to create them. Peptone applied to concrete at 1% by mass of cement has been demonstrated in studies to diminish concrete strength [19].

*Bacterial suspension preparation:* bacterial cultures were cultured for 7 days to guarantee sporulation before being placed in a falcon tube 50 mm and centrifuged at 10 000 rpm for 10 minutes before being added to the cement mortar. Finally, the vegetative cells and spores were harvested by re-suspending the cell pellets in a sterile 0.9% NaCl solution. The pure plate count technique and optical density of bacterial cultures were employed to create culture suspensions with a final cell density of  $2 \times 10^9$  CFU/mL, which were subsequently utilized in 0.5% concentration of the cement weight.

*Mortar mixes:* the mortar mixture was weighed and stirred for five minutes using a mechanical mixer. After that, water was poured, and the mixing procedure took 10 minutes. By weight, the sand/cement ratio was 1:3. The ratio of water to cement was 0.45. To test the effect of adding bacteria to the mortar mix, a control mortar mix was made. Specimens were prepared for mortar mixing with the addition of calcite-producing *Bacillus sphaericus* (bacterial mortar). Bacteria were added at a rate of 0.6% of the cement's weight. At a ratio of 0.5 bacteria by weight, three organic bio-mineral precursor chemicals, calcium lactate (CL), peptone (P), and yeast extract (Y), were added. Table 1 shows the proportions of experimental mortar mixtures. For several testing, the mortar was cast in molds. Remolded test specimens were stored in a damp towel. Every day, the specimens were sprayed with water to keep them wet [23].

Table 1

Experimental mortar mixes proportions

| Code | Bacteria                   | Nutrition       | Sand/cement | Water/cement | Bacteria addition/cement | Organic compound/cement |
|------|----------------------------|-----------------|-------------|--------------|--------------------------|-------------------------|
| C    | Control                    | 0.0             |             |              | 0.0                      | 0.0                     |
| BSCL | <i>Bacillus sphaericus</i> | Calcium lactate | 3:1         | 0.45         | 0.6%                     | 0.3%                    |
| BSP  |                            | Peptone         |             |              |                          |                         |
| DSYE |                            | Yeast extract   |             |              |                          |                         |

*Setting time:* Vicat equipment for cement paste was used to conduct initial and final setting time tests [24]. There was no increase to the cement. Only before the setting time experiments, a standard water/cement ratio was tested on cement. To examine the influence of bacteria and organic additives on setting time, cement pastes were mixed with three organic additives as given in Table 2.

*The rate of water absorption:* speed of water absorption is a measure of the capillary forces exerted by the pore structure causing fluids to be drawn into the body of the material. In this experiment, the speed of water increases in the mass of samples due to water absorption at certain times when only one surface of the specimen is exposed to water. Mortar samples were dried in an oven at 70 °C for 3 days and then cooled for ages 3, 7, 28,

90 and 120 days of casting. The method of curing was moist curing. The sides of the mortar samples were covered with epoxy resin in order to allow the flow of water in one direction [25]. The end of the samples was sealed with tightly attached plastic sheet and protected in position by an elastic band. The initial mass of the samples was taken after which they were kept partly immersed to a depth of 10 cm in water. The readings were started with the initial mass of the sample for a period of 2 hours from first contact with water. Also, the readings were started with the initial mass of the sample at selected times after first contact with water (typically 1, 5, 10, 20, 30, 60, 110 and 120 min). Samples were removed and excess water was blotted off using paper towel and then weighed. The gain in mass ( $m$ , kg/s) at time ( $t$ , s), exposed area of the specimen ( $a$ , m<sup>2</sup>), and density of water ( $d$ ), were used to obtain the rate of water absorption ( $I$ , m/s<sup>0.5</sup>) as per the equation:

$$I = \frac{\Delta m}{ad}. \quad (1)$$

*Compressive strength test:* the compression test was conducted of the prepared mortar. Test specimens with dimensions of 70×70×70 mm were cast. All specimens were provided with sufficient time for hardening and cured. Three specimens were prepared for each age. After the specified period (3, 7, 28, 90 and 120 days) all the specimens were tested for its maximum load in the compression testing machine. The cubes were tested on hydraulic machine with a 3000 kN capacity as according to code specifications.

*Flexural strength test:* test specimens with dimensions of (160×40×40 mm) were cast cured using moisturized wet cloth. The flexural specimens were subjected to three-point loading test. The flexural strength was determined for 28, 90 and 120 days, using a flexural testing machine with a capacity of 15 ton to determine the maximum load before failure.

Average of three tested specimens for each age was taken. The flexural strength is calculated using following formula [26; 27]:

$$\text{Flex.Strength} = \frac{3PL}{2d_1d_2^2}, \quad (2)$$

where  $P$  – the maximum applied load to the specimen, N;  $d_1$  – the width of the specimen, mm;  $d_2$  – the depth of specimen, mm.

## Results and discussion

*Setting times:* the obtained results from the initial and final setting times of control and bacterial cement paste are shown in Table 1 and illustrated in Figure 3. The initial setting times for all specimens achieved the limits of ASTM C403. Each specimen has different initial setting time and final setting time according to nutrition. The final setting times for all specimens achieved the limits of ASTM C403 except for BSCL and BSY. When nutrition addition was calcium lactate or yeast extract, the final setting time exceeds the code limits (10 hours). Final setting times for BSCL and BSY were 120 and 121% compared to the code limit [24]. Calcium lactate and yeast extract contain of calcium phosphate and zinc in its raw materials respectively, which they can retard setting times.

*The rate of water absorption:* the influence of bacteria and different nutrients on the water absorption of mortar after 2 hours was investigated. It was observed that with the inclusion of bacteria, the rate of water absorption of mortar decreased as shown in Table 3 and Figure 4. Noting that the use of calcium lactate as a nutrient resulted in further reduction in the rate of water absorption. At the ages of 3, 7 & 28 days, it was observed that the rate of water absorption of all bacterial specimens have smaller gain of water absorption than that of control mixture, almost cutting it by half, which aligns with what previous literature. At 90 days the specimens with yeast extract and peptone for nutrients had a smaller gap in water absorption compared to the control specimen, while the specimen containing yeast extract continued to cut the gain of water absorption by half. At the age of 120 days, it was observed that the rate of water absorption of all bacterial specimens after 2 hours has smaller gain of water absorption than that of control mixture and became semi-impermeable. Microbial induced calcite precipitation is responsible for filling up the pores in mortar and hence decreasing water absorption of bacterial mortar specimens.

Table 2

Initial and final setting times for different specimens

| Sample name            | Initial time | Final time |
|------------------------|--------------|------------|
| Control sample         | 135          | 345        |
| BSCL (calcium lactate) | 101          | 395        |
| BSY (yeast extract)    | 99           | 399        |
| BSP (peptone)          | 168          | 384        |

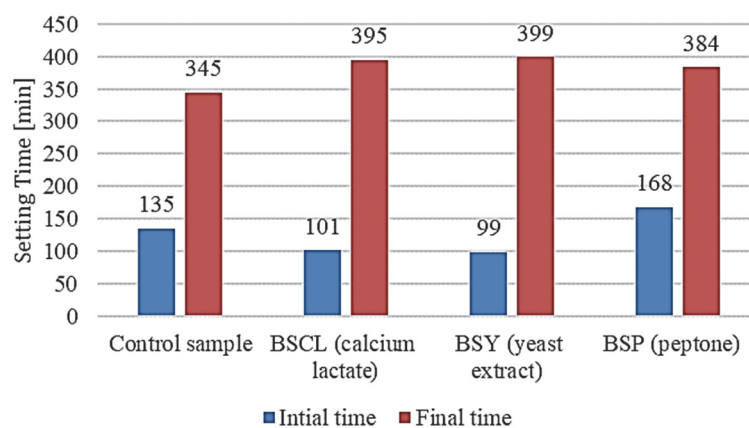


Figure 3. Initial and final setting times for different specimens

Table 3

Rate of water absorption for bacterial and control mortar after 2 hours

| Sample name            | Rate of water absorption/ $10^{-7}$ , $m/sec^{0.5}$ |      |      |      |      |
|------------------------|---|------|------|------|------|
|                        | Age, days   |      |      |      |      |
|                        | 3   | 7    | 28   | 90   | 120  |
| Control sample         | 2.2   | 1.55 | 0.8  | 0.3  | 0.25 |
| BSCL (calcium lactate) | 1.4   | 0.65 | 0.3  | 0.12 | 0.03 |
| BSY (yeast extract)    | 1.2   | 1.1  | 0.3  | 0.21 | 0.16 |
| BSP (peptone)          | 1.34  | 1.05 | 0.37 | 0.26 | 0.21 |

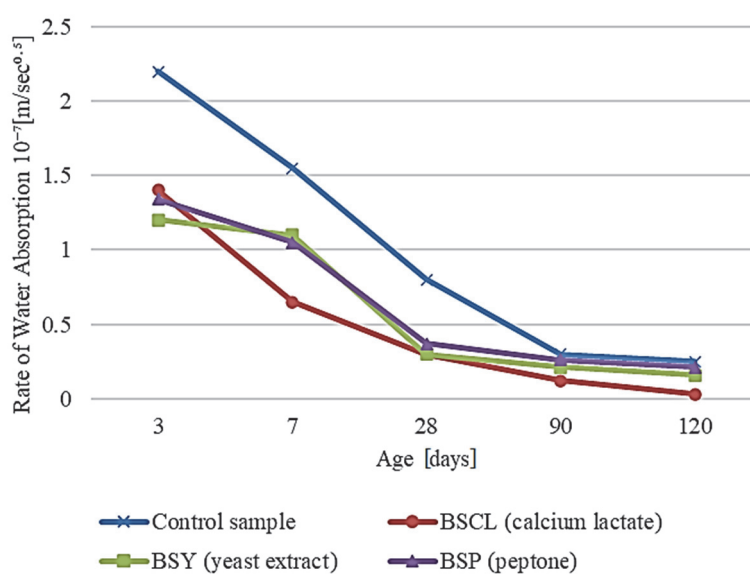


Figure 4. Rate of water absorption for bacterial and control mortar after 2 hours

*Compressive strength:* the results from the compressive strength test have shown an increase in strength for the bacterial mortar when compared to control mortar. Table 4 and Figure 5 showed a significant increase in strength of control and bacterial mortar over time. At the age of 3 days, the compressive strength value of BSY was equal to that of control specimen, but the compressive strength of BSCL and BSP became 108 and 108.39% of the compressive strength of the control sample.

At 7 days of age BSCL, BSY and BSP starts to show an increase in compressive strength by achieving 106.33, 116.37 and 113.7% of the compressive strength for the control specimen respectively.

At 28 days of age BSY and BSP showed a decrease in compressive strength by 8.3 and 13.29% while BSCL showed an increase of 0.9% in comparison to the control specimen.

At 90 days of age BSCL, BSY and BSP starts to show an increase in compressive strength by achieving 104.64, 122.68 and 125.7% of the compressive strength for the control specimen respectively.

At 120 days of age BSCL, BSY and BSP starts to show an increase in compressive strength by achieving 111.97, 137.1 and 126.5% of the compressive strength for the control specimen respectively.

This proved significant activity of bacteria until age of 120 days. Calcite precipitation induced by bacteria is responsible for filling up the pores in mortar and hence increasing bonds in the microstructure which resist loads significantly and hence compressive strength was increased compared to of control mortar. After 120 days, bacterial mortar with yeast extract proves to have higher compressive strength.

Table 4

Compressive strength for bacterial and control mortar specimens, N/mm<sup>2</sup>

| Sample name            | Age, days |       |       |       |       |
|------------------------|-----------|-------|-------|-------|-------|
|                        | 3         | 7     | 28    | 90    | 120   |
| Control sample         | 20.5      | 26.2  | 34.9  | 43.5  | 50.1  |
| BSCL (calcium lactate) | 22.14     | 27.86 | 35.23 | 45.52 | 56.11 |
| BSY (yeast extract)    | 20.5      | 30.49 | 32    | 53.37 | 68.69 |
| BSP (peptone)          | 22.22     | 29.79 | 30.26 | 54.65 | 63.36 |

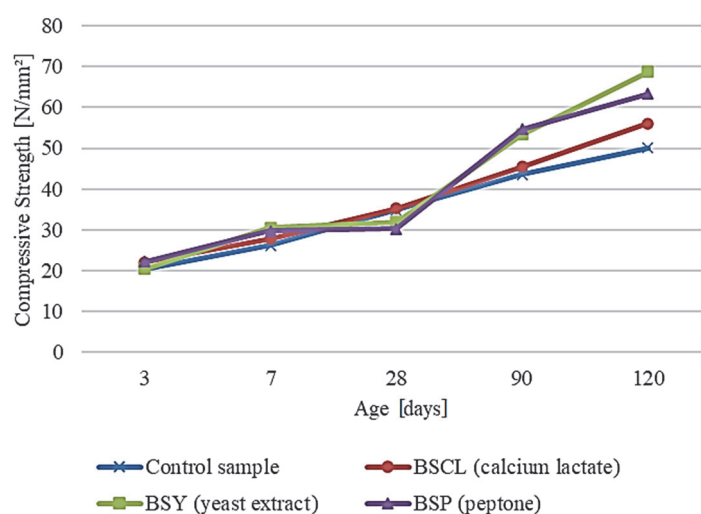


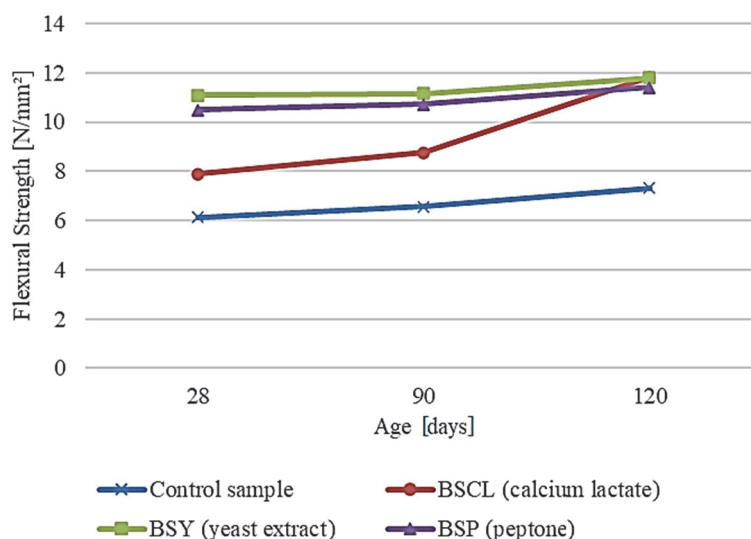
Figure 5. Compressive strength for bacterial and control mortar specimens

Table 5

Flexural strength for bacterial and control mortar specimens, N/mm<sup>2</sup>

| Sample name            | Age, days |       |       |
|------------------------|-----------|-------|-------|
|                        | 28        | 90    | 120   |
| Control sample         | 6.13      | 6.56  | 7.31  |
| BSCL (calcium lactate) | 7.88      | 8.75  | 11.81 |
| BSY (yeast extract)    | 11.09     | 11.15 | 11.8  |
| BSP (peptone)          | 10.5      | 10.71 | 11.4  |





**Figure 6.** Flexural strength for bacterial and control mortar specimens

*Flexural strength:* bacterial and control mortar were tested. It was noticed that flexural strength value of BSP was higher than that of flexural strength value of BSCL at age of 28 and 90 but it decreased at the age of 120 days as illustrated in Figure 6 and Table 5.

Results of flexural strength test revealed that there is an increase in the strength for the bacterial mortar when compared to the control mortar as illustrated in Figure 6. At the age of 28 days, the flexural strength value of BSCL, BSY and BSP were 128.54, 180.91 and 171.28 % of flexural strength of control mortar respectively. At the age of 90 days, the flexural strength value of BSCL, BSY and BSP were 133.38, 169.96 and 163.26 % of flexural strength of control mortar respectively. At the age of 120 days the flexural strength value of BSCL, BSY and BSP were 161.55, 161.42, and 155.95% of flexural strength of control mortar respectively. Microbial induced calcite precipitation is responsible for filling up the pores in mortar and hence increased the flexural strength as observed in previous research. Generally, bacterial mortar proved to have a higher flexural strength.

## Conclusion

Several conclusions could be derived from the results obtained in this investigation as follows:

1. The bacteria nutrition acts as an accelerator for cement pastes for initial setting time for all bacterial mortar compared to control mortar, while acts as a retarder of cement pastes for final setting time for all bacterial mortar. With yeast extract acting as the strongest accelerator of the initial setting times and retarders for the final setting times. Initial and final setting for all mortar were within limit according to the American code for design and construction of concrete structures ASTM C403.

2. The rate of water absorption of all bacterial specimens with different types of nutrients after 2 hours has smaller gain of water absorption than that of control mixture and specimen BSCL became semi-impermeable after 120 days which aligns with previous results that showed a decrease in water absorption.

3. Significant activity of bacterial mortar, biochemically induced calcium carbonate precipitation is responsible for filling up the pores in mortar which in turn decreases rate of water absorption of bacterial mortar and decreases permeability.

4. Compressive strength for all bacterial mortar increased compared to the control specimen's compressive strength. Compressive strength of specimen containing yeast extract achieved the highest compressive strength at 120 days age with an increase of 126.5% compared to the control specimens

5. Compressive strength for BSY and BSP showed a decrease by 8.3 and 13.29% while BSCL showed an increase of 0.9% in comparison to the control specimen at 28 days of age.

6. All bacterial mortar specimen showed a similar increase in flexural strength of about 160% under different types of nutrients

Therefore, the calcium-producing microbes are responsible for filling the pores in the cement mortar, thus reducing the rate of water absorption and increasing the compressive strength and flexural strength of the bacterial cement mortar, with yeast extract being the best option for increasing both compressive and flexural strength.

## References

1. Ganendra G., De Muynck W., Ho A., Charalampous Arvaniti E., Hosseinkhani B., Ramos J.A., Rahier H., Boon N. Formate oxidation-driven calcium carbonate precipitation by methylocystis parvus OBBP. *Appl. Environ. Microbiol.* 2014;80(15):4659–4667. <http://doi.org/10.1128/AEM.01349-14>
2. Zhang X., Jin Z., Li M., Qian C. Effects of carrier on the performance of bacteria-based self-healing concrete. *Construction and Building Materials.* 2021;305:124771. <https://doi.org/10.1016/J.CONBUILDMAT.2021.124771>
3. Vekariya M.S., Pitroda J. Bacterial concrete: new era for construction industry. *Int. J. Eng. Trends Technol.* 2013; 4(9):4128–4137. Available from: <http://www.ijettjournal.org/volume-4/issue-9/IJETT-V4I9P181.pdf> (accessed: 09.01.2022).
4. Seifan M., Samani A.K., Berenjian A. New insights into the role of pH and aeration in the bacterial production of calcium carbonate (CaCO<sub>3</sub>). *Appl. Microbiol. Biotechnol.* 2017;101(8):3131–3142. <http://doi.org/10.1007/s00253-017-8109-8>
5. Patil K., Waghare B., Salve R. Effect of bacterial calcite precipitation on compressive strength of mortar cubes. *Int. J. Eng. Adv. Technol.* 2013;2(3):486–491. Available from: <http://www.ijeat.org/attachments/File/v2i3/C1186022313.pdf> (accessed: 09.01.2022).
6. Zhang W., Zheng Q., Ashour A., Han B. Self-healing cement concrete composites for resilient infrastructures: a review. *Composites Part B: Engineering.* 2020;189:107892. <https://doi.org/10.1016/J.COMPOSITESB.2020.107892>
7. Qian C., Zheng T., Zhang X., Su Y. Application of microbial self-healing concrete: case study. *Construction and Building Materials.* 2021;290:123226. <https://doi.org/10.1016/J.CONBUILDMAT.2021.123226>
8. Mohammed H., Ortoneda-Pedrola M., Nakouti I., Bras A. Experimental characterisation of non-encapsulated bio-based concrete with self-healing capacity. *Construction and Building Materials.* 2020;256:119411. <https://doi.org/10.1016/J.CONBUILDMAT.2020.119411>
9. Roghanian N., Banthia N. Development of a sustainable coating and repair material to prevent bio-corrosion in concrete sewer and waste-water pipes. *Cement and Concrete Composites.* 2019;100:99–107. <https://doi.org/10.1016/J.CEMCONCOMP.2019.03.026>
10. Tian Z., Tang X., Xiu Z., Zhou H., Xue Z. The mechanical properties improvement of environmentally friendly fly ash-based geopolymers mortar using bio-mineralization. *Journal of Cleaner Production.* 2022;332:130020. <https://doi.org/10.1016/J.JCLEPRO.2021.130020>
11. Wang J., Ersan Y.C., Boon N., De Belie N. Application of microorganisms in concrete: a promising sustainable strategy to improve concrete durability. *Appl. Microbiol. Biotechnol.* 2016;100(7):2993–3007. <http://doi.org/10.1007/s00253-016-7370-6>
12. Nain N., Surabhi R., Yathish N.V., Krishnamurthy V., Deepa T., Tharannum S. Enhancement in strength parameters of concrete by application of Bacillus bacteria. *Construction and Building Materials.* 2019;202:904–908. <https://doi.org/10.1016/J.CONBUILDMAT.2019.01.059>
13. Anderson R.K.I., Jayaraman K., Voisard D., Marison I.W., Stockar U. Von Heat flux as an on-line indicator of metabolic activity in pilot scale bioreactor during the production of *Bacillus thuringiensis var. galleriae*-based biopesticides. *Thermochimica Acta.* 2002;386(2):127–138. [https://doi.org/10.1016/S0040-6031\(01\)00709-2](https://doi.org/10.1016/S0040-6031(01)00709-2)
14. Zhang L.V., Nehdi M.L., Suleiman A.R., Allaf M.M., Gan M., Marani A., Tuyan M. Crack self-healing in bio-green concrete. *Composites Part B: Engineering.* 2021;227:109397. <https://doi.org/10.1016/J.COMPOSITESB.2021.109397>
15. Restuccia L., Reggio A., Ferro G.A., Tulliani J.M. New self-healing techniques for cement-based materials. *Procedia Structural Integrity.* 2017;3:253–260. <https://doi.org/10.1016/J.PROSTR.2017.04.016>
16. Edvardsen C. Water permeability and autogenous healing of cracks in concrete. *ACI Materials Journal.* 1999;96(4): 448–454. <https://doi.org/10.14359/645>
17. Hearn N. Self-sealing, autogenous healing and continued hydration: What is the difference? *Mater. Struct. Constr.* 1998;31(8):563–567. <http://doi.org/10.1007/bf02481539>
18. Achal V., Mukherjee A., Reddy M.S. Microbial concrete: way to enhance the durability of building structures. *J. Mater. Civ. Eng.* 2011;23(6):730–734. [http://doi.org/10.1061/\(ASCE\)MT.1943-5533.0000159](http://doi.org/10.1061/(ASCE)MT.1943-5533.0000159)
19. Jonkers H.M., Thijssen A., Muyzer G., Copuroglu O., Schlangen E. Application of bacteria as self-healing agent for the development of sustainable concrete. *Ecol. Eng.* 2010;36(2):230–235. <http://doi.org/10.1016/j.ecoleng.2008.12.036>
20. Wang J.Y., De Belie N., Verstraete W. Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete. *J. Ind. Microbiol. Biotechnol.* 2012;39(4):567–577. <http://doi.org/10.1007/s10295-011-1037-1>
21. Basaran Bundur Z., Kirisits M.J., Ferron R.D. Biomineralized cement-based materials: impact of inoculating vegetative bacterial cells on hydration and strength. *Cem. Concr. Res.* 2015;67:237–245. <http://doi.org/10.1016/j.cemconres.2014.10.002>
22. Lee H.X.D., Wong H.S., Buenfeld N.R. Self-sealing of cracks in concrete using superabsorbent polymers. *Cement and Concrete Research.* 2016;79:194–208. <https://doi.org/10.1016/J.CEMCONRES.2015.09.008>
23. Chahal N., Siddique R. Permeation properties of concrete made with fly ash and silica fume: influence of ureolytic bacteria. *Construction and Building Materials.* 2013;49:161–174. <https://doi.org/10.1016/J.CONBUILDMAT.2013.08.023>
24. Kumar Jogi P., Vara Lakshmi T.V.S. Self healing concrete based on different bacteria: a review. *Materials Today: Proceedings.* 2021;43:1246–1252. <https://doi.org/10.1016/J.MATPR.2020.08.765>
25. Naji Givi A., Abdul Rashid S., Aziz F.N.A., Salleh M.A.M. The effects of lime solution on the properties of SiO<sub>2</sub> nanoparticles binary blended concrete. *Composites Part B: Engineering.* 2011;42(3):562–569. <http://doi.org/10.1016/j.compositesb.2010.10.002>
26. Jang I., Son D., Kim W., Park W., Yi C. Effects of spray-dried co-cultured bacteria on cement mortar. *Constr. Build. Mater.* 2020;243:118206. <http://doi.org/10.1016/j.conbuildmat.2020.118206>
27. Tayebani B., Mostofinejad D. Self-healing bacterial mortar with improved chloride permeability and electrical resistance. *Constr. Build. Mater.* 2019;208:75–86. <http://doi.org/10.1016/j.conbuildmat.2019.02.172>