



Research article

Bioremediation of mortar made from Ordinary Portland Cement degraded by *Thiobacillus thioeparus* using *Bacillus flexus*Reginah Wangui Ngari^{a,*}, Joseph Karanja Thiong'o^a, Jackson Muthengia Wachira^b, Genson Muriithi^b, Daniel Karanja Mutitu^b^a Department of Chemistry, Kenyatta University, Nairobi, Kenya^b Department of Physical Sciences, University of Embu, Embu, Kenya

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ABSTRACT

Cement is widely used as a construction material in the construction industry. However, there are challenges affecting its durability efficacy. Cement mortar/concrete is subject to degradation by aggressive ions such as sulphates and chlorides. Sulphates can be introduced into the concrete or mortar by Sulphur producing bacteria of the species *Thiobacilli*. Microbiologically induced CaCO₃ precipitation (MICP) has found its application in bioremediating cement based materials. It has been found to be environmental friendly. However, no work has been reported on bioremediation of biodegraded cement based materials. This paper presents findings of possible bioremediation of mortars after undergoing biodegradation. *Bacillus flexus*, a beneficial bacterium was used. The control mortars were prepared using Ordinary Portland Cement (OPC). The test mortars were prepared and cured in a solution of *Thiobacillus thioeparus*, a Sulphur oxidizing bacteria, deleterious bacterium for 14, 28, 56 and 90 days. Compressive strength analysis was conducted on the 14th, 28th, 56th and 90th day of curing. Results showed that the lowest compressive strength was recorded on the 90th day as 31.02 MPa. This was a 34.17 % loss in compressive strength. Another category of mortar cured in *Thiobacillus thioeparus* for 28 days was bioremediated for 28 days using *Bacillus flexus* solution. Compressive strength and Scanning Electron Microscopy (SEM) analyses were then done. The results show a compressive strength of 45.83 MPa at the 56th day. This represents a 99.91 % strength recovery from biodeterioration. The SEM analysis results revealed a denser material. This was due to massive precipitation of calcium carbonate in the mortar matrix and pores/voids for bioremediated mortars as opposed to the biodegraded mortars. The results further revealed reduced ettringite crystals on the bioremediated mortars. *Bacillus flexus* could perhaps be used in restoring lost compressive strength as well as in sealing voids in degraded concrete in sewer lines and other cement based materials. This could improve on its efficacy with minimal repair.

1. Introduction

Degradation of cement based materials is a major durability limitation. This leads to premature failure of the material. Presence of deleterious agents such as sulphates, chlorides in the mix media and within the environment of cement based material can have adverse deleterious effect on it. Repair and maintenance of these structures is an expensive affair. Currently, repair uses epoxy-mortars and water repellent materials. The efficacy of these materials is only limited to a short time [1]. Presence of micro-cracks/voids provides a pathway for ingress of the degrading agents, such cracks can be sealed using biological methods. The metabolic conversion of calcium acetate using *Bacilli*

bacteria has given promising results [1, 2, 3]. This metabolism involves the conversion of organic salt via microbial respiration to deposit calcium carbonate.

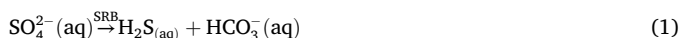
Deterioration of cement made materials caused by microorganisms is termed as Microbiologically Induced Deterioration (MID) has been as a result of the acid formed [5]. Acids arise from microbial activities and affect cement based materials such as sewer pipeline compromising on their structural integrity [5, 6]. Design measures have been put in place to counter the effect of MID so as to protect these materials. These measures include; use of tricalcium (C₃A) free cement, use of fly ash as a pozzolana, and use of low water to cement ratio (w/c = 0.35), use of blended cements, Sulphur resisting cement [6]. However, these measures

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have not guaranteed 100 % efficacy of the material. Repairing degraded concrete is not only expensive but also does not restore the material to its original state.

In sewer systems, deterioration is initiated when hydrogen sulphide (H_2S) is converted into sulphuric acid by sulphur oxidizing bacteria. The hydrogen sulphide is produced when sulphur reducing bacteria (SRB) reduce sulphates as given in Eq. (1) [7]:



Bacteria strain of *Thiobacillus* has been found to be effective in oxidation process (4). The conversion involves the reaction Eqs. (2), (3), (4), and (5)



The acid formed is termed as biogenic sulphuric acid and is a degrading agent. The presence of this acid decreases the pH value of the cement based material to below 2 [8]. This acidic environment favors the decomposition of cement hydration products and subsequent dissolution [9]. Studies by Huber *et al.* [10], demonstrated that cement based material deterioration by the biogenic acid was comparable to the deleterious effect of chemical sulphuric acid of a concentration of 0.2 mmol/L [11]. Its presence within the cement matrix results in reactions of calcium silicate hydrate and calcium hydroxide with it. This is demonstrated in equations 3 -4 [12,13]:



This results in formation of gypsum, which increases the volume of the material by 124 % since it is expansive [12, 13]. Subsequently, the gypsum formed as shown in Eqs. (3) and (4) reacts with the tricalcium aluminate (C_3A) in cement forming ettringite and which is destructive [5]. This ettringite ($C_6AC_6A\bar{S}_3H_{32}H_{32}$) causes the volume of the material to increase by 227–700 % due to expansion [13, 14]. This is shown in Eq. (5):



The increased volume raises the internal pressure resulting in peeling and development of micro-cracks and the integrity of the cement based material is compromised [6, 14]. These micro-cracks act as pathways for ingress of deleterious substances such as chlorides, sulphates and carbon (IV) oxide into the material compromising on its durability. Remediation of cement based material by way of bacteria (bioremediation) has not been reported. However, the use of Microbiologically Induced Calcite Precipitation (MICP) in improving compressive strength has been reported [3, 25], though the use of the same in bioremediation has not been documented. This paper reports the use of the same in bioremediating bio-degraded mortar.

Table 1. Requisite amounts for the liquid culture for DSM 18181.

Reagent	Requisite amount (grams)
$(NH_4)_2SO_4$	0.10
K_2HPO_4	4.00
KH_2PO_4	4.00
$MgSO_4 \cdot 7H_2O$	0.10
$CaCl_2$	0.10
$FeCl_3 \cdot 6H_2O$	0.02
$MnSO_4 \cdot H_2O$	0.02
Agar	12.00
$Na_2S_2O_3 \cdot 5H_2O$	10.00

2. Materials and methods

2.1. Materials

OPC (42.5 MPa) test cement that conforms to Kenya Standard KS EAS 18:1–2017 [15] was used in this investigation. Analytical grade (AR) chemicals and bacteria nutrients were sourced from Highway Laboratory Chemical Equipment, Nairobi, Kenya. *Bacillus flexus* (DSM 1320) and *Thiobacillus thioparus* (DSM 18181) were acquired from Germany, Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.

2.2. *Thiobacillus thioparus* microbial culture

Pure spores of *Thiobacillus thioparus* (DSM 18181) were cultivated from the University of Embu, Kenya (Microbiology laboratory). The procedure adopted was as outlined in the supplier's manual. To prepare 1000 ml of liquid culture, requisite amounts of analytical grade chemicals were accurately dissolved in 1000 ml of distilled water, pH of 7. Table 1 gives the requisite amounts of the reagents that were used in preparing the liquid culture:

The media was sterilized at 121 °C by autoclaving, after which the media was allowed to cool to room temperature. The media pH was adjusted to 6.6 using a mixture of Na_2CO_3 and $NaHCO_3$. The pure spores of *Thiobacillus thioparus* were added followed by 10.0 g of $Na_2SO_3 \cdot 5H_2O$. 12.0 g of agar was added; this was the source of bacterial nutrients. Incubation was conducted in a shaker for 5 days and at a temperature of 30 °C. Bacterial concentration of 1.0×10^7 cells mL^{-1} was used in this investigation and the microbial solution was stored in sterilized container. This solution was used in casting and curing the microbial mortar prisms.

2.3. Microbial cultivation of *Bacillus flexus*

Bacillus flexus (DSM 1320) pure spores were cultivated from the University of Embu, Kenya (Microbiology laboratory). The procedure adopted was as per the supplier's manual, the *Bacillus flexus* bacterial solution was cultivated using the necessary quantities of nutrients. Table 2 gives the necessary amounts of the reagents that were used in preparing the liquid culture:

In order to form the liquid medium used, 5.00 g of peptone was applied to 3.00 g of meat extract and 3.95 g of calcium acetate in a liter of distilled water. Media sterilization was initially performed at a temperature of 121 °C by autoclaving for 20 min and then cooled to room temperature. To adjust the pH of this media to 9.7, 1 M solution of Na_2CO_3 (1.0 mL in 10.0 mL) was prepared by adding 5.30 g of anhydrous Na_2CO_3 and 4.20 g of $NaHCO_3$ were mixed and added to the stock culture. Bacteria spores were then added to this mixture. Incubation was carried out by way of shaker incubator and at 130 rotations per minute for a period of 72 h at a temperature of 30 °C. Optical density (OD) was determined using Atomic absorbance spectrophotometer (G10S UV-Vis, USA). In order to obtain 1.0×10^7 cells mL^{-1} as the concentration to be used in this investigation, this test determined the volume of culture solution to be used in the mixing phase. In the spectrophotometer set at 600 nm, 0.5 mL of both the blank and microbial solution was mounted separately and the OD was noted.

2.4. Mortar prism preparation

40 mm × 40 mm × 160 mm were cast as per KS EAS 148: 1–2017 [15]. To prepare a mortar of w/c of 0.5, 450 g of OPC cement were placed in the mix basin of an automatic programmable mixer model number CH 8224lohningent. 225.0 ml of distilled water was added. The mix basin and its content was then clamped onto the automatic programmable mixer and allowed to run for 3 min. 1350 ± 5 g of standard sand was placed in an automatic pour-trough little by little until all was added while the

Table 2. Requisite amounts for the liquid culture for DSM 1320.

Reagent	Amount (grams)
NaHCO ₃	4.20
Na ₂ CO ₃	5.30
Calcium acetate	3.95
Meat extract	3.00
Peptone	5.00

mixer was still running at a speed of 30 revolutions per minute (rpm). The machine was allowed to run for 10 min. Once the mortar was mixed it was poured in to steel moulds of 40 mm × 40 mm × 160 mm. Using a trowel, the mortar paste was scooped from the automatic programmable mixing basin and placed in a compaction mould. The mould was gently lifted off the vibrating table. The excess mortar was removed using a straightedge spreader. The surface of the mortar was smoothed using the same straightedge spreader held almost level. The prisms were left in the mould for 24 h then demoulded for curing in distilled water. They were labeled OPC-H₂O-H₂O. To prepare the second category the bacterial solution was used both as the mix and curing media, the mortar was labeled as OPC-TT-TT and the third set was cast as the second category but after curing for the first 28 days the prisms were transferred to *Bacillus flexus* solution for a further 28 days and were labeled as OPC-TT-BF.

2.5. Determination of compressive strength

Compressive strength test was performed in accordance with KS EAS 148:1–2017 [15]. This was done at 14th, 28th, 56th and 90th day. For bioremediation purposes, the mortars were considered at 56th day of curing and comparison done. For each category of prisms, three prisms were removed from the curing tank; any deposits wiped off and covered with a damp cloth. Their identities were noted down and the prism placed on the testing machine with one face on the supporting rollers and with its longitudinal axis normal to the supports. Load was applied vertically by means of the loading roller at a rate of 50 N/s until failure to obtain prism halves. The halves were crushed smoothly by applying load

at a rate of 2400 N/s. Final compressive strength measurement was taken as the average strength of the three prisms.

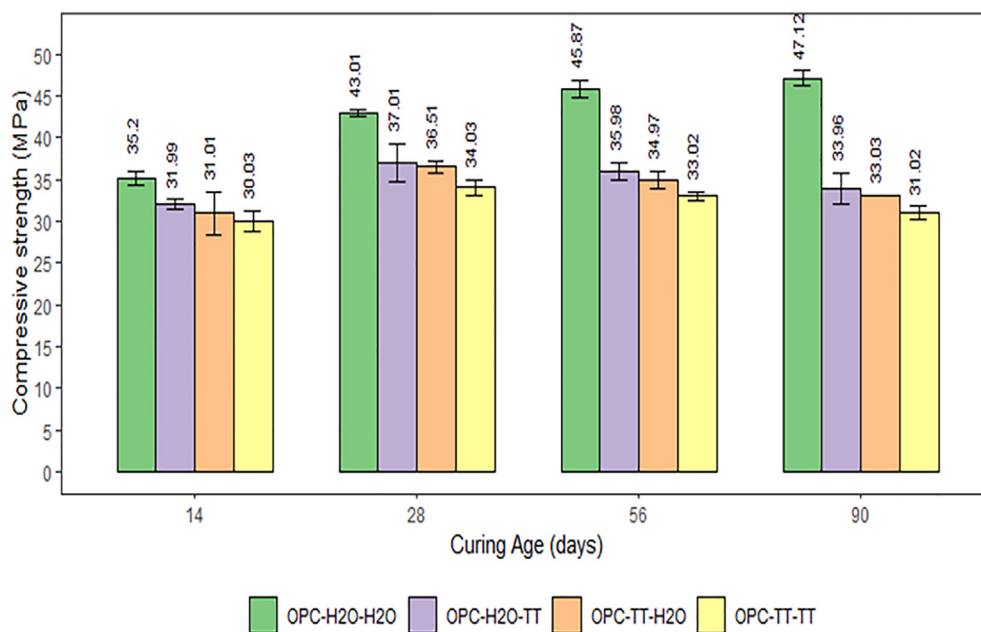
2.6. Scanning electron microscopy (SEM)

The analysis was done on each category of mortar prisms. SEM model used in this test was Ziess Ultra Plug FEG-SEM. The procedure described by Scrivener *et al.* [16]) was used in the sample preparation for this analysis. To stop the hydration process, isopropanol alcohol was used. SEM analysis was conducted after 56th day of curing for each category of mortars.

2.7. Determination of water absorption

Water absorption tests were carried out on control, degraded and bioremediated mortars. The control mortars were prepared using distilled water as both mix and curing media and were labelled as OPC-H₂O-H₂O. The degraded mortars were cast and cured in *Thiobacillus thio-parus* solution and were denoted as OPC-TT-TT. The last set was the bioremediated and they were cast with *Thiobacillus thio-parus* solution and cured in *Bacillus flexus* solution and labeled as OPC TT-BF. The test was carried according to the protocol given by Achal *et al.* [17]. To determine the water absorption, mortars cured were on the 28th day dried in an oven at 100 °C for a period of 24 h after which they were submerged in water with the 40 mm × 40 mm face facing downward. The mortar prisms were removed at regular intervals of 0.25 h, 0.5 h, 1 h, 1.5 h, 3 h, 5 h, 8 h, 24 h, 72 h, 96 h, 120 h, 144 h and 168 h [17]. The new mass of the prism was noted once the submerged surface was dried with a clean towel and the prism re-submerged immediately. This was observed up to the 168th hour. For each mortar category, three samples were subjected to this test simultaneously and triplicate results obtained. Their average was reported. Eq. (6) was used to determine the percent of water absorbed [17]:

$$\% \text{ water absorption} = \left[\frac{w_2 - w_1}{w_1} \right] \times 100 \quad (6)$$

**Figure 1.** Compressive strength with varied curing age.

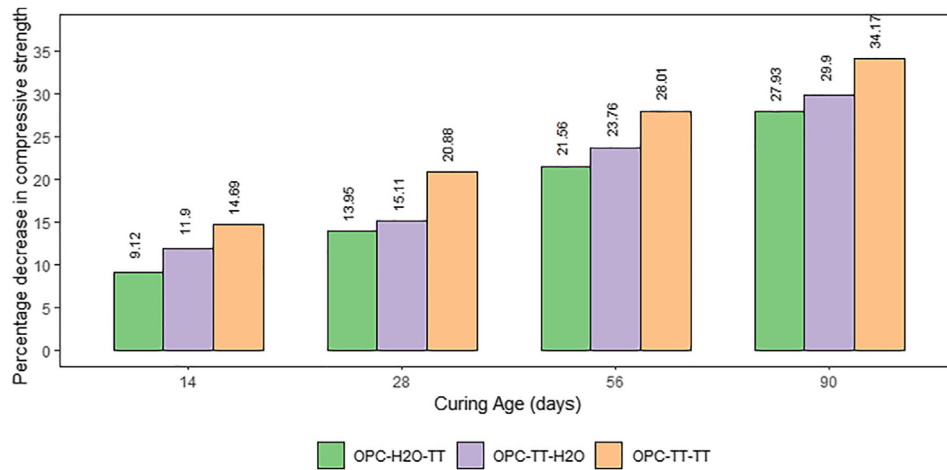


Figure 2. Percentage decrease in Compressive strength with curing Age.

where w_2 is the mass of saturated specimen, w_1 the mass of the dried specimen.

3. Results and discussions

3.1. Compressive strength results

Figure 1 represents results of compressive strength of the control and microbial mortars. The compressive strength of the control mortars, OPC-H₂O-H₂O, was noted to have improved with curing period with the highest noted at 90th day as 47.12 MPa. This was attributed to the continued hydration reactions with curing period [17]. However, the strength did not increase appreciably after 28 days of curing. This was attributed to slow cement hydration process since OPC gains much of its strength by 28th day of curing [17]. Ngari et al. [24] and Muthengia et al. [25] made similar observations. They attributed the same to early hydration reactions of OPC (by 28th day of curing). It was observed that the microbial mortars compressive strength was below that of the control mortars across all the categories. This was supported by SEM images Figures 3 and 5, from 3 and 5, CSH is observed and it is the cement hydration product majorly responsible for cement strength development

[19]. However, this was missing in the biodegraded mortar, Figure 4. From Figure 2, it is clear that the highest percentage decrease in compressive strength was recorded at 90 days of curing as 34.17 % for OPC-TT-TT category of mortars. This perhaps was due to the degradation effect of *Thiobacillus thioeparus* increased with duration of curing. This could further show that, deleterious effect was influenced by the mix media during prism casting. Additionally, the most affected mortars were OPC-TT-TT category and the least affected were the OPC-H₂O-TT category. This was attributed to the deleterious/deterioration effect of *Thiobacillus thioeparus*. This was expected due to MID process by *Thiobacillus thioeparus* [4, 26]. The bacterium being a sulphur oxidizing bacteria degraded the mortars.

3.2. SEM results

The SEM images for OPC-H₂O-H₂O, OPC-TT-TT and OPC-TT-BF are given in Figures 3, 4 and 5 respectively. For the control sample, ettringite was seen as needle - shaped crystals. This ettringite was the hydration product formed when tricalcium aluminate complexes with gypsum. The gypsum added to give cement the desired setting qualities [17]. Tricalcium aluminate reacts faster with water compared to tricalcium silicate

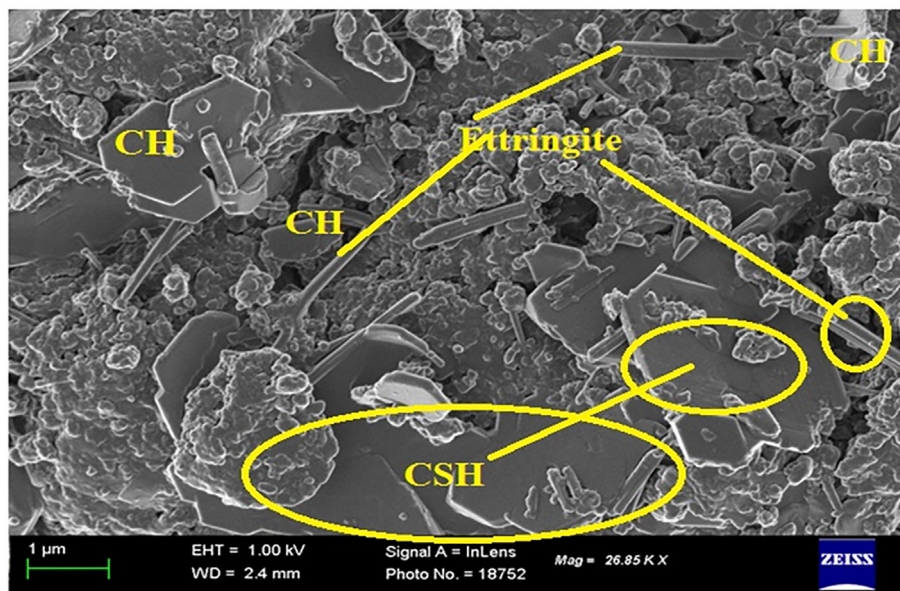


Figure 3. SEM micrograph for control mortar, OPC-H₂O-H₂O.

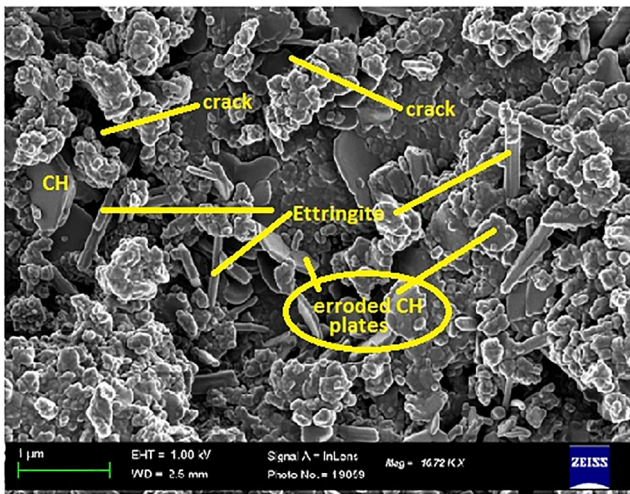


Figure 4. SEM image for the biodegraded mortar, OPC-TT-TT.

and that would result in flash setting [18]. Sulphate added complexes with tricalcium aluminate to give ettringite. This ettringite passivates aluminate crystals and is formed as given Eq. (5) [17]. From the SEM image for OPC-TT-TT, massive deposits of ettringite are observed, eroded calcium hydroxide (CH) plates and micro-cracks. These were perhaps due to the metabolic activity of *Thiobacillus thio-parus* which is a deleterious bacterium. Sulphur oxidizing bacteria have been documented to develop biogenic sulphuric acid in the sewer system [19, 20]. *Thiobacillus thio-parus* bacteria being a sulphur oxidizing bacteria, oxidized hydrogen sulphide gas to produce the same acid. This acid attacks the CH and C-S-H, which are major products of cement hydration reaction to produce gypsum which in turn reacted with tricalcium aluminate phase of cement to give secondary ettringite [21]. These reactions are given in Eqs 5, 6, 7:



The observed micro-cracks/voids were due to excessive internal pressure that resulted from gypsum and ettringite formed as shown in Eqs. (5) and (7) [2]. This exposed the mortar to possible degradation as these micro-cracks act as pathways (since they are inter-linked/interconnected) for aggressive agents' ingress. Micro cracking of cement made material was reported by Joshi *et al.* [22]. They [22] attributed this to the buildup of gypsum and ettringite within the pore system of the cement matrix. Meas and De Belie [23] made similar observation. From Figure 4, massive destruction of the cement mortar matrix was observed in the microbial mortars involving *Thiobacillus thio-parus*. The observed eroded CH plates were due to the aggressive effect of the biogenic sulphuric acid. The bacteria have been found to attack new cement made pipe surfaces [5]. This results in production of hydrogen sulphide gas has been identified as the major deleterious agent in both sewer and waste water treatment systems [7]. This is due to formation of biogenic sulphuric acid which is considered to be responsible for the degradation [7]. Muthengia *et al.* [3], associated cracking and spalling of mortar matrix to the expansive gypsum and ettringite. This has been demonstrated in Eqs. (2) and (3). OPC-TT-BF micrograph show ettringite and micro-cracks/voids undergoing healing by *Bacillus flexus*. Calcite formed from MICP process is deposited during the healing process.

3.3. Water absorption

Water absorption tests were carried up to 168th hour and the findings presented in Figure 6. From the findings, OPC - TT -TT exhibited the higher percent gain in water absorption as compared with the control and the remediated mortars, OPC-H₂O-H₂O and OPC-TT-BF respectively. This could perhaps be due to massive deterioration of the cement mortar matrix by *Thiobacillus thio-parus*. This deterioration resulted in formation of multiple micro-cracks which made the mortar extensively porous. The porous material increased the absorption of water. This observation was similar to Munyao *et al.* [2] findings. Lower gain in percent water absorption observed with the control and remediated mortar prisms was attributed to a denser microstructure due to continued hydration reactions and MICP process

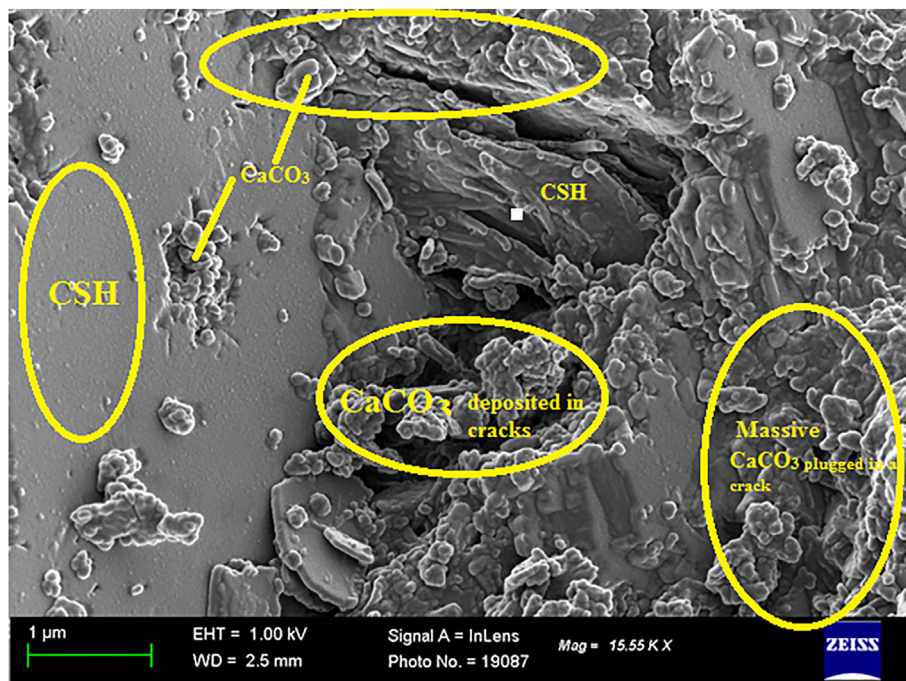


Figure 5. SEM image for bioremediated mortar, OPC-TT-BF.

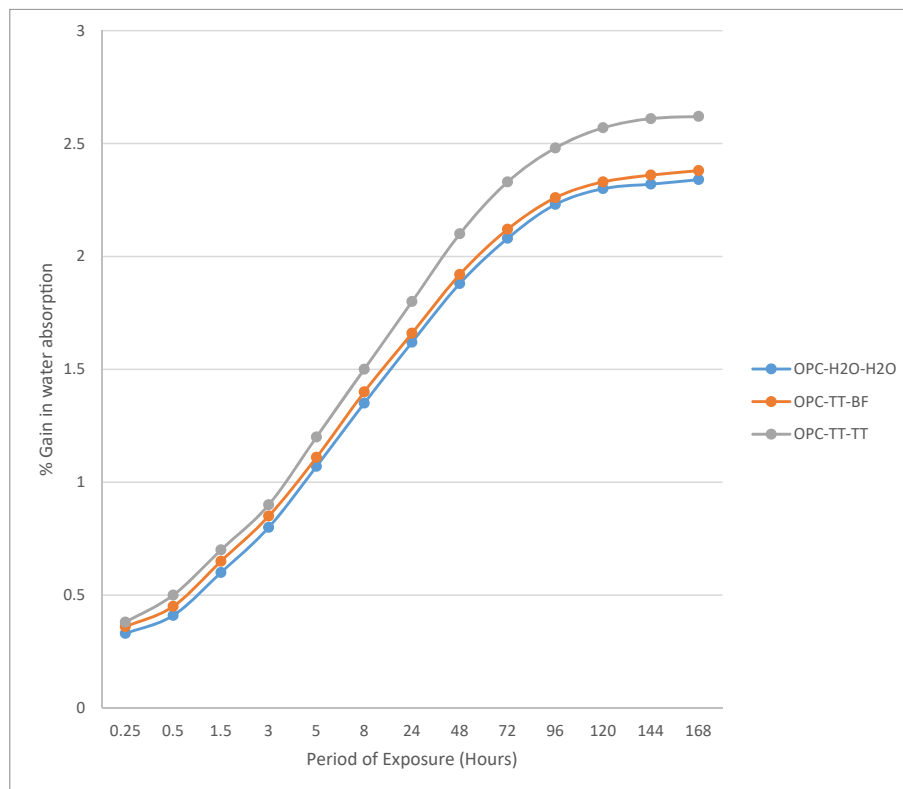


Figure 6. % Gain in water absorption.

respectively. The hydration products perhaps sealed the pores and the calcite formed from MICP process possibly sealed the pores and micro-cracks as is evident from the SEM image, Figure 3. *Bacillus flexus* being a calcite precipitating bacteria precipitated calcite that deposited in the pores and in the micro-cracks/voids. Figure 3 shows massive biodeposition of calcite. It was further noted that after 96 h the increase in water absorption was not very appreciable for both the control and bacterial mortars. At this time, saturation of mortars perhaps could have reduced capillary suction.

4. Conclusion

From the investigations reported in this study, the following conclusions have been drawn:

1. *Thiobacillus thioparus* degrade the mortar microstructure. This degradation perhaps could compromise on the integrity of cement made materials due to ingress of aggressive agents through the micro-cracks.
2. *Thiobacillus thioparus* decrease compressive strength of the mortar while it increases water absorption.
3. *Bacillus flexus* could be incorporated in concrete in sulphate laden environment like the sewer systems to aid in mitigating the deleterious/degradation effect of species *Thiobacilli*.

Declarations

Author contribution statement

Reginah Wangui Ngari, Joseph Karanja Thiong'o, Jackson Muthengia Wachira, Genson Muriithi, Daniel Karanja Mutitu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

The authors do not have permission to share data.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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