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Original Article

Effect of Temperature on the Self-Healing Efficiency of Bacteria and on that of Fly Ash in Concrete

Millicent W. Njau[1], John Mwero[2], Zachary Abiero-Gariy[3], Viviene Matiru[4]

[1]*Department of Civil Engineering, Pan African University Institute for Basic Sciences, Technology, and Innovation (PAUISTI) hosted at Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya*
[2]*Department of Civil & Construction Engineering, University of Nairobi, Nairobi, Kenya*
[3]*Department of Civil Engineering, Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya*
[4]*Department of Botany, Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya*

[1]millicentnjau1@gmail.com

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Abstract *- Self-healing is a technique used to repair cracks in concrete autogenously or autonomously. Several investigations have been conducted to improve the self-healing efficiency, which will allow for the application of the technique on a larger scale by simulating real conditions. The study aims to investigate the effect of temperature on the efficiency of crack healing. Autonomous self-healing was induced by incorporating Bacillus thuringiensis to the concrete mix as a microbial self-healing agent and calcium nitrate as its nutrient source. Autogenous self-healing concrete was developed using fly ash as a partial cement substitute. The mechanical properties of both concrete mixes were studied, and a comparison was made with those of the control concrete mix. The crack surface was examined for concrete samples cured at different temperatures (25oC to 45oC at intervals of 5oC) to assess the self-healing process. According to the findings, the compressive strength of fly ash concrete samples was improved at 90 days by 3.34%, while the split tensile strength was improved from as early as 7 days by 1.88% to 3.53% at 90 days. The inclusion of the bacteria and its nutrient source improved the split tensile strength of concrete but reduced its compressive strength at 90 days. Both self-healing techniques were accelerated when the concrete specimens were cured at slightly elevated temperatures. The optimum temperature for efficient bacterial self-healing was found to be 40oC. The results show that at 45oC, the healing rates were between 25%-45%, while at 25oC, the healing rates were between 10-30% for concrete samples containing fly ash. For the samples containing the microbial healing agent, a crack 0.6mm wide was completely healed in 18 days when the concrete specimen was cured at 40oC, while larger cracks up to 0.8mm showed up to 40% crack closure in 28 days when exposed to the bacteria's optimum temperature for calcite precipitation. In conclusion, the self-healing efficiency is increased at slightly elevated temperatures. The results obtained for microbial self-healing suggest that optimal conditions are required for the practical application of this technique; thus, it can be adopted in areas where the atmospheric conditions are close to optimum.*

Keywords *- Bacillus thuringiensis, Class C fly ash, Compressive strength, Self-healing index, Split tensile strength.*

1. Introduction

Concrete is an important material used for construction that has been utilized extensively around the world. It is used for heavy constructions such as dams, roads, buildings, and other civil structures due to its strength and durability. However, due to its low tensile strength, the major problem that affects the durability of concrete is its high probability of cracking, which non-load factors could cause. These could be external or internal mechanisms that include chemical reaction attacks, thermal cracks, and shrinkage cracks. The cracks do not necessarily affect the structure's performance. However, they could increase the probability of ingress of aggressive fluids that affect the structure's durability and service life [1]–[4]. Hand mending cracks are common; however, they can be ineffective since microcracks are challenging to detect. The extent of damage, depth, and location inside the concrete makes it difficult to repair all the cracks. The cost incurred to repair the cracks is also very high [5], [6].

A few approaches have been proposed to prevent cracks from forming and progressing at an early age. Some of these techniques include the use of silicon-based polymers, chlorinated rubber, acrylic resins, and epoxy systems.

However, their application is limited due to high cost, moisture sensitivity, non-compatibility with concrete, and low heat resistance [3]. As a result, recent research has identified the self-healing mechanism as a viable solution to concrete crack formation.

For decades, many researchers have carried out numerous studies on concrete crack repair to understand the self-healing technique better. There are two types of self-healing techniques, namely autonomous and autogenous healing. There are two processes through which autogenous healing could occur. First, the carbonate and calcium ions in the water and cement, respectively, react directly to form calcium carbonate ($CaCO_3$) crystals. Second, some pozzolanic materials or anhydrous cement particles continue to hydrate due to exposure to humid conditions. The $CaCO_3$ formed then acts as a product to heal cracks in concrete [7].

Previous research has demonstrated that when sufficient humidity is provided, the efficiency of autogenous healing is high when the cracks have a width ranging from 0.01-0.1mm. For early age cracks, cracks up to 0.35mm show generally good healing. Specimens with supplementary cementitious materials (SCMs) show considerable crack healing of about 32% for cracks of width between 0.3mm and 0.5mm [8]–[11]. However, autonomous healing has been considered an alternative pathway to attain a higher degree of crack closure.

Incorporating engineered additions that are not conventional into the concrete matrix to attain crack closure is known as autonomous self-healing [7]. Chemically based healing agents were first employed, but they had disadvantages such as having different thermal expansion coefficients and environmental, health, and concrete hazards. An alternative technique to heal cracks using environmentally friendly processes was considered. This technique is known as Microbially induced calcium carbonate precipitation (MICP) [12]. The precipitation of calcium carbonate is induced through this method within the concrete matrix. This technique can potentially plug minuscule cracks in porous media where conventional concrete cannot reach, calcium carbonate acting as a biogenetic cement [13].

Reference [14] originally introduced the usage of microorganisms for self-healing. An organic nutrient source and non-ureolytic bacteria were added before mixing and became an integral part of the concrete. When cracks emerge, microorganisms bece and mineralize fill the cracks. Under the respiration of the bacteria, the substrate is converted metabolically, precipitating calcium carbonate as shown in Equation 1 below:

$$\text{Nutrient source} + O_2 \xrightarrow{Baceria} CaC + CO_2 + H_2O \quad (1)$$

From the study in reference [12], these non-ureolytic bacteria could heal up to 0.46mm in 100 days of healing. Cracks of widths between 0.1mm-0.3mm achieved 85% or more healing after 20 days of healing in the study by Luo et al. [1] for cement mortar samples. Cracks of width between 0.3-0.5mm attained 50-70% healing in 20 days, while those of 0.8mm attained less than 30% healing rates in 20 days.

In practical engineering, the environmental conditions vary greatly, with some structures immersed in water for long periods while others are exposed to moist environments and others to varying temperature conditions. The bacteria adopted for self-healing are selected to precipitate calcite and survive in high pH conditions. However, the restart of their metabolic activity after they are incorporated into the concrete depends on the temperature, nutrient source, and water content. It is unlikely that a particular bacterial species will be discovered that will be viable for self-healing under all exposure conditions. Therefore, focusing on a bacteria's restart is required to determine the necessary conditions for successful bacterially induced calcification. This is important since diverse atmospheric conditions exist worldwide, affecting the bacteria's performance.

To summarize, autogenous and autonomous self-healing mechanisms cannot be understood entirely due to a significant lack of knowledge in their effectiveness in different environmental conditions. Understanding this is crucial, especially if the technology can be applied in the real world. As a result, this research aims to explore the effects of different temperature conditions on the efficiency of the self-healing process.

2. Materials and Methods

The study aimed to induce self-healing by autonomous and autogenous methods. For autonomous self-healing, bacterial self-healing in the concrete matrix was induced using $CaCO_3$ forming bacteria. *Bacillus thuringiensis* ATCC 33679 attained from the American Type Culture Collection (ATCC) and grown in the PAUISTI microbiology laboratory was selected for this study. Fly ash replaced twenty percent of the cement by weight to induce self-healing by continued hydration.

2.1 Fly Ash and Cement

This study used fly ash imported from India. The cement used was Class 42.5N Ordinary Portland cement locally obtained, meeting the requirements of BS EN 197-1:2000. An X-ray fluorescence (XRF) test was conducted to obtain the fly ash's chemical properties, and these properties were compared to those of cement. According to the study by reference. [15], substituting 15-20% of cement with fly ash in the gel paste system reduces mesopores. It increases the volume of Calcium silicate hydrate (C-S-H) gel improving autogenous self-healing. When using Class C fly ash in structural concrete mixes, 20 to 35 percent is used as a percent of total cementitious material [16]. Therefore, 20% was adopted for this study.

2.2 Study Bacteria

Bacillus thuringiensis is a gram-positive, spore forming, facultative bacteria. It is rod-shaped, with a length and width of 0.2-1µm and 2-5 µm, respectively. It is considered a urease-negative organism whose colonies have around to irregular form. It is primarily found in the soil [17].

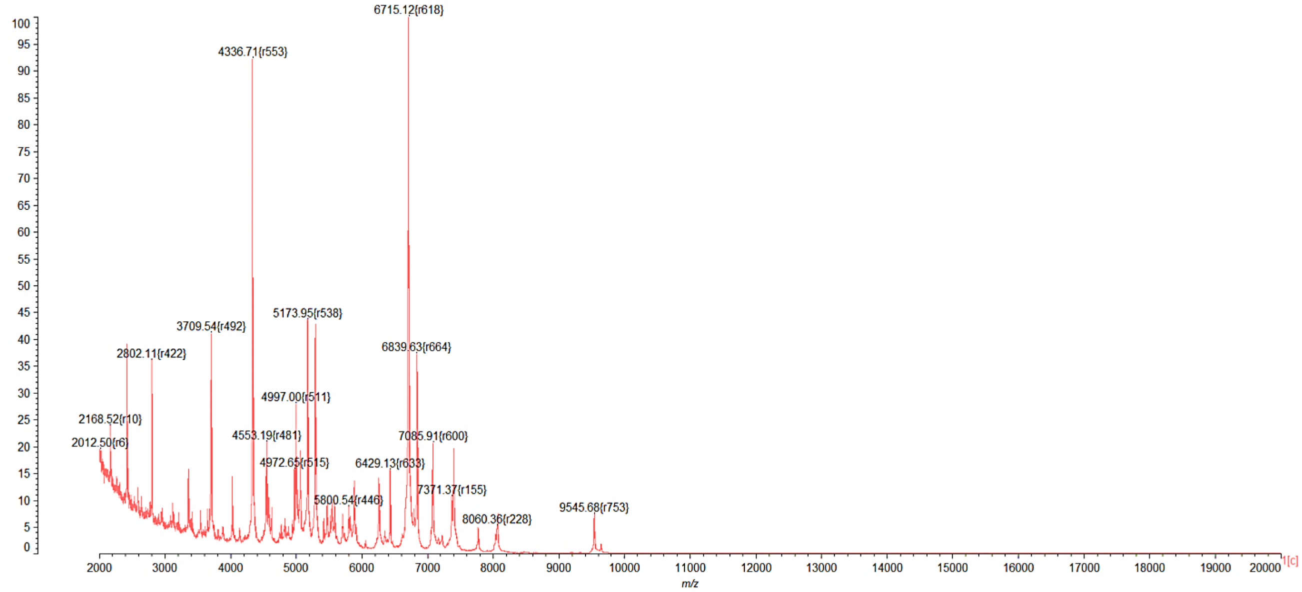

Fig. 1 Maldi-tof report for *Bacillus thuringiensis* ATCC 33679

2.3 Coarse and Fine Aggregates

This study's coarse and fine aggregates were locally acquired and prepared per BS 882:1992.

2.4 Preparation of the Bacterial Healing Agent

The bacterial strain *Bacillus thuringiensis* ATCC 33679 with a 99.90% sequence similarity of 16s rRNA gene with Bacillus thuringiensis, as shown in Figure 1, was used for this research work.

Its self-healing effectiveness is not well established as it has only been used as a coculture with other non-ureolytic bacteria or, in some cases, with ureolytic bacteria. The strain was grown on a nutrient agar plate and incubated at 35ºC for 24 hours. It was then cultured further in nutrient broth at 35ºC for 24 hours. The sub-culture was then inoculated at 1:100 (bacterial stock to the nutrient broth). The samples were then incubated at 35ºC in a shaker incubator operating at 150rpm for 24 hours. The suspension was then stored at 4ºC until further use. The absorbance of the cell inoculum was 0.8084, which is approximately 6×10^8 cells/mL bacterial cells. Maldi-tof was used to confirm the identity of the bacterium further. The bacterial solution was fixed at 13L/m^3 of concrete to keep the microbial solution quantity constant in the mix.

2.5 Preparation of the concrete specimens

Crushed gravel as coarse aggregates, naturally available river sand as fine aggregates, Class 42.5N Ordinary Portland cement that conforms to the requirements of BS EN 197-1:2000, and Class C fly ash that conforms to the requirements of ASTM C-618 were used to prepare the different concrete mixes. The mix design used is as shown in Table 1.

The batches of concrete were prepared, including the mix with bacteria per BS EN 12390-2:2019. The calcium nitrate was added during dry mixing while the bacterial suspension was added to the mixing water. Both cylindrical specimens (100mm diameter and 200mm height) and cubic specimens (100mm ×100mm×100mm) were cast and demolded after 24 hours. They were then cured at room temperature.

2.6 Testing of the Specimens

Tests to determine the compressive and splitting tensile strength were conducted in triplicate on the specimens at 7, 14, 28, 56, and 90 days. The compressive and splitting tensile strength tests were conducted per BS EN 12390-3:2019 and BS EN 12390-6:2019, respectively. The results were reported in averages. Changes in concrete strength were evaluated against control measurements.

Table 1. Mix design

Mix Ingredients	Control mix (per m^3)	Bacterial mix(perm^3)	Fly ash mix (per m^3)
Water/cement ratio	0.55	0.55	0.55
Fine aggregates (kg)	648.50	648.50	648.50
Coarse aggregates (kg)	1198.96	1198.96	1198.96
Cement (kg)	347.54	347.54	278.03
Fly ash (Kg)	-	-	69.51
Bacterial Solution (L)	-	13.00	-
Calcium Nitrate (w/w cement)	-	8.69	-

2.7 Creation of artificial cracks

It would take a while for cracks to appear on the concrete naturally, and since cracks can affect a structure's service life, there was a need to have the ability to simulate the concrete crack's real state in the laboratory. This was done by developing cracks under spitting tensile load. The cylindrical specimens were cured for 28 days at room temperature then loaded under a splitting tensile load to develop cracks ranging from 0.4mm to 1.0mm. The cracks formed were properly marked to be measured before and after the healing process. This was a simple approximate method to control the crack widths. Therefore, within a reasonable time frame, a large number of specimens were pre-cracked.

2.8 Self-healing curing conditions

After the cylindrical specimens were pre-cracked, they were immersed in water at different temperatures (25ºC, 30ºC, 35ºC, 40ºC, and 45ºC) to quantify crack healing under different temperatures conditions. For 28 days, the changes in the crack width were obeyed two on microscope closing.

2.9 Self-healing effect characterization

Crack healing was assessed by analyzing the crack closure rate.

$$\text{Crack cloing ate} = 1 - \qquad (2)$$

Where w_i represents the crack width at pre-cracking, and w_t represents the crack width after the healing process.

The self-healing index was also used to assess self-healing by taking all cra measures of specific types f concrete. Reference [18]and [9 rmend the use of the self-healing index of crack closure.

$$\beta = 1 - \frac{t}{A_i} \qquad (3)$$

A_i denotes the area between the horizontal axis and the bisecting line, while A_t represents the area

between the plotted graph's horizontal axis and the polyline that connects all crack width points remaining, as shown in Figure 2 [9], [18].

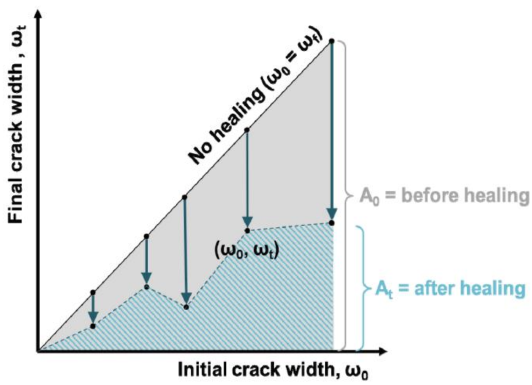

Fig. 2 Concept for evaluating crack closure using the methods described in [9], [18]

3. Results and Discussions

3.1 Chemical Composition of Fly Ash

From Table 2, fly ash had a combined percentage of 71.87% for SiO_2+Al_2O_3+Fe_2O_3, which was greater than 70%, ASTM C618's minimum requirement for a good pozzolana.

Table 2. Composition of fly ash and cement

Compound	w/w percentage (%)	
	Fly ash	Cement
CaO	21.53	62.74
Al_2O_3	13.33	5.45
SiO_2	45.16	24.62
P_2O_5	0.70	0.44
SO_3	1.55	2.96
K_2O	2.57	0.61
TiO_2	1.06	0.19
Fe_2O_3	13.38	2.74

The presence of alumina, silica, and iron oxide above the minimum requirement for a good pozzolana indicates fly ash's ability to form cementitious compounds when mixed with the free lime of the ordinary Portland cement the presence of moisture. The SiO_2 content is also greater than 35% as per the requirements of ASTM C-618. The SiO_2 in the fly ash is required for the pozzolanic reaction with calcium hydroxide, a by-product of the hydration of Portland cement minerals and water, to produce additional hydration products. Therefore, sufficient amounts of SiO_2 are required for this reaction.

According to ASTM C618, The fly ash used in this work is classified as Class C fly ash. Type C Fly ash is both pozzolanic and cementitious. The cementitious properties are due to the high CaO content (above 20%). The research conducted in reference [19] shows that high amounts of calcium will allow the fly ash to react with water to produce hydrates when a calcium hydroxide source is not present. When large quantities of mineral additives are used as a cement substitute, the self-healing rate may decrease as the availability of calcium hydroxide limits the pozzolanic reaction. Therefore, the study in reference [20] recommends using calcareous fly ash to increase the calcium content.

3.2 Compressive Strength

Table 3 shows the average compressive strength values for the three concrete mixes. The average compressive strength values were plotted over time in Figure 3 to compare the three mixes. Incorporating calcium nitrate at 2.5% w/w of cement and the microbial solution in the bacterial specimens increased the 7- and 14-day strength by 2.52% and 4.05%, respectively. The strength at 28 and 56 days was less than that of control concrete by 1.62% and 1.44%, respectively. This amount is less than that reported in reference [21], who reported a 10% decrease in strength at 28 days when 3% of calcium nitrate was used, and reference [22], who reported a 17.4% decrease when 3% calcium nitrate was used at 28 days. The 90-day strength, on the other hand, was 18% lower than the 56-day strength.

Table 3. Compressive strength results

Test time (days)	Control Mix	Fly ash mix	Bacterial mix
7	29.92	25.52	30.68
14	36.23	31.54	37.69
28	42.91	40.70	42.21
56	43.79	43.65	44.05
90	44.37	45.85	36.13

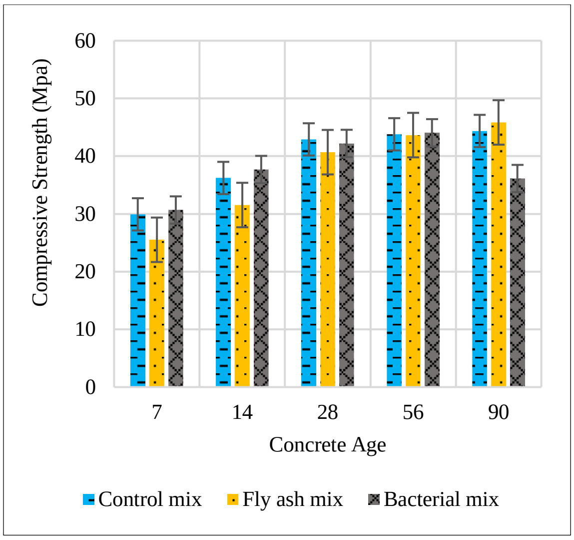

Fig. 3 Compressive strength of the different concrete specimens at 28 days

The bacteria have minimal influence on cement hydration and are only used for their calcification potential. The accelerated hydration was mainly attributed to calcium nitrate. There was an increase in the calcium ions concentration due to the incorporation of calcium nitrate. Thus, the cement hydration process was accelerated [21]–[23]. Compared to the control concrete samples, the strength at later ages is lower, which may be due to the consumption of large amounts of water at an early age during hydration [24]. The nature of crystals formed by the microorganism reaction is probably responsible for the reduction in strength after 90 days. The calcite crystals produced might have been powder-like and soft instead of hard crystals, which could affect the concrete's compressive strength [25]. According to reference [26], the rate of production and size of calcite crystals could lead to desirable or undesirable strength properties, and in this case, the effect was negative. Calcium carbonate polymorphs such as vaterite, aragonite, and calcite can form due to biomineralization. The calcite crystal's nature could vary, which would impact the concrete's compressive strength, especially if the changes in the morphology of the precipitates occur over time.

Figure 3 shows that the compressive strength improves at later stages for concrete specimens with Class C fly ash compared to control concrete specimens. The strength of the mix with fly ash was 45.846Mpa at 90 days which is greater than that of the control mix samples (44.366Mpa) at 90 days. The improvement was slight and probably due to the pozzolanic reaction that occurred at a later age. During the pozzolanic reaction of fly ash, calcium hydroxide is produced when Portland cement hydrates are consumed, producing calcium aluminate hydrate and calcium silicate hydrate. As a result of the slower rate of pozzolanic reaction and the dilution effect of fly ash at the initial stages, the early age compressive strength was less than that of the control concrete samples to about 28 days [27], [28]. Because of the high calcium content, Class C fly ash has both pozzolanic and cementitious properties, so the strength reduction was minimal after 28 days [29], [30]. Therefore, it can provide cementing action at an early age.

3.3 Split Tensile Strength

Table 4 shows the three concrete mixes' average split tensile strength results. The average split tensile strength values were plotted over time in Figure 4 to compare the three mixes. The split tensile strength of bacterial concrete was less than the control concrete's strength by 1.46% and 2.60% at 7 and 14 days, respectively but was higher at 28 days by 8.14%. At 56 and 90 days, it was a little lower than that of control concrete samples by 0.89% and 2.30%, respectively.

Table 4. Split tensile strength results

Test time (days)	Control Mix	Fly ash mix	Bacterial mix
7	2.017	2.055	1.988
14	2.136	2.261	2.081
28	2.584	2.853	2.794
56	2.917	3.028	2.891
90	3.075	3.183	3.004

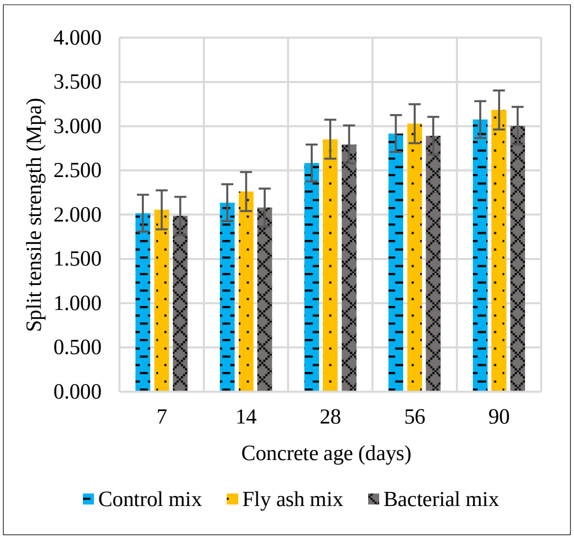

Fig. 4 Split tensile strength of the different concrete specimens at 28 days

Microbiologically meditated calcium carbonate precipitation still occurs as the specimens are exposed to oxygen dissolved in the water. The $CaCO_3$ produced and the calcium salts fill the concrete pores improving the bio-concrete matrix integrity causing its densification, thus significantly increasing its split tensile strength at later stages as was reported in other studies [7], [31], [32].

The concrete samples containing fly ash had a higher split tensile strength than those of control concrete at all ages studied. This trend is similar to that reported in reference [33] and [29]. Class C fly ash is pozzolanic and helps improve the aggregate-paste bond by densifying the transition zone and forming a more hydrated gel that fills the concrete capillary, improving the concrete strength. The micro aggregate effect of fly ash is also beneficial to concrete as the microbeads in the fly ash can properly disperse in concrete and firmly combine with the gel formed during the hydration of cement, thus promoting the concrete density [27], [34].

3.4 Effect of Temperature on the Crack Healing Efficiency

Figure 5 depicts the change in the visual appearance of cracks of various widths for the different cases studied. The precipitates formed first appeared on the crack edges then increased gradually until they healed the crack in the cement paste system.

3.4.1 Autogenous Self-healing

Table 5 shows the initial width (IW) and final crack width (FW) at pre-cracking and after 28 days of curing for the three concrete mixes. Figures 6-8 show the crack closure rate of control concrete samples with cracks of different widths between 0.4mm and 1mm at different curing temperature conditions. The results show that for the different crack width ranges, the increase in curing temperature increases the healed crack width. The crack closure rate was between 25% and 45% at 45ºC. At lower temperatures, the crack width healed was minimal.

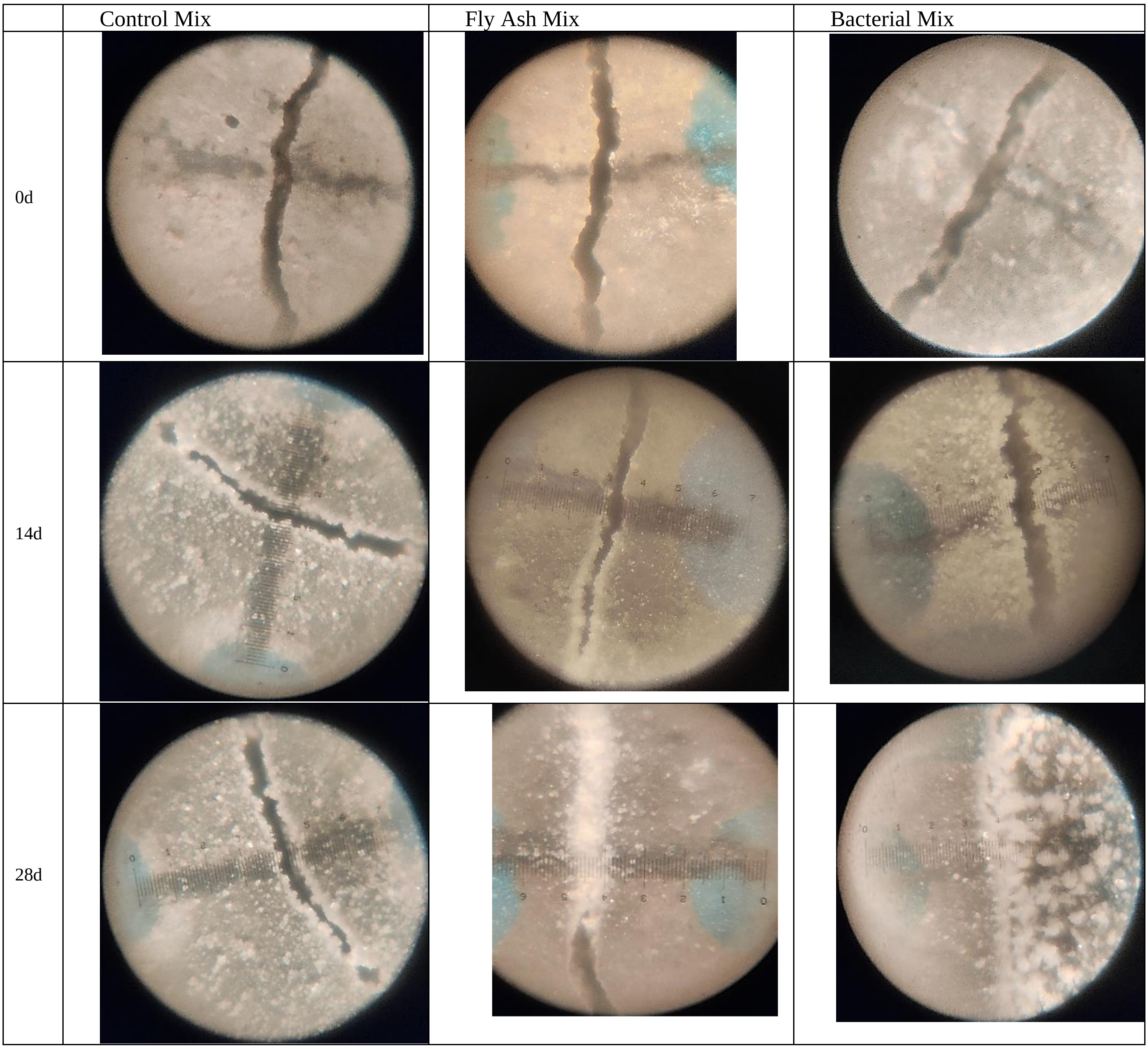

Fig. 5 Example of crack closing evolution for a 0.7mm crack for each case studied

Table 5. Initial and final crack width at different temperatures for the different concrete mixes

Curing temperature		25 ºC		30 ºC		35 ºC		40 ºC		45 ºC	
Concrete mix	Crack width (CW) range (mm)	IW	FW	IW	FW	IW	FW	IW	FW	IW	FW
Control mix	0.4<CW<0.6	0.5	0.45	0.5	0.4	0.4	0.3	0.6	0	0.6	0.45
	0.6<CW<0.8	0.7	0.7	0.7	0.7	0.7	0.6	0.7	0.5	0.7	0.5
	0.8<CW<0.1	1	0.85	1	1	0.9	0.85	1	0.6	0.9	0.5
Fly ash mix	0.4<CW<0.6	0.5	0.45	0.5	0.4	0.5	0.4	0.6	0.55	0.5	0.3
	0.6<CW<0.8	0.7	0.5	0.8	0.5	0.6	0.45	0.8	0	0.6	0.45
	0.8<CW<0.1	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.7	0.8	0.5
Bacterial mix	0.4<CW<0.6	0.5	0.5	0.5	0.4	0.5	0.3	0.6	0	0.6	0.4
	0.6<CW<0.8	0.7	0.7	0.7	0.7	0.75	0.65	0.7	0.6	0.7	0.6
	0.8<CW<0.1	0.8	0.6	0.9	0.9	1	1	1	0.6	0.9	0.75

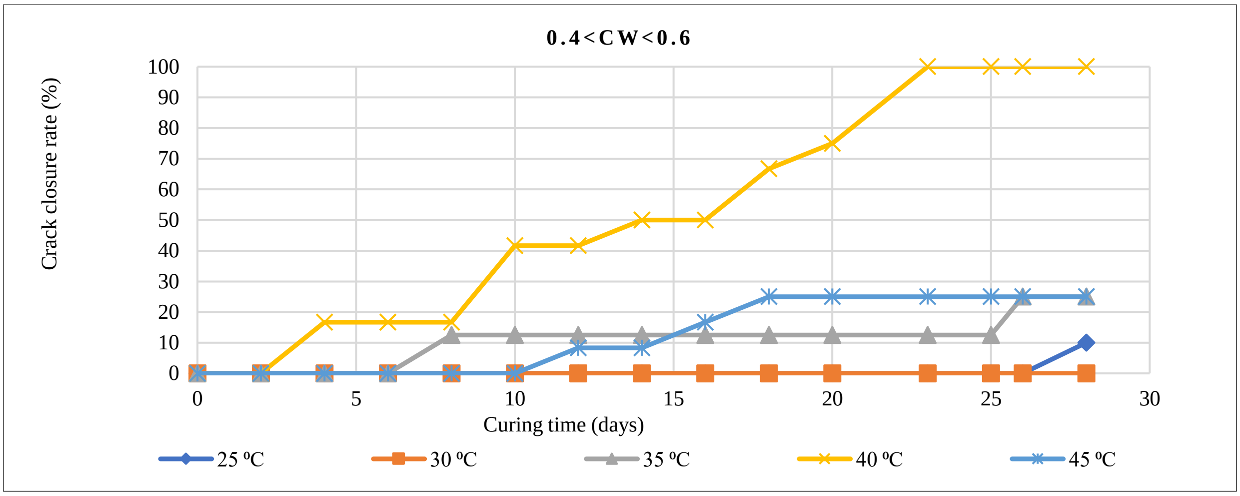

Fig. 6 Crack closure rate for control samples with crack widths ranging from 0.4mm to 0.6mm

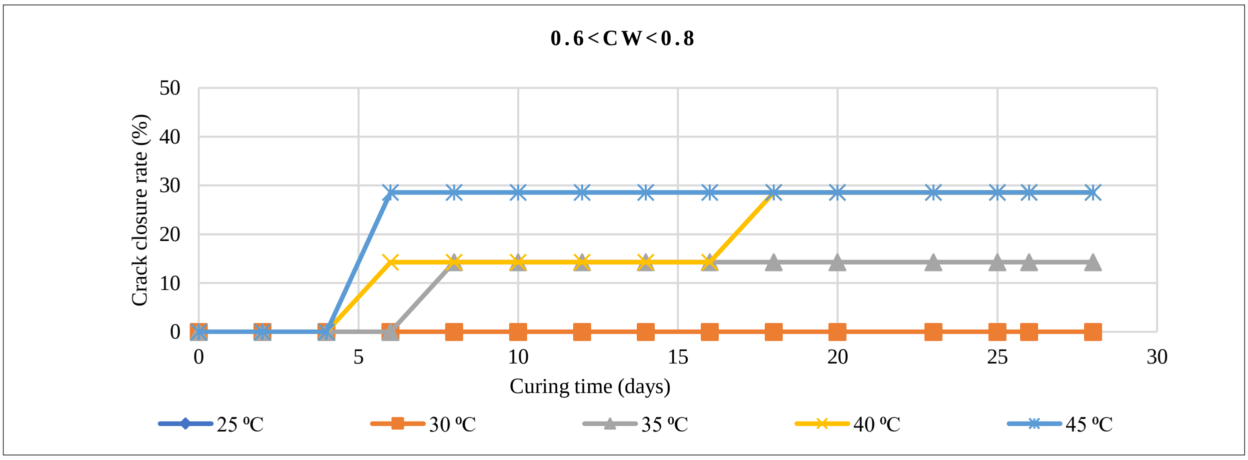

Fig. 7 Crack closure rate for control samples with crack widths ranging from 0.6mm to 0.8mm

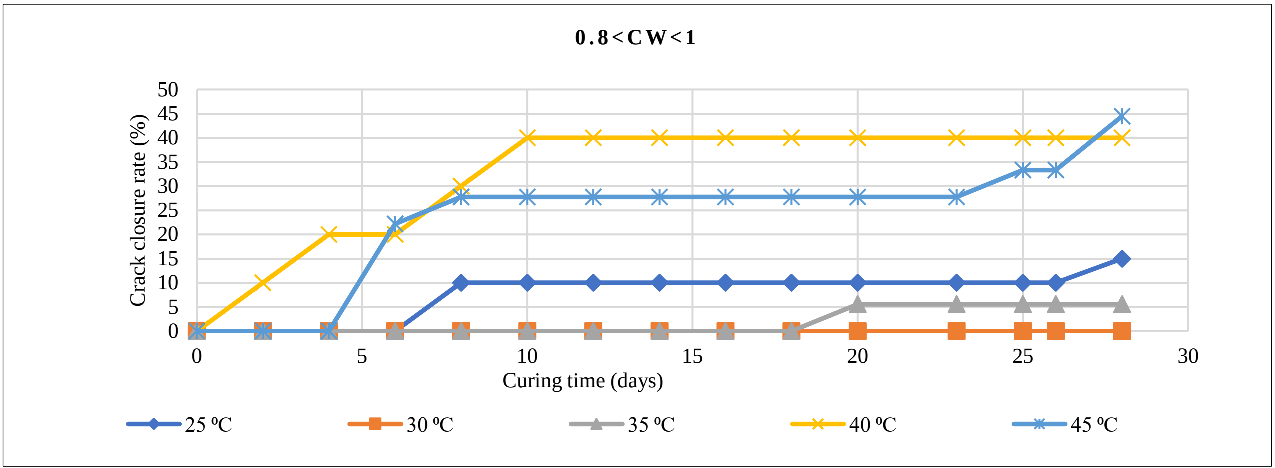

Fig. 8 Crack closure rate for control samples with crack widths ranging from 0.8mm to 1.0mm

Figures 9-11 show the crack closure rate of concrete samples with fly ash as a partial cement replacement with cracks of different widths between 0.4mm and 1mm at different curing temperature conditions. Like the control concrete samples, the healed crack width increased with temperature for the different crack width ranges. The crack closure rate was highest at 45 degrees and was between 25% and 40% at this temperature. At 25 degrees, the crack closure rate was between 10% and 30% as hydration continued even at lower temperatures.

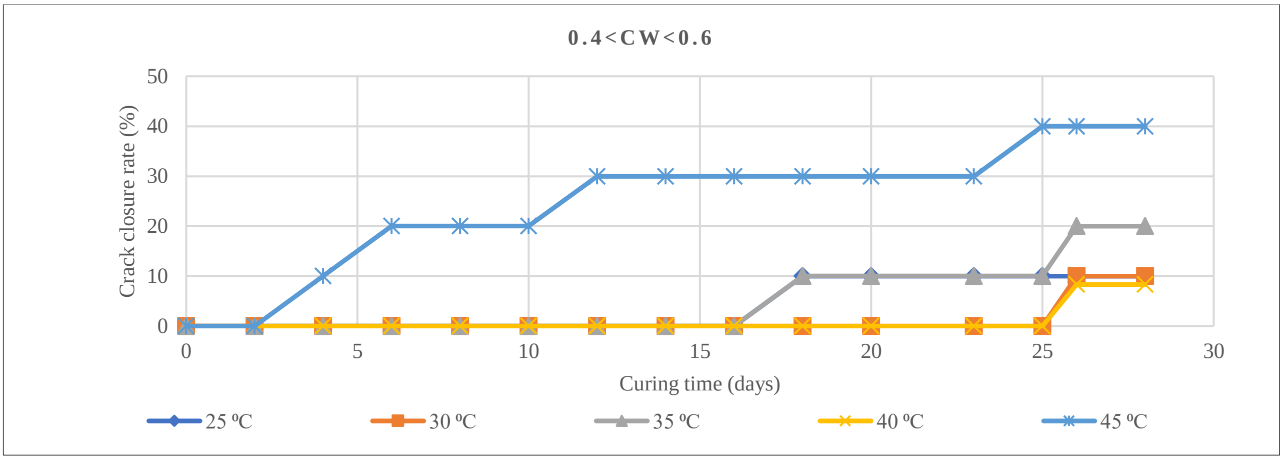

Fig 9 Crack closure rate for fly ash concrete samples with crack widths ranging from 0.4mm to 0.6mm

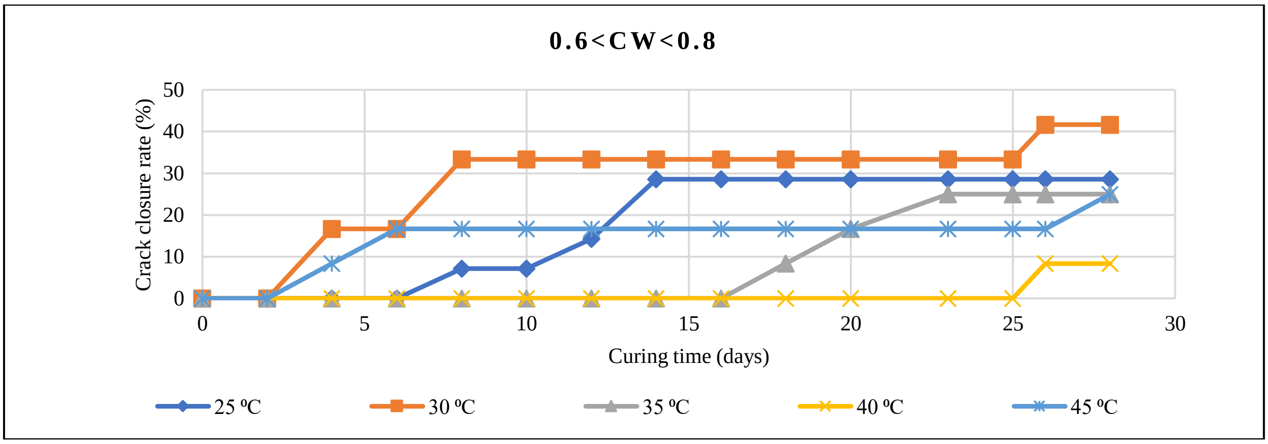

Fig. 10 Crack closure rate for fly ash concrete samples with crack widths range of stall sm 0.6mm to 0.8mm

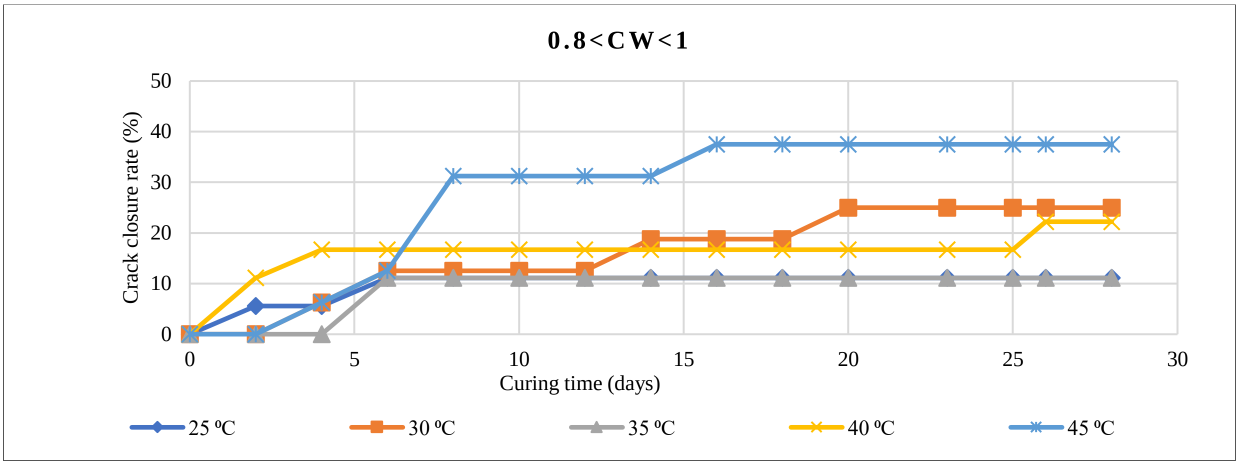

Fig. 11 Crack closure rate for fly ash Conca left samples with crathe leftrightangcementrom 0.8mm to 1.0mm

The autogenous self-healing technique can be expom twomation ofstals from carbonate ions resent in water that seeps into the crack aleft-right ions from theleft-riht of cment, as shown in Equations 4 and 5 [7].

$$Ca^{2+} + C \leftrightarrow CaCo_3 \quad (4)$$
$$C + HCO_3^- CaCO_3 + H^+ \quad (5)$$

The second involves hydrating the unhydrated cement particles on the crack surface. Fly ash hydrates slower than cement; therefore, some of it may be available in the concrete matrix in an unreacted state [35]. Fly ass the $Ca(OH)_2$ suppli, ed uring cement hydratin, as shown in Equation 6, reducing the amount of $CaCO_3$ precipitated.

$$3Ca(OH)_2SiO_2 \rightarrow 3CaO \cdot iO_2 + 3H_2O \quad (6)$$

Hence, using supplementary cementitious materials (SCMs) with high CaO content favors the precipitation of calcite which makes them very effective for healing cracks if the crack width is over 0.25mm [35], [36]. The CaO content in Class C fly ash is high, making it a very effective self-healing material. The key self-healing components for concrete made with this material are calcite and C-S-H.

Temperature accelerates the rate of hydration of OPC and the pozzolanic reaction of fly ash. Some of the pozzolanic reaction effects on hydration are accelerated at elevated temperatures. These include the production of more calcium silicate hydrate and the release of Si and Al into the pore solution, which are accelerated [37]. Hence, the formation of hydration products is sped up, and the crack healing rate increases.

Previously, references [8] and [9] reported that autogenous healing from cement is only limited for cracks of widths between 0.01-0.1mm, and no healing occurred even for early age cracks of widths above 0.45mm. However, from this study, considerable healing was observed for cracks between 0.4mm and 1mm. As a result, a conclusion can be made that the efficiency of autogenous self-healing is improved at elevated temperatures.

3.4.2 Autonomous Self-healing

Table 5 shows the initial and final crack width at pre-cracking and after 28 days of curing for the three concrete mixes. Figures 12-14 show the crack closure rate of the bacterial concrete samples with cracks of different widths between 0.4mm and 1mm at different curing temperature conditions. The results show that the healed crack width increased with an increase in curing temperature up to 40 degrees, then reduced for the different crack width ranges. The smaller cracks between 0.4mm and 0.6mm had completely healed at 18 days. For the larger cracks, up to 40% of closure had occurred in 28 days. As with the other mixes in this study, the smaller cracks healed faster than larger cracks.

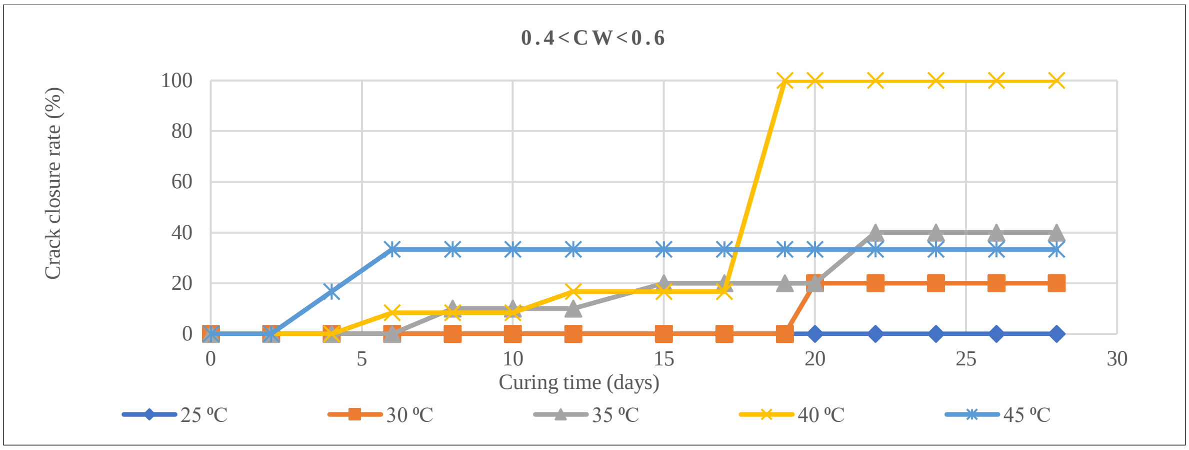

Fig. 12 Crack closure rate for bacterial concrete samples with crack widths ranging from 0.4mm to 0.6mm

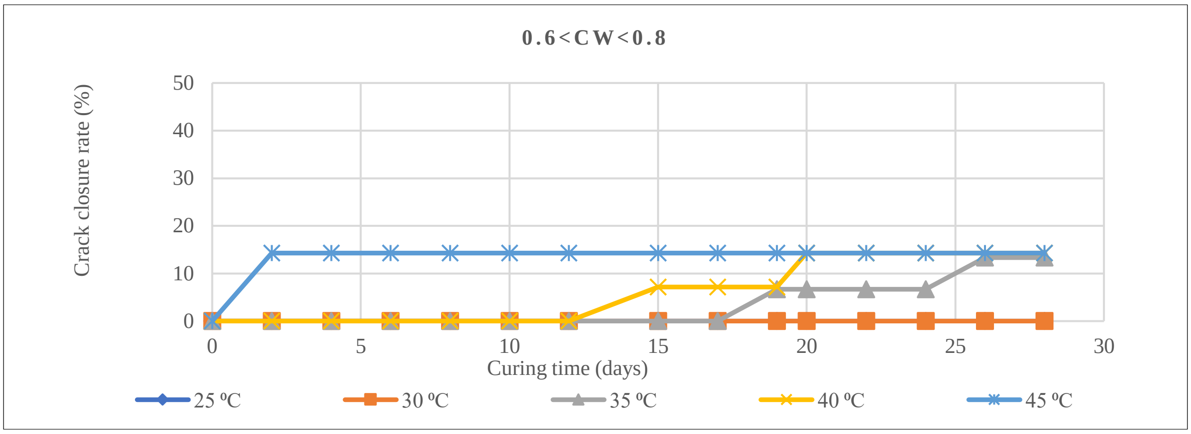

Fig. 13 Crack closure rate for bacterial concrete samples with crack widths ranging from 0.6mm to 0.8mm

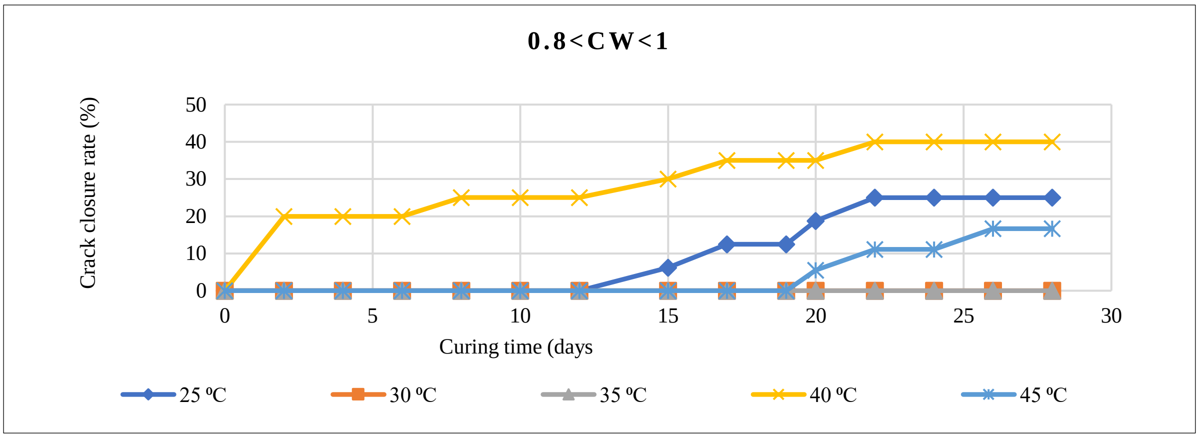

Fig. 14 Crack closure rate for bacterial concrete samples with crack widths ranging from 0.8mm to 1.0mm

For bacterial-based healing, healing could have occurred from either the hydration of unhydrated cementitious products available in the microstructure of the hardened concrete or the precipitation of calcium carbonate from bacterial exposure [38]. During the mixing process, *Bacillus thuringiensis* and calcium nitrate were added to the concrete. When the cracks develop, the bacteria are activated by oxygen and water penetrating the crack. Their metabolic activity is then restarted. Due to the substrate's metabolic conversion under the bacteria's respiration, calcium carbonate is formed, filling the cracks [1].

Bacillus thuringiensis has a two-stage growth cycle. The first is the vegetative cell cycle, where the bacterium multiplies, but when nutrients are depleted or environmental conditions become adverse, they form spores. The spores allow them to survive in adverse conditions, and they only germinate and restart a vegetative cell cycle under favorable conditions [17], [39]. The metabolic restart is dependent on the temperature, nutrient source, and the presence of water. Temperature influences carbonate precipitation by changing the enzyme activity. Thus, increased precipitation efficiency is noted at elevated temperatures due to increased reproduction and enzyme activity. When the temperature exceeds the optimum temperature for the bacteria's activity, the enzymes are denatured, leading to a loss of activity [40]. This would explain the decline in healing efficiency beyond 40 degrees. The effect of temperature on calcite precipitation and reaction time is different for different bacteria [4], [41]. Thus, their performance in different environmental conditions would be different. Reference [42] reported that in 100 days, crack width of 0.46mm was healed by non-ureolytic bacteria. According to the findings of this study, crack widths of 0.4-0.6mm were completely healed in 28 days when concrete specimens were cured at 40 degrees. Thus, it can be concluded that exposing the bacteria to their optimum conditions improves healing efficiency.

3.4.3 Self-Healing Index of Crack Closure

Reference [9] and [18] recommended using the self-healing index of crack closure by considering all crack width measures for a particular concrete type. When this technique is used, similar conclusions could be drawn as with the crack closure rate. The data in Table 5 was used to plot the graphs in Figure 15. From figure 15, the graphs show that for the control mix specimens, most of the points are close to the bisecting line of "no healing". For the samples with bacteria, the samples cured at 40 degrees moved furthest from this bisecting line showing that most healing occurred under these conditions.

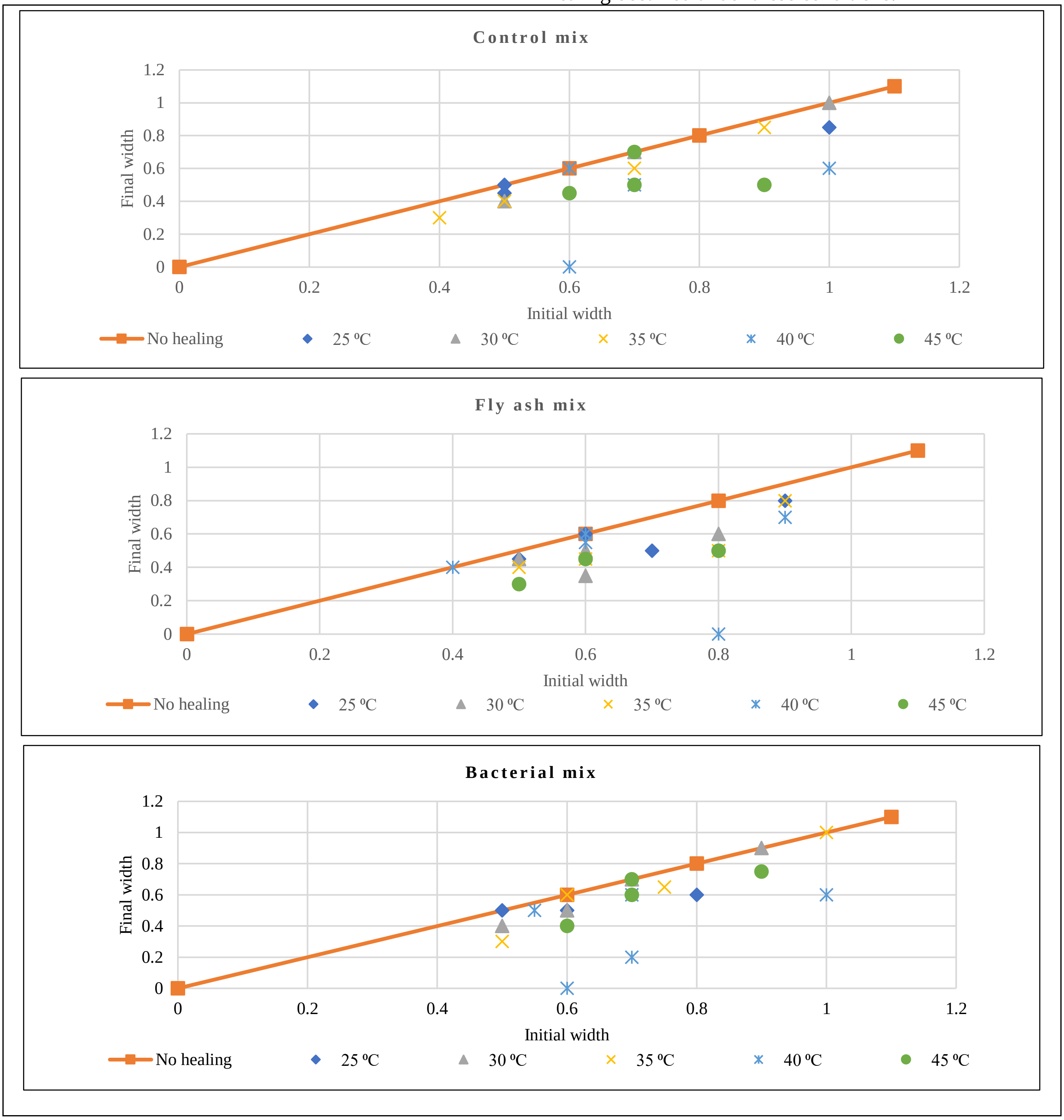

Fig. 15 Representation of crack width at 28 days for different curing temperatures compared to the initial crack width

Equation 3 was used to determine the self-healing index of crack closure β using the graphs in Figure 15. Figure 16 depicts the results.

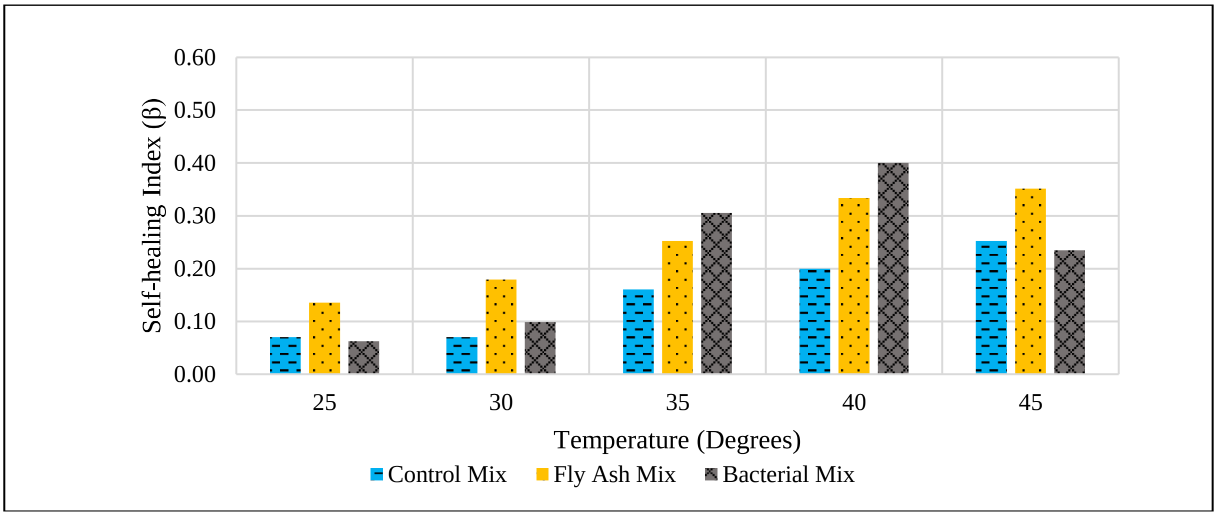

Fig. 16 Self-healing index of crack closure for concrete cured at different temperatures

This graph in Figure 16 was used to compare the efficiency of autonomous and autogenous healing using OPC and fly ash at different temperatures. From the graph, the cracks of specimens healed at high temperatures healed faster than those healed at a lower temperature. The specimens with fly ash replacing cement at 20% were healed much faster than the control concrete and the bacterial concrete, which could be due to the large amount of CaO, which favored the precipitation of more calcite when Ca^{2+} reacted with CO_3^{2-} in the water at 25ºC, 30ºC, 35ºC, and 45ºC. However, at 40ºC, the bacterial concrete exhibits higher healing efficiency. The maximum healing efficiency obtained was 40%, 35%, and 25% of crack healing for bacterial, fly ash, and control concrete, respectively.

4. Conclusion

The effects of temperature on self-healing efficiency were explored in this study by both autonomous and autogenous techniques. After specific curing days, the compressive, split tensile strength, crack closure rate, and self-healing ratio were studied. Based on this study's findings and analyses, the following conclusions were reached:

- Concrete's compressive and splitting tensile strength increases when cement is partially replaced by Class C fly ash.
- Incorporating *Bacillus thuringiensis* as a microbial healing agent and calcium nitrate as its nutrient source accelerated cement hydration, improving the early age compressive strength. However, the use of this healing agent lowered the compressive strength at 90 days by 18%. This healing agent increased the concrete's split tensile strength at all ages.
- The crack closure rate increased with increasing temperature for the bacterial, fly ash, and control mixes studied. For the bacterial mix, the optimum temperature for calcite precipitation was 40ºC. At this temperature, the bacteria showed better overall healing efficiency.
- The self-healing index of crack closure increased with increasing temperature in temperature for the bacterial, control, and fly ash mixes up to 40 degrees centigrade then reduced. The index was highest at 40 degrees for the bacterial mix, showing that this could be the optimum temperature for the bacteria to precipitate calcite. The fly ash used in this study showed the highest overall healing efficiency at all temperatures except the temperature at which the bacteria was exposed to its optimum temperature for calcite precipitation.

By determining the necessary conditions for successful bacterially induced calcification, the bacteria that would be most efficient for self-healing purposes can be determined according to the environmental conditions that the concrete will be exposed to. The study bacteria would effectively precipitate calcium carbonate for self-healing in environments where the temperature is close to optimum. However, it is critical to identify and address microorganism reactions that may have an undesirable long-term impact on the concrete's mechanical properties when used for self-healing purposes.

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