https://erjm.journals.ekb.eg
ISSN: 1110-1180

DOI: ---------

ERJ
Engineering Research Journal
Faculty of Engineering
Menoufia University

Effect of Bacillus Megaterium Bacteria and Different Calcium Sources on Strength and Permeation Properties of Concrete

Randa F. Esmail[1], Rateb N. Abbas[2], and Noha M. Sorour[3],and Amal A. Nasser[4*],

[1] *Department of Civil Engineering, Faculty of Engineering, Menoufia University, Egypt*
[2]*Department of Microbial Biotechnology, Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt*
[3]*Department of Industrial Biotechnology, Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt*
[4*]*Department of Civil Engineering, Faculty of Engineering, Menoufia University, Egypt.*
**(AMAL.NASER@sh-eng.menofia.edu.eg)*

ABSTRACT
Different methods were used to repair concrete cracks. Recently, microbial induced calcium carbonate precipitation is presented as a self-healing technique for concrete manufacture, which is proposed as an eco-friendly method. It is a bio-mineralization process. Microbial activities induced precipitation that can heal cracks. Effect of bio-mineralization in concrete by incorporation of ureolytic species such as *Bacillus Megaterium* is investigated. Fresh and hardened tests were carried out on concrete at various ages. "*Bacillus Megaterium*" (*Bm*) with a cell density of 2×10^9 CFU/mL was used when added to mixtures with two ratios of 0.5% and 0.25% of cement weight. Calcium lactate, Calcium acetate, and Calcium format were added as nutrition to bacteria by 0.25% and 0.125% of cement weight. Test results indicated that the inclusion of *Bm* in concrete enhanced properties. Bacteria addition and all nutritions used revealed varied decreases in the rate of water absorption and capillary permeability coefficient. *Bm* 0.5% with acetate nutrition concrete mix had a maximum increase in compressive strength at all ages. It had compressive strength value 152.99% of that of the compressive strength of the control concrete mixture at 28days. *Bm* 0.5% with lactate nutrition concrete mix had flexural strength value 162.74% of that of control concrete flexural strength at 28days. Enhancement of properties was due to calcite deposition on the bacteria cell surfaces within the pores. SEM imaging indicated the formation of calcite crystals in different shapes according to the added nutrients of bacterial concrete specimens.

Keywords: *Biomineralization; Calcium Carbonate Precipitation; Bacillus Megaterium;Self-Healing Concrete.*

1. Introduction

Concrete is the most widely used construction material. It has adequate compressive strength and low tensile strength. The low tensile strength is a major cause of crack propagation. Cracks cause shortens lifetime of structures [1, 2]. Conventional methods have been carried out for managing of concrete micro-cracks, such as using of organic polymers (epoxy, siloxane, acrylics, and polyurethanes). Recently, microorganisms are used to improve the overall behavior of concrete by microbial mineral precipitation resulting from metabolic activities. This process is termed Bio-mineralization [3]. Microbial induced calcite precipitation is a promising technique in the field of concrete research, which is associated with biological mineralization [4, 5]. Bio-mineralization is achieved through ureolytic bacteria possessing urease enzyme. The mechanism includes a series of reactions; the urease enzyme catalyzes the hydrolysis of urea into ammonia and carbon dioxide thereby increasing the alkalinity of the surrounding medium. The negatively charged bacterial cell wall attracts the positively charged Ca^{2+} ion. Subsequently, the calcium ion reacts with the CO_3^{2}- and results in precipitation of $CaCO_3$ on cell surface [6]. Calcium concentration, concentration of dissolved inorganic carbon (DIC), the pH of the surrounding solution, available nucleation sites, saturation index, $[Ca^{2+}]/[CO_3^{2-}]$ ratio and bacterial species are the factors that control the mineralization of calcium carbonate [7].

Stone restoration and soil strengthening are new techniques of microbial-induced calcium precipitation [8]. The effectiveness of this technique led to its implementation for crack repair. Repair application includes surface treatment for concrete, enhancement of mechanical properties and improves the durability of cementitious materials [6]. Calcite deposition on cell surfaces was proved to plug the pores and improve strength [9]. Bacterial cells proved to have a potential for sequestering atmospheric CO_2 and fixing it into different carbonate minerals as calcite, magnesite, and dolomite [10]. *Bacillus* species can produce a binding filler material (calcite) due to their urease activities. Hence, concrete capillary pores decrease leading to improve strength and durability [11]. In concrete manufacture, incorporated bacteria should be alkali resistant to endure the high pH of cement reaction. Also, endospore forming should withstand mechanical stresses induced during concrete mixing. Bacterial endospores can form in approximately 6–8 h after being exposed to adverse conditions. They are metabolically inactive, dehydrated and may remain viable for periods up to 200 years. Concrete cracks make an open path for entering water and air, which germinate endospores into active vegetative cells within 90 min. Those activated cells start precipitating calcite to heal micro cracks in concrete [12]. Bacterial based healing process was previously applied externally. A bacterial solution is sprayed manually on service cracks appeared to naked eye. This required a regular observation which is a time-consuming process. Henk M. Jonkers investigated immobilized bacteria in concrete mix. This was the first proof of with bacterial producing calcite into concrete matrix. The incorporation of bacteria can heal cracks in the microstructure, which increases the structure lifespan [13]. Calcite forming ability was conducted in model *Bacillus* species such as *B. subtilis, B. cohnii, B. pasteurii, B. pseudofirmus, B. alkalinitrilicus, B. sphaericus* LMG 22257 and *B. megaterium*) for enhancement of concrete properties and durability [14]. Surface treatment of using *B.megaterium* SS3 is effective and eco-friendly of bio deposition of coherent precipitation that improves building materials durability [7]. Selected Egyptian microorganisms *Bacillus megaterium* proved to enhance mechanical and physical properties of cement mixes. It promotes micro-cracks self-healing, which improved durability of cement mixes. *Bacillus megaterium* were added to rich cement mortar as a matrix of RC lamina. Reinforced laminates were loaded in flexural testing machine. Compared to non-bacterial mortar, *B.megaterium* mixes can heal and restore the material to its original state when damaged [15].

The main objective of the present experimental program is to investigate the effect of incorporation of *Bacillus Megaterium* and different nutrients of bacteria on the physical and mechanical properties of bacterial concrete. *Bacillus megaterium* with cell density of 2×10^9 CFU/mL was used. Three different nutrients were added to bacterial mixes Calcium lactate, Calcium acetate and Calcium formate. Also, the effect of super plasticizer addition on the bioconcrete properties was investigated and its characterization was confirmed using SEM analysis.

2. Materials and Methods

2.1 Bacterial Strain and Growth Conditions

Bacillus megaterium was originally isolated from the alkaline soil samples located at Wadi EL-Nitron, Behera governorate, Egypt. It was identified using MALDI-TOF Biotyper (Matrix-Assisted Laser Desorption Ionization Time-Of-Flight, Bruker) identifying the ribosomal proteins using mass spectrometry [16]. *B. megaterium* has a rod-shaped, gram positive; endospore forming species of bacteria having doubling time of about 1.6 h is chosen for the investigation. The culture was grown in autoclaved Nutrient broth (NB) (Peptone 10 g/L, yeast extract 10 g/L, sodium chloride 5 g/L) [17]. The pH was adjusted to 7.5 and cultures were aerobically incubated in 2L Erlenmeyer flasks using a rotary shaking incubator at 150rpm for 7days at 30°C. Growth and sporulation yield of bacteria was regularly checked and quantified using microscopic analysis and pour-plate count method. All microbiological assays were carried out in Microbial Biotechnology Laboratory, Microbial Biotechnology Department, Genetic engineering and Biotechnology Research Institute, University of Sadat city, Egypt.

2.2 Bacterial Nutrients

Calcium based nutrients were used. Calcium lactate has a white color. It is crystalline salt with formula $C_6H_{10}CaO_6$, consisting of two lactate anions $H_3C(CHOH)CO^{-2}$ for each calcium cation Ca^{2+}. It forms several hydrates. The most common of that nutrients, is pentahydrate $C_6H_{10}CaO_6{\cdot}5H_2O$. Calcium formate is Calcium salt of formic acid. It is inflammable and forms orthorhombic crystals. Its color is white-to-yellow crystals or crystalline powder. Calcium acetate chemical formula is $Ca(C_2H_3O_2)_2$ with the standard name calcium acetate. It is a white powder and has slight acetic acid odor.

2.3 Cement Mixes Material

Portland cement was obtained from La Frage Company. The cement type is CEM I, 42.5N Grade as requirements of Egyptian Standard Specifications (ESS) (4756-1/2007) [18]. Fine aggregate is natural siliceous sand nominal size of 4.75 mm. Coarse aggregate is crushed limestone. Aggregate properties comply with Egyptian Standard Specifications (E.S.S, 1109-2002) [19] and Egyptian Code for Concrete Structures (ECP, 203-2018) [20]. Potable water free from organic substances was used for mixing and curing of concrete. Water/cement ratio used in mixtures was 0.45. Commercially available Super plasticizer called Viscocrete 3425 (from Sika Company). It is aqueous solution of modified polycarboxylates according to ASTM-C- 494 Types G and F and BS EN 934 part 2: 2001. Super plasticizer was used in concrete mixtures with 1% of cement weight.

2.4 Mix Proportion

Sand/cement ratio was 1:3 by weight. The sand/coarse aggregate ratio was 1:1.5 by weight and water/cement ratio was 0.45. A control concrete mixture was prepared to investigate the effect of bacteria addition and different nutrients. There were two stages of work; the first stage was conducted by using *Bacillus megaterium*. It was added to concrete mixtures with two ratios (0.5% and 0.25% of cement weight). Calcium lactate, Calcium formate, and Calcium acetate were added as nutritions to bacteria by 0.25% and 0.125% of cement weight.

The second stage was conducted by using *Bacillus megaterium*. It was added to concrete mixtures with two ratios (0.5% and 0.25% of cement weight). Calcium lactate was added as nutrition to bacteria by 0.25% and 0.125% of cement weight. Super plasticizer was used in concrete mixtures with 1% of cement.The mix proportions of experimental concrete mixes for first and second stages are given in Tables (1). The concrete mixtures were cast in molds for different tests.

2.5 Mixing, Casting, and Curing of Concrete Mixtures

Concrete mixtures were mixed in a mechanical mixer. Mixtures were carried out as following.

- Liquid component was weighed and mixed together prior to adding to the dry materials.
- All dry materials were weighted and mixed in the pan for about five minutes.
- Liquid component was gradually added in the pan. The mixing continues for another 4-6 minutes depending on consistency of the mix.
- Cubes and prisms concrete molds were filled in three layers and compacted on a vibrating table.
- Samples were demolded after 24 hours of casting. They were wrapped with wet cloth for curing. The specimens were kept moist by spraying water every day.

3. Experimental Tests

3.1 Physical Properties

3.1.1 The Rate of Water Absorption

Water absorption test is determined to investigate water penetration in concrete [21]. Speed of water absorption is an indicator of capillary forces exerted by the microstructure pores, which cause drawn of fluids into the material. Speed of water absorption of concrete was measured by an increase of mass samples, when only one surface of the specimen is exposed to water. Three cubes (70*70*70 mm) of each age were tested to determine the velocity of water absorption at the ages 7, 28, 90, and 120 days. Concrete samples were dried in an oven at 110^{o}C for 24 hours and then they were air cooled. The sides of samples were painted with epoxy resin in order to allow the flow of water in one direction. The end of the samples was sealed with a plastic sheet stretch. Sample was weighed. Then, the initial mass of the samples was taken after partly immersed in 0.5cm depth of water. The readings were recorded at selected times after first contact with water (typically 1, 5, 10, 20, 30, 60, 110, 120 min, and 24 hours). Then, samples were removed; excess water was blotted off and weighed. Samples were replaced again in water for the chosen time. The gain in mass (Δm, kg/s) at time t (s), exposed area of the specimen (a, m^2), and density of water (d), were used to obtain the rate of water absorption (I, $m/s^{1/2}$) as per the equation (1) [22].

$$I=\Delta m / (a*d) \qquad \text{Equation (1)}$$

Table (1): Concrete Mixes Proportions (kg/m^3).

Mixes	Sample Code	Cement	Sand	Coarse Aggregate	Water	Bacteria/ Cement(%)	Calcium Lactate/ Cement(%)	Calcium Formate/ Cement(%)	Calcium Acetate/ Cement (%)	Super plasticizer /Cement (%)
Control And Bacterial Concrete Mixes	C0	350 kg	775 kg	1163 kg	157.5 kg	0.0	0.0	0.0	0.0	0.0
	BMCL 0.5					0.5	0.25	0.0	0.0	0.0
	BMCL0.25					0.25	0.125	0.0	0.0	0.0
	BMCF0.5					0.5	0.0	0.25	0.0	0.0
	BMCF0.25					0.25	0.0	0.125	0.0	0.0
	BMCA0.5					0.5	0.0	0.0	0.25	0.0
	BMCA0.25					0.25	0.0	0.0	0.125	0.0
	BMCLSP0.5					0.5	0.25	0.0	0.0	1
	BMCLSP0.25					0.25	0.125	0.0	0.0	1

3.1.2 Capillary Permeability Coefficient

Coefficient of water absorption may be considered a measure of permeability of water. It was determined by measuring the rate of water uptake by dry concrete cubes in a period of 24 hour. Three cubes (70*70*70 mm) of each age were tested to determine the velocity of water absorption at the ages 7, 28, 90, and 120 days. The concrete samples were dried at 110^{o}C in an oven for 24 hours and then were air cooled. The same procedure as in previous section was conducted. After immersing for 24 hours, the specimens were taken out of the water. The amount of water absorbed during the time was calculated for concrete samples after 7, 28, 90, and 120days using the equation (2) [22].

$K= Q^2/(A^2 \times t)$ Equation (2)

Where,

K: the coefficient of water absorption (m^2/sec)

Q: is the quantity of water absorbed (m^3) by the dried samples in 24 hour and

A: is the surface area (m^2) of concrete samples through which water penetrates.

3.2 Mechanical Properties

3.2.1Compressive Strength

For compressive strength test three cubes (70*70*70 mm) of each age were tested and average values are reported to determine the compressive strength at the ages7, 28, 90, and 120 days. The test was done according to Egyptian Standard Specifications (E.S.S, 2421-2005) [23]. All the specimens were tested in a Compression Testing Machine of 2000 kN capacity. They were tested up to failure [24].

3.2.2 Flexural Strength

Three prisms (40*40*160 mm) of each age were tested and average values are reported to determine the flexural strength at the ages 28, 90, and 120 days. Each prism is tested using three point-loading test on the Flexural Testing Machine. The load is applied through the roller placed at the middle (central point load).

3.3 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) is a verified tool to visualize the surface structure of biomaterials and morphology of bacterial concrete [11]. Scanning Electron Microscope is a highly robust analyzing system that involves the use of electron beam to generate superlatively magnified surface images of the focused surface in of the investigated sample. The image is produced as a raster scan image whose targeted resolution can be more than 1nm. It was done after 120 days in National Research Center, Cairo, Egypt.

4. Tests Results and Discussion

4.1 Physical Properties

4.1.1 The Rate of Water Absorption

The influence of bacteria and different nutrients (Calcium lactate, Calcium formate and Calcium acetate) on the rate of water absorption of concrete mixtures during 2 hours was investigated. At age of 7, 28, 90, and 120 days the relation between the rate of water absorption and time during 24 hours showed that all bacterial specimens had smaller gain of water absorption than that of control mixture.

At age of 7 days, it was observed that BMCL0.25 had the least water absorption after 24 hours. This value of BMCLSP0.5 was 48.35 % of water absorption value of control mixture. At the same ratio of bacteria (0.5%), it was observed that BMCL0.5 had the highest water absorption after 24 hours and BMCLSP0.5 had the least water absorption. At the same ratio of bacteria (0.25%), it was observed that BMCF0.25 had the highest water absorption after 24 hours and BMCL0.25 had the least water absorption as shown in Figure (1). BMCL0.25 gained 52.75 % of water absorption of control mixture.

At age of 28 days, it was observed that BMCLSP0.25 had the least water absorption after 24 hours. It was 50.6% of water absorption of control mixture. At the same ratio of bacteria (0.5%), it was observed that BMCLSP0.5 had the least water absorption. It was 54.32% of water absorption of control mixture. At the same ratio of bacteria (0.25%), it was observed that BMCA0.25 had the highest water absorption after 24 hours and BMCLSP0.25 had the least water absorption as shown in Figure (1).

At age of 90 days, it was observed that BMCA0.5 had the least water absorption after 24 hours. It was 75.34% of water absorption of control mixture. At the same ratio of bacteria (0.5%), it was observed that that BMCF0.5 had the highest water absorption after 24 hours. At the same ratio of bacteria (0.25%), it was observed that BMCL0.25 had the least water absorption as shown in Figure (1). It was 83.56% of water absorption of control mixture.

At age of 120 days, it was observed that among all mixes BMCL0.25 and BMCLSP0.5 had the least rate of water absorption after 24 hours. They had the same value of rate of water absorption. It was 50.7% of rate of water absorption value of control mixture as shown in Figure (1).

Inclusion of bacteria resulted in concrete cubes surface consolidation this due to calcite precipitations. Biogenic crystals precipitated within the pores of concrete microstructure and resulted in water absorption reduction. As the bacterial cells are about 1-4 um dimensions, bacteria lied easily in the surface pores with larger diameters. Rapid diffusion of bacterial cells might be occurred inside porous concrete mix, which fills voids and clogs pores inside microstructure. Entrapped bacterial biomass also leads to decrease porosity [7]. Deposition of a calcite layer on surface or inside pores of concrete specimens decrease water absorption and permeability. Inclusion of bacteria decreased the water uptake compared to control mixtures [21]. After 90, and 120days of casting concrete, same nutrient bacterial concrete specimens with (0.5%) bacteria had rate of water absorption after 2 hours lower than that of (0.25%) bacterial concrete specimens. high bacterial ratio results to more precipitation of calcium carbonate. calcite precipitation occurred near the surface of cracked concrete cubes, because of oxygen presence [24].

Calcite precipitation in concrete pores sealed water ingress, which led to water absorption decrease of bacterial concrete [25].

Concrete cubes amended with fly ash (0%, 10% and 20%) with *Bacillus megaterium* ATCC 14581 cells absorbed nearly 3.5 times less water than that of control cubes. Microbial deposition of calcite layer on concrete surface specimens resulted in substantial decrease of water uptake [26]. Increase of cell concentration decreases permeability due to deposition of calcium carbonate precipitation [27].

4.1.2 Capillary Permeability Coefficient

The coefficients of capillary permeability of all bacterial concrete and control concrete mixtures after

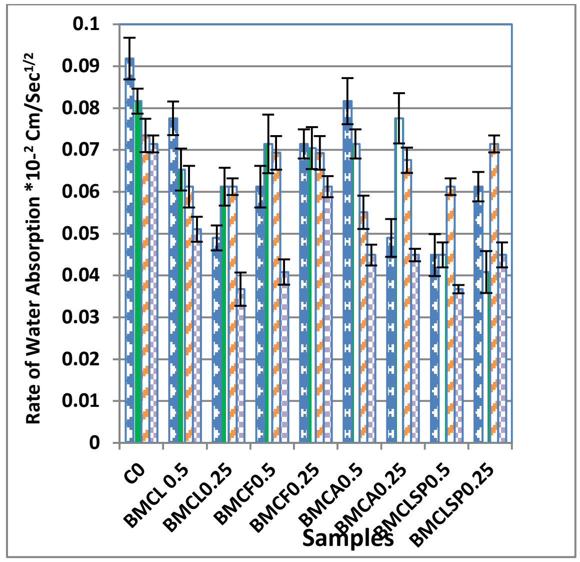


Figure (1): Rate of Water Absorption for Bacterial and Control Concrete after 24 hours.

24 hours at the age of 7, 28, 90, and 120 days were investigated to study the influence of bacteria and different nutrients (Calcium Lactate, Calcium Formate and Calcium Acetate) on permeability of concrete mixtures. It was noticed that at all ages capillary permeability coefficient for all bacterial concrete specimens decreased compared to control concrete specimens as illustrated in Figure (2). At age of 7 days, among all mixes BMCLSP0.5 had the least capillary permeability coefficient after 24 hours. It was 23.87% of capillary permeability coefficient of control mixture. At age of 28 days, it was observed that among all mixes BMCLSP0.25had the least capillary permeability coefficient after 24 hours. It was 30.22% of capillary permeability coefficient of control mixture. At age of 90 days, it was observed that among all mixes BMCA0.5 had the lowest capillary permeability coefficient after 24 hours. It was 56.16 % of capillary permeability coefficient of control mixture. At age of 120 days it was observed that among all mixes BMCLSP0.5had the lowest capillary permeability coefficient after 24 hours. It was 50.7% of capillary permeability coefficient value of control mixture. Siddique et al., revealed that capillary water absorption of concrete was significantly influenced by the addition of bacteria. Reductions in sorptivity of concrete specimens were in the range of 50-70% at 28 and 56 day respectively, compared to nonbacterial concrete specimens. The biological precipitation of calcite resulted in densed section and decrease capillary water uptake [25]. Bacterial concrete sorptivity was low compared to control concrete. Capillary water absorption was

reduced for about 60% after 60 days of control concrete [28]. Calcite deposition makes a barrier layer led to reduction in permeability to any substances. And thus improves impermeability and the durability of structures. This improves resistance of cementitious materials towards degradation [29]. Bacterial crystallization of calcium carbonate block pores of concrete. Capillary sorption of concrete mix is reduced [30].

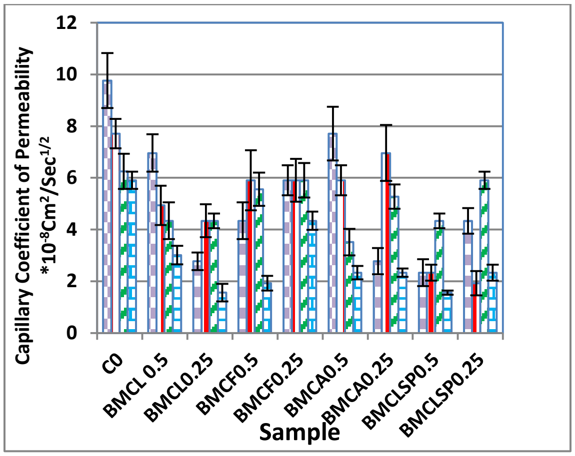


Figure (2): Capillary Permeability Coefficient (K) for Bacterial and Control Concrete after 24hours.

4.2.2Mechanical Properties

4.2.2.1 Compressive Strength

Test results revealed that improvement of compressive strength for all concrete specimens at all ages as shown in Figure (3). For calcium lactate mixtures, it was observed that, compressive strength values of BMCL0.5 were greater than that of compressive strength values of BMCL0.25 and control mixture at all ages. Vijay et al., investigated the effect of *Bacillus subtilis*. They reported that the maximum increase in compressive strength was 12%, observed with the addition of 0.5% of calcium lactate in concrete due to plugging pores with bacterial calcite precipitation [31].

For calcium formate mixtures, test results showed that compressive strength values of BMCF0.25 were greater than that of BMCF0.5 values and control mixture as shown in Figure (3). Ganendra et al., investigated the effect of calcium formate concentration and bacterial cell densities on calcium carbonate precipitation. They used 10^6, 10^7, 10^8, and 10^9 *Methylocystis parvus* OBBP cells liter^{-1} concentrations and 0.5, 2.5, 5, and 10 g of calcium formate liter^{-1}. Results showed that maximum calcium carbonate precipitation was obtained from calcium formate concentration 5g/litre and cultures 10^9cells/mL. They concluded that a high calcium formate addition increased salt stress imposed on bacterial cells, which decrease their activity [32].

For calcium acetate mixtures, it was noticed that, compressive strength values of BMCA0.5 were greater than that of BMCA0.25 values and control mixture at all ages.

For calcium lactate mixtures with or without super plasticizer, it was noticed that compressive strength values of BMCL0.5 were greater than that of compressive strength values of BMCL0.25, BMCLSP0.5, BMCLSP0.25, and control mixture at all ages. Maximum increment was at 120 days. Compressive strength values of BMCL0.25, BMCLSP0.5, BMCLSP0.25, and control mixture were 82%, 87.82%, 77.1%, and 75.5% of compressive strength value of BMCL0.5 mixture at 120days, respectively.

Compressive strength values of BMCLSP0.5 were greater than that of compressive strength values of BMCLSP0.25 and control mixture. Compressive strength values of BMCLSP0.25 and control mixture were 87.8% and 86.3% of compressive strength value of BMCLSP0.5 at 120days, respectively.

Both cells surface and polycarboxylate super plasticizers are negatively charged. Yet, the interaction of bacterial cells with polycarboxylate super plasticizers is unknown [33]. Increase in negatively charged might reduce deposition of Ca^{2+} cations from the environment on the cells surface. Then bacteria are probably gradually formed into endospores. These materials do not share in the process of hydration and therefore, reduce the compressive strength.

At the ages of 7days, it was noticed that compressive strength value of BMCL0.25 and BMCF0.5 was lower than that of control mixture. Compressive strength value of BMCL0.25 and BMCF0.5 were 82.27% and 75.9% of compressive strength value of control mixture, respectively. *B. megaterium* has a slower ability to obtain the precipitation in early age [3].

At the ages of 28 days, it was noticed that compressive strength value of BMCL0.25 increased and became higher than that of compressive strength values of control mixture; also it became equal compressive strength for BMCLSP0.25. At the ages of 90 and 120 days, it was noticed that, compressive strength values of BMCL0.25 recorded another increment and became higher than that of compressive strength values of BMCLSP0.25 and control mixture as shown in Figure (3). It was 105.87% and 108.15% of compressive strength value of control mixture.

At the same ratio of bacteria (0.5%), it was noticed that, compressive strength values of BMCA0.5 had the highest compressive strength among all. It was136.04%, 152.99%, 130.48%, and 134.9% of compressive strength value of control mixture at 7, 28, 90, and 120 days, respectively.

At the same ratio of bacteria (0.25%), at the ages of 7 and 28days, it was noticed that BMCF0.25 had the highest compressive strength among all mixes. At the age of 90 days, it was noticed that, BMCL0.25 had the highest compressive strength among all mixes. At the age of 120 days, it was noticed that compressive strength values of BMCA0.25 increased and became greater than that of compressive strength value of BMCL0.25, BMCF0.25, and BMCLSP0.25 mixtures. Compressive strength values of BMCLSP0.25 and, BMCF0.25 became comparable as shown in Figure (3). From above it was noticed that BMCA0.5 had maximum increment in compressive strength at all ages as shown in Figure (3). Calcium acetate is believed to be a better source of calcium when applying MICP technology to reinforced concrete materials to improve the mechanical properties of microbial concrete [34]. Nagarajan et al., concluded that the addition of *Bacillus megaterium* bacteria increases the compressive strength of concrete. In standard grade concrete the compressive strength is increased up to 10.92% at 28 days by addition of *Bacillus magaterium* bacteria when compared to conventional concrete [35]. Nain et al., stated that *Bacillus megaterium, Bacillus subtilis*, and their consortia showed increased compressive strength by 22.5%, 14.3%, and 15.8% respectively when compared to that of conventional concrete [23]. Achal et al., calcite precipitation investigated the effects of *Bacillus megaterium* ATCC 14581 on compressive strength of fly ash-amended mortar and concrete. At the fly ash concentrations of 10%, 20%, and 40% in mortars, bacterial cell enhanced mortar compressive strength by 19%, 14%, and 10%, respectively, compared to control specimens [26]. Girish et al., concluded concrete cured by distilled water and used *Bacillus subtilis* with 10^8 cells/ml concentration has achieved 52 MPa compressive strength, whereas *Bacillus megaterium* with 10^5 cells/ml concentrations has achieved 57 MPa compressive strength compared to normal concrete strength 44 MPa. Concrete cured by nutrient (peptone) solution and used *Bacillus subtilis* of 10^2-10^4 cells/ml concentration achieved compressive strength 49 MPa, whereas *Bacillus megaterium* with 10^3 had only 44 MPa as same as normal concrete[36]. Andalib et al., investigated the optimum concentration of bacteria by using five different cell concentrations of *Bacillus megaterium* (10 x10^5 to 50 x10^5 cfu/ml). It was concluded that strength was obtained in the case of 30 x 10^5 cfu/ml at different ages. The strength of highest grade of bacterial concrete (50 MPa) had improved by (24%) as compared to lowest grade (30 MPa) (12.8%) due to calcification mechanism [11]. Siddique et al., revealed that addition of bacteria in silica fume (SF) concrete enhances the compressive strength at all ages. Maximum strength of bacterial concrete was observed with 10% SF and was about 12% more than that of the concrete with same silica fume replacement at 56day. Improvement in strength of concrete with bacteria might be due to deposition of the calcite in the pores, subsequently reduction in pores, compact microstructure obtained, made concrete dense and thereby increasing the strength [25]. The bacteria when cultured and added with nutrient broth in the water mix. Bacteria act as a site of nucleation. The bacterial cell surface have negatively charged groups, attracts divalent ions such as Ca^{2+} and Mg^{2+}. Urease enzyme catalyzes the hydrolysis of urea into ammonia and carbonate. Carbonate reacts with Ca^{2+} and thereby forming calcium calcite precipitate on bacterial surface [3, 6]. Calcite plugs the pores and micro cracks. It causes better packing and compaction of the concrete mix around them which gave the specimens much higher strength than controlled concrete specimen [3, 12, 13, 37]. Also, calcium carbonate acts as a catalyst for cement hydration and enhances hydrolyzation at faster rate and hence increases the compressive strength of concrete [3].

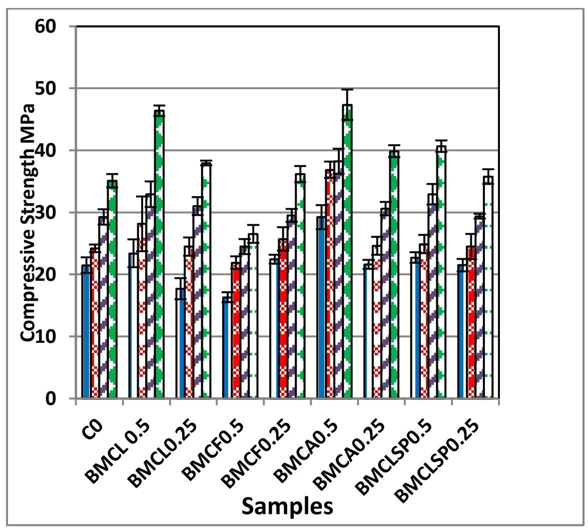


Figure (3): Compressive Strength for All Bacterial and Control Concrete mixes.

4.2.2 Flexural Strength Test

Test results revealed that improvement of compressive strength for all concrete specimens at all ages as illustrated in Figure (4). At all ages, it was noticed that, control mixture achieved least flexural strength value while BMCL0.5 achieved the highest flexural strength value. It was 162.74%, 136.11, and 145% of flexural strength of control specimens at age of 28, 120, and 180 days, respectively. Flexural strength values of all bacterial concrete specimens increased compared to control specimens. For calcium lactate concrete mixtures, it was noticed that flexural strength values of BMCL0.5 was higher than that of flexural strength values of BMCL0.25 and control mixture at all ages. For calcium formate mixtures, test results revealed that flexural strength values of BMCF0.25 recorded increment in all ages with respect to flexural strength values of BMCF0.5 and control concrete mixture as illustrated in Figure (4). For calcium acetate mixtures, test results showed that flexural strength values of BMCA0.5 recorded increment in all ages with respect to flexural strength values of BMCA0.25 and control concrete mixture as illustrated in Figure (4). For calcium lactate mixtures with or without super plasticizer test results showed that flexural strength values of BMCL0.5 were greater than those of flexural strength values of BMCL0.25, BMCLSP0.5, BMCLSP0.25, and control mixture. Flexural strength values of BMCL0.2.5 recorded increment in all ages with respect to flexural strength values of BMCLSP0.25 and control concrete mixture. Flexural strength values of BMCLSP0.5 recorded increment in all ages with respect to flexural strength values of BMCLSP0.25 and control concrete mixture. At the same ratio of bacteria (0.5%), it was noticed that BMCL0.5 had the highest compressive strength among all mixes. At the same ratio of bacteria (0.25%), it was noticed that BMCL0.25 had the highest compressive strength among all mixes. Biomineralization leads to overall compaction of concrete matrix and therefore, resists bending failure [6]. Nagarajan et al., concluded that the addition of *Bascillus megaterium* bacteria showed significant improvement in the split tensile strength and flextural strength than the conventional concrete [35]. Vighnesh et al., investigated the effects of *Bacillus megaterium* MTCC 3353 on flexural strength of metakaolin amended concrete of grade (25 Mpa and 40 Mpa), cement was partially substituted by metakaolin (10, 12.5 and 15%) for bacterial specimens (BS). It was concluded that BS + 10% and BS + 12.5% were the optimal for the two mixes, respectively, and offered a greater strength than control at 28 days. It can be attributed to the bacterial action and reactions of metakaolin. In Mix 1, BS + 10% provided 5 MPa (19.04%), while the other cases offered 4.6 MPa (9.53%) compared to typical concrete (4.2 MPa). In Mix 2, BS + 12.5% provided 7.2 MPa (20%), while the others resulted in 6.4 MPa (6%) and 6.8 MPa (13.3%) respectively compared to ordinary concrete (6 MPa) [38]. Durga et al., concluded that the self-healing concretes (using *Bacillus halodurans* with a concentration of 10^5 cells/ml of water cured for 14days and 28days had a 11% and 28% higher flexural strength than the conventional concrete, respectively [39].

Shashank et al., investigated the effect of three different bacterial species *(Bacillus subtilis, Bacillus sphaericus and Bacillus pasteurii*) for different bacterial cell concentrations, 10^4 to 10^7on flexural strength for 28 days. It was concluded that as cell concentration increased, the strength of the concrete also got increased. But for 10^4 and 10^5 a slight increase was observed compared to 10^6 and 10^7 where a maximum of 36% increase was observed [27]. Ganesh et al., investigated the effect of bacterial and fiber combination on the properties of GGBS based geopolymer concrete to be used as paver block. Bacterial combinations such as *Bacillus Subtillis and Bacillus Sphaericus* and high modulus glass fibers and low modulus polypropylene fibers were incorporated to produce hybrid fiber reinforced bacterial geopolymer concrete. It was concluded that Incorporation of 0.75% of glass fiber and 0.25% of polypropylene fiber, along with the bacteria increased the flexural strength 53.70% than the conventional geopolymer concrete without fiber and bacteria [40]. Nain et al., concluded that once the pores were plugged, the flow of nutrients and oxygen to bacteria cells stopped, gradually cells form into endospores and acts as organic fibre, thus increasing strength. Bacteria from genus *Bacillus* are not only proved to be efficient in crack healing ability but also play a great role to increase the strength [3].

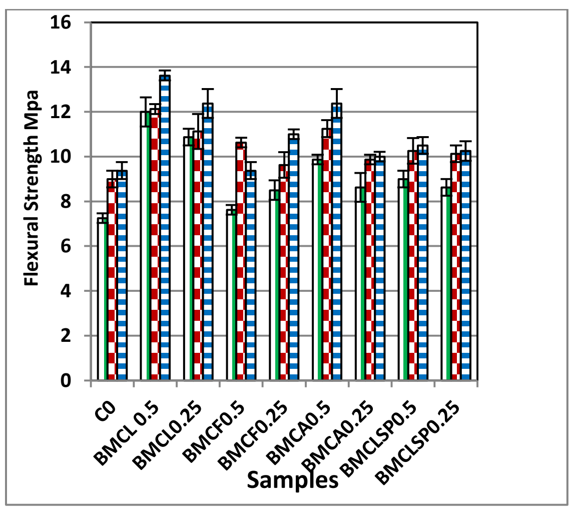


Figure (4): Development of Flexural Strength for All Bacterial and Control Concrete mixes.

4.3 Scanning Electron Microscopy (SEM)

A scanning electron microscope (SEM) is a type of electron microscope that produces image of a sample by scanning it with a focused beam of electrons. The electrons interact with electrons in the sample, producing various signals that can be detected, which contains information about the sample's surface topography and composition. The electron beam is generally scanned in a raster pattern, and the beam's position is combined with the detected signal to produce the image. Figure (5) shows the microstructure and surface morphology of bacterial concrete. Calcite crystals help in bridging concrete cracks as shown in Figure (5.a, b) which was proven by Girish et al., [36]. Calcite is precipitated as result of chemical reaction; precipitation shapes are different according to the added nutrient. Crystals observed as a barrier using Calcium lactate and super plastisizer Figure (5.c, d). Rhombohedra morphology of calcite crystals was observed using calcium Formate as shown in figure (5.e, f). Those crystals have SEM imaging in many papers [15, 16, 28]. SEM image examination revealed that bacterial concrete matrix using a super plasticizer was more compact and dense.

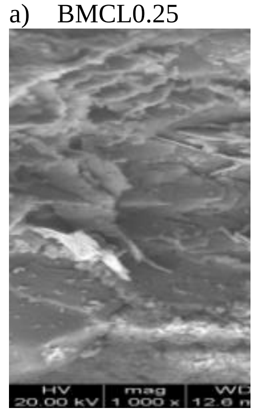
5. Conclusions

Based on the present experimental investigations, the following conclusions are drawn:

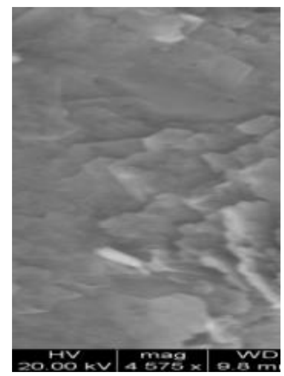
1. Inclusion of *Bacillus megaterium* in concrete and all nutrition's used showed varied decrease in rate of water absorption and capillary permeability coefficient.
2. *Bacillus megaterium* and different organic compounds had positive effect on compressive strength. Among all mixes**,** mix *Bacillus megaterium* 0.5% with acetate nutrition (BMCA-0.25) had maximum increment in compressive strength at all ages. It had compressive strength values of 136.04%, 152.99%, 130.48%, and 134.9% that of compressive strength of control concrete mixture at 7, 28, 90, and 120 days, respectively.
3. Inclusion of bacteria and organic additives enhanced flexural strength. *Bm* 0.5% with lactate nutrition concrete mix had maximum increase in flexural strength at all ages. It had flexural strength values 162.74%, 136.11% and 145% that of flexural strength of control concrete mixtures at 28, 90 and 120 days, respectively.
4. SEM image examination revealed that bacterial concrete matrix precipitation shapes are different according to the added nutrient.

a) BMCL0.25

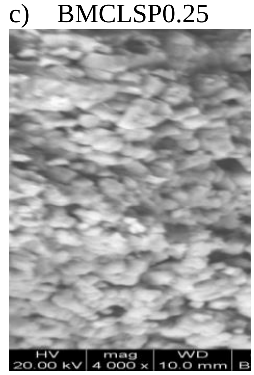
b) BMCL0.25



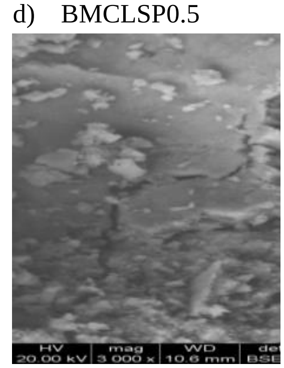
c) BMCLSP0.25



d) BMCLSP0.5



e) BMCF0.25



f) BMCF0.25

Figure (5): SEM Analysis of *Bacillus Megaterium* Bacterial Concrete (a), (b) 0.25% with Lactate Nutrition, (c), (d) Lactate Nutrition Using Super plasticizer, and (e), (f) 0.25% with Formate nutrition.

6. References

[1] Prabowo, H., (2020), "Durability Based Service Life Prediction of Bacterial Concrete", Journal of Physics: Conference Series, Vol. 1469, pp. 1-6.

[2] Jacob, C. S. , and Jose, V., (2019), "Study on Mechanical Properties of Self-Healing Self Curing Concrete", National Conference on Structural Engineering and Construction Management, Proceedings of SECON'19, Angamaly, India, pp. 935-943.

[3] Nain, N., Surabhi, R., Yathish, N. V., Krishnamurthy, V., Deepa, T., and Tharannum, S., (2019), " Enhancement in Strength Parameters of Concrete by Application of *Bacillus* Bacteria", Construction and Building Materials, Vol. 202, `pp. 904-908.

[4] Stanaszek-Tomal,E.,(2020),"Bacterial Concrete as a Sustainable Building Material?", Sustainability, Vol. 12, pp. 1-13.

[5] Joshi, S., Goyal, S., and Reddy, M. S.,(2018), "Influence of Nutrient Components of Media on Structural Properties of Concrete during Biocementation", Construction and Building Materials, Vol. 158, pp. 601-613.

[6] Chaurasia, L., Bisht, V., Singh, L.P., and Gupta, S., (2019), "A Novel Approach of Biomineralization for Improving Micro and Macro-Properties of Concrete", Construction and Building Materials, Vol. 195, pp.340–351.

[7] Dhami, N. K., Reddy, M. S.,and Mukherjee, A., (2013), "*Bacillus Megaterium* Mediated Mineralization of Calcium Carbonate as Biogenic Surface Treatment of Green Building Materials", World J Microbiol Biotechnol, DOI 10.1007/s11274-013-1408-z.

[8] Joshi, S., Goyal, S., and Reddy, M. S.,(2018), "Influence of Nutrient Components of Media on Structural Properties of Concrete during Biocementation", Construction and Building Materials, Vol. 158, pp. 601-613.

[9] Krishnapriyaa, S., Babub, D.L. V., Prince, A. G.,(2015), "Isolation and Identification of Bacteria to Improve the Strength of Concrete", Microbiological Research, Vol. 174, pp.48–55.

[10] Kaur, G., Dhami, N. K., Goyal S., Mukherjee, A., and Reddy M. S.,(2015), "Utilization of Carbon Dioxide as An Alternative to Urea in Biocementation", Construction and Building Materials, Vol. 123 pp. 527–533.

[11] Andalib, R., M. Z., Hussin, M. W., Ponraj, M., Keyvanfar, A., Mirza, J., and Lee, H-S.,(2016), "Optimum Concentration of *Bacillus Megaterium* for Strengthening Structural Concrete", Construction and Building Materials, Vol. 118 , pp.180–193.

[12] Krishnapriyaa, S., Babub, D.L. V., Prince, A. G.,(2015), "Isolation and Identification of Bacteria to Improve the Strength of Concrete", Microbiological Research, Vol. 174, pp.48-55.

[13] Vashisht, R., and Shukla. A., (2020), "Potential Application of Bacteria to Improve the Self-Healing and Strength of Concrete", Journal of Building Pathology and Rehabilitation, Vol.5, No.10.

[14] Vashisht, R., Attri, S., Sharma, D., Shukla, A., and Goel, G., (2018), "Monitoring Biocalcification Potential of *Lysinibacillus* sp. Isolated from Alluvial Soils for Improved Compressive Strength of Concrete", Microbiological Research, Vol. 2o7, pp.226-231.

[15] Zahran, M. A., and Nasser, A.A., (2014), "Self-healing of Cracked Concrete with Bacterial Approach", Eng Res J, Vol. 37, No. 2, pp. 255–264.

[16] Ahmed S. O., Nasser, A. A., Abbas, R.N., Kamal, M. M., Zahran, M. A., and Sorour, N. M., (2021), " Production of bioconcrete with improved durability properties using Alkaliphilic Egyptian bacteria", 3 Biotech (2021) 11:231.

[17] Seng, P., Rolain, J. M., Fournier, P.E., LaScola, B., Drancourt, M., and Raoult, D.,(2010),"MALDI-TOF-Mass Pectrometry Applications in Clinical Microbiology", Future Microbial, Vol. 5, No. 11, pp. 1733-1754.

[18] Egyptian Standard Specifications, "Composition, specifications and conformity criteria for common cements", Part 1, ESS,

4756-1, 2007.

[19] Egyptian Standards Specification, "concrete aggregates from natural sources", ESS 1109-2002.

[20] Egyptian Code for Concrete Structures, 203-2018.

[21] Pokhrel, U.,(2019) , "Role of Mixing of Two Bacteria on Compressive Strength, Water Absorption and Microstructure of Mortar Cubes", M.s Thesis, Faculty of the North Dakota State University of Agriculture and Applied Science, Fargo, North Dakota, 77p..

[22] Givi, A. N., Rashid, S. A., Aziz, F. N. and Salleh, M. A. M., (2010), "The Effects of Lime Solution on the Properties of SiO2 Nanoparticles Binary Blended Concrete", Composites: Part B, Elsevier Journal, Vol. 42, pp. 562-569.

[23] Egyptian Standard Specifications, "Cement physical and mechanical tests", Part 2: Determination of fitness, Rep. No. 2421, 2005.

[24] Ramadas, A., Shobana, K.,and Samuel, T.M.,(2019), "A Review on Self-Healing Concrete", International Research Journal of Engineering and Technology(IRJET), Vol. 6, Issue.3, pp.762-766.

[25] Siddique, R., Jameel, A., Singh, M., Barnat-Hunek, D., Kunal., Aït-Mokhtar, A., Belarbi, R., and Rajor, A., (2017), "Effect of Bacteria on Strength, Permeation Characteristics and Micro-Structure of Silica Fume Concrete", Construction and Building Materials, Vol. 142 , pp.92–100.

[26] Achal, V., Pana, X., and zyurtb , N., (2011), "Improved Strength and Durability of Fly Ash-Amended Concrete by Microbial", Ecological Engineering , Vol. 37, `pp. 554–559.

[27] Shashank, B. S., and Nagaraja P. S., (2021) , "Durability Studies on Low-Strength Bacterial Concrete", Advances in Sustainable Construction Materials, Vol.124, pp 639-650.

[28] Kadapurea, S. A., Kulkarnib, G., Prakashc, K.B., and Kadapured, P. S., (2020) , "Mechanical and Durability Performance of Sustainable Bacteria Blended Fly Ash Concrete: An Experimental Study", International Journal of Sustainable Engineering, Vol. 13, No. 1, pp. 45-53.

[29] Chahal, N., Siddique, R., and Rajor, A., (2012), "Influence of Bacteria on the Compressive Strength, Water Absorption and Rapid Chloride Permeability of Concrete Incorporating Silica Fume", Construction and Building Materials, Vol. 37, pp. 645–651.

[30] Kaur, G., Dhami, N. K., Goyal S., Mukherjee, A., and Reddy M. S.,(2015), "Utilization of Carbon Dioxide as An Alternative to Urea in Biocementation", Construction and Building Materials, Vol. 123 pp. 527–533.

[31] Vijay, K., and Murmu, M., (2019), "Effect of Calcium Lactate on Compressive Strength and Self-Healing of Cracks in Microbial Concrete", Front. Struct. Civ. Eng, Vol. 13, No. 3, pp. 515–525.

[32] Ganendra, G., De Muynck, W., Ho, A., Arvaniti, E. C., , Hosseinkhani, B., Ramos, J. A., Rahier, H., and Boon, N., (2014) , " Formate Oxidation-Driven Calcium Carbonate Precipitation by Methylocystis parvus OBBP", Applied and Environmental Microbiology,Vol. 80, No. 15, pp.4659-4667.

[33] Azima, M., and Bundur, Z. B., (2019), "Influence of Sporasarcina Pasteurii Cells on Rheological Properties of Cement Paste", Construction and Building Materials, Vol. 225, pp.1086–1097.

[34] Zhang, Y., Guo, H. X., and Cheng, X. H., (2015), "Role of Calcium Sources in the Strength and Microstructure of Microbial Mortar", Construction and Building Materials, Vol.77. pp. 160–167.

[35] Nagarajan, V., Prabhu, T. K., Shankar, M. G., and Jagadesh, P.,(2017), " A Study on the Strength of the Bacterial Concrete Embedded with *Bacillus Megaterium*", International Research Journal of Engineering and Technology (IRJET), Vol. 4, Issue,12, pp. 1784-1788.

[36] Girish, Soumya,T., Girish, S., (2020), "An Experimental Study on Self-Remediating Bacterial Concrete", Trends in Civil Engineering and Challenges for Sustainability, Vol. 99, pp. 283-293.

[37] Jose, S., Reshma, G. V., Rodrigues, A., John, J.,and Venu, D.,(2018) "A Study on Strength Characteristics of Bacterial Concrete" International Research Journal of Engineering and Technology (IRJET), Vol. 5, Issue, 3, pp. 2939- 2942.

[38] Vighnesh, R., Kumar, P,R, S., Poornima, V., Venkatasubramani, R., and Sreevidya, V., (2020), "Improvements in Mechanical and Durability Parameters of Bio-Engineered Concrete with Metakaolin as A Partial Substitute for Cemen", European Journal of Environmental and Civil Engineering, Published online, https://doi.org/10.1080/19648189.2020.1767696.

[39] Durga, C. S., Ruben, N., Chand, M. S. R., and Venkatesh, C., (2019), "Evaluation of Mechanical Parameters of Bacterial Concrete ", Annales de Chimie - Science des Matériaux, Vol. 43, No. 6, pp. 395-399.

[40] Ganesh, C., Muthukannan, M., Kumar, A. S., and Arunkumar, K., (2021), "Influence of Bacterial Strain Combination in Hybrid Fiber Reinforced Geopolymer Concrete subjected to Heavy and Very Heavy Traffic Condition", Journal of Advanced Concrete Technology, Vol. 19,pp. 359-369.