Direct Research Journal of Engineering and Information Technology
Vol. 9, (2) Pp.56-60, March 2022
ISSN 2354-4155
DOI: https://doi.org/10.26765/DRJEIT10955575
Article Number: DRJEIT10955575

http://directresearchpublisher.org/drjeit/

Full Length Research Paper

Effect of Ureolytic *Bacillus subtilis* Bacteria on Concrete

Magaji Sani[1], Umar A. Masari[1], and Muhammad Dikko A.[2]

[1]Department of Civil Engineering, Federal Polytechnic, Kaura-Namoda, Zamfara State, Nigeria.
[2]Department of Food Technology, Federal Polytechnic, Kaura-Namoda, Zamfara State, Nigeria.
Corresponding author E-mail: umarmasari79@gmail.com

Received 3 February 2022; Accepted 17 March 2022; Published 30 March 2022

ABSTRACT: The application of concrete is rapidly increasing worldwide and therefore the development of bacterial mediated concrete is urgently needed for environmental reasons. As presently, about 8% of atmospheric carbon dioxide emission is due to cement production, mechanisms that would contribute to longer service life of concrete structures would make the material not only more durable but also self-repair, i.e., the autonomous healing of cracks in concrete. In this study application of ureolytic Bacillus syphilis bacteria for grade M30 concrete at various concentrations has been shown to have reduce water absorption and increase compressive strength. Microbial self-healing of cracks in the concrete shows promising results at the laboratory seepage test scale. However, their self-healing efficiency needs to be further proven in larger concrete elements, and under non-ideal conditions. Further research should be conducted with the application of Energy Dispersive Spectrometer (EDS) and Scanning Electron Microscope (SEM) should be employed to investigate the morphology and chemical constituents of self-healing products and to observe the self- healing process and detect the components of the precipitation.

Keywords: Ureolytic bacteria, building cracks, *bacillus subtilis*, self-healing, structural failure, concrete, MICP, compressive strength

INTRODUCTION

Concrete is one of the massively used construction and building materials, and has a great susceptibility of forming cracks. These cracks develop due to several factors like rate and quantity of drying, drying shrinkage, tensile strength and strain, elasticity, creep etc. Formation of these cracks results in significant reduction in concrete service life and high renewal costs. The formation of cracks and their succession under the effect of tensile stresses is a major disadvantage of concrete making it exposed to toxic environment due to entry of detrimental compounds (Mayur and Pitroda, 2013; Grishma, 2014). Manufactured materials like epoxies are used for crack remedy. Yet, they pose their own drawbacks. They are not only expensive but also alter the appearance of the structure to a certain extent and need consistent upkeep. Consequently, bacteria induced calcium carbonate (calcite) precipitation is recommended as an alternative. When the concrete hardens, cracks are healed by bacterial reaction as water ingresses through the cracks. As bacteria in concrete carry out process of self-healing of cracks, this concrete is also known as Self-Healing Concrete (Jonkers *et al*., 2010; Mounika *et al*., 2018; Shubham *et al*., 2019).

Addition of bacteria as an agent for crack repair in the concrete mixture would not only prove economical but also save the environment from the harmful effects of using other chemical processes of crack treatment. Using bacteria as binder and fillers in concrete would also improve the performance of concrete in terms of its strength and durability (Jonkers and Schlangen 2008; Jonkers *et al*., 2010).

The objectives of this study to develop bacterial concrete by introducing the bacteria of bacillus family, find optimum dosage of bacteria required for bacterial concrete, increase compressive strength of concrete and remediate the cracks developed in concrete.

Approach in crack healing

In the context of civil engineering research, healing is the phenomenon of restoration of concrete structure from a state of damage. In case of crack healing, chemical and physical processes result in the introduction of secondary products that block or seal the cracks. To some extent, concrete is capable of healing cracks autogenously, which is called self-healing or autogenous healing (Hearn, 1998). Engineered healing is warranted when autogenous healing is not adequate. Engineered self-healing is a process in which new substitute is incorporated into the cement material which is stimulated on crack propagation resulting in sealing of crack (Mihashi and Nishiwali, 2012; Wu *et al*., 2012).

Autogenous healing in concrete

Autogenous healing is a natural process of crack repair that occur in concrete in the presence of moisture and the absence of tensile stress. The repair is by a combination of mechanical blocking by particles carried into the crack with the water and the deposition of calcium carbonate from the cementitious material. Autogenous healing of cracks was reported due to the formation of calcium carbonate precipitation as consequence of water leakage through cracks (Clear, 1985). Formation of calcite under wet conditions in cracks of concrete was also cited as a probable healing mechanism. Swelling and hydration of cement paste, blocking of flow path by water impurities or by concrete particles broken from crack surface, and precipitation of calcium carbonate crystals were suggested as possible chemical and physical mechanisms contributing to the autogenous healing (Guppy, 1988). Formation of calcite in the crack seems to be the sole cause for the autogenous healing and the crystal growth rate is dependent on the width of crack and water pressure, whereas concrete composition and water hardness have no influence on autogenous healing (Edvardsen, 1999).

Applications of MICCP in civil engineering

Supplementation of concrete with healing agents to stimulate the healing action to repair cracks is known as engineered self-healing. Many researchers used different healing agents and proposed their mechanisms on cementitious materials. One of the new healing agents used is the application of Microbial induced calcite precipitation (MICP). The potential and effectiveness of MICCP technology by using bacteria have been used widely in various fields. Several authors have reported the potential of MICCP property in the application of remediation of heavy metal and radio nuclide contaminated ground water (Fujita *et al*., 2004), soil bioclogging (Tittelboom *et al*., 2010), restoration of stone monuments (Daskalakis *et al*., 2015; Rodriguez-Navarro *et al*., 2003) and durability enhancement of concrete structures (Achal *et al*., 2011a & b). To reduce the permeability and increase the shear strength of soil, MICCP was performed by using Bacillus sp. with urea and calcium solution by Chu *et al*. (2012).

METHODOLOGY

The preliminary work required to be carried out is to select the suitable bacteria, select the process of cultivation, mix proportion of cement sand and aggregate in concrete along with water and bacterial solution. The study majorly focuses on two aspects which deal with testing the compressive strength and surface healing capacity of bacterial solution on concrete surface. Following sub-sections describe the methods in detail.

Cultivation of bacteria

The pure culture of bacteria *bacillus subtilis* was conserved on nutrient agar slants that form irregular desiccated white colonies on them. Inoculation of four different colonies of the bacteria into nutrient broth each of 2 ml in ml conical flask is carried out. It is incubated at the temperature of 37^0C and 170 rpm in orbital shaker incubator. The medium composition used for bacterial culture's growth consists of NaCl, Peptone, yeast extract. Processes include sterilization, inoculation and incubation. Sterilization is done to achieve sterile environment. It's done in an autoclave or pressure cooker at a temperature of 121^0C for 25 to 30 minutes. Inoculation is the streaking of the bacteria on to the media with the help of an open loop. Incubation is done to grow and maintain the bacterial cultures. Figure 1 shows the process of bacteria cultivation carried out in Food Technology laboratory (Magaji *et al*., 2022).

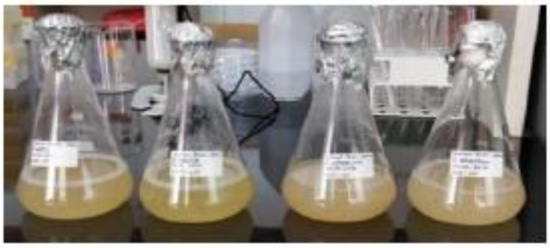

Figure 1: Cultivated bacterial solution.

Experimentation work on concrete

With the normal concrete, i.e. 15ml, 30ml, 45ml, 60ml bacterial solution i.e. liquid broth is added to the conventional concrete. Then the results are compared with results from normal concrete, after gaining the highest strength we can find out the optimum dosage of bacteria we used. For each combination and normal concrete 3 blocks are casted for 7- and 28-days' compressive strength test. Total 30 concrete blocks were casted and tested for the present study. The cube samples were casted on the moulds of size 15x15x15 cm. After about 24 hours the specimens were de-moulded and kept in water curing tank. Tests were done as per British Standard codes, BS 8110.

Compressive strength of bacterial concrete

Mix design can be defined as the process of selecting suitable ingredients of concrete and determining their

Figure 2: Dry mix of aggregates

Figure 3: Concrete cubes casting

Figure 4: Curing of cubes

Figure 6: cubes with intentional cracks

Figure 8: Crack filling process in cubes

relative proportions with the objective of producing concrete of certain minimum strength and durability as economically as possible. In our investigation we have made M30 grade of concrete as per BS 8110: Part 1: 1985. Further, we have poured the concrete in the cube moulds and five different samples were made which are as follows:

1. Conventional concrete of grade M30
2. Concrete with 15 ml bacterial solution
3. Concrete with 30 ml bacterial solution
4. Concrete with 45 ml bacterial solution
5. Concrete with 60 ml bacterial solution

There are different methods of mixing the bacterial solution in the concrete which are; direct mixing, indirect mixing and injection method. In this study direct method of mixing was employed. The compressive strength was determined using compressive testing machine.

Bacterial solution as a crack healing or water proofing agent

More five different samples of concrete cubes were kept separately from the ones used for the compressive strength test. These samples of concrete cubes were used for permeability or crack healing test. Intentional cracks of same dimensions were developed on the surface of each casted cube (Figures 2, 3,4,5 and 6). The main purpose of this experiment was to estimate the time taken for certain amount of seepage that takes place through these intentional cracks. Ponding of concrete cubes was done (Figure 5) and the amount of water loss was noted. Once the actual seepage is determined, then bacterial solution was applied on the surface of concrete cubes. This was done in order to check the effect of *Bacillus Subtilis* in different quantities of 15m, 30ml, 45ml and 60ml.

RESULTS AND DISCUSSION

Compression test performed on concrete cubes

Table 1 shows the results of compressive strength tests conducted on all the concrete blocks of different *Bacillus Subtilis* concentration. Universal testing machine was used to assess the compressive strength. It was observed from above Table 1, that combination with 60ml bacterial solution in concrete gives better result for compressive strength than any other combination. For both 7 days and 28 days' compressive strength test, the maximum value was obtained when 60ml of *Bacillus Subtilis* solution was added to M30 grade of concrete. The results also revealed that the combination with 15ml of *Bacillus Subtilis* for both at 7 and 28 days gives higher compressive strength than conventional concrete. It could also be observed that the compressive strength of bacterial concrete with 60ml bacterial solution at 7 days is greater than that of conventional concrete at 28 days. This implied that the compressive strength of concrete showed significant increase with the addition of bacteria as recorded by Mounika *et al*. (2018) and Shubham *et al*. (2019). The Effect of bacterial concrete is shown in Figure 7. With increase in ml of bacteria in concrete, compressive strength of cubes increases and seems to maintain constant value from the optimum value with insignificant increase. This implied that the concentration of 45ml is considered as optimal value of bacteria in concrete (Mounika *et al*., 2018).

Result for ponding of concrete cubes tests

Based on the test conducted, Table 2 compiles the results which depict that layer of *Bacillus Subtilis* on the concrete cubes surface gives good results for protection of seepage through the intentional cracks developed on the cubes. It can be observed from the results of seepage test that upon application of the *Bacillus Subtilis* layer on the concrete cubes with the intentional crack developed, it was witnessed that the seepage time was drastically increased from 1hr 10min for the conventional to 4hr 50min. This implied that the application of bacterial solution can be used as a crack-healing (Shubham *et al*., 2019). It was also noticed the application of the selected bacteria control seepage through the intentional cracks developed on the cubes. This is as a result of the formation of calcium carbonate precipitation (Clear, 1985) and this application of Microbial induced calcite precipitation (MICP) can serve as waterproofing material as noticed by Shubham *et al*.

Table 1: Result of compressive strength test.

Bacterial concrete.(Mix)	Compressive Strength AT 7 DAYS (N/mm^2)	Compressive Strength AT 28 DAYS (N/mm^2)
0ml	32.28	38.82
15ml	34.10	42.41
30ml	36.38	45.07
45ml	38.70	49.55
60ml	40.58	50.20

Table 2: Seepage of test results

Cubes no.	Type of top coat	Time for seepage1	Time for seepage2	Time for seepage3	Ave time for seepage
Cube A	Conventional	1hr 10min	1hr 15min	1hr 20min	1hr 15min
Cube B	B. Subtilis	4hr 48min	4hr 50min	4hr 52min	4hr 50min

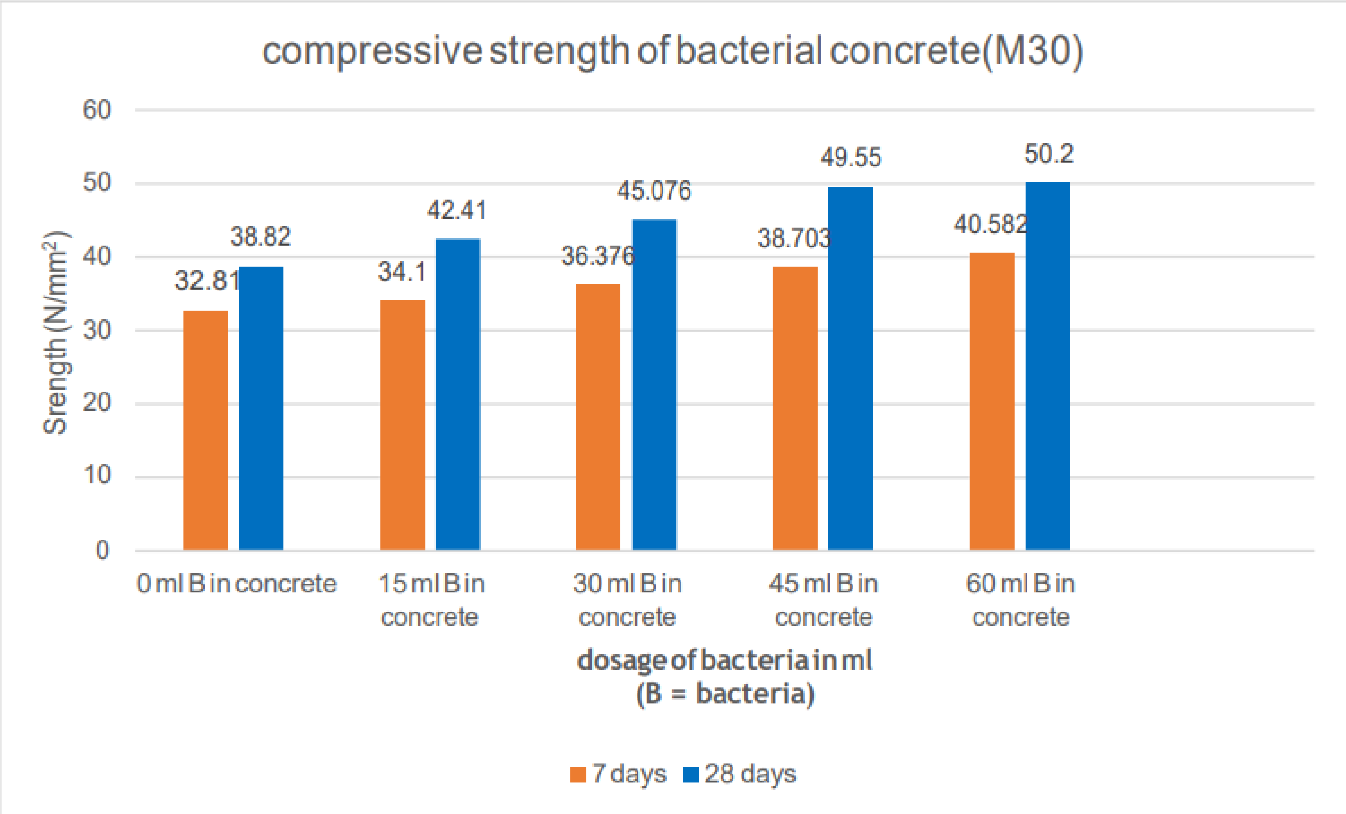

Figure 7: Compressive strength of bacterial concrete (M30) for 7 and 28 days.

(2019) as consequence of water leakage through cracks.

Conclusion

The compressive strength was found to increase with bacterial addition and this increase is mainly due to deposition of microbial induced calcium carbonate precipitation on the microorganism cell surfaces and within the pores of the mortar. It was noticed that in normal mortar, the compressive strength was increased with the increase in bacterial cell concentration up to 10^6 cells/ml. Maximum increase in compressive strengths was achieved at 10^6 cells/ml. The percentage increase in compressive strength of 45ml and 60ml bacterial concrete using B. Subtilis for 7 days is higher than conventional concrete. Application of bacterial solution in concrete produce calcium carbonate precipitation deposit which naturally heal up concrete cracks and serve as waterproofing material.

Bacterial concrete is fairly new concept. It is easy to prepare and requires basic knowledge of bacteria and cultures. It is a very innovative idea to make use of these natural phenomena of bacteria to improve concrete’s performance. The solution is not only reliable but also increases durability of the structure making it maintenance free. It thus requires due attention in order to make a more sustainable infrastructure to move towards a more eco-friendly construction practices.

Recommendation

Further research should be conducted with the application of Energy Dispersive Spectrometer (EDS) and Scanning Electron Microscope (SEM) should be employed to investigate the morphology and chemical constituents of self-healing products and to observe the self- healing process and detect the components of the precipitation.

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