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Evaluation of effects of multi-varied atmospheric curing conditions on compressive strength of bacterial (bacillus subtilis) cement mortar

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HIGHLIGHTS

- Curing conditions effects on compressive strength of bio cement mortar.
- Combination of varying temperature and relative humidity on bacteria activities.
- Combination of varying wind speed and sunlight exposure on bacteria activities.
- Compressive strength comparison at 7 and 28 days.

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ABSTRACT

Addition of calcite-producing bacteria is a relatively new technique used globally to produce self-healing concrete/cement mortar as well as to increase compressive strength and durability. Due to different atmospheric conditions around the world, the performance of these bacteria will vary due to different curing conditions. In this study, the effects of different atmospheric curing conditions on the compressive strength of bio cement mortar (BCM) are examined. The atmospheric conditions (curing) are varied by combining different temperature and relative humidity, and wind speed and sunlight exposure time. The microorganism used is Bacillus subtilis, and it is added to concrete mortar through direct mixing. The concentration of the bacteria solution is 10^9 cells/ml and two volumes of 30 ml and 50 ml are applied. Two control specimens - water-cured bio cement mortar (BC) and water-cured cement mortar (NC), are made to serve as baselines for comparison and evaluation of the effects of the curing conditions. The results showed that increased temperature, relative humidity and wind speed increased the compressive strength of BCM. However, increased sunlight exposure time decreased the compressive strength. The results indicated that atmospheric conditions influence the performance of bacteria in BCM. The BCMs had higher 7 and 28 days compressive strength than the control sample (water-cured cement mortar). The minimum and maximum 7 day increments were 53.7% and 120.6% respectively, while the 44.9% and 130.6% respectively were the 28 day. The results show that calcite-producing bacteria improves the compressive strength of cement mortar, however, the environmental curing conditions of the produced BCM will influence the calcite-producing ability of the bacteria.

1. Introduction

For centuries, concrete has been extensively used as the main construction material for structures such as buildings, roads, dams and other civil structures worldwide. The availability of vast quantity of its constituent materials (cement, water, coarse and fine aggregates) has made it an advantageous construction material. The hardened-state properties of compressive strength, durability, malleability as well as compatibility with steel reinforcement bar have made it a construction choice material. In spite of all the advantages of concrete for construction, its vulnerability to deterioration and damage can be hastened by occurrence of cracks, hence, loss of its desired properties (compressive strength, permeability, durability) over a period of time. The existence of cracks in concrete structures can be caused by shrinkage, plastic settlement

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and thermal contraction which do not necessarily affect the performance of the structure. However, these cracks lead to infiltration of gaseous chemicals and water. Thus, causing harm to the concrete structure [1–4], and requiring substantial maintenance cost [5].

Considering that civil structures are the most important asset of any society [6], several methods have been proposed to curb commencement and progression of cracks at the early stages in concrete. These methods involve strategies using epoxy systems, acrylic resins, chlorinated rubber, polyurethane and silicone-based polymers. However, problems such as non-compatibility with concrete, high cost, moisture sensitivity, low heat resistance have limited the application of these methods [7,8]. As a result, recent studies have emerged with the concept of bio concrete as a viable solution to crack formation in concrete. The procedure of bio concrete involves addition of microbial organisms to concrete to produce calcium carbonate ($CaCO_3$) that seals up the cracks. The calcium compounds in the concrete are converted to calcium carbonate by the bacteria through respiratory effect [5]. This was demonstrated when Xu and Yao [9] suggested that incorporation of bacteria as a healing agent in concrete matrix impels $CaCO_3$ formation upon cracking. Bacillus cohnii spores were incorporated as $CaCO_3$ precipitant for self-healing strategy of concrete cracking. Cracks with a range of 0.1–0.4 mm width were sealed completely by layers of $CaCO_3$ precipitates. Wiktor and Jonkers [10] integrated bacterial spores (Bacillus alkalinitrilicus) into concrete as a self-healing agent. During casting of the concrete, multiple cracks of 1.0 mm width were inflicted by stretching embedded steel. The authors stated that the bacterial-integrated concrete indicated crack-healing as much as 0.46 mm width while the control specimen had only 0.18 mm. Similarly, Qian et al. [11] discovered that $CaCO_3$ precipitated in crack openings when bacteria-loaded pottery sand was added to concrete. In their later study, they stated that bacteria sealed most 0.3 mm cracks within 5 days and all the surface cracks by the 20th day [12]. Recently, Zhang et al. [13] reported the sealing of 0.79 mm width crack at 28 days. Their study involved utilising expanded perlite as bacteria carrier in concrete through immobilization of Bacillus cohnii. Jadhav et al. [14] applied self–healing Sporosarcina pasteurii bacteria to seal up cracks caused by brittleness of geopolymer concrete.

Evidently, the potentials of calcite-producing bacteria to sealing up of cracks in concrete have been shown. In addition to crack sealing in concrete, the activities of these microorganisms have been shown in several studies to fill up tiny voids in concrete to increase the compressive strength properties of concrete. The 28 day compressive strength of cement mortar was increased by 25% when calcite precipitating bacteria was added [15]. They concluded that the bacteria activity produced calcite within pores of the cement mortar. Achal et al. [16] noted a 17% improvement in the compressive strength of cement mortar cubes treated with Sporosarcina pasteurii. The authors stated that microbial calcite precipitated within the mortar plugs pores, subsequently enhancing the compressive strength of the mortar cubes. Bundur et al. [17] increased the compressive strength of cement mortar by incorporating vegetative bacterial cells of Sporosarcina pasteurii to cement mortar. A 23.5% increase in compressive strength was reported by Bensal et al. [18] by applying halophilic bacteria Exiguobacterium mexicanum isolated from sea water to concrete. Kalhori and Bagherpour [19] reported a 30% increase of the compressive strength of sandcrete. The $CaCO_3$ precipitating bacterium was Bacillus subtilis. Recently, Shashank et al. [20] utilised a Bacillus family bacteria that is resistant to high alkaline environment of concrete to increase the compressive strength by as much as 36%.

Furthermore, these microorganisms have improved other concrete properties like permeability, porosity and durability. For example, Ramakrishnan et al. [21] confirmed increased resistance to permeability, alkali, freeze-thaw attack and shrinkage in concrete with bacterial cells. Achal et al. [16] observed decreased water permeability of bio-treated cement mortar, so was reported by De Muynck et al. [22] and Kim et al. [23]. Nosouhian et al. [24] stated that the chloride penetration of concrete reduced when bacteria strained were added to the mixing water. Siddique et al. [25] stated that calcite precipitating bacteria reduced concrete's water absorption and porosity properties as well as chloride permeability.

These above studies have shown the existence of abundant studies on bio concrete. However, limited studies have investigated the influence of concrete curing conditions on the activities of these microorganisms. The activities of these microbial organisms to produce calcite are often affected by factors such as bacteria amount, growth media and environmental conditions. The precipitation of calcium carbonate in media depends on factors that include pH [26], presence of nucleation site [27], dissolved inorganic carbon [28] and calcium ions concentration [29]. In fact, the concentration of dissolved inorganic carbon depends on several climatic parameters such as temperature and the partial pressure of carbon dioxide [30,31]. In addition, Wood et al. [32] showed that ultraviolet exposure significantly decreased the recovery of bacterial spores. Since bio concrete is a concept applied globally, the atmospheric condition varies at different locations. Furthermore, climate change is altering the atmosphere (temperature, wind, relative humidity, rainfall etc.) at an alarming rate. Thus, the activities of the microbial organisms will vary at different locations. In addition, the existence of limited information on effect of atmospheric conditions on microbial activities in concrete and the lack of any standard concrete design code on application of bio concrete [33] make this study remarkably important.

Therefore, this study investigates the effects of different atmospheric conditions on the activities of the microorganism (Bacillus subtilis) in bio cement mortar (BCM) through curing conditions. These effects are evaluated by assessing the compressive strength attained on the 7th and 28th days. This involves curing the BCM in different atmospheric conditions. The atmospheric condition factors considered are temperature, sunlight, relative humidity and wind speed. BCM and control cubes are prepared and cured in a combination of varying temperature and relative humidity, and sunlight exposure and wind speed. The control samples (sample with and without bacteria) were water-cured at room temperature. Compressive strength test is carried out on the 7 and 28 days on the BCM cubes to evaluate the effect of different atmospheric conditions. The results provide adequate information regarding microbial activities in concrete at various locations with regards the atmospheric factors.

2. Materials

2.1. Preparing of microorganism (Bacillus subtilis)

Pure culture of bacillus substilis was obtained locally in Kuala Lumpur, Malaysia. The media used for culturing contained 10 g tryptone, 5.0 g yeast extract and 10.0 g NaCl in 950 ml H_2O. After inoculating in a laminar flow, the broth culture of B. substilis was prepared on a rotating shaker at 150 rpm and 37 °C for 48 h. The broth was introduced into the cement mortar by direct incorporation into the mixing water. The concentration of the bacteria solution is 10^9 cells/ml.

2.2. Properties of cement

Ordinary Portland cement (OPC) was used in this research. The cement used is a product from cement industries of Malaysia berhad (CIMA) that is available in local markets. This cement has BS 12

Table 1
Physical properties and chemical constituents of Cement.

Physical properties	
Colour	Grey
Specific gravity	3.0
Chemical constituents (%)	
CaO	63.8
SiO_2	21.3
Al_2O_3	3.78
Fe_2O_3	3.75
MgO	1.77
SO_3	3.0
Na_2O	–
K2O	–

1996 specifications [34], and it is a high quality multi-purpose product which is suitable for most concrete works. The physical properties and chemical constituents of the OPC cement as obtained from the manufacturer are shown in Table 1.

2.3. Properties of fine aggregates

The fine aggregate was locally obtained river sand that passed through 600 μm sieve with fineness modulus of 2.5. The density of the fine aggregate is 1750 Kg/m^3. Sieve analysis was conducted on the fine aggregate, and the grading curve is shown in Fig. 1. The fine aggregate conforms to BS 882 [35]. It was washed with water and air dried to ensure that the water/cement ratio was not being affected.

2.4. Mix proportion and test specimens

The cement mortar applied in this study has a mix ratio of 1:2 by weight, while the water/cement ratio is 0.45. The cement mortar matrix is designed to attain a 28 day compressive strength of 25 N/mm^2. The test cubes are 50 × 50 × 50 mm. The fine aggregate and cement are thoroughly mixed with water. Two different volumes of 30 ml and 50 ml of the bacteria (Bacillus subtilis) spore solutions are prepared. A total of 156 test specimens are made. The control specimens (cubes with and without bacteria) are water-cured. The water-cured BCM control specimens are labeled BC, while the water-cured cement mortar control specimens are labeled NC. Vibration machine is applied to compact the cubes during casing.

2.5. Curing conditions

All the sample specimens are removed from moulds 24 h after casting. These samples are then place in their various curing conditions. Combinations of curing conditions are applied to evaluate the effects of different climatic conditions on the activities of the microorganisms in the BCM. The climatic factors combined are varying temperature and relative humidity, and sunlight exposure time and wind speed. The temperatures applied are 10 °C, 26 °C and 40 °C, and relative humidity are 50%, 72% and 95%. These conditions are achieved by using Digital Automatic Incubators that control and maintain the specified temperatures and humidity.

The wind speeds are 0 m/s, 3 m/s and 6 m/s, while sunlight exposure times are 2 h, 5 h and 8 h. Cleanroom Air Showers are used to provide the specified wind speed, while the sunlight exposure time is done and regulated manually. Tables 2 and 3 provide more details on the curing conditions of the specimens. In addition, control sample specimens of cement mortar cubes without bacteria (NC) and cement mortar cubes with bacteria (BC) were water-cured to compare results to those cured in combined atmospheric conditions.

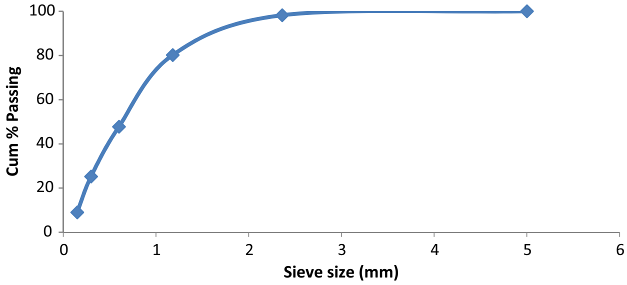


Fig. 1. Grading curve of the fine aggregate.

Table 2
Temperature and relative humidity curing conditions (combination).

Case	Temperature (°C)	Relative humidity (%)
1	10	50
2	10	72
3	10	95
4	26	50
5	26	72
6	26	95
7	40	50
8	40	72
9	40	95

Table 3
Wind speed and sunlight exposure time curing conditions (combination).

Case	Wind speed (m/s)	Sunlight exposure time (hrs)
1	0	2
2	0	3
3	0	8
4	3	2
5	3	3
6	3	8
7	6	2
8	6	3
9	6	8

2.6. Test procedure

The climatic effect on the efficiency of the bacteria activity in the concrete is measured by determining the compressive strength of the cubes. The compressive strength is determined according to BS 1881-116 [36]. The 7 and 28 days compressive strengths are measured after curing in different conditions. To reduce error and obtain better results, three samples are subjected to the same condition and their average compressive strength is obtained as the final reading.

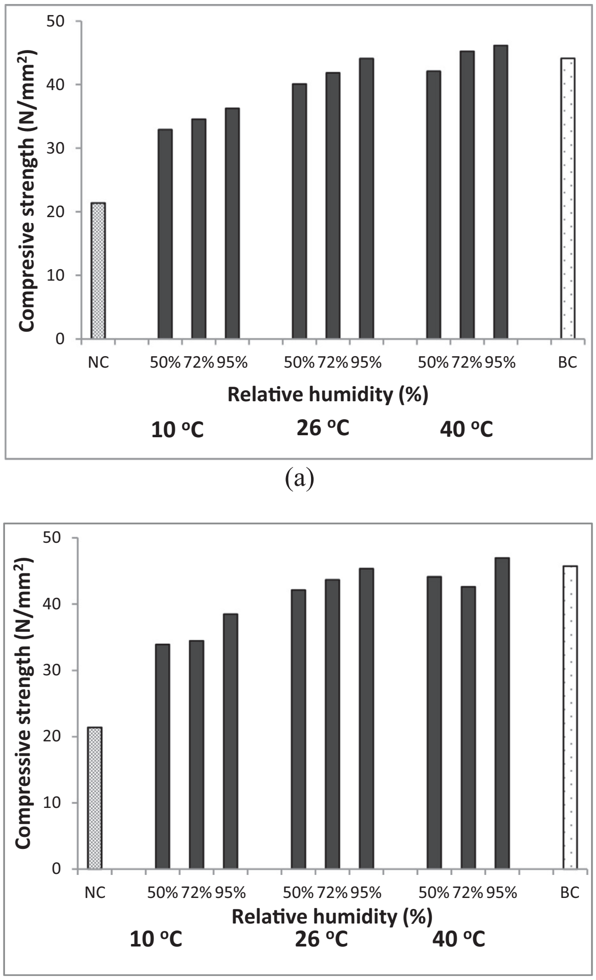
3. Experimental results

The results reported in this study are based on the compressive strength of BCM cubes cured in different curing condition combinations. This involved combining different atmospheric conditions to evaluate their effects on bacteria activities in BCM. The bacteria (bacillus subtilis) in the BCM produce $CaCO_3$ through respiration to fill the micro spores in the mortar, thereby increasing the compressive strength. The atmospheric conditions combined in this study are temperature and relative humidity, and wind speed and sunlight exposure time.

3.1. Effect of temperature and relative humidity

A combination of various temperatures and relative humidity are applied as curing conditions of the BCM cubes. The compressive strengths of the BCM are taken on the 7 and 28 days. Figs. 2 and 3 show the 7 and 28 days compressive strengths of the BCM cubes respectively, containing 30 ml and 50 ml bacterial spore solutions. In addition, the compressive strength values of the control samples (BC and NC) are also indicated in Figs. 2 and 3. The 7 and 28 days compressive strength of NC are 21.4 N/mm^2 and 27.4 N/mm^2 as shown in Figs. 2 and 3. The 7 day compressive strengths of BC with 30 ml and 50 ml bacterial spore solutions are 44.1 N/mm^2 and 45.7 N/mm^2 respectively, while the 28 day values are 52.8 N/mm^2 and 55.7 N/mm^2 respectively. All the BCM, irrespective of curing condition, had higher compressive strengths than the control sample NC which was without bacteria and was water-cured. The 7 day compressive strength of BCM cured in 85% relative humidity and 40 °C increased by 130% of the control specimen NC (Fig. 2(b)). This is due to the calcite produced by the activities of the bacteria in the BCM cubes.

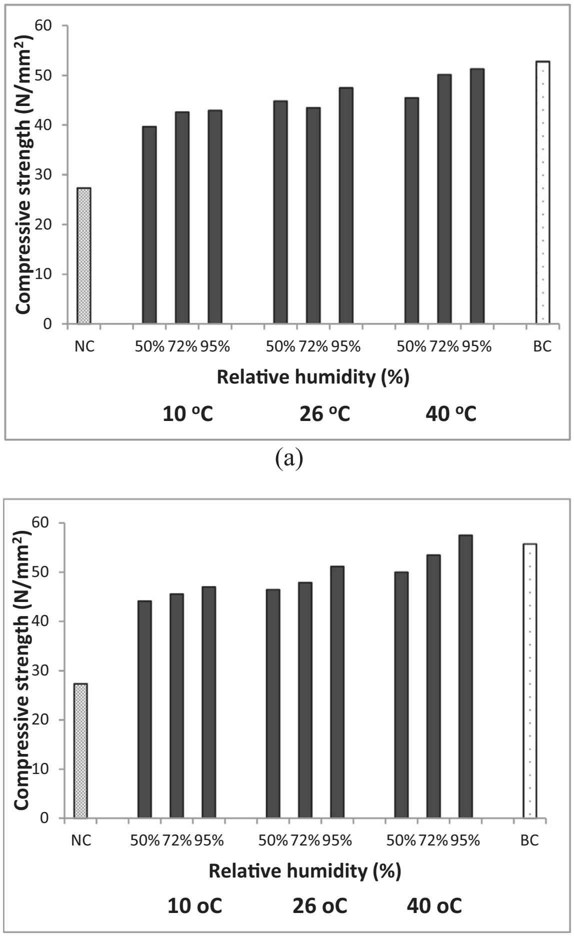
In Fig. 2, the 7 day compressive strengths of the BCM cubes with 30 ml and 50 ml are presented. The trend shows that the compressive strength of BCM increased with increased bacterial spore solution. The increased compressive strength is due to higher amount of bacterial spores, thus more produced calcite in the mortar and leading to increased compressive strength. This is similar to findings of Chahal et al. [3] where different concentrations of bacteria were applied. Furthermore, Fig. 2 shows that increased temperature increased the 7 day compressive strength of the BCM cubes. For example, in Fig. 2(a), the compressive strength increased by 21% from 32.9 N/mm^2 to 40 N/mm^2 when temperature increased from 10 °C to 26 °C at 50% relative humidity. Similarly, in Fig. 2 (b), the compressive strength increased by 24% from 33.9 N/mm^2 to 42.1 N/mm^2 when temperature increased from 10 °C to 26 °C at 50% relative humidity. The highest temperature considered in this study was 40 °C which is close to the optimum culturing temperature of the bacteria (Bacillus subtilis) [37–39]. In addition, Fig. 2 indicates that increased relative humidity increased strength with both volumes of bacterial spore solutions. At 10 °C, Fig. 2(a) shows that the 7 day compressive strength of the BCM increased from 32.9 N/mm^2 to 36.3 N/mm^2 when the relative humidity was increased from 50% to 95%. The bacteria activity increased due to increased relative humidity [40], thus producing more calcite to increase the compressive strength. It should be noted that for both volumes of bacterial spore solutions, the 7 day compressive strength of BCM cured in combined atmospheric conditions can exceed that of water-cured BCM. This is achievable by combining the suitable temperature and relative humidity shown in Fig. 2.



(a)

(b)

Fig. 2. 7 day compressive strength using temperature and relative humidity. (a) 30 ml of bacteria spore solution; (b) 50 ml of bacteria spore solution.



(a)

(b)

Fig. 3. 28 day compressive strength using temperature and relative humidity. (a) 30 ml of bacteria spore solution; (b) 50 ml of bacteria spore solution.

Fig. 3 presents the 28 day compressive strength of BCM with 30 ml and 50 ml of bacterial spore solutions in different curing conditions as well as the control samples (BC and NC). The 28 day compressive strengths of the 30 ml and 50 ml water-cured control sample BC are 52.8 N/mm^2 and 55.7 N/mm^2 respectively. These values amount to 92.6% and 103% respectively, increments in compressive strength of the water-cured control sample NC that is 27.4 N/mm^2. It is observed in Fig. 3 that increased temperature of the curing condition of BCM increased compressive strength. For example, in Fig. 3(b), the compressive strength increased from 44.2 N/mm^2 to 50 N/mm^2 at relative humidity of 50% when temperature increased from 10 °C to 40 °C. This indicates that the activities of the bacteria in the BCM are still sensitive to temperature, thus providing increased strength at 40 °C. Similarly, relative humidity increment can be seen to increase the compressive strength of the BCM. At 10 °C in Fig. 3(b), the compressive strength increased from 44.2 N/mm^2 to 47 N/mm^2 when the relative humidity increased from 50% to 95%. The increased relative humidity increased the bacteria activity in the cement to produce more calcite.

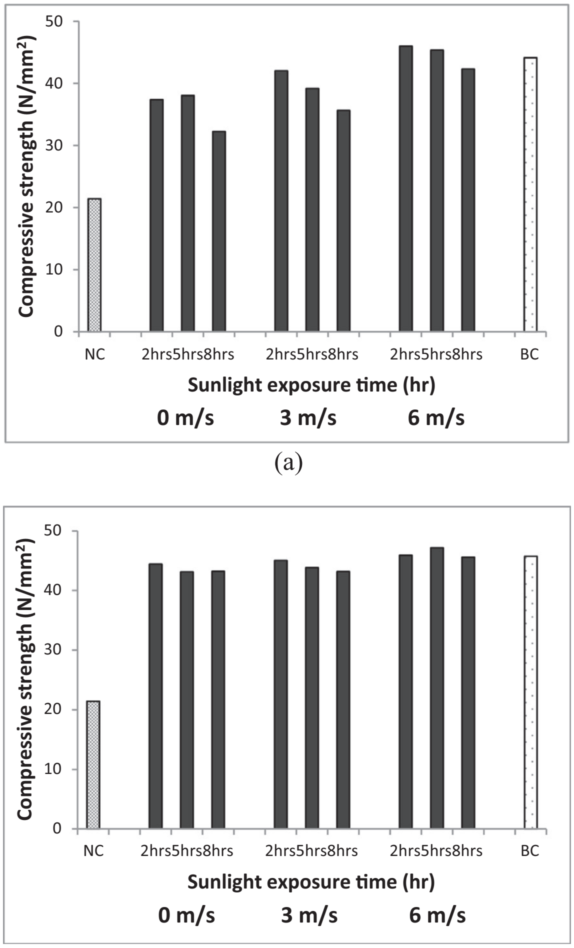
The minimum 28 day compressive strength of the BCM as shown in Fig. 3 for both 30 ml and 50 ml bacteria spore solutions are 39.7 N/mm^2 and 44.2 N/mm^2 respectively. These values indicate a minimum of 44.9% increment of the water-cure cement mortar strength. The maximum strengths are 51.3 N/mm^2 and 57.5 N/mm^2 for the 30 ml and 50 ml bacteria spore solutions respectively, giving a maximum increment of 110%.

From Figs. 2 and 3, it is clearly shown that the curing conditions of BCM significantly affected the compressive strength at the 7 and 28 days. At both days, the compressive strength of all the BCM is higher than water-cure cement mortar. Increasing either or both temperature and relative humidity increased the compressive strength of BCM. The 28 day compressive strength of water-cured BC has the highest compressive strength when 30 ml of bacteria spore solution is applied. On the other hand, BCM cured at 40 °C and 95% relative humidity has the highest compressive strength when 50 ml bacteria spore solution is applied.

3.2. Effect of wind speed and sunlight exposure time

The effects of combining varying wind speeds and sunlight exposure time conditions are explained in this section. The BCM cubes are cured in varying condition combinations to evaluate their effects on the compressive strength. Figs. 4 and 5 show the 7 and 28 days compressive strengths of the BCM cubes respectively. In addition, the strengths of control samples (BC and NC) are also presented in Figs. 4 and 5.

From Fig. 4, it is observed that BCM with both volumes (30 ml and 50 ml) of bacteria spore solution have higher 7 day compressive strength than the water-cured control sample cement mortar (NC). Also, BCM with 50 ml (Fig. 4(b)) bacteria spore solution has higher 7 day compressive strength than the 30 ml. (Fig. 4(a)). The trend in Fig. 4 shows decreased 7 day compressive strength as the sunlight exposure time increased. For example, at 3 m/s (Fig. 4(a), the compressive strength decreased from 42 N/mm^2 to 35.6 N/mm^2 when the sunlight exposure time is increased from 2 h to 8 h. This amounts to 15% decrement in compressive strength. Exposure to sunlight limits bacteria activities, thus lesser calcite is produced in the BCM. This is similar to the conclusion made by Wood et al. [32] when they discovered that ultraviolet exposure significantly decreased the recovery of bacteria spores. Contrary to this, increased wind speed increased the 7 day compressive strength of BCM. At 2 h sunlight exposure (Fig. 4(a)), the compressive strength increased from 37.4 N/mm^2 to 46 N/mm^2 when the wind speed increased from 0 m/s to 6 m/s, resulting to 23% increment in the compressive strength. The least 7 day compressive strength (32.3 N/mm^2) from the BCM (Fig. 4(a)) indicates a 17% increment of the NC compressive strength. The increased compressive strength is due to calcite produced by the activities of the bacteria (Bacillus subtilis) in the BCM. The 7 day compressive strength of combined atmospheric conditions cured BCM can exceed that of water-cured control sample BC. In Fig. 4(a), at wind speed of 6 m/s and sunlight exposure times of 2 h and 5 h, the compressive strength atmospheric conditions cured BCM (46 N/mm^2 and 45.3 N/mm^2) exceed the water-cured control sample BC (44.1 N/mm^2). Also, Fig. 4(b) shows that wind speed at 6 m/s, and 2 h and 5 h sunlight exposure times provided 45.9 N/mm^2 and 47.2 N/mm^2 compressive strengths which are higher than the BC value of 45.7 N/mm^2.

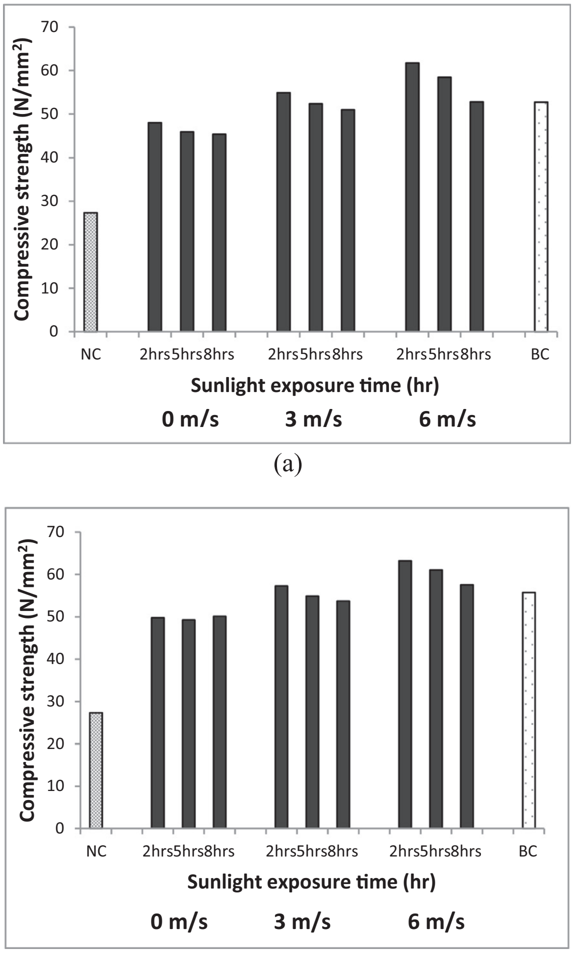


(a)

(b)

Fig. 4. 7 day compressive strength using wind speed and sunlight exposure time. (a) 30 ml of bacteria spore solution; (b) 50 ml of bacteria spore solution.

Fig. 5 shows the 28 day compressive strength of the BCM cured with combinations of atmospheric conditions. A higher amount of bacteria spore solution provided higher compressive strength as seen in Fig. 5(a) and (b). At 28 days, the compressive strength decreased as the sunlight exposure time increased. For example, in Fig. 5(b), compressive strength decreased from 57.2 N/mm^2 to 53.7 N/mm^2 when sunlight exposure time increased from 2 h to 8 h at 3 m/s wind speed. This amounts to 6.1% decrement in compressive strength. However, increased wind speed increased compressive strength. At 2 h sunlight exposure (Fig. 5(a)), the compressive strength increased from 48 N/mm^2 to 61 N/mm^2 when wind speed increased from 0 m/s to 6 m/s. Some combined atmospheric curing conditions provided higher compressive strength than the water-cured control sample BC. Examples are BCM cured in 6 m/s wind speed (Fig. 5(b)) had higher compressive strength than BC.



(a)

(b)

Fig. 5. 28 day compressive strength using wind speed and sunlight exposure time. (a) 30 ml of bacteria spore solution; (b) 50 ml of bacteria spore solution.

The results shown in Figs. 4 and 5 clearly shows that wind speed and sunlight exposure time have significant influence on the compressive strength of BCM. From the results above, increased wind speed has positive effect on the compressive strength by increasing it while increased sunlight exposure time decreased the compressive strength of BCM.

4. Conclusions

This paper evaluates the effect of different atmospheric conditions on the activities of calcite-producing bacteria (Bacillus subtilis) in BCM by examining the 7 and 28 days compressive strengths. A total of 156 cement mortar cubes with and without bacteria were prepared. These cubes were subjected to different atmospheric conditions by combining varying temperatures and relative humidity, and wind speeds and sunlight exposure time.

The results presented in this paper showed that atmospheric conditions influence the compressive strength of BCM. In addition, the results showed that the compressive strength attained by all the BCMs were higher than the water-cured control sample cement mortar (NC). The temperature of 40 °C and relative humidity of 95% increased the28 day strength of BCM by 110%, while wind speed of 6 m/s and sunlight exposure time of 2 h resulted to 133% increase. The quantity of bacteria spore solution added to the BCM influences the compressive strength. It was observed that increased temperature, relative humidity and wind speed were beneficial to the BCM as they increased the compressive strength. However, increased sunlight exposure time was detrimental to the BCM as it decreased the compressive strength.

Furthermore, the results showed that the value of compressive strength of water-cured control sample BC can be attained if the BCM is cured under suitable atmospheric conditions (temperature, relative humidity and wind speed). The strength of BCM (compared to BC) was increased by 3.2% at 40 °C and 95% relative humidity, and by 13.5% at 2 h sunlight exposure time and 6 m/s wind speed. This study has shown that calcite-producing bacteria improves the compressive strength of concrete, however, the environmental curing conditions of the produced bio concrete will influence the calcite-producing ability of the bacteria.

Declaration of Competing Interest

None.

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