Civil and Environmental Research
ISSN 2224-5790 (Paper) ISSN 2225-0514 (Online)
Vol.10, No.7, 2018

Evaluation of the Effect of Bacillus Pumilus Precipitate on the Strength and Durability of Concrete

Oriola, Folagbade Olusoga Peter*
Department of Civil Engineering, Nigerian Defence Academy, Kaduna, Nigeria

Sani, John Engbonye
Department of Civil Engineering, Nigerian Defence Academy, Kaduna, Nigeria

Anebi, Morgan Adah
Department of Civil Engineering, Nigerian Defence Academy, Kaduna, Nigeria

Abstract
Microbiologically induced calcite precipitate (MICP) also called bio-mineralization is a process by which living organism's forms inorganic solids. The basic principles of MICP involves the formation of urease by the Bacillus species and the hydrolyze urea produce ammonia and carbon dioxide, and the ammonia released to the surroundings subsequently increases pH leading to accumulation of insoluble calcite ($CaCO_3$). Bacillus pumilus is a common soil bacterium which can induce the precipitation of calcite. The effect of different concentrations of B. pumilus on the compressive strength and durability of concrete was studied. It was found that the concrete treated with bacteria (i.e. concrete mixed with bacteria and concrete cured in bacteria suspension) performed better than the control concrete. Though the treated concrete were stronger than the control in terms of compressive strength, the strength increase was more pronounced at early ages (7 & 14 days) than at later ages (21 & 28 days); the best performance at 28days was an increase of about 6% above the compressive strength of the control for concrete mixed with bacteria, at a suspension density of $1.5x10^8$ cells/ml. The durability performance improved with increase in the concentration of B. pumilus, though there was no significant difference between the performance of concrete mixed with bacteria and concrete cured in bacteria suspension. At 28 days, the weight loss was 16.5%, 11.8%, 17.8% and 14.2% for concrete mixed with bacteria at concentration of 0, 1.5E8, 12E8 and 24E8 cells/ml respectively. Similarly, at 28 days, the weight loss was 16.5%, 15.5%, 15.7% and 15% for concrete cured in cementation reagent containing various B. pumilus suspension at density of 0, 1.5E8, 12E8 and 24E8 cells/ml respectively. Furthermore, the general trend for loss of weight under acidic condition was increase in loss of weight with duration of immersion but the rate of loss decreased with age. The depositions of calcite inside the micro cracks of concrete by B. pumilus were analyzed under scanning electron microscope (SEM). The unique imaging and microanalysis capacities of SEM established the presence of calcite precipitation inside cracks, bacterial impressions and new calcite layer on the concrete surface of concrete. This calcite layer improves the compressive strength and impermeability of the concrete, thereby increasing its resistance to acidic environment especially at $1.5 x 10^8$cells/ml B. pumilus suspension.
Keywords: Bacillus *Pumilus*, Concrete, Compressive strength and Durability

1. Introduction

Ordinary Portland Cement (OPC) Concrete which is the mixture of cement, fine aggregate, coarse aggregate and water is without argument, the most commonly used construction material because it is cheap, easy to make and convenient to place or cast to shape. However, it has limitations of deteriorating due to micro cracks and pores which are formed within the concrete matrix. (Claisse *et al.,* 1997, Ravindranatha *et al.*, 2014a). These micro cracks causes concrete to be susceptive to ingress of liquid and gases which can attack the constituents of concrete. Degradation mechanisms of concrete often depend on the way potentially aggressive substances can penetrate into the concrete, possibly causing damage. The permeability of the concrete is dependent on the porosity and on the connectivity of the pores. The more open the pore structure of the concrete, the more vulnerable the material is to degradation mechanisms caused by penetrating substances. Khan (2003) confirmed that durability of concrete is related to the characteristics of its pore structure.

Furthermore OPC Concrete have a very high potency to crack which can easily leads to reduction in the strength and durability of concrete (Chahal *et al.,* (2012) and Arunachalam *et al.,* (2010). Therefore, the requirements for high durability for structures exposed to harsh environments such as sea buoys, offshore structures, tunnels, highway bridges, sewage pipes and structures for solid and liquid wastes containing toxic chemicals and radioactive elements may not be achieved using OPC concrete. However, based on continuous research carried out, various modifications have been made to try to overcome these deficiencies of cement concrete; one of such developments is bacteria concrete. Due to microbial activities of bacteria, microbiologically induced calcite precipitation (MICP), a highly impermeable calcite layer is formed which fills the micro cracks and pores in concrete and contributes to increase in the strength and improvement in the performance of concrete structure with excellent resistance to corrosion and deterioration. It has been observed that the action of bacteria can continue even after cracking of concrete as the dormant bacteria may reactivate and restart the production of precipitates as water is introduced into the concrete through the crack, this has the potential of sealing the crack (Senot, 2017, Jonkers, 2011, Wiktor and Jonkers, 2011). Thus MICP Concretes have both high quality pre-cracking and post cracking qualities. Thus, microbial concrete presents a potentially enormous lengthening in service-life of infrastructure, substantially reduces the maintenance costs and also considerably increases the safety of structures (Ravindranatha *et al.*, 2014b).

Previous studies on aerobic microorganism (i.e. Bacillus *pasteurii* and Pseudomonas *aeruginosa*) showed that there was significant improvement (of up to 18%) in compressive strength of cement mortar [Ramakrishnan *et al.,* (1998); Ramakrishnan *et al.,* (2001)]. Rafat and Navneet*,* (2011) also found that types of bacterial culture and medium composition had a great impact on calcium carbonate crystal morphology where pure culture resulted in a more pronounced effects. It has been hypothesized that almost all bacteria are capable of $CaCO_3$ production because precipitation occurs as a byproduct of common metabolic processes such as photosynthesis, sulfate reduction, and urea hydrolysis. Even the effect of bacteria on various parameters in concrete proves to be beneficial development and the compressive strength is increased significantly.

Though all bacteria are capable of $CaCO_3$ production, recent researches have shown that only some specific species of bacteria can be useful to enhance the durability and strength of concrete structures; those that can survive in the alkaline environment inside concrete (Senot, 2017). Therefore this study focuses on determining the effect of bacillus *pumilus* precipitate on the strength and durability of concrete. *B. pumilus* is a common soil bacterium which thrives in alkaline soil environment and is expected to survive in concrete and induce the precipitation of strong and durable calcite in concrete since other bacteria has been used successfully for bacteria concrete like *Baccilus species* (Muynck *et al.,* 2008; Achal *et al., 2009; Henk and Erik, 2009), Sporosarcina species (Park et al., 2010; Chahal et al., 2012) and Shewanella species. (Ghosh and Mandal, 2006).*

2.0 Materials and Methods

2.1 Materials

2.1.1Cement: Ordinary Portland Cement of 42.5 grade available in local market was used in the investigation.

2.1.2 Fine Aggregate: Natural river sand having specific gravity of 2.60 was used.

2.1.3 Coarse Aggregate: 20mmmaximum aggregate size, crushed angular granite from local quarry was used as coarse aggregate in this investigation.

2.1.4 Water: Tap water (potable) was used for mixing and curing during the laboratory investigation.

2.1.5 Bacillus *pumilus*: The *B. pumilus* been aurease-producing bacteria was used for this study and it is classified according to American Type Culture Collection as ATCC 27142. The role of bacteria (*B.* pumilus) is to produce enzyme urease through its metabolic activities under proper cultivation process. The enzyme urease triggers the MICP biochemical reaction of hydrolyzing urea.

2.1.6 Cementation Reagents: Cementation reagents serve as raw materials for calcite formation in the MICP process. The cementation reagent that was employed in this study comprise of solution 20g of urea ($CO(NH_2)_2$), 2.8g of calcium chloride ($CaCI_2$), 3g of nutrient broth, 10g of ammonium chloride (NH_4CI), and 2.12g sodium bicarbonate ($NaHCO_3$) per litre of deionized water (DeJong *et al*., 2006; Qabany *et al*., 2011; Stocks-Fischer *et al*., 1999; Stoner *et al*., 2005).

2.1.7 Sulfuric acid: Sulfuric acid was used to provide the acid environment required for the short time durability test. It comprises of Ammonia (NH_3) -0.0005%, Arsenic (As) -0.00001%, Chloride (Cl) -0.0002%, Heavy metals (Pb)-0.0001%, Iron (Fe)-0.0001%, Nitrate (No_3)-0.00002%, Oxygen absorbed (O)-0.00015%, Non-volatile residue-0.0025%, and weighs 1.84g/ml

2.2 Methods

2.2.1 Concrete production

In all, 98 cubes of concrete were casted, there were 7 sets of 14 cubes, the first set was for control, three sets was for concrete mixed with various suspension density of *B. pumilus* and the other three sets was for concrete immersed in various suspension density of *B. pumilus*. The targeted Class of concrete was C25/30 with a mix ratio of 0.55/1:2:3 and the size of the mold used was 100mm cubes. Cubes after casting were de-molded after 24 hours.

2.2.2 Control concrete

The control samples were produced in accordance to BS EN 12390-2:2000, cured in water at ambient temperature (about 28^{o}C) and compressive strength test conducted at 7, 14, 21 and 28 days respectively.

a) Concrete mixed with bacteria

The same mix for the control was adopted but 250mlof various suspension density (1.5 x 10^8 cells/ml, 12.0 x 10^8 cells/ml, and 24.0 x 10^8 cells/ml) of *B. pumilus* solution were used to replace equal volume of water for the water cement ratio so as not to alter the workability of the concrete. The cast cubes were de-molded and cured in water at ambient temperature (about 30^{o}Cand compressive strength test was conducted at 7, 14, 21 and 28 days respectively.

b) Concrete cured in cementation reagent

Concrete cubes were cast as per that of the control and de-molded after 24hr, they were thereafter cured in cementation reagent solution in which 250ml of various suspension density (1.5 x 10^8 cells/ml, 12.0 x 10^8 cells/ml, and 24.0 x 10^8 cells/ml) of *B. pumilus* were added into three separate curing tank which was used for the curing of the cubes. The added suspension density of *B. pumilus* is to enhance the production of calcite precipitation that seal up the pore space around the concrete cube. Compressive strength test was conducted at 7,14, 21 and 28 days respectively.

2.2.3 Compressive Strength Test

The most common test carried out on concrete is the compressive test. The compressive test is easy and relatively inexpensive to carry out (Mindess *et al*., 2003). Testing standard requirements use different geometries of specimens to determine the compressive concrete strength. The most used standards are cylinders with slenderness equal to two (150mm dia. x 300mm length) or cubes of 150mm or 100mm sides. For this research, 100mm cubes were used. Compressive strength test was carried out for the various types of concrete (control, bacterial mixed and concrete cured in cementation reagent) for the ages of 7, 14, 21 and 28 days in accordance to BS 1881-116:1983. The compressive test was conducted in the Civil Engineering laboratory of Nigeria Defence Academy, Kaduna. Fig. 1 shows the picture of the ADR 2000BS Analogue Type Compression Testing Machine used.

2.2.4 Durability test resistance against acid attack

After 28 days curing, the cubes were removed from water, allowed to dry in the laboratory for 24 hrs and then immersed in 10% solution of H_2SO_4. (in a ratio of 942ml of distilled water to 58ml of 98% concentrated sulfuric acid) The cubes were removed in groups from the solution and tested after 7, 14, 21 and 28days after immersion. The response of the cubes to the solution was evaluated through the change in appearance, weight, compressive strength and thickness that was observed. Fig. 2 shows the concrete been immersed in solution of H_2SO_4 while Fig. 3 shows the effect of the sulphuric acid on the concrete. The percentage weight lost is calculated thus:

Weight loss % = $\frac{Loss\ in\ specimen\ weight}{Initial\ specimen\ weight} \times 100$

2.2.5 Scanning electron microscopy (SEM)

The deposition of calcite inside the micro cracks of concrete by bacteria was analyzed under scanning electron microscope (SEM). SEM is a powerful magnification tool that utilizes focused beams of electrons to obtain information. The high resolution, three dimensional images produced by SEMs provide topographical, morphological and compositional information makes them invaluable in a variety of science and industry application

3.0 Discussion of Results

3.1 Compressive strength test for concrete mixed with bacteria

The compressive strength tests results at various stepped densities of *B. pumilus suspension* for concrete mixed with bacteria *is* shown in Fig. 4. The graph shows the comparison between the control concrete and the concrete mix with various stepped densities of *B. pumilus suspension.* The compressive strength generally shows an increasing trend with increase in curing days except at 24 x10^8cells/ml suspension density which showed an unexpected decrease after 21 day curing. The improvement in strength at early age may be as a result of the calcite precipitate form as *B. pumilus* produce enzyme urease through its metabolic activity and the enzyme urease triggered the MICP biochemical reaction by hydrolyzing urea ($CO(NH_2)_2$). The ammonium (NH_4^+) produced increased the pH and caused the bicarbonate (HCO_3^-) to precipitate with calcium ion (Ca^{2+}) from the calcium chloride supplied in order to form the calcium calcite ($CaCO_3$). Fig. 4 shows some deposit of calcite precipitate on the surface of the concrete. It would however appear that precipitation decreased with increase in *B. pumilus* suspension.

Table 1 shows the percentage difference in compressive strength of the bacteria concrete when compared to the control at different ages:

Results as presented in Table 1 indicates that apart from the very highest suspension density of 24E8 cells/ml *B. pumilus*, concrete mixes with bacteria addition performed better at early age than the control in terms of compressive strength. However, the compressive strengths at 28 days were not significantly different, the highest compressive strength increase was achieved with an addition of 1.5E8 cells/ml of *B. pumilus* suspension and it was 6.3% above the compressive strength of the control. With an additive of *B. pumilus* suspension density of 24E8 cells/ml, the compressive strength was lower than that of the control at all ages; this implies that there is a concentration that the addition of *B. pumilus* suspension is no longer beneficial to compressive strength.

A two-way analysis of variance (ANOVA) for the compressive strength test for concrete mixed with bacteria is summarized in Table 2. The results show that the effect of curing days and *B. pumilus* suspension density on the compressive strength were statistically significant (F_{CAL} = 35.03571> F_{CRIT} = 3.862548) for curing days and (F_{CAL} = 10.60714> F_{CRIT} = 3.862548) for *B. pumilus* suspension density. The effect of curing days was more pronounced than that of *B. pumilus* suspension density.

3.2 Compressive strength test for concrete immersed in cementation reagent containing various Bacillus *pumilus* suspension density

The compressive strength tests results at various stepped densities of *B. pumilus suspension* for concrete immersed in bacteria *is* shown in Fig. 5. The graph shows the comparison between the control concrete and the concrete immersed at various stepped densities of *B. pumilus suspension* containing the cementation reagent. The compressive strength generally shows an increasing trend with increase in curing days except at 24 x10^8cells/ml concentration which decreases after 21 day curing.

Figure 5 revealed that the general trend of the growth of compressive strength was increase with age, however, a decrease was observed after 21 days for a curing solution of 24E8 cells/ml of *B. pumilus suspension* density. Furthermore, concrete cured in solution of bacteria with a concentration of 1.5E8and 12E8cells/ml of *B. pumilus suspension* density performed generally better than the control at all ages but with *B. pumilus suspension* density of 24E8 cells/ml the bacteria concrete performed worse than the control after 14days. Table 3 shows the percentage difference between the compressive strength of the bacteria cured concrete and that of the control at different ages.

From Table 3, curing in cementation reagent containing various *B. pumilus* suspension density caused significant increase in compressive strength of concrete at 7 and 14 days curing but the increase reduced after 14 days with only a very small amount of strength increase above that of control at 28 days for only the 1.5 x 10^8cells/ml concentration of bacteria.

From Figure 4 and 5 there is a significant increase in the compressive strength of concrete treated with bacteria up to 14 days as against the control concrete. Concrete treated in 1.5 x 10^8cells/ml and 12 x 10^8cells/ml bacteria concentration performed better than 24 x 10^8cells/ml. This is attributed to the behavior of microbial cells within the concrete matrix. During the initial curing period, microbial cells obtain good nourishment because the concrete was still porous (Jagadeesha *et al.,* (2013)). It is also possible that as the pH of the specimen remained high, cells were in inactive condition and as curing period was increased, it started growing slowly. Upon cell growth, calcite would precipitate on the cell surfaces as well within the concrete matrix which might be attributed to various ions present in media. Thus, the concrete becomes less porous and permeable and increasing the compressive strength. This explains the behavior of the increased compressive strength at the age of 14 days in concrete treated with bacterial. However the percentage increase in 21 and 28 days was not as significant as on 7 day and 14 day. This is attributed to the fact that as hydration of concrete advances, most of the pores which result in reduction in compressive strength in earlier days will gets filled up with hydration products of cement. The improvement in the compressive strength of concrete specimens based on MICP produced by *B. pumilus* is supported by previous studies (Ramakrishnan, *et al*., 2005; Bang, *et al.,* 2010 and Ghosh *et al*., 2005). Irrespective of the type of bacteria concrete treatment adopted, it was found that concrete mixed or immersed in 1.5 x 10^8cells/ml and 12 x 10^8cells/ml bacterial concentration performed better than 24 x 10^8cells/ml.

A two-way analysis of variance (ANOVA) for the compressive strength test for concrete immersed in bacteria is summarized in Table 4. The results show that the effect of curing days and *B. pumilus* concentration on the compressive strength were statistically significant (F_{CAL} = 37.5283> F_{CRIT} = 3.862548) for curing days and (F_{CAL} = 8.275472> F_{CRIT} = 3.862548) for *B. pumilus* concentration. The effect of curing days was more pronounced than that of *B. pumilus* concentration.

3.3 Durability test results for concrete mixed with bacteria

The variation of percentage loss in strength for concrete immerse in Sulphuric acid for concrete mixed with bacteria is shown in Figure 6. The result shows that the percentage loss in weight of the control concrete is higher compared to the bacteria concrete. Concrete mixed with 1.5 x 10^8cells/ml *B. pumilus* concentration gave the lease percentage weight loss beyond 7 days curing period. The percentage weight loss for the control concrete recorded a value of 8.6% for 7 days curing which decreases to a value of 4.8 % at 24.0 x 10^8cells/ml while at 28 days curing the value decreases from 16.5 % for the control concrete to 11.8 % at 1.5 x 10^8cells/ml *B. pumilus suspension.*

A two-way analysis of variance (ANOVA) for the durability test for concrete mixed with bacteria is summarized in Table 5. The results show that the effect of curing days and *B. pumilus* concentration on the compressive strength were statistically significant (F_{CAL} = 53.02532> F_{CRIT} = 3.862548) for curing days and (F_{CAL} = 10.90638> F_{CRIT} = 3.862548) for *B. pumilus* concentration. The effect of curing days was more pronounced than that of *B. pumilus* concentration.

3.4 Durability test results for concrete immersed in cementation reagent containing various Bacillus *pumilus* suspension density

The variation of percentage loss in strength for concrete immerse in sulphuric acid for concrete immersed in bacteria is shown in Figure 7. The result of durability test on concrete immersed in bacteria shows that lower percentage weight loss is achieved at higher bacteria concentration of 24 x 10^8cells/ml for all the curing period.
The percentage weight loss for the control concrete recorded a value of 8.6% for 7 days curing which decreases to a value of 4.0 % at 24.0 x 10^8cells/ml while at 28 days curing for concrete immersed in cementation reagent containing various *B. pumilus* suspension density the value decreases from 16.5 % for the control concrete to 15.0 % at 24.0 x 10^8cells/ml *B. pumilus suspension.*

From Figure 6 and 7, there was a significant loss in weight of control concrete when immersed in sulfuric acid as compared to concrete treated with bacteria; this is due to the action of sulphuric acid on concrete. The sulphuric acid converts the calcium hydroxide in concrete to calcium sulphate which can be leached out of the concrete. Though there is a loss in weight in concrete mixed with bacteria and concrete immersed in bacteria, but not as high as that of the control concrete. This is due to the presence and action of *B. pumilus* and the Cementation reagents that is responsible for the production of calcite, which helps to reduce the effect of sulfuric acid on the concrete. The higher the bacterial dosage, the higher the calcite formation and the better the durability performance.

A two-way analysis of variance (ANOVA) for the durability test for concrete mixed with bacteria is summarized in Table 6. The results show that the effect of curing days and *B. pumilus* concentration on the compressive strength were statistically significant (F_{CAL} = 165.0041> F_{CRIT} = 3.862548) for curing days and (F_{CAL} = 20.27432> F_{CRIT} = 3.862548) for *B. pumilus* concentration. The effect of curing days was more pronounced than that of *B. pumilus* concentration.

3.5 Scanning electron microscope (SEM)

To determine the influence of MICP on the microstructure both the control and MICP samples were examined under the scanning electron microscope (SEM). The examination was made on the samples collected from the cubes tested at 28 days. No precipitate was observed in the control samples (see Fig. 8a) wherein,pores can be easily seen inside it. A clear calcite precipitation was found on the crack remediated area in the samples containing the bacteria cells (see Fig. 8b and c). The SEM analysis of concrete mixed and immersed with *B. pumilus* has revealed distinct calcite crystals embedded in concrete. High calcium amounts in it confirmed that calcite was present in the form of calcium carbonate due to bacteria. Fig. 8b shows the presence of crystalline calcium carbonate associated with bacteria. The deposition of calcite serves as barrier to reduce porosity and thus improves impermeability and strength of the concrete similar observations were made by (Chahal *et al.,* (2012) and Abo-El-Enein *et al.*, (2013))

4.0 Conclusion

Based on the results of this investigation the following conclusions are made:

1. *B. pumilus* treated concrete showed improved compressive strength at early age above that of untreated samples. Scanning revealed that this is due to the deposition of the calcite material by the bacterial activity. Improvement in compressive strength after 28days over that of the control was only noticeable at low bacteria concentration.
2. The compressive strength of *B. pumilus* treated concrete decreases with higher concentration of *B. pumilus*, while there is improvement of the durability of *B. pumilus* treated concrete with higher suspension density of *B.pumilus*.
3. The optimum *B. pumilus* cells suspension density which leads to the highest improvement of the compressive strength of concrete treated with *B. pumilus* at 28 days curing is 1.5 x 10^8 cells/ml while the optimum *B. pumilus* suspension density that improved the durability of concrete at 28 days curing is 24.0 x 10^8 cells/ml

5.0 Recommendation

B. pumilus suspension density of 1.5x10^8cells/ml is recommended for bacteria concrete irrespective of the method of use either the *B. pumilus* suspension density is used in the mixing of concrete or if the concrete is casted and immersed in cementation reagent containing various Bacillus *pumilus* suspension density.

References

Abo-El-Enein, S.A., Ali, A.H., Talkhan, F. N.and Abdel-Gawwad, H.A. (2013).“Application of microbial biocementation to improve the physico-mechanical properties of cement mortar”. Housing and Building National Research Center. Production and hosting by Elsevier B.V. All rights reserved. HBRC Journal (2013) 9, 36–40.http://dx.doi.org/10.1016/j.hbrcj.2012.10.004

Achal V., Mukherjee A., Basu P. C. & Reddy M. S. (2009).Lactose mother liquor as an alternative nutrient source for microbial concrete production by *Sporosarcina pasteurii*. *Journal of Industrial Microbiology and Biotechnology*, 36, 433-438.

Arunachalam, K.D., Sathyanarayanan, K.S., Darshan, B.S. and Raja, R.B., (2010) "Studies on the characterization of Bio sealant properties of Bacillus sphaericus". *International Journal of Engineering Science and Technology*, vol.2, no.3, pp. 270–277.

Bang, S.S., Lippert, J.J. Yerra, U., Mulukutla, S., and Ramakrishnan, V., (2010).*Microbial calcite, a bio-based smart nanomaterial in concrete remediation".*International Journal of Smart and Nano materials. Vol. 1, No. 1, pp. 28-39

BS 1881: Part 116: *Method of determination of compressive strength test*, British Standards Institution, London, 1983

BS EN 12390–2: (2000): Testing hardened concrete – Part 2: Making and curing specimens for strength tests, British Standards Institution, London, UK.

Chahal N., Siddique R., Rajor A., (2012). "Influence of bacteria on the compressive strength, water absorption and rapid chloride permeability of fly ash concrete". *Construction and Building Materials,* vol. 28, no 1, pp 351-356

Claisse, P. A., Elsayad, H. A., and Shaaban I. G. (1997). "Absorption and sorptivity of cover concrete". *Journal of Materials in Civil Engineering,* 9, 105-110.

DeJong J. T., Fritzges M. B., and Nüsslein K., (2006)."Microbially Induced Cementation to Control Sand Response to Undrined Shear". J. Geotech Geoenviron. Eng., vol. 132, pp. 1381-1392.

Ghosh, P., Mandal, S., Chattopadhyay, B.D., and Pal, S. (2005) *Use of microorganism to improve the strength of cement mortar,"*Cement and Concrete Research, International Journal of Scientific Research in Science, Engineering and Technology ISSN 35: 1980-1983

Ghosh P. and Mandal S. (2006).Development of bioconcrete material using and enrichment cultureof novel thermophilic anaerobic bacteria, *Indian Journal of Experimental Biology*. 44, pp. 336-339

Henk M. J. and Erik S. (2009).A two component bacteria-based self healing concrete. *Concrete Repair, Rehabilitation and Retrofitting II*, London, Taylor & Francis Group.

Jagadeesha, K.B.G., Prabhakara, R., Pushpa, H.,(2013) *Effect of bacterial calcite precipitation on compressive strength of mortar cubes."*International Journal of Engineering and Advance Technology ISSN: 2249-8958, Volume -2, Issue -3

Jonkers, H. (2011). "Self-healing concrete".Ingenia, issue 46, pp 39-43.

Khan, M. I. (2003).*"Isoresponses for strength, permeability and porosity of high performance Mortar."Building and Environment,* 38, 1051-1056.

Mindess, S., Young, J.F., and Darwin, D. (2003)."*Concrete.*"Prentice Hall, Pearson Education, Inc. United States of America.

Muynck W .D., Cox K., Belie N .D. and Verstraete W. (2008). Bacterial carbonate precipitation as alternative surface treatment for concrete. *Construction and Building Materials*, 22, pp. 875-885.

Park S. J., Park Y. M., Young Chun W. Y., Kim W. J., and Ghim S. Y. (2010). Calcite-Forming Bacteria for Compressive Strength Improvement in Mortar, *Journal of Microbiology and Biotechnology*, 20 (4), pp. 782–788

Qabany, A. A., Mortensen, B., Martinez, B., Soga, K., and Dejong, J. (2011)."Microbial carbonate precipitation correlation of s-wave velocity with calcite precipitation."Geo-Frontiers 2011, pp. 3993- 4001.

Rafat S., and Navneet K.C., (2011) *Effect of Ureolytic bacteria on Concrete Properties, Construction and Building Materials,"*International Journal of Scientific Engineering and Applied Science(IJSEAS), vol . 25, pp 3791-3801

Ramakrishnan, V., Bang, S. S., and Deo, K. S. (1998). *"A novel technique for repairing cracks in high performance concrete using bacteria."Proceedings of International conference on high performance high strength concrete,* Perth, Australia, 597-618.

Ramakrishnan, V., Panchalan, R.K. and Bang, S.S. (2001) *Improvement of concrete durability by bacteria mineral precipitation,"*The South Dakota School of Mines and Technology, SP-225-2

Ramakrishnan, V., Panchalan, R.K., Bang, S.S., and City, R. (2005) *Improvement of concrete durability by bacterial mineral precipitation,"International Conference on Fracture.*

Ravindranatha, L., Kannan, N., andLikhit, M.L., (2014b) *Self-Healing Material Bacterial Concrete,"*IJSEAS vol. 3

Ravindranatha, L., Udaya Shankar, H.N., and Suhas S.V., (2014a) *Effect of Bacteria on Strength of Concrete,"* Proceedings of the 3rd World Conference on Applied Sciences, Engineering and Technology, Kathmandu, Nepal, ISBN 13: 978-81-930222-0-7, pp 228-231

Stocks-Fischer, S., Galinat, J. K., and Bang, S. S. (1999). *"Microbiological precipitation of CaCO3."Soil Biology and Biochemestry,* v.31, 1563-1571.

Senot, S. (2017). *"Can self-healing mechanism help concrete structures sustainable?"* Procedia Engineering, 171, pp 238-249.

Stoner, D. L., Watson, S. M., Stedtfeld, R. D., Meakin, P., Griffel, L. K., Tyler, T. L., Pegram, L. M., Barnes, J. M., and Deason, V. A. (2005). "Application of stereo lithographic custom models for studying the impact of biofilms and mineral precipitation on fluid flow." Appl. Environ. Microbiol., Vol. 71, No. 12, pp. 8721-8728

Wiktor, V., Jonkers, H.M. (2011). *"Quantification of crack-healing in novel bacteria-based self- healing concrete"*. Cement and Concrete Composites, vol. 33, pp 763-770.

Figure 1: Compressive testing machine

Fig 2: Cubes immersed in Sulphuric acid

Fig3: The effect of Sulphuric acid on concrete

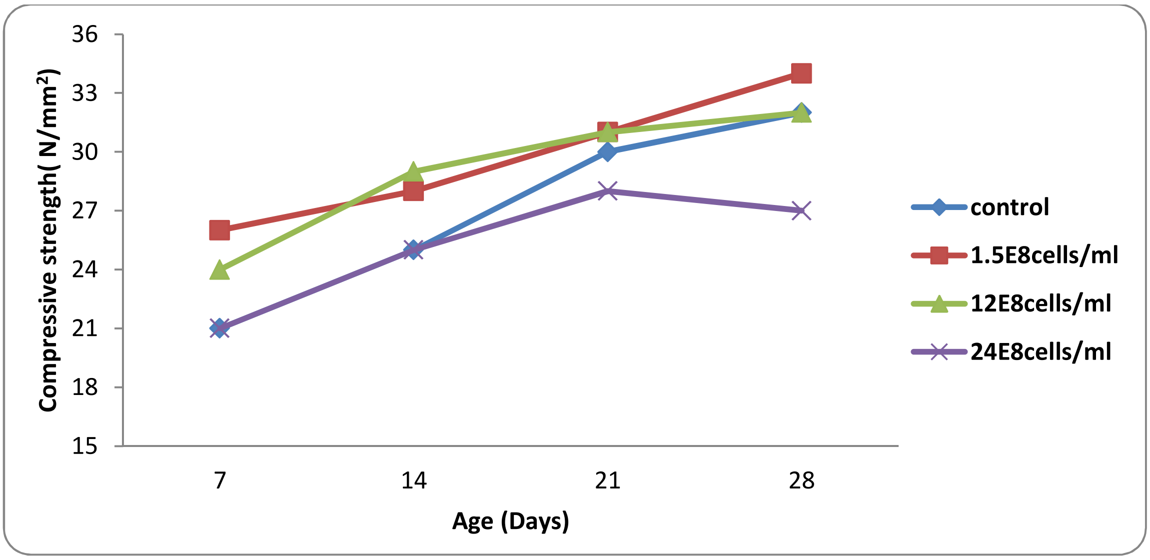


Figure 4: Comparison of compressive strength results of control and concrete made with various Bacillus *pumilus* suspension density

Table 1: Percentage Difference Between the Compressive Strength of Bacteria Mix Concrete Compared with that of Control at Different Ages

Age (Days)	Concentration of Bacteria			
	0 (Control)	**1.5E8 cells/ml**	**12E8 cells/ml**	**24E8 cells/ml**
7	0.0	23.8%	14.3%	0%
14	0.0	12%	16%	0%
21	0.0	-3.3%	3.3%	-10%
28	0.0	6.3%	0%	-9.4%

Figure 4: Precipitate on surface of concrete

Table 2: Two-way analysis of variance results for Compressive strength test for concrete mixed with bacteria

ANOVA						
Source of Variation	*Sum off Squares*	*df*	*MS*	F_{CAL}	*P-value*	F_{CRIT}
Curing Days	163.5	3	54.5	35.03571	2.72E-05	3.862548
Bacteria Treatment	49.5	3	16.5	10.60714	0.002598	3.862548
Error	14	9	1.555556			
Total	227	15				

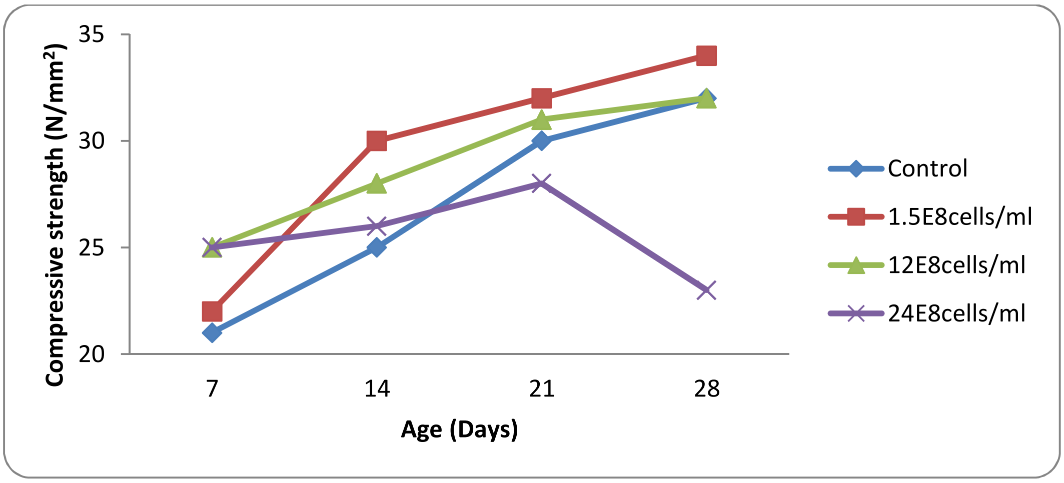


Figure 5: Comparison of compressive strength results of control and concrete immersed in cementation reagent containing various Bacillus *pumilus* suspension density

Table 3: Percentage Difference Between the Compressive Strength of Bacteria Solution Cured Concrete Compared with that of Control at Different Ages

Age (Days)	Concentration of Bacteria			
	0 (Control)	1.5E8/ml	12E8/ml	24E8/ml
7	0.0	4.8%	14.3%	0%
14	0.0	20%	16%	0%
21	0.0	6.7%	3.3%	-6.7%
28	0.0	6.3%	0%	-15.6%

Table 4: Two-way analysis of variance results for compressive strength test for concrete immersed in cementation reagent containing various Bacillus *pumilus* suspension density

ANOVA						
Source of Variation	*Sum off Squares*	*df*	*MS*	F_{CAL}	*P-value*	F_{CRIT}
Curing Days	207.1875	3	69.0625	37.5283	2.05E-05	3.862548
Bacteria Treatment	45.6875	3	15.22917	8.275472	0.005912	3.862548
Error	16.5625	9	1.840278			
Total	269.4375	15				

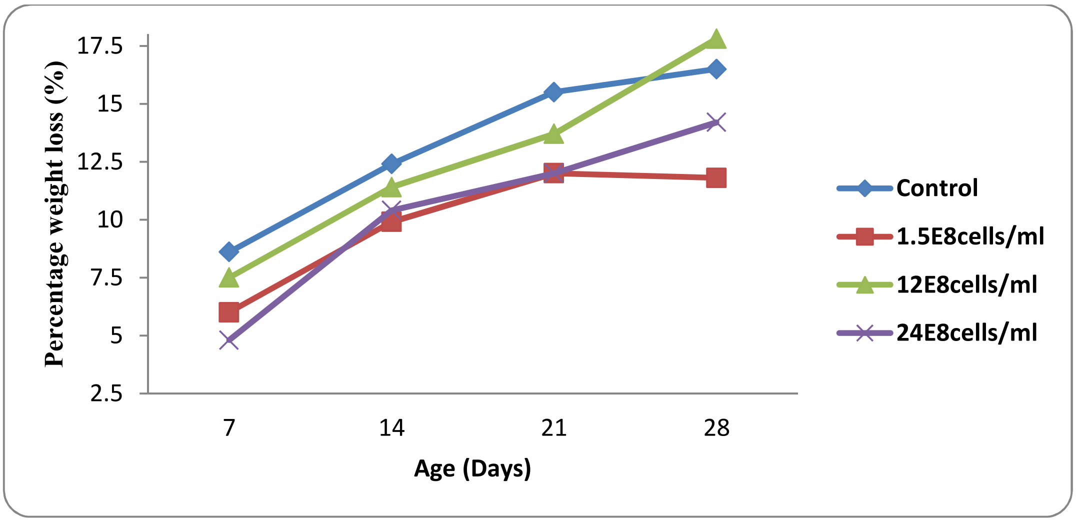


Figure 6: percentage weight loss comparison between control concrete and concrete mixed in various concentrations of bacteria

Table 5: Two-way analysis of variance results for durability test results for concrete mixed with bacteria

ANOVA						
Source of Variation	*Sum off Squares*	*df*	*MS*	F_{CAL}	*P-value*	F_{CRIT}
Curing Days	158.8219	3	52.94063	53.02532	4.81E-06	3.862548
Bacteria Treatment	32.66688	3	10.88896	10.90638	0.002361	3.862548
Error	8.985625	9	0.998403			
Total	200.4744	15				

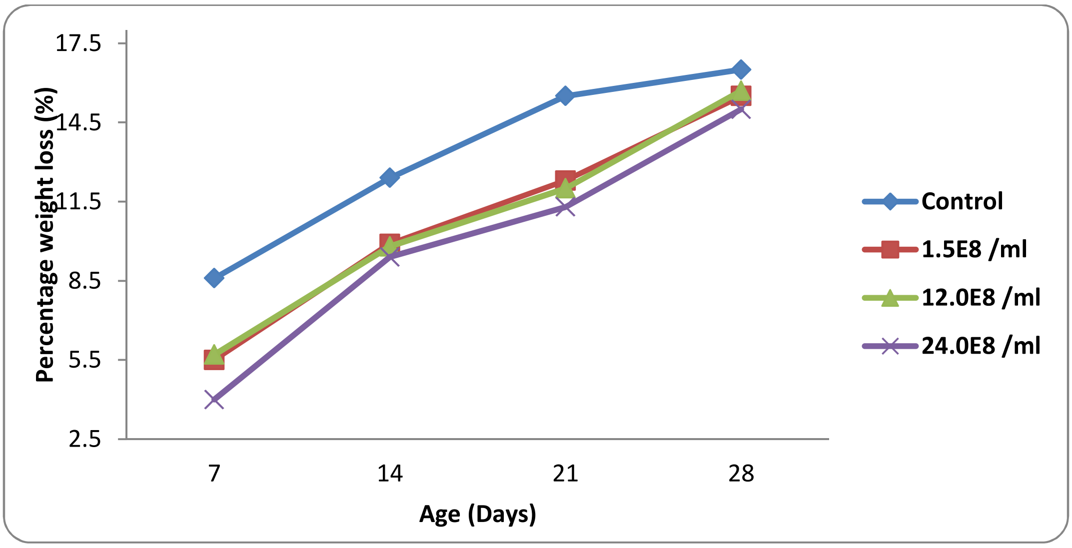
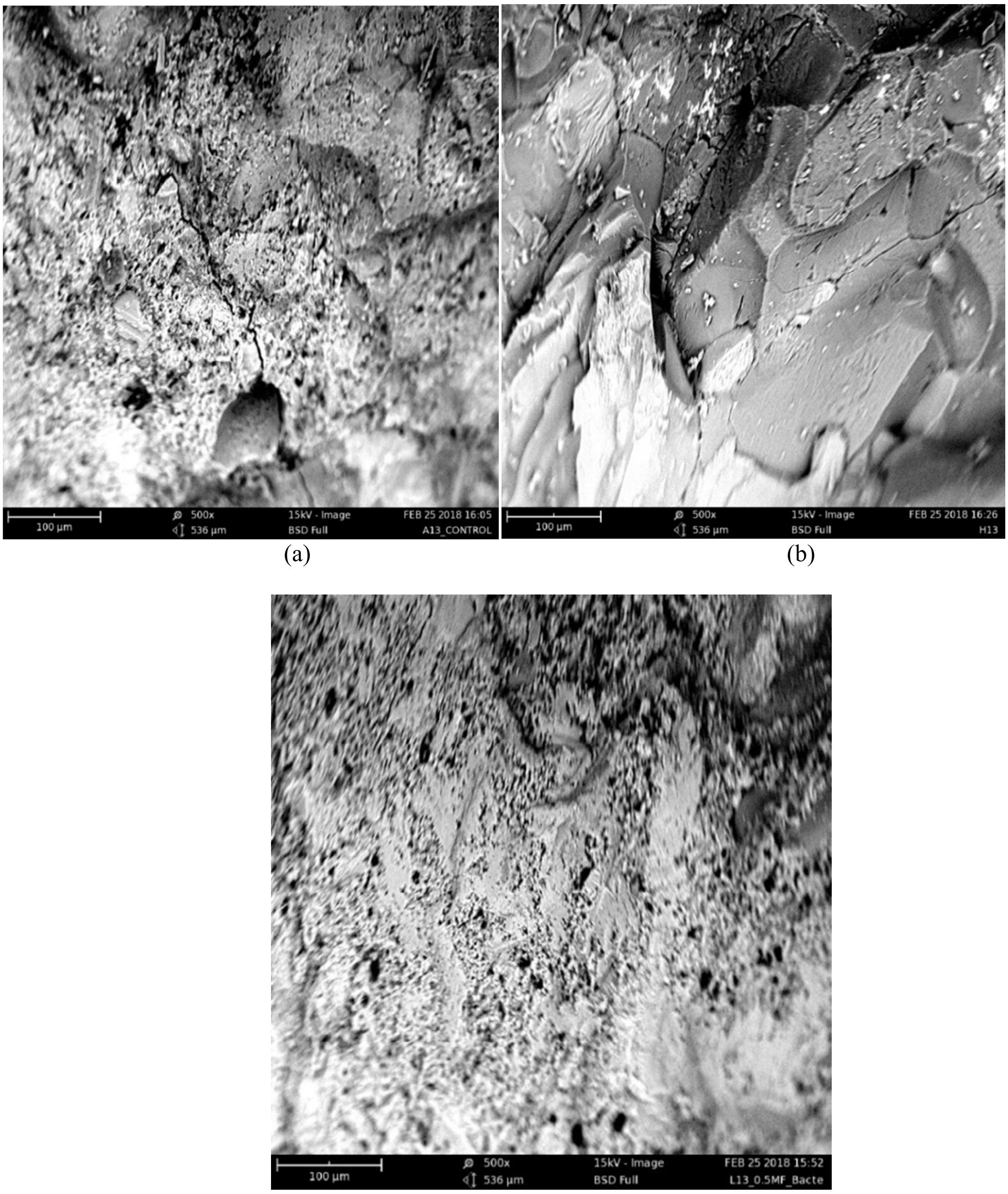


Figure 7: percentage weight loss comparison between control concrete and concrete immersed in cementation reagent containing various Bacillus *pumilus* suspension density

Table 6: Two-way analysis of variance results for durability test results for concrete immersed in cementation reagent containing various Bacillus *pumilus* suspension density

ANOVA						
Source of Variation	*Sum off Squares*	*df*	*MS*	F_{CAL}	*P-value*	F_{CRIT}
Rows	203.505	3	67.835	165.0041	3.49E-08	3.862548
Columns	25.005	3	8.335	20.27432	0.000242	3.862548
Error	3.7	9	0.411111			
Total	232.21	15				



(a) (b)

(c)

Figure 8: SEM images of (a) control concrete (b) concrete mixed with bacteriaand (c) concrete immersed in cementation reagent containing various Bacillus *pumilus* suspension density