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# The Effect of Bio-Deposition on Mechanical Performance of Cement-Based Materials

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**ABSTRACT:** Applications of concrete are expanding rapidly all over the world, in a way that approximately 7% of the entire $CO_2$ emit into the atmosphere by human pertains to the interventions made to nature in order to produce concrete. Hence, the need for producing types of concrete that are more compatible with sustainable development is felt more than ever. Finding novel methods for making cement-based materials to reduce cement consumption and the resulting pollution are of great importance among researchers. Improving the properties of these materials using microbial calcite sediments is an environment-friendly technique with an acceptable performance. To improve the compressive strength of cement mortar, microbial calcite sediments produced by Bacillus Subtilis and Sporosarcina Ureae bacteria types were used in this study. Recent studies were also investigated in this paper. To examine the effect of the culture environment, two general designs (with and without extra urea) with different concentrations of zero to $10^6$ cells per milliliter were developed. Moreover, to study the effect of the curing method, two processing curing schemes (in distilled water and in a mineral-containing nutrient environment) were considered. The results show the proper effectiveness of this method, in a way that up to 35% increase in the samples' compressive strength may be observed depending on bacterium type and curing method.

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## 1- Introduction

Concrete is the most widely used construction material at the present age. Ease of access to ingredients, simple production, and easy formability and molding of fresh concrete have led to the popularity of this material in construction industry [1]. On the other hand, production of cement is a highly energy-consuming process with a great level of pollution [2]. That is why conducting research on methods of reducing cement consumption in the construction industry has drawn the attention of researchers throughout the world. In this context, pozzolans such as fly ash, silica fume, and ground granulated blast furnace slag may be mentioned [3].

Mechanical properties of cement-based materials may also be improved using microbiological methods. The adoption of microbiological science has gained increasing popularity in the construction industry due to its great capability in overcoming the limitations of traditional methods. This modern, environment-friendly technology may be employed to repair damaged concrete structures. For instance, Hydrolytic enzymes, such as Lipase, that break Ester bonds were used to remove the resin coatings of old Aclyric [4]. Furthermore, Thiobacilli (sulphur oxidizing bacterium) was used to clean concrete surfaces damaged by lichen [5]. Another promising application of this technology is the use of microorganisms that can cause minerals to deposit in the concrete composition in order to improve mechanical properties and also make bacterial concrete. Under certain circumstances, some bacteria can decompose urea leading to the sedimentation of carbonate minerals (calcium carbonate) in the environment after a sequence of chemical reactions. This can affect mortar structure and improve its properties [6]. In this study, the effect of adding different concentrations and compositions of Sporosarcina Ureae and Bacillus Subtilis bacteria as well as different curing schemes on the compressive strength of mortar are studied, while recent studies are also investigated.

## 2- Literature Review

In recent years, numerous studies have been conducted regarding the effect of adding microorganisms on the principal properties of concrete, such as compressive strength. The effects of the existence of bacteria or carbonate crystals produced by them on concrete texture requires further examination and analysis given the close relationship between the microstructure status and the properties of hardened concrete.

Ramachandran et al. [7] used the microbial sequestration process of calcium carbonate for improving the compressive strength of the mortar prepared with ordinary Portland cement. They investigated the effect of the added substance, as well as the type and quantity of such bacteria as Pasteuri and Aerugenosa on properties of cement paste. In addition, they examined both the effect of live and dead cells in order to study the effect of the biological mass status on compressive strength. Mortar designs made with low concentrations of Pasteurii bacterium exhibited improved compressive strength compared with the reference mortar samples

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(without bacterium). The 28-day compressive strength for samples made with the reference mortar and those containing the aforementioned bacterium were approximately 55 MPa and 65 MPa, respectively. They attributed the increased compressive strength to the existence of organic materials resulting from the microbial biological mass in the mortar.
Nevertheless, with the biological mass expanding, reduced strength was reported, especially for dead cells. The researchers believed that increased mortar porosity as a result of the decomposition of the existing organic materials in the course of time.
Wahhabi et al. [8] used alkaliphile bacterium of bacillus type that was separated from soil to produce sediments inside the mortar sample in a study conducted on the compressive strength of cement mortar. After the samples were tested, a 15% increase in the compressive strength was reported compared with the control samples.
Nosouhian et al. [9] adopted Bacillus Subtilis, Sporosarcina Pasteurii, and Bacillus Sphaericus bacteria in a research performed to investigate the effect of processing using biological sediments on concrete durability. In a part of this research, they performed microbial sequestration process on concrete samples that were processed in the water up to 27 days by adding bacteria and nutrient substances (urea and calcium salt) to the curing environment. They, thus, compared the compressive strength of the mixes at different ages. At all ages, the compressive strength of the samples processed via microbial sequestration process was greater than that of the control samples. For example, over 90 days, the compressive strength of all samples processed by this method was 2.26 % higher on average compared with control samples. Nonetheless, this was unexpected given the previous studies [10] addressing the limitation of bacteria penetrating into the concrete surface. They also observed a considerable reduction in the water absorption of the samples processed by this technique compared with reference samples.
Siddique et al. [11] adopted a sort of urease bacterium separated from marble production waste in a laboratory, and examined the compressive strength and permeability of the concrete made with the cement obtained from baghouse filters. The concentration of the bacterium solution was constant in all designs ($10^5$ cells per milliliter). Over different periods,about 10 % increase in compressive strength was observed for the samples made with ordinary cement. Additionally, the samples containing bacteria and cement replacement materials exhibited a better performance in all substitution percentages compared with the samples without bacteria containing cement substitutes. In another research in the same year [12], they examined the effect of Bacillus Aerius bacterium on the properties of the concrete containing rice husk ash. This urease bacterium was added to concrete mixing water with a concentration of $10^5$ cells per milliliter. The results indicated the positive effect of using the bacterium on the compressive strength in all mixtures. In both of these studies, the effect of using the bacterium on the water absorption of plans was reported to be positive.

## 3- Materials and Methods

### 3- 1- Preparing Bacterium Solution to Be Used in the Mortar

The freeze-dried samples of Bacillus Subtilis and Sporosarcin Ureae bacteria acquired from the Persian Type Culture Collection were cultured in a culture environment containing urea (25 grams per liter), sodium bicarbonate (2.12 grams per liter), ammonium chloride (10 grams per liter), nutrient broth (3 grams per liter), and bihydrous calcium chloride (25 grams per liter) in an incubator at a temperature of 28 degrees centigrade.
After reaching the concentration of $10^6$ cells per millimeter, the thinned solutions from the resulting combination were prepared with ratios of 100, 10, and 1 volumetric percentage of distilled water and were used as mortar mixing water.

### 3- 2- Preparing Urea-Calcium Chloride Environment to Be Used in the Mortar

According to the previously defined experimental plan, half of the samples should be kept in the urea-calcium chloride environment up to seven days after their preparation. Then, they should be transferred to the water.

### 3- 3- Mortar Samples

Ordinary Portland cement type 1-425 of Sepahan Esfahan cement factory was used to make the samples. The liquid culture environment contained bacteria with the concentration of $10^6$ cells per milliliter with ratios of 100, 10, and 1 volumetric percentage and was thinned using distilled water and was utilized as mortar mixing water. To investigate the effect of urea concentration on the activity of bacteria, the quantity of urea in the half of the solutions made was twice as much (50 grams per liter in pure bacterium solution). Cubic samples of mortar were made according to ASTM C109 with dimensions 50×50×50 millimeters with the water-to-cement ratio of 0.5 (the bacteria solution was taken into account) and they were then compacted. The sample mixture designs and specifications are presented in Tables 1 and 2. The chemical composition of the used cement is shown in Tables 3 and 4.

### 3- 4- Preparing Bacterium Solution to Be Used in the Mortar

The Cubic mortar samples were made with different bacterium concentrations. Moreover, the control sample was made with the same water-to-cement ratio without using bacteria. To examine the effect of the bacterium solution on the mechanical properties of the hardened mortar, the compressive strength test was conducted on the above samples for 7 and 28 days.

**Table 1. Mixture designs**

| Mix Code | Cement (weight ratio) | Water (weight ratio) | Bacterium Solution (weight ratio) | Sand (weight ratio) |
|---|---|---|---|---|
| CSW - CSM | 1 | 0.5 | 0 | 2.52 |
| 0.01 UW | 1 | 0.45 | 0.05 | 2.52 |
| 0.01 UM | 1 | 0.45 | 0.05 | 2.52 |
| 0.01 W | 1 | 0.45 | 0.05 | 2.52 |
| 0.01 M | 1 | 0.45 | 0.05 | 2.52 |
| 0.1 UW | 1 | 0.4 | 0.1 | 2.52 |
| 0.1 UM | 1 | 0.4 | 0.1 | 2.52 |
| 0.1 W | 1 | 0.4 | 0.1 | 2.52 |
| 0.1 M | 1 | 0.4 | 0.1 | 2.52 |
| 1 UW | 1 | 0 | 0.5 | 2.52 |
| 1 UM | 1 | 0 | 0.5 | 2.52 |
| 1 W | 1 | 0 | 0.5 | 2.52 |
| 1 M | 1 | 0 | 0.5 | 2.52 |

**Table 2. Mixture specifications**

| Mix Code | Specification |
|---|---|
| CSW | No bacterium - No extra urea – Cured in water |
| CSM | No bacterium - No extra urea – Cured in culture environment |
| 0.01 UW | 1% bacterium - With extra urea – Cured in water |
| 0.01 UM | 1% bacterium - With extra urea – Cured in culture environment |
| 0.01 W | 1% bacterium - No extra urea – Cured in water |
| 0.01 M | 1% bacterium - No extra urea – Cured in culture environment |
| 0.1 UW | 10% bacterium - With extra urea – Cured in water |
| 0.1 UM | 10% bacterium - With extra urea – Cured in culture environment |
| 0.1 W | 10% bacterium - No extra urea – Cured in water |
| 0.1 M | 10% bacterium - No extra urea – Cured in culture environment |
| 1 UW | 100% bacterium - With extra urea – Cured in water |
| 1 UM | 100% bacterium - With extra urea – Cured in culture environment |
| 1 W | 100% bacterium - No extra urea – Cured in water |
| 1 M | 100% bacterium - No extra urea – Cured in culture environment |

**Table 3. Chemical composition of the cement used in this study**

| Chemical | $SiO_2$ | $Al_2O_3$ | $Fe_2O_3$ | CaO | MnO |
|---|---|---|---|---|---|
| Content (%) | 21.83 | 4.34 | 2.21 | 65.34 | 0.13 |

**Table 4. Chemical composition of the cement used in this study (continue)**

| Chemical | MgO | $P_2O_5$ | $Na_2O$ | $K_2O$ | $SO_3$ | L.O.I |
|---|---|---|---|---|---|---|
| Content (%) | 2.17 | 0.12 | 0.36 | 0.63 | 2.57 | 0.91 |

## 4- Results And Discussions

The compressive strength experiment results on cubic mortar samples of 5×5×5 centimeters containing different concentrations of Sporosarcin Ureae bacterium are illustrated Figures 1 to 4 in 7 and 28 days in normalized form. Compressive strength results of the control samples cured in water and culture environment at these periods are also presented in Figures 5 and 6.

As can be seen, the sample exhibits the greatest increase in strength compared with control samples at the concentrations of $10^4$ and $10^5$ cells per milliliter. (during 7 days up to 38 % and 28 days up to 35 % increase compared with the corresponding control sample, depending on the type of curing and the existence or non-existence of extra urea). This increased strength may be mainly attributed to the existence of calcium carbonate sediments resulting from microbial activities. This also agrees with the outcomes of previous studies [11-13].

In light of these results, the type of bacteria, as well as the type of processing environment, are considered to be two effective factors in the process of gaining strength, in a way that Sporosarcin Ureae and Bacillus Subtilis bacteria displayed different performances in relation to their processing environment. In other words, the samples containing Sporosarcin Ureae that were cured in the water gained greater strength compared with the samples that were processed in a nutrient environment. This was opposite for Bacillus Subtilis bacterium. The mixtures made using this bacterium in a nutrient environment exhibited greater acquisition of strength compared with the mixtures cured in the water. One reason for this difference in performance can be the unwanted, potential reproduction of other microorganisms in the mortar mixture (the bacteria existing in stone materials, cement, and even water) by being placed in a nutrient environment, in addition to bacterium type.

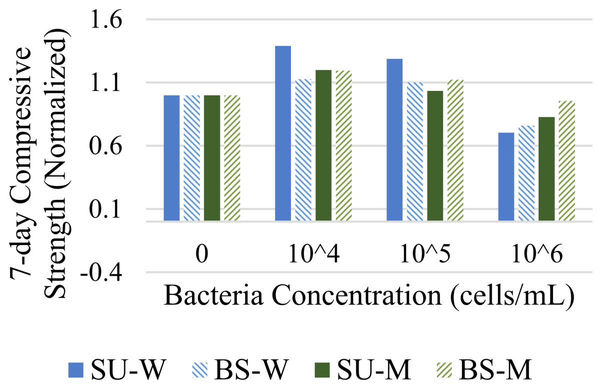


**Fig. 1. Normalized Seven-Day Compressive Strength for the Mixes without Extra Urea**

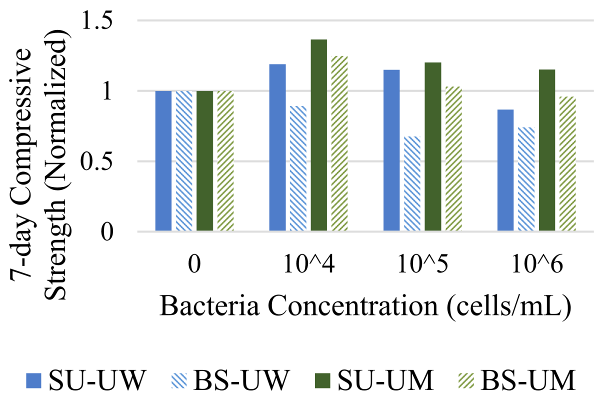


**Fig. 2. Normalized Seven-Day Compressive Strength for the Mixes with Extra Urea**

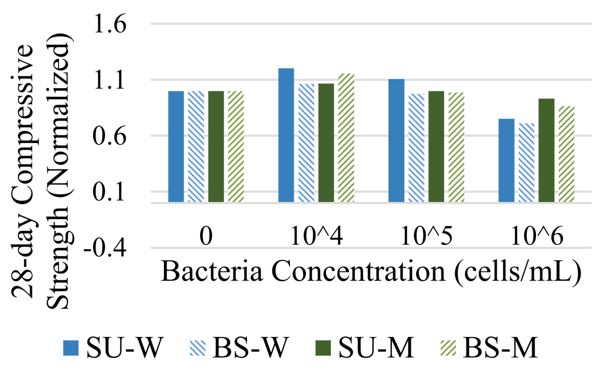


**Fig. 3. Normalized 28-Day Compressive Strength for the Mixes without Extra Urea**

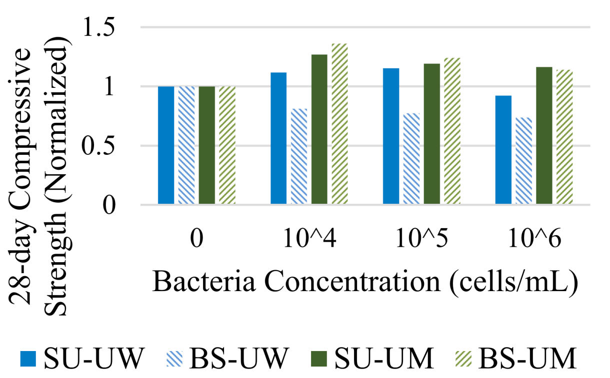


**Fig. 4. Normalized 28-Day Compressive Strength for the Mixes with Extra Urea**

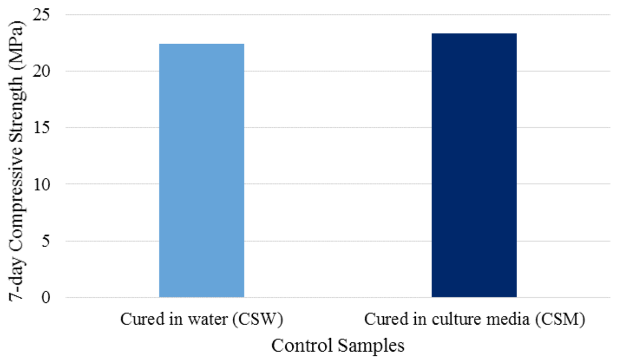


**Fig. 5. 7-Day Compressive Strength for the Control Samples**

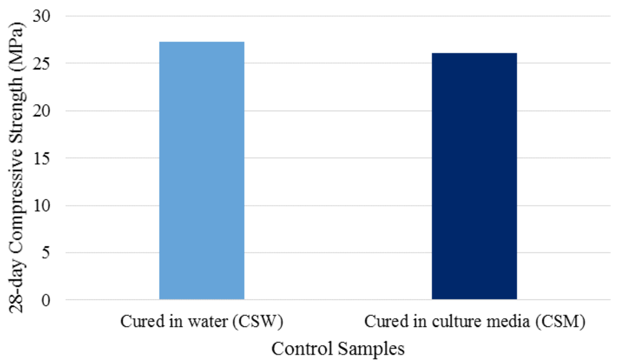


**Fig. 6. 28-Day Compressive Strength for the Control Samples**

However, the compressive strength of the design containing Bacillus Subtilis bacterium reached the lowest level among the mixes being compared to all mixes containing extra urea. Consequently, the existence of urea probably reduces the activity of this bacterium excessively or leads to the generation of low-strength by-products.

With respect to the existence of extra urea, it may be observed given the variation trend of compressive strength that the existence of extra urea at the age of 7 days results in increased strength compared with the same mode without extra urea. However, this difference in strength over 28-day-period fades away to a great extent. In other words, the existence of extra urea in the samples may somehow have a quick-setting effect, i.e. it speeds up the strength acquisition at early periods. However, strength at longer periods does not differ much with the mode where there is no extra urea.

Furthermore, taking an overall view allows one to see that the variations of compressive strength at concentrations of more than $10^4$ cells per milliliter have a decreasing trend, insofar as compressive strength values are comparable with those of the control samples at the concentration of $10^6$ cells per milliliter. What accounts for this may be the disruption of the integrity and cohesion of cement matrix as a result of the excessive activity of the bacteria at high bacteria concentrations.

## 5- Conclusion

It becomes clear in light of the conducted experiments that the activity of microorganisms can improve the compressive strength of cementitious mortar. This agrees with the results of previous studies [13]. Of course, when bacterium concentration exceeds a certain limit, compressive strength begins to decrease. In the present study, the optimal concentration is $10^4$-$10^5$ cells per milliliter. It should be mentioned that the optimal value was achieved to be 105 cells per milliliter in previous studies [11, 12]. It is also obvious that the type of the microorganism used plays a decisive role in how mortar properties change, in a way that Sporosarcin Ureae bacterium seems to outperform Bacillus Subtilis bacterium.

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