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THE USE OF MICROORGANISMS IN THE BIOCONCRETE DEVELOPMENT

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Abstract.
The most popular building material worldwide is thought to be concrete. However, a variety of reasons, including overloads, temperature changes, shrinkage, earthquakes, and others, can cause concrete to crack. These fissures increase the concrete's permeability while decreasing the structure's strength and lifespan. As a result, the significance of "self-healing concrete" is increasingly emphasized, with particular focus on bioconcrete, a unique kind of concrete that has the capacity to self-heal microcracks caused by overloads. This capability stems from the unique properties of the bacteria employed in the concrete mix, which include the capacity to survive in severe environments that are comparable to those found in the concrete environment. in addition to their capacity to precipitate the substance that seals the fissures, calcite (calcium carbonate, or CaCO3). The purpose of this study is to evaluate the effectiveness of self-healing Bacillus mycoides added to Portland cement mortar. This bacterium was isolated from Geidam, Yunusari, Damaturu, Dapchi and Gashua soils. following seven days of curing, the load percentage concept caused the specimens to crack, and tests were conducted 21 days following the cracking.
The mortar was tested using a compression test, an acid fizz test, and bacterial sporulation tests conducted inside mortar specimens. The results showed that one possible method for reducing cracking is the use of self-healing bacteria. Compared to the negative control without bacteria, the rate of healing with bacteria was higher.

Key words: *Bioconcrete, mortar, calcite, precipitation, self-healing, shrinkages, cracks, permeability and incorporated.*

Introduction

Due to its strength, durability, and affordability, concrete is one of the most widely used building materials in the world, J. McCormag and R. Brown (2013), A. Neville (1996). However, it is susceptible to cracking due to a variety of variables, including temperature, shrinkage, overloads, earthquakes, human activity, and more, G. Souradeep and H. W. Kua (2016). These fractures reduce the structure's longevity and quality, which raises the concrete's porosity H. Jonkers, A. Thijssen (2010).

The stability of the structure is unaffected by

microcracks, but they have the potential to grow into a network of smaller and larger cracks, which could alter the concrete's permeability and allow chemicals and water to enter the mixture, causing corrosion and a gradual loss of tensile strength before failing, G. Souradeep and H. W. Kua (2016), W. Virgine, H. Jonkers (2011), A. Albughadi, M. Abou-ZEID (2016). Because of this, fixing the fractures is crucial to reducing porosity and boosting the structure's durability. The current maintenance procedure repairs the cracks after they occur and propagate. Because the fissures are undetectable or in an unobtrusive position, the protocols are costly, time-consuming, and in certain situations, impossible, G. Souradeep and H. W. Kua (2016), A. Albughadi, M. Abou-ZEID (2016). For instance, repairs account for about half of yearly construction budgets in Europe, E. Cailleux, V. Pollet (2009). Because concrete has the capacity to mend itself, a novel method for patching concrete cracks known as "self-healing concrete" was developed. This concept was influenced by the way wounds mend in the human body, G. Souradeep and H. W. Kua (2016).

A form of self-healing concrete that is inexpensive, sustainable, and environmentally friendly, bioconcrete (a concrete made from microorganisms, cement, lime, and iron rods) contains self-healing agents without sacrificing the concrete's original qualities while it is ssbeing mixed, H. Jonkers and E. Schlangen (2008). In order to survive in the concrete paste, this new method relies on the development of spores and bacteria that create minerals that are initially present in hostile environments like soil, rocks, alkaline lakes, etc. H. Jonkers and E. Schlangen (2007). When cracks appear, the penetrating moisture and oxygen will activate bacteria within the concrete, causing calcite to precipitate out to fill the fissures.

Bacillus was restricted using a number of techniques, which have effectively stabilized the bacteria's metabolic activity over an extended period of time by lowering the rate of bacterial activity, A. Fathy et al. (2014).

One of the processes involves encapsulating the healing ingredient to render it immobile. Crack prevented this from succeeding, A. Fathy et al. (2014). Using diatomaceous earth (DE) as an immobilizing agent was an additional technique, S. Bang et al. (2001). Diatomaceous is a soft, siliceous silt that occurs naturally and has particles that range in size from 10 to 200 µm. Fossil remains of diatoms with extremely porous, light, and chemically stable skeletons are referred to as diatomaceous, N. Degirmenci (2009). These diatom pores have the capacity to house microorganisms, nutrients, water, and oxygen, all of which can eventually support life, S. Bang et al. (2001).

All of the earlier research has demonstrated how effective self-healing concrete is at removing cracks. The current work expands on previous findings by using mortar as the material and soil-isolated Bacillus bacteria as the self-healing agent. Because of their capability to live and resist high temperatures, their ability to create spores, their ability to activate when exposed to moisture, and their ability to live and survive in strong alkaline environments, these bacteria were selected to lessen the requirement for a mobilizing agent. By consuming calcium lactate and generating calcite, the crack's sealing agent, the bacteria act as a catalyst.

This study also analyses the effect of reducing the pH level of mortar specimens by replacing a percentage of cement with silica fume
.

Methodology

Selection of potential sources for bacterial isolation

Bacterial isolates with the required characteristics were searched from soil samples collected from areas with harsh conditions such as Geidam, Yunusari, Damaturu, Dapchi and Gashua.

Cultivation of bacteria and selection of bacterial colonies

About 1 gram of soil from each of the aforementioned soil sources was added to 10 millilitres of nutrient broth with pH values ranging from 9 to 12, and the mixture was then incubated for three days at 37 degrees Celsius. Following a three-day incubation period, each sample was well shaken to allow the soil particles to precipitate. One millilitre of the supernatant was serially diluted with nine millilitres of sterile normal saline. Each sample was then diluted up to 10-7 using a further series decimal dilution procedure.

To screen for viability and identify isolated colonies of bacteria that were tolerant of the pH values that it was cultured in, 100 microliters of different dilutions

were dispersed across the surface of nutrient agar. After that, the nutrient agar was incubated at 37°C for 48 hours. Bacterial colonies were initially selected and isolated from each soil sample based on variations in colony form and color. To guarantee purity, each colony is moved into a different nutrient agar plate and subcultured five (5) times. Each isolate is assigned a numerical code.

Characterization of bacteria
Tolerance to pH 14

Each bacterial pure colony was cultured in nutrient broth at pH 14 at 37°C for 24 hours, and then cultured over nutrient agar to check for viability for another 24 hours.

Hydrolysis of urea

Potential isolates that were able to tolerate high pH levels were cultured over urea base agar supplemented with 5% urea and incubated for 24 hours at 37ºC to test for their ability to produce an enzyme called urease.

Morphology of cell and spore formation

Gram stain procedures was used to examined the morphology of the potential isolates and its ability to form spore was tested using the malachite green stain according to the regular microbiology protocol. Specimens were examined using light microscopy at 40x magnification.

Thermal stability

The potential bacterial isolates were further cultured in nutrient broth and incubated at 70ºC for 24 hours. The growth of the bacteria was recorded after incubation as scores with negative signs (-) indicating no growth and positive signs (+) indicating growth.

Formation of calcite and acid fizz test

Each potential isolate was grown in a flask containing 200ml of mineral salt media (MSM) supplemented with 5% calcium lactate pentahydrate ($[CH_3CH(OH)COO]_2Ca{\cdot}5H_2O$) and incubated in an orbital shaker at 100 rpm for 3 days at 37ºC. At the end of the incubation period the culture was centrifuged at

14,000rpm for 10 minutes. The supernatant was collected, calcite produced was examined by CO_2 gas generated after adding 2 drops of 10% HCl acid the formation of bubbles indicated the presence of calcite.

Materials

The cement used is Ashaka Cement with a fineness modulus of 485 m2/kg. The sand used was well-graded fine aggregate passing through sieve No.8. Tap water and grade 9 silica fume were used. The precursor used for inducing bacteria to precipitate calcite was calcium lactate. Diatomaceous earth (DE) with a pH value of 9.0 – 10.5 and a median particle size of 20 – 25 microns was used.

Phase I preparation

Bioconcrete cubes measuring 5 cm^3 were prepared according to regular protocol using the materials found in the proportions in Table 1 with a water/cement + silica ratio of 0.7. The bacterial type used in the cubes was chosen based on previously carried out pilot studies to determine the bacteria with the highest potential for healing crack. Preparation of the bioconcrete cubes was followed by curing in tap water for about 2 weeks.

Table 1 (Phase 1 bioconcrete content)

Cube ID	Control	Bioconcrete
Cement (g)	205	205
Silicafume (g)	45	45
Water (ml)	135	135
Sand (g)	360	360
Calcium lactate (g)	5	55
Bacterial load (CFU/blend)	5	$2.4x10^{12}$

Phase I cracking and compression

ASTM C109 was used for compression testing. After curing for 7 days, a cube from each of the blends (control and bioconcrete) was loaded until it failed to determine the ultimate load. Each control and bioconcrete cubes were exposed to 80% of its respective load just to initiate the formation of microcracks. A group of control and bioconcrete cracked cubes were immersed in tap water for more three weeks (21 days) to allow the bacteria starts calcite precipitation and crack healing, while another group of cracked cubes were loaded until it failed to assess the compressive strength of cubes that had cracks. At the end of the three weeks, each cube was loaded to failure to determine the compressive strength after calcite precipitation.

Phase II preparation with diatomaceous (DE)

Bioconcrete cubes with and without diatomaceous earth (DE) were prepared following regular protocol according to the specifications in Table 2 with a water/cement + silica ratio of 0.5; the cubes that contained diatomaceous earth (DE) were prepared by displacing a portion of the sand with diatomaceous earth. Preparation of the bioconcrete cubes was followed by curing in tap water for 1 week.

Table 2 (Phase II bioconcrete content)

Cube ID	Control -DE	Control +DE	Bioconcrete -DE	Biocontrol +DE
Cement (g)	465	465	465	465
Silicafume (g)	95	95	95	95
Water (ml)	500	500	500	500
Sand (g)	1475	1435	1475	1435
Calcium lactate (g)	14.5	14.5	14.5	14.5
DE (g)	0	75	0	75
Bacterial load (CFU/blend)	0	0	$2.4x10^{10}$	$2.4x10^{10}$

Phase II cracking and compression

Compression test was conducted in accordance with ASTM C109. After curing for 7 days, a cube from each blend (control and bioconcrete, both with and without diatomaceous earth was loaded until failure to determine the ultimate load. Then, each control and bioconcrete cube was exposed to 80% of its respective ultimate load to induce the formation of microcracks. A group of cracked cubes were then immersed in tap water for another week (7 days) to allow the bacteria to begin calcite precipitation and crack healing, while another group of cracked cubes was left in tap water for three weeks (21 days). At the end of each period, each cube was loaded to failure to determine the compressive strength after precipitation of the calcite.

Results

Bacterial isolation

Tolerance to alkaline pH (12 – 14)

Thirty-five bacterial isolates that can tolerate pH 12 were successfully obtained. These isolates were cultivated in nutrient broth with pH value 14 in order to reduce the number of isolates and to select the highest tolerability to high pH values. Eighteen isolates out of the 35 were able to tolerate pH 14.

Gram reaction and sporulation

The 18 isolates that tolerated high pH levels were stained using Gram stain and malachite green stain and 10 out of 18 isolates were found to be Gram positive spore-formers.

Urease production

Urease test was conducted for ten (10) of these isolates, and nine out of these ten isolates were able to turn the color of the media from light orange to deep pink, thus indicating positive results for urease production.

Thermal stability

After being cultivated in nutrient broth, the nine bacterial isolates were incubated for twenty-four hours at 80 degrees Celsius. Six isolates grew normally and could withstand high temperatures. After a day at 80°C, growth was measured by looking at the media's turbidity. The six isolates underwent additional testing for calcite formation.

Formation of Calcite

Only two of the six isolates that were tested produced a positive result for calcite production; these two isolates came from the mud extracted from ponds and the legume soil sample.

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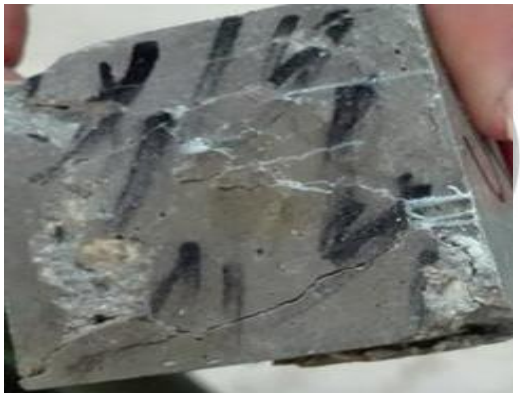
Bacterial identification

Using the API 50 CH kit, the two isolates that produced the required results were identified as



Bacillus mycoides for the bacteria isolated from the legume soil and *Bacillus circulans* for the bacteria isolated from the fish pond muck.

Using the API 50 CH kit, the two isolates that produced the required results were identified as *Bacillus mycoides* for the bacteria isolated from the legume soil and *Bacillus circulans* for the bacteria isolated from the fish pond muck.

Phase I results

Only *Bacillus mycoides*, out of the two found bacteria, demonstrated robust and encouraging results in feasibility trials on crack healing. *Bacillus mycoides* was thus employed in every experiment that was part of this study. As is common for normal, phase I results showed that the control group's greatest compressive strength increased by 5.9% after 28 days as the curing period increased.

Comparing the bioconcrete's compressive strength after curing to the day it cracked, there was a noticeable 15.4% improvement. Calcite precipitation was the primary cause of this, as demonstrated by the cubes' macroscopic analysis in Figure 1.

Table 3 Compressive strength of bioconcrete before and after calcite precipitation

Cube ID	Number of days after cracking	Control	Bioconcrete
Compressive strength of cracked cubes	On the day of cracking	31.24	25.63
	21 days after cracking	33.77	23.92
Percentage of compressive increases		6.9%	16.4%

Fig. 1 Calcite precipitation on bioconcrete surface

Fig. 2 Calcite precipitation on bioconcrete surface

Phase II Results

Phase II's goal was to evaluate the effects of diatomaceous earth-induced bacterial immobilization on the crack-healing process. After cracking for 10 and 28 days, the compressive strength of the control and bioconcrete cubes was assessed. According to the results, the bioconcrete cubes without diatomaceous earth had a higher compressive strength than the control cubes, which also lacked diatomaceous earth. These findings aligned with those from phase I. regarding the bioconcrete enhanced with diatomaceous earth, the rise in compressive strength after 28 days of cracking was much higher than the percentage of increase in the compressive strength of the control cubes that contained diatomaceous earth. Table 4 and 5 show the results obtained.

Table 4 The compressive strength of the bioconcrete in comparison with the control after 7 and 21 days of cracking

Cube ID	Compressive strength (N/mm2)		
	First day of cracking	10 days after cracking	28 days after cracking
Control - DE	35.66	41.24	51.32
Control + DE	40.67	53.22	54.32
Bioconcrete – DE	45.82	49.60	51.23
Bioconcrete + DE	42.33	47.57	49.70

Table 5. The percentage of compressive strength increase after 7 and 21 days of cracking.

Cube ID	Percentage of compressive strength increase (%)	
	7 days after cracking	21 days after cracking
Control - DE	8.56	19.93
Control + DE	10.52	12.83
Bioconcrete - DE	15.15	22.65
Bioconcrete + DE	10.62	14.62

Discussion and conclusion

Because many building material destructions were caused by cracks or microcracks that formed in the construction materials as a result of various environmental variables, bioconcrete became important. The foundation of the bioconcrete concept is the concrete's inherent capacity to mend itself, which is derived from bacteria included into the paste.

The bacteria are enriched with oxygen and water when the microcracks form in the concrete. As a result, they are able to sprout and begin using the nutrients, such as calcium lactate, that are present in the concrete to initiate the calcite precipitation process, which is ultimately in charge of microcrack healing. Accordingly, there are many studies that are interested in developing bioconcrete using different +types of bacterial cells.

Spore-forming thermotolerant, alkaline-tolerant, and urease-producing bacteria were effectively identified in this work; these traits are thought to be essential for bacterial cells to survive used concrete material and precipitate calcite. The best source of isolates was determined to be the soil in which fava bean legumes were cultivated after samples from several geographic and environmental sources were investigated.

Furthermore, it was discovered that adding more *Bacillus* bacteria to the concrete matrix improved calcite precipitation because more live bacteria

would have a greater cumulative effect on the healing of mortar specimens (result not included). The bacteria isolated from the soil of legumes demonstrated the most promising results in calcite precipitation out of all the bacteria isolated from the different sources.

The results of the compression test showed that the concrete cubes with bacteria had a higher compressive strength than the control specimens after curing. This suggests that the bacteria's healing mechanism strengthens the material. Following the measurement of compressive strength for both bioconcrete and regular concrete (control), it was discovered that the bioconcrete cubes' percentage increase in compressive strength (caused by calcite precipitation and curing time) was greater than that of the control cubes (caused by curing time alone).

The results of the compressive strength test make it evident that batches containing diatomaceous earth had a higher compressive strength than batches without diatomaceous earth because diatomaceous earth protected the bacterial spores from heat and mechanical stress and allowed for more flexible nutrient storage for calcium lactate, which raised the rate of bacterial metabolic activity and, consequently, the amount of calcite precipitation. Because of the calcite precipitation and curing period, the addition of diatomaceous earth to bioconcrete also boosted its compressive strength.

Recommendation

Long-term testing and exposure conditions should be covered in future research. The conversion of spores to vegetative cells will be significantly hampered by rapid chloride permeability testing; therefore, permeability testing with only water should be taken into consideration. The suitability of employing self-healing mechanisms for structures submerged in seawater should be the subject of future research.

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