



Performance Evaluation of Cement Mortar Subjected to Bacillus Coagulans Bacteria Calcite Precipitate

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ABSTRACT

Under the suitable condition, Bacteria, can precipitate calcium carbonate that can improve the strength and durability of cement mortar. The growth of the bacteria is facilitated by Nutrients Broth Growth Medium (NBGM) that contain the required bacteria growth nutrients. In this paper, the concentration of Bacillus Coagulans bacteria and the amount of NBGM on the strength and durability of cement mortar was assessed. The mix ratio of the cement mortar is three-part sand and one part ordinary Portland cement, with 0.5 water to cement ratio. The assessments were made at the age of 3, 7, 14 and 28 days after mixing of the bio-cement mortar and casting of the bio-cement mortar cubes. The NBGM used contained 3g of nutrient broth, 20g of urea, 10g of NH₄Cl and 2.12g of NaHCO₃, all per litre of distilled water. The adopted bacterial cells concentration are 1.5x10⁸ cells/ml, 6.0x10⁸ cells/ml and 1.2x10⁹ cells/ml. For each bacterial concentration, NUB of 0%, 30%, 40% and 50% replacement of the mixing water was used. The results show that the 28 days compressive strength of the control specimen as well as for the three respective bacterial concentration is 24.08 N/mm², 24.53 N/mm², 24.14 N/mm² and 24.21N/mm². It was established that the strength increase with an increased amount of nutrients broth growth media, with the highest compressive strength of 28.02 N/mm² for the mortar with lowest bacteria concentration of 1.5x10⁸ cells/ml. The water absorption was also found to be decreasing with an increasing amount of the NUB. This is a confirmation of increased durability. The improvement in strength and durability was also evident in the SEM results.

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INTRODUCTION

Today, due to the increase in population and urbanization, the need to provide safe and environmentally friendly structures has become a necessity. Concrete is widely used as construction material around the world because of its

resistance, durability and low cost in comparison with other construction material (Khaliq and Ehsan, 2016; Siddique et al., 2016). Cement is one of the most important components of the concrete since it provides properties of compaction. As the demand for supplementary

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cementitious materials is increasing day by day, it is very important to find suitable technology to ensure the improvement of the properties of structures.

Bio-cement a recently discovered novel product of bio-mineralization based microbial induced calcite precipitation (MICP) process, is widely used to improve the durability of concrete (Achal et al., 2011). To date, several studies have demonstrated the positive influence of microbial compounds on cement mortar and concrete properties. The MICP process is an effective and eco-friendly technology that can be applied to solve various environmental problems (Achal et al., 2012; Anbu et al., 2016; De Muynck et al., 2010; Mansi Aggarwal. and Pandey., 2018; Mitchell et al., 2010; Muyideen et al., 2019; Schwantes-Cezario et al., 2019; Shashwati and Sunita, 2019). MICP refers to the formation of calcium carbonate from a supersaturated solution due to the presence of their microbial cells and biochemical activities (Bosak, 2011). Calcium carbonate is one of the most well-known minerals that bacteria deposit by the phenomenon called bio-cementation or MICP. Such deposits have recently emerged as promising binders for protecting and consolidating various building materials (Achal et al., 2010). It should be noted that bacteria has been in existence, however many of their biotechnological applications are not yet widespread (Bang et al., 2001). Although most researchers are concerned in the calcite precipitation and the process is not yet clear and defined. The effect of multi-component growth media, containing germination and sporulation aids for the bacteria aerobic oxidation pathway, on the basic properties of fresh and hardened cement mortar instead of the potential self-healing efficiency in a structural service was investigated by Xin Chen et al. (2019) and also Chidara et al. (2014) altered the

chemical dosage proportions of the nutrient growth medium to achieve early strength in concrete.

To permit the germination of bacterial spores, growth of bacterial cells, precipitation of calcite and sporulation of bacteria with the cement mortar, nutrients are needed, typically consisting of carbon and nitrogen sources. Thus, in this study, the induced calcite precipitation process is utilized in cement mortar to improve its compressive strength.

MATERIALS AND METHODS

Materials

Microorganism

To stimulate calcite mineralization, which is a by-product of microbial metabolic activity, a urease producing bacteria was used to induce the calcite precipitate. This MICP process is known as Urea Hydrolysis. The bacteria species used for the study is *Bacillus Coagulans*. It is classified as ATCC 7050 in the American Type Culture Collection, endospore-forming (spore is formed within the cell) bacteria, it is a Gram-positive rod-shaped bacterium, grows optimally at 37°C and pH in the range 5.5 to 6.2. It is obtained from the Department of Microbiology laboratory Ahmadu Bello University, Zaria which was isolated from lateric soil.

Nutrient Broth Growth Medium

The growth medium was prepared using a mixture of chemicals at varying proportions which is based on initial microbiological studies (Chahal and Siddique, 2013). Chemical used include the following per liter of distilled water: 3 g of nutrient broth (Bacto); 20 g of urea; 10 g of NH_4Cl and 2.12 g of NaHCO_3 .

Cement and Sand

The cement used in this research work is 42.5 N Portland Limestone Cement



conforming to BS EN 197-1:2000. Locally available clean, well-graded, natural river sand having fineness modulus of 2.76 and nominal maximum size of 5 mm was used conforming to BS EN 196-1:2005.

Method

Preparation of Microbial cement mortar

The mortar was prepared using a cement-sand ratio of 1:3 by weight. On 76 mm cubic moulds were prepared. Cement and sand were thoroughly mixed, adding along with grown culture at w/c ratio of 0.5 of different cells concentration of B. Coagulans corresponding to 1.5×10^8 cells/ml, 6.0×10^8 cells/ml and 1.2×10^9 cells/ml, which the nutrient growth medium was also added at varying percentage (%) replacement of mixing water (30%, 40% & 50%). Cubes were cast and compacted using rammer. After demolding all specimens were cured until compressive strength at the intervals of 3, 7, 14 and 28 days. Control specimens were also prepared similarly.

Compressive Strength

Compressive strength test was carried out on harden cement mortar and bio-cement mortar by BS EN 196-1 (2005). The compressive strength test was conducted on cubes of 76 mm × 76 mm × 76 mm mixed mortar using the compressive testing machine (Denison) at the concrete laboratory of A.B.U, Zaria. The failure load was observed and recorded and the compressive strength was determined in N/mm^2 .

Water Absorption

Water absorption test is carried out to determine the water absorption value of mortar, expressed in percentage. This property is particularly important in concrete used for water-retaining structure or watertight basement, as well as being

critical for durability. This test was performed on 50mm x 50mm x 50mm cube specimens prepared in the laboratory. Water absorption measurements were done by weighing the saturated specimens (W_s) and dried specimens in the oven at 110 °C for 24 h (W_d) at curing times of 3, 7, 14 and 28 days. The water absorption was determined in %.

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) has become an important tool for the study of microstructures, hydration progress and structural morphology. This test was conducted to determine the microstructure of the sample and to examine its matrices. The mortar cubes considered for the test were controlled cement mortar cube for 28 days curing period and optimal bio-cement mortar cube for 28 days curing period. The SEM was conducted at the multipurpose laboratory of Umaru Musa Yar'adua University Katsina, Katsina State, Nigeria.

RESULTS AND DISCUSSION

Compressive Strength

The compressive strength results of the control cement mortar and the bio-cement mortar mixtures containing bacterial are shown in Fig. 1, 2 and 3. At 28 days, the compressive strength of the control cement mortar is $24.08 N/mm^2$, whereas that of the cement mortar with NBGM of 30%, 40% and 50% are 24.53, 25.57 and $28.02 N/mm^2$ respectively at bacterial concentration of 1.5×10^8 cells/ml. At 28 days, the compressive strength results of the cement mortar with NBGM of 30%, 40% and 50% at bacterial concentration of 6.0×10^8 cells/ml are 24.14, 24.18 and $25.33 N/mm^2$ respectively. Likewise, for bacterial concentration of 1.2×10^9 cells/ml with 30%, 40% and 50% NBGM are respectively 24.21, 24.35 and $25.05 N/mm^2$ respectively.

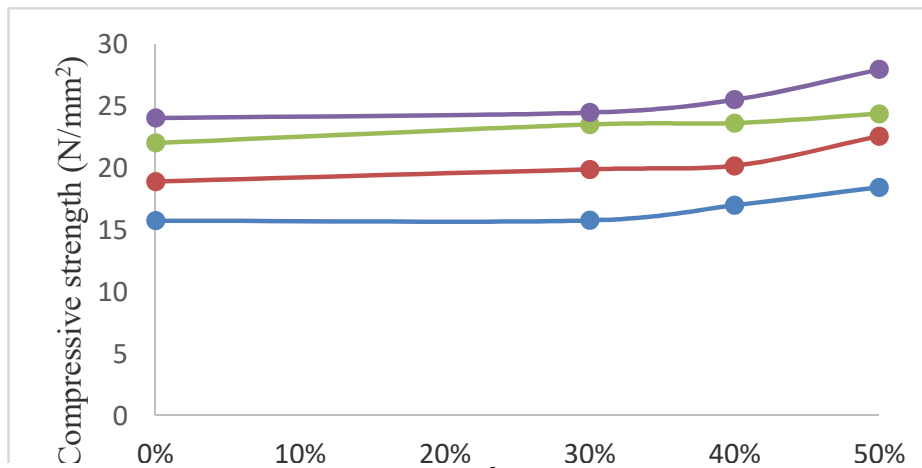


Fig 1: Compressive Strength Result for 1.5×10^8 cells/ml B. Coagulans

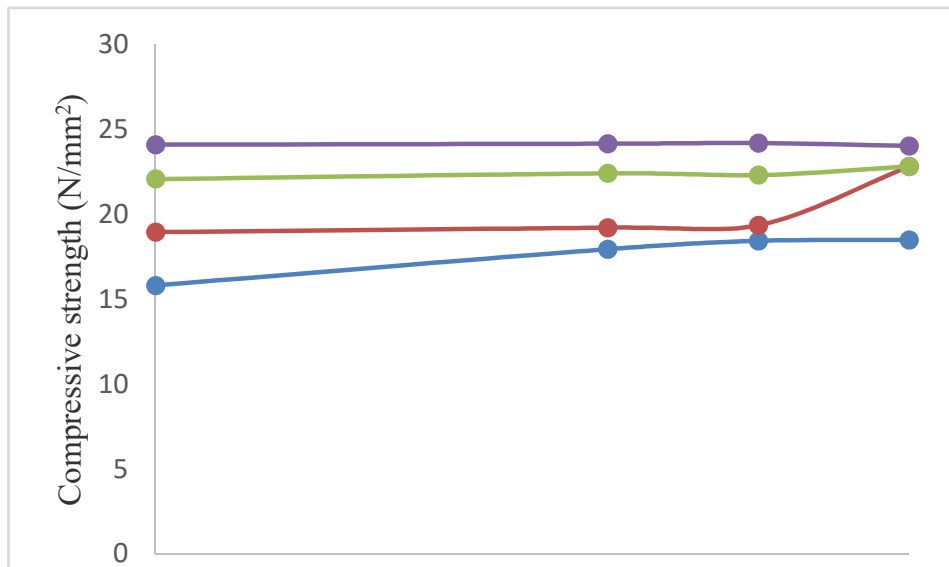


Fig 2: Compressive Strength Result for 6.0×10^8 cells/ml B. Coagulans

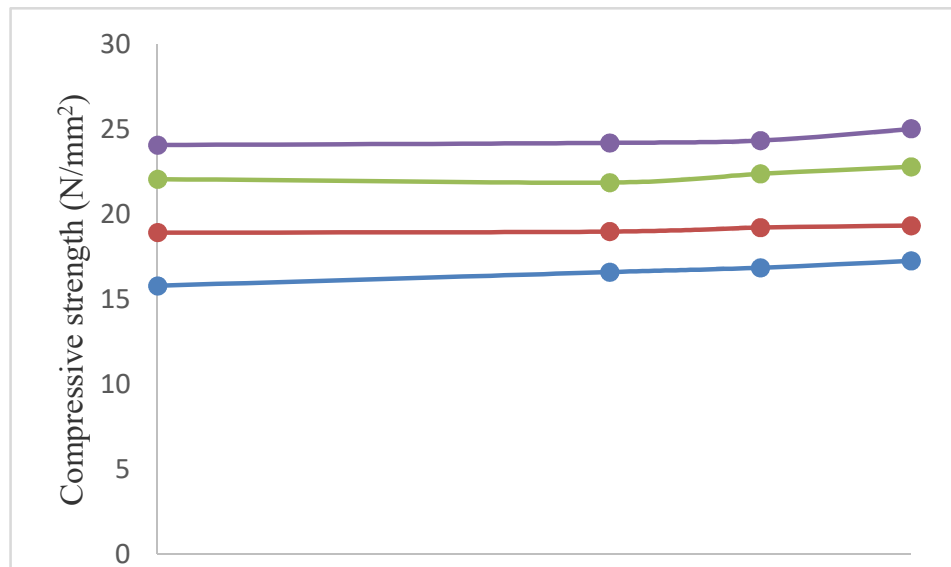


Fig 3: Compressive Strength Result for 1.2x10⁹ cells/ml B. Coagulans

The percentage replacement of the NBGM with curing days affected the strength, compared to the control at either age. However, the strength improvement is due to the deposition of calcium carbonate on the cement mortar, as bacteria can precipitate in calcite form. In the biomineralization process, bacteria serve as nucleation sites, through which calcium carbonate precipitates with the bacteria. Bacteria cell surfaces have negatively charged group that act as scavengers for divalent cation (Ca²⁺) by binding them onto their cell surfaces at neutral pH, which makes ideal nucleation sites for calcite deposition. Subsequently, the bound cation (metal ions) reacts with anions (carbonate) to form calcium carbonate in an insoluble form.

The number of spores and viable cells increases at the same rate, but the spore-to-cell ration depends on the composition of the nutrient medium (NUB) and the NUB is typically consisting of carbon and nitrogen sources. The continues precipitation of the calcium carbonate occurs at the bacterial cell surface if there is

sufficient concentration of calcium ion (Ca²⁺) and carbonate ion (CO₃²⁻) in solution (Qian et al., 2010).

Water Absorption

The results shown in Figure 4, 5 and 6, illustrates that the water absorption of cement mortar with or without bacterial cells decreases with a time of curing up to 28 days, this is due to the continuous hydration and accumulation of hydrated products which fill the open pores of the specimens. Also, the water absorption values of cement mortar specimens mixed with bacterial cells at 1.5x10⁸ cells/ml, 6.0x10⁸ cells/ml and 1.2x10⁹ cells/ml are lower than those of control specimens. This is attributed to that bacterial biomass and microbial calcite precipitation within the pores and on the surface of cement mortar and also percentage replacement of the NBGM with curing days affected the water absorption, compared to the control at either age. However, the improvement is attributed to the nitrogen and carbon source in the growth medium which permit the germination of bacterial spores, growth

of bacterial cells, precipitation of calcite and sporulation of bacteria.

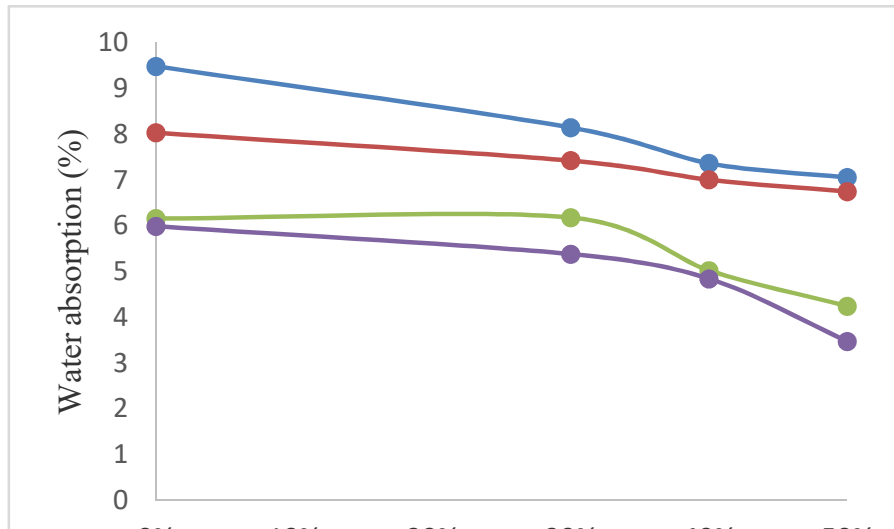


Figure 4: Water absorption Result for 1.5×10^8 cells/ml B. Coagulans

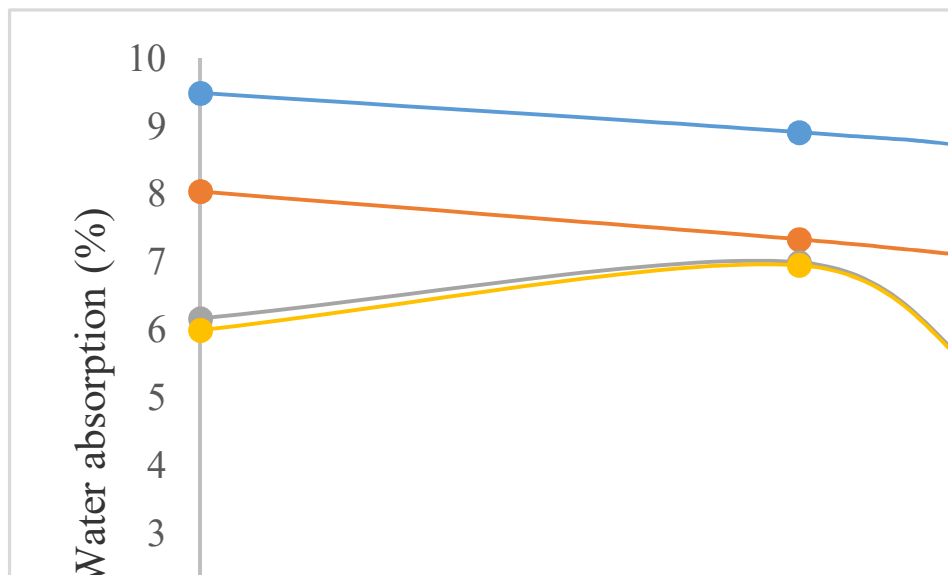


Figure 5: Water absorption Result for 6.0×10^8 cells/ml B. Coagulans

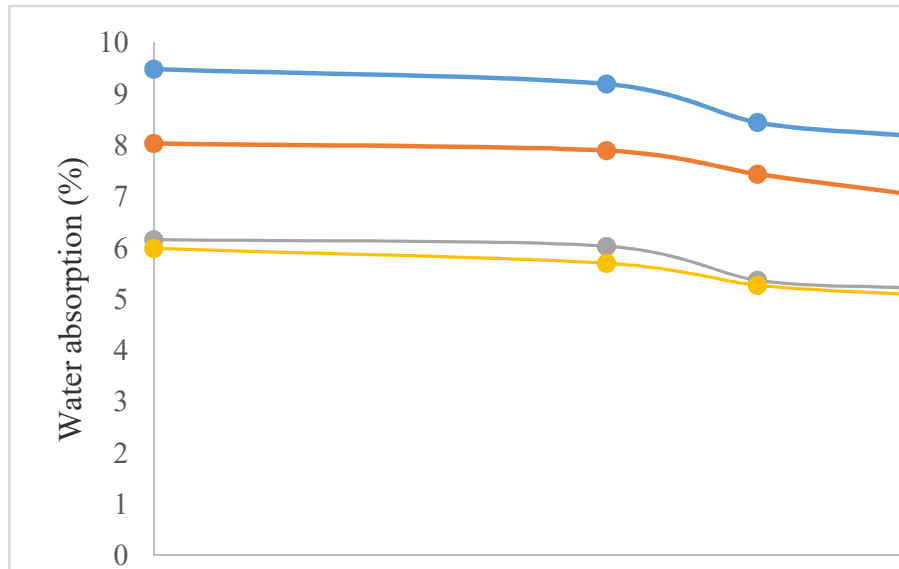


Figure 6: Water absorption Result for 1.2×10^9 cells/ml B. Coagulans

Scanning Electronic Microscopy (SEM)

To determine whether the increase in compressive strength of the specimens prepared with bacteria could be attributed to the microbial calcite precipitation, the mortar samples were taken off and examined under SEM. Figure 7 is a scanning

electron micrograph of the matrix of bacteria-free cement mortar while Figure 8 shows micrographs of the specimen prepared with *Bacillus Coagulans*. The sample showed calcite crystals grown all over and precipitated.



Figure 7: Cement Mortar at 28 days (Control)

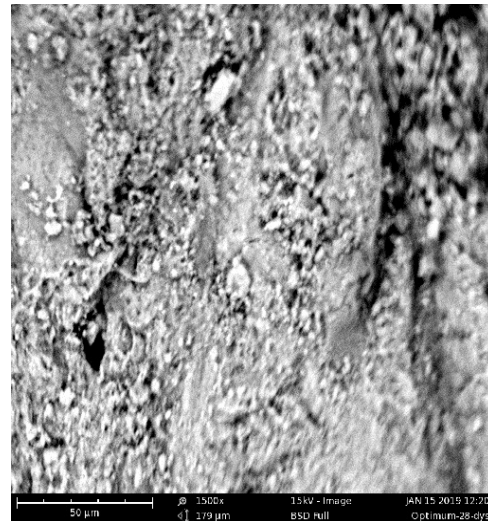


Figure 8: Optimal Bio-Cement mortar



CONCLUSION

1. Based on the result of the study, it is concluded that the compressive strength of the bacteria modified cement mortar were improved due to the deposition of the calcite by the bacterial activity.
2. The compressive strength increases with the concentration of bacterial cells while a decrease in strength improvement was observed when cement mortar mixed with 6.0×10^8 cells/ml and 1.2×10^9 cells/ml.
3. The addition of bacterial cells with an increment of the nutrient broth growth media (NUB) to the cement mortar improves the compressive strength of the mortar concerning control.
4. Therefore, the optimum bacterial cells concentration which leads to the highest improvement in cement mortar gives higher compressive strength of 28.02 N/mm^2 at 28 days is 1.5×10^8 cells/ml with 50% growth media.
5. The compressive strength of the bacteria modified cement mortar were improved due to the deposition of the calcite within the pores of the cement-sand matrix as indicated from the microstructure obtained from scanning electron microscopy (SEM) examination.
6. Based on the result of the study, it is concluded that the water absorption of the bacteria modified cement mortar was improved due to the deposition of the calcite by the bacterial activity.
7. The water absorption decreases with the concentration of bacterial cells while an increase in water

absorption improvement was observed when cement mortar mixed with 6.0×10^8 cells/ml and 1.2×10^9 cells/ml.

8. The addition of bacterial cells with an increment of the nutrient broth growth media (NUB) to the cement mortar improves the water absorption of the mortar concerning control.
9. Therefore, the optimum bacterial cells concentration which leads to the highest improvement in cement mortar gives lower water absorption of 3.46% at 28 days is 1.5×10^8 cells/ml with 50% growth media.

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