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ORIGINAL ARTICLE

The influence of Calcite-Producing Bacteria on Cement Mortar Compressive Strength and Durability Characteristics

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ABSTRACT:

Background: The phenomenon of microbiologically induced calcite precipitation (MICP) is a part of the chemical process called biomineralization. Microorganisms form inorganic solids like calcite by such process. Bacillus spp. are common soil bacteria that may induce calcite precipitation. The microbial sealant properties of $CaCO_3$ improve mortar and concrete's compressive strength.

Methods: This study examined the impact of locally isolated Calcite-Producing bacteria on mortar characteristics.
Eight isolates of Bacillus spp. have been prepared in suspensions adjusted to absorbance of 1 at 600 nm, that contain 2% urea and 25 mmoll-1 calcium chloride, and applied to prepare mortar cubes. The ratio of cement: sand: water mix was 1:3:0.5. Control cubes were prepared using the same mix ratio and containing water instead of bacterial suspension. Compressive strengths for all cubes on days 7 and 28 were measured. A durability tests and water absorption test for the cubes were performed.

Results: Bacterial mortar cubes showed a compressive strength about 39 to 81% and 9.6 to 60.2% higher than the control on the 7th day and 28th day, respectively. Bacterial mortar cubes resist H_2SO_4 attack at pH ≥ 2, while control cubes couldn't resist at pH ≤ 6. After treatment with 5% $MgSO_4$, all bacterial cubes showed higher compressive strengths. Water absorption of bacterial cubes ranged from 2.4-4.8%, while the control cubes absorbed water with a percentage of 7.2%.

Conclusion: These species are promising contenders for MICP involved investments, and can be used in a many of construction applications.

KEYWORDS: Calcite precipitation; Urolytic bacteria; Bacillus spp.; mortar; Compressive strength, durability; Gaza strip.

INTRODUCTION:

The significant ability of microorganisms to precipitate calcite introduces a noticeable progress in all branches of concrete and mortar technology (Vempada, Reddy, Rao, & Sasikala, 2011). Calcite is a coarse crystal that easily adheres to the surfaces forming shells. In addition to its ability to constantly expand upon itself, it is insoluble in water. There is an inherent ability to precipitate calcite continuously within the mortar or concrete matrix through the bacteria (Ramachandran, Ramakrishnan, & Bang, 2001; Ramakrishnan, Panchalan, Bang, & City, 2005). Studies reported that *Sporosarcina pasteurii* (formerly known as *Bacillus pasteurii*) as confining bacteria that precipitates calcite (Chahal, Rajor, & Siddique, 2011). However, many studies have emphasized the importance of bacteria, primarily those belonging to the genus *Bacillus*, as significant contributors in this phenomenon. These bacterium species are also urease positive (Ali, Karkush, & Al Haideri, 2020; Elmanama & Alhour, 2013; Tayyem, Elhello, & Elmanama, 2021).

The phenomenon that known as Microbial Induced Calcite Precipitation (MICP) can be invested to enhance the overall strength and characteristics of cement mortar and concrete samples. Moreover, a promising sustainable and repair applications can be accomplished based on MICP. The majority of building structures, highway bridges, slopes, and dams are erected and repaired using concrete (Bernardi, DeJong, Montoya, & Martinez, 2014; Javadi, Badiee, & Sabermahani, 2018; Vempada et al., 2011). The production of bio-cement using MICP harbored bacteria improves the strength and durability of building materials that face adverse effects of the surrounding environment (Bernardi et al., 2014; Javadi et al., 2018).

It can be used to repair cracks in mortar and concrete. Increasingly, it can be applied to prohibit a variety of geotechnical issues, such as sand's ability to liquefy and soil erosion (Moosazadeh et al., 2019; Salifu, MacLachlan, Iyer, Knapp, & Tarantino, 2016). Gaza Strip region suffers from a shortage of construction materials along with several related problems including the intrusion of underground water and increased humidity, especially in refugee camps and areas near the Sea beach. This causes wall cracks, foundation concrete deterioration, and external wall painting and plastering could get badly affected (Enshassi, Kumaraswamy, & Al-Najjar, 2010). In this study, several *Bacillus spp*. have been used in different conditions to measure and compare their effect on the compressional strength, durability, and water absorption of mortar cubes.

METHODOLOGY:

Materials, microorganisms, and media used

Ordinary Portland Cement CEM II B-LL 32.5N and clean, dry, obtained locally, well-graded, natural sand (with 0.1 mm to 0.6 mm diameter size) were utilized. Chemical composition of the utilized cement is shown in table 1 (Andrade & Sanjuán, 2021).

Table 1: Chemical compositions of cement utilized in the study

Chemical Composition, %						
AL_2O_3	CaO	Fe_2O_3	MgO	SiO_2	SO_3	others
4.30	55.96	2.20	2.40	16.83	3.06	1.14

Aerobic and alkaliphilic bacteria belong to *Bacillus* spp. that was previously isolated and identified by numbers (3, 7, 8-1, 8-2, 8-3, 8-4, 9, and 10) according to their source of isolation (Table 2) (Tayyem, Elhello, & Elmanama, 2021), have been cultured in nutrient broth (HiMedia, India). Bacterial cultures were incubated at 37°C using the WIS-20 shaking incubator (WITEG Labortechnik, Germany); until an absorbance value of 1 at wavelength 600 nm was obtained.

Table 2: Identification of bacterial isolates

Isolate no.	Closely related to	Similarity %
3	*Paenibacillus alvei*	90.3
7	*Lysinibacillus spaericus*	98.1
8-1	*Lysinibacillus spaericus*	98.1
8-2	*Lysinibacillus spaericus*	94.2
8-3	*Bacillus licheniformis*	94
8-4	*Lysinibacillus spaericus*	98.1
9	*Paenibacillus residui*	93.8
10	*Bacillus mycoides*	94.3

Estimation of Calcite Production

To investigate calcite precipitation, microbiologically mediated sand plugging was used. A plastic column (height=15 in.; diameter=3 in.) was backed with 50 ml of bacterial culture (OD_{600}=1.0) containing 2% urea and 25 mM $CaCl_2$, that were combined with 100 g sterilized coastal sand, the bottommost side of the column was clogged using Whatman filter paper. A control reaction was packed in a column in which sterile sand was mixed with the growth media only (without bacteria). Each column was immersed daily with the bacterial suspension of one of the eight isolates at the ambient temperature to imitate the natural environmental conditions. The experiments of the sand columns were terminated after 8 days (Figure 1). Prior to the estimation of the calcite concentration,

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ISRAA UNIVERSITY JOURNAL OF APPLIED SCIENCE (IUJAS)

the microbiological sand column was separated into three layers (the upper, middle, and bottom layer), each of which was grounded and sieved through a 45 mm diameter mesh. By using the EDTA titration method, the precipitated calcite from each layer was determined. A sand sample (1 gm) from each portion was dissolved with 1 ml of 1 N HCl, 10 ml of ammonium buffer pH 10, and 5 ml of distilled water. Erichrome black T indicator was added as five drops and the mixture was finally titrated against 0.05 M EDTA. The amount of calcite present as calcium carbonate was calculated.

Figure 1: Application of Cementation reactants into a sand core for calcite estimation.

Mix proportions and procedure

Bacterial suspensions containing 2% urea and 25 mM calcium chloride were used to mix cement and sand. The cement: sand: the bacterial suspension ratio was 1:3:0.5. A cubic molds of 50 mm x 50 mm x 50 mm were used to prepare the mortar cubes. Control cubes were prepared under the same conditions using a sterile broth media as a liquid source.

A second trial using bacterial suspensions in a phosphate buffer, and a sterile buffer solution was used to prepare control cubes. The components were mixed properly using Mlx40-D mortar mixer (CAD Instrumentation, France). The total number of mortar cubes prepared was 135. The cubes were

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casted and compacted. After demolding, each test cube was cured at room temperature in its corresponding bacterial suspension (in broth or buffer), while control cubes were cured with a sterile broth or buffer depending on the trial. The curing solutions were replaced every 7 days. Figure 2 A shows a mortar cube after curing with calcite precipitation at the surface.

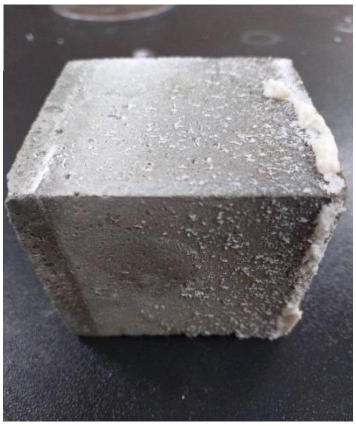
Compressive strength of mortar cubes after microbial cementation

The compressional strengths for the cubes of the were determined on the 7th and 28th days ages (Figure 2B), using an automatic compression testing machine (Comptest 3000, Eie Instruments Pvt. Ltd., India). In total, 54 cubes were tested for their compressive strength. All tests were done in replicates.

Durability assessment: Compressive strength after sulfate treatment

Regarding durability assessment, a total of 54 mortar cubes (50 mm x 50 mm x 50 mm) were prepared in triplicate for each type of bacteria and the control, immersed in their corresponding curing solution for 28 days. The compressive strength was measured for the cubes on day 28, and the remaining cubes were placed in magnesium sulfate solution (50 g of $MgSO_4$ in 1000 ml distilled water), another compressive strength test was performed on the 90th day.

A



B

Figure 2: A: shows a mortar cube after curing with the bacterial suspension, and B: shows the application of the compressive strength test.

Durability assessment: acid resistance test

Serial pH grades of H_2SO_4 solution were prepared for the evaluation of the acid resistance of the cement mortar. The pH solutions were as follows; 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, and 5.5. A drop of each H_2SO_4 solution was dripped on the mortar and a stereomicroscope Olympus (Figure 3A) was used to observe the mortar carefully for 2 min to see whether air bubbles appeared (Figure 3B). If there were no air bubbles, it was possible to deduce that the mortar could withstand corrosion from this pH of the H2SO4 solution before being exposed to the next pH of the H2SO4 solution. The first pH value at which air bubbles were not generated, was recorded as the acid resistance value. For example, if air bubbles are not generated at pH=3.0, and the last pH showed air bubbles generation was 2.5, the acid resistance value of the mortar is 3.0 (Chunxiang, Jianyun, Ruixing, & Liang, 2009). A total of 27 cubes were tested (triplicate for each type of bacteria and the controls).

A

B

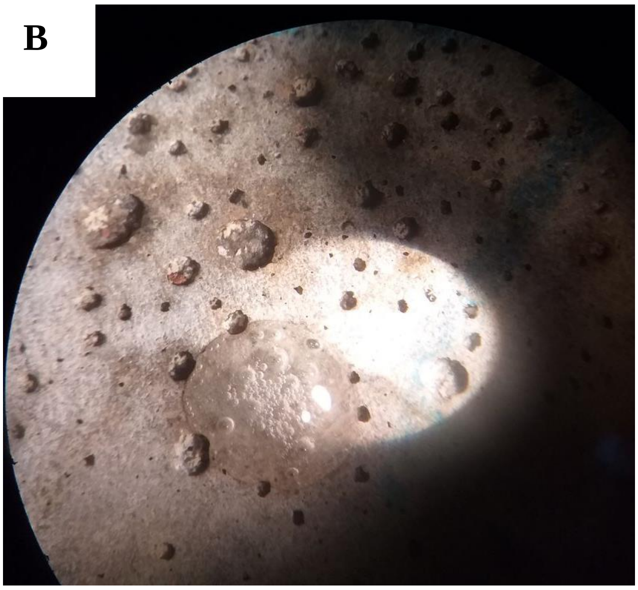


Figure 3: A: investigation of mortar cubes under the stereomicroscope, and B: shows the reaction of acid attack to the mortar cube under the microscope represented as bubbles.

Water Absorption capacity Test

Water absorption capacity was measured for 27 cubes of bacterial suspension and control. Cubes were molded and treated in water from a tap for 28 days, saturated overnight in water and weighed. The cubes were then desiccated in an oven at 100 ^{o}C for 24 hours, cooled, and reweighed. Water absorption was calculated using the following formula:

$$\%\, Water\ Absorption = \frac{(W_{saturation} - W_{oven\ dried})}{W_{oven\ dried}} \times 100$$

ISSN:2523-0522
Volume 7: issue 2, October 2024
ISRAA UNIVERSITY JOURNAL OF APPLIED SCIENCE (IUJAS)

Where $W_{saturation}$ is the weight of cubes after saturation in water for 24 h and $W_{Oven\ dried}$ is the weight of cubes after oven drying for 24 hours (Sarda (Sarda, Choonia, Sarode, & Lele, 2009).

RESULTS and DİSCUSSİON

Estimation of Calcite Production in Sand Core

The included isolates were locally recovered and identified as their relativeness to *Bacillus spp*. (Tayyem et al., 2021). The quantification of the calcium carbonate produced by the isolates was carried out using the EDTA titration method (V Achal, Mukherjee, Basu, & Reddy, 2009; Association & Association, 1995; Stocks-Fischer, Galinat, & Bang, 1999). Results showed the presence of high concentrations of calcium in all the bacterial-treated sand columns in the form of calcite or calcium carbonate. The control column showed a lower content of calcite. The highest concentration of calcite was found in the sand column consolidated with the isolates 8-4, 10, 9, and 8-2, which was clearly observed from the results of EDTA titration (Figure 4). For all sand cores, the highest levels of calcite crystals were formed in the upper portion (surface), and the flow rate of the infused media became slower over time.

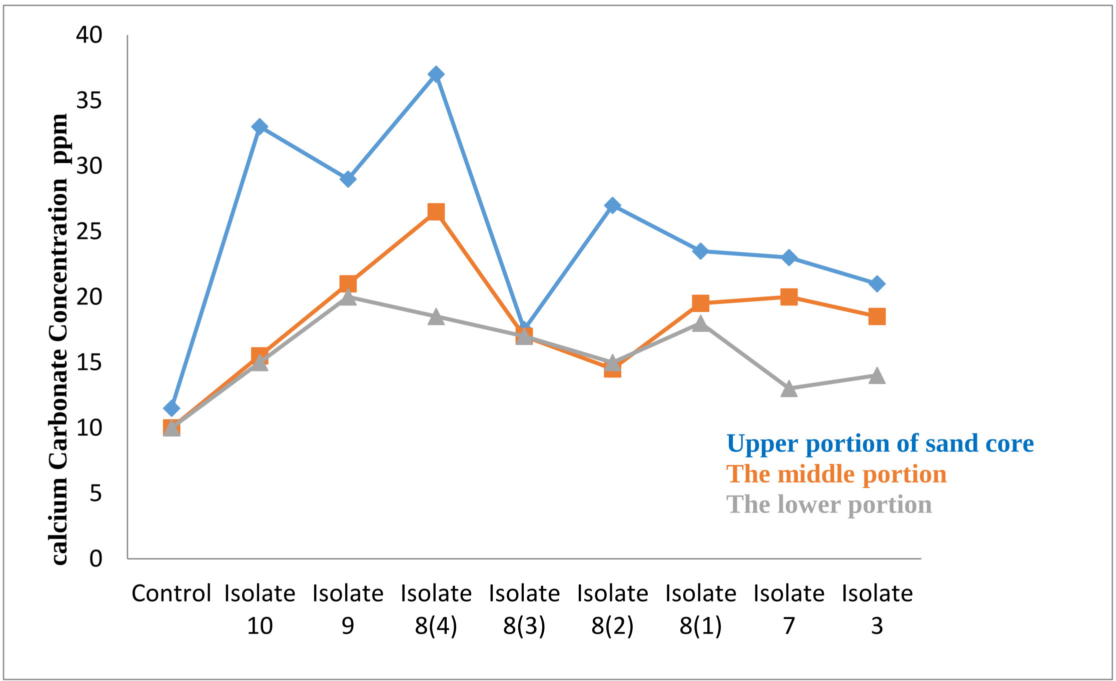


Figure 4: Quantification of $CaCO_3$ precipitation in the different layers of sand cores.

This was in agreement with (V Achal et al., 2009; Varenyam Achal, Mukherjee, & Reddy, 2011; Park, Park, Chun, Kim, & Ghim, 2010). This is attributed to the fact that the isolates used are aerobic in nature. The variability in the extent of calcite production among isolates depends on several factors, including pH of growth medium, type of nutrient source, urease production capacity, bacterial generation time, incubation time, and the initial source of calcium (V Achal et al., 2009; Varenyam Achal et al., 2011; Hadi, Abbas, Almajed, Binyahya, & Al-Salloum, 2022; Wu, Hu, Zhang, Xue, & Zhao, 2019).

The influence of different strains on the compressive strength of mortar

The compressive strength results of mortar cubes consolidated with calcite-forming bacteria at the age of 7 and 28 days are presented in Table 3. The 7-day and 28-day bacterial mortar cubes showed higher compressive strength as compared with control samples. The 7-day strength test of cubes consolidated with 8-2 bacteria indicated the highest increase of compressive strength (81.4%) when compared to that of the control cubes. The same isolate showed the highest increase in compressive strength of the 28 -day bacterial mortar cubes.

Table 3: Compressive strength of mortar cubes consolidated with different isolates of calcite producing bacteria in broth media against control cubes

Bacterial Mortar cubes	Compressive strength in KN			
	Average of 7th day compressive strength (KN)		Average of 28th day compressive strength (KN)	
	Strength	Strength increase %	Strength	Strength increase %
Control	32.2	0	46.7	0
3	49.5	53.7	58.8	25.9
7	47.4	47.2	62.8	34.5
8-1	50.3	56.2	71.8	53.7
8-2	58.4	81.4	74.8	60.2
8-3	45.1	40.1	51.2	9.6
8-4	44.8	39.1	64.1	37.3
9	49.8	54.7	58.6	25.5
10	46.2	43.5	63.3	35.5

Table 4 presents the compressive strength of the mortar cubes that prepared with bacteria suspended in a buffer at the 7^{th} and 28^{th} days, The 7-day and 28-day bacterial mortar cubes indicate higher compressive strength relative to the control samples in most of the isolates. The 7-day strength test for cubes consolidated with 8-1, 9, and 8-2 bacteria presented the highest improved compressive strength (40.1%, 40.1%, and 39.5%, respectively) relative to the control. While the 28–day strength test showed that the highest improved compressive strength of the mortar cubes was for the isolate 7 (37.1%), followed by the isolate 8-4, and 8-2 (strength increase 29.6% and 27.4%, respectively). However, mortar cubes consolidated with the isolate 9 did not indicate a significant improvement in

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ISRAA UNIVERSITY JOURNAL OF APPLIED SCIENCE (IUJAS)

compressive strength at age of 28 days.

The compressional strength results of bacterial mortar cubes treated in the broth medium indicated a stronger strength at the 7 and 28 days when compared with mortar cubes, which were prepared with buffer-based bacterial suspension.

These findings are in good agreement with several studies (Balam, Mostofinejad, & Eftekhar, 2017; Jagadeesha Kumar, Prabhakara, & Pushpa, 2013; Mondal & Ghosh, 2018; Ngari, Thiong'o, Wachira, Muriithi, & Mutitu, 2021; Park et al., 2010; Priyom, Islam, Islam, & Shumi, 2022; Sahoo, Sathyan, Kumari, Sarkar, & Davis, 2016; Salmasi & Mostofinejad, 2020). MICP is an effective phenomenon that can be invested in surface consolidation in porous media as mortar cubes. During the initial curing period, the bacterial cells incorporated within the porous mortar matrix still can get nourishment, however can grow slowly in a totally new environment. Later, as the bacteria grow, calcite may deposited on the surface of the cells as well as within the mortar matrix. Over a long curing period the cement mortar becomes less porous and impermeable with no more spaces for bacterial growth. This leads to an increase in the compressive strength (Jagadeesha Kumar et al., 2013). This decrease in pores in the bacterial cubes due to calcite precipitation explains the increased compressive strength of the mortar cubes cured with broth media compared to those cured with buffer solutions.

Table 4: Compressive strength of mortar cubes consolidated with different isolates of calcite producing bacteria suspended in a buffer against control cubes

Mortar cubes treated with bacteria suspended in buffer	Compressive strength in KN			
	Average of 7th day compressive strength (KN)		Average of 28th day compressive strength (KN)	
	Strength	Strength increase %	Strength	Strength increase %
Control	32.9	0	45.6	0
3	43.4	31.9	51.7	13.4
7	39.5	20.1	62.5	37.1
8-1	46.1	40.1	55.3	21.3
8-2	45.9	39.5	58.1	27.4
8-3	45.2	37.4	53.3	16.9
8-4	42.6	29.5	59.1	29.6
9	46.1	40.1	45.1	-1.1
10	43.8	33.1	57.9	27.0

Compressive strength of sulfate treated bacterial mortar cubes

The sulfate-treated bacterial mortar cubes showed an increase in compressive strength as compared with that of the control cubes (Table 5), with a maximum increase of 50.7% and tolerance to sulfate

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attack of isolate 3. The most sensitive mortar cubes for sulfate attack are those prepared with isolate 8-1 suspension. This suggests that bacterial mortar cubes can resist hard environmental conditions by holding higher durability characteristics.

Acid resistance of mortar cubes

Figure 5 summarizes the data obtained from the acid resistant test for mortar cubes consolidated with bacterial isolates suspended in a buffer. The acid resistance of the bacterial cubes with isolates 3, 8-1, 8-2, and 8-3 indicated the best values as compared to the control samples. This was in agreement with several studies (Javadi, Badiee, & Sabermahani, 2018; Munyao, Thiong'o, Wachira, Mutitu, & Mwirichia, 2020; Nguyen, Ghorbel, Fares, & Cousture, 2019; Ramakrishnan, Panchalan, Bang, & City, 2005; Salmasi & Mostofinejad, 2020).

Table 5: Compressive strength of mortar cubes consolidated with different isolates of calcite producing bacteria suspended in a buffer and treated with sulfate solution against control cubes

Mortar cubes with bacteria suspended in buffer	Compressive strength in KN			
	Average of 28th day compressive strength (KN)		Average of 90th day compressive strength (KN)	
	28 days of curing in bacteria containing buffer solutions		62 days of sulfate treatment	
	Strength	Strength increase %	Strength	Strength increase %
Control	45.6	0	59.72	0
3	51.7	13.4	90.0	50.7
7	62.5	37.1	81.1	35.8
8-1	55.3	21.3	62.8	5.2
8-2	58.1	27.4	82.7	38.5
8-3	53.3	16.9	63.7	6.7
8-4	59.1	29.6	69.5	16.4
9	45.1	-1.1	65.3	9.3
10	57.9	27.0	70.1	17.4

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Volume 7: issue 2, October 2024
ISRAA UNIVERSITY JOURNAL OF APPLIED SCIENCE (IUJAS)

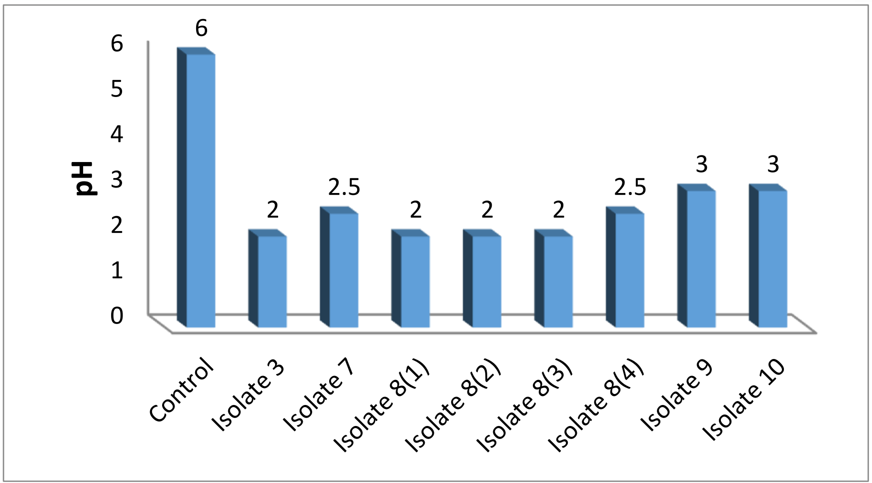

Fig. 5: Sulfate attack test of mortar cubes consolidated with different isolates of calcite producing bacteria suspended in a buffer.

Water absorption test

The water absorption test was conducted on mortar cubes prepared using phosphate buffer suspension of bacteria to determine the rise in the mortar cubes' resistance to water infiltration. Calcium-treated Bacterial mortar cubes showed a significant decrease of water absorption in comparison with that of the control samples (Table 6). Mortar cubes containing isolates 8-4 showed the highest reduction of water absorption (66.7%), followed by cubes containing isolates 8-2 (65.3%). While cubes prepared with 8-3 isolate suspension showed the lowest reduction of water absorption (23.6%).

Table 6: Percentage of water absorption of mortar cubes consolidated with different isolates of calcite producing bacteria suspended in a buffer.

Mortar cubes	Wt (saturated)	Wt (dry)	% Water absorption	% Reduction in water absorption
Control	263.2	245.4	7.2	0
3	267.2	258.2	3.5	51.4
7	279.9	272.8	2.6	63.9
8-1	273.2	265.9	2.7	62.5
8-2	282.7	275.9	2.5	65.3
8-3	265.8	251.9	5.5	23.6
8-4	272.0	265.6	2.4	66.7
9	261.7	255.0	2.6	63.9
10	256.7	244.8	4.8	33.3

The lower gain of water absorption in bacterial mortar cubes when compared to control affirms the ability of bacteria to improve mortar's ability to resist the negative effect of humidity. Similar findings were also reported in previous studies (Varenyam Achal et al., 2011; De Muynck, De Belie, & Verstraete, 2010; Munyao et al., 2020; Ngari et al., 2021). The bacterial presence results in a significant decrease in water uptake (Varenyam Achal et al., 2011).

In conclusion, the isolated bacteria is able to calcify microorganisms that have positive effect on the mortar characteristics, namely: compressive strength, durability, and resistance to water penetration (water absorption). Hence, the microbial addition can provide a local and cheap alternative, high quality, cost effective, environmentally safe, and easy to use mortar sealant.

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CONFLİCT of INTEREST
No conflict of interest declared

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