ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY (AAIT)
SCHOOL OF CHEMICAL AND BIO ENGINEERING
BIOCHEMICAL ENGINEERING STREAM

Optimization of Parameters to Improve the Strength of Concrete Cube Using Bacteria Species

A thesis submitted to the Research and Graduate School of Addis Ababa Institute of Technology, School of Chemical and Bio engineering in partial fulfillment of the requirements for the attainment of the Degree of Master of Science in Chemical Engineering under Biochemical Engineering Stream

By Eman Negash

Advisor: Dr Solomon Kiros

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ADDIS ABABA, ETHIOPIA

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

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DECLARATION

I declare that this thesis for the M.Sc. Degree at Addis Ababa University, here by submitted by me, is my original work and have not previously been submitted for the degree at this or any other university, and that all resources of materials used in this thesis have been duly acknowledged.

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This thesis has been submitted for examination with my approval as University Advisor.

Name: Dr. Solomon Kiros

Signature: __________

Date: __________

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Finally, I greatly thank to Ethiopia Biodiversity Institute give me the isolated bacteria with great appreciate and Ethiopia Industry Development Institute (ELIDI) allow me work Scanning Electron Microscopy (SEM).

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ANOVA	Analysis of variance
$CaCO_3$	Calcium carbonate
CO_2	Carbone dioxide
CA	Coarse Aggregate
DF	Degree of Freedom
ELIDI	Ethiopia Lither Industry Development Institute
ES	Ethiopian Standard
FA	Fine Aggregate
MPa	Mega Pascal
MEOR	Microbial Enhanced Oil Recovery
MICP	Microbiologically Induced Carbonate Precipitation
OPC	Ordinary Portland cement
PH	potential of hydrogen
RSM	Response Surface Methodology
SEM	Scanning Electron Microscopy
UTM	Universal Testing Machine
XRD	X-ray diffraction

ABSTRACT

Concrete is the most commonly used building material, but the cracks in concrete create problem it was main reason for a decreased service life of concrete structures. Most research done used chemical method which not effective on cost and environmental. So, need of a new technology to overcome the bottle necking of concrete has led to the development of a very special concrete known as Bacterial Concrete. The objective of this thesis was optimizing of parameters to improve the strength of concrete using bacteria bacillus species (*Bacillus subitilis*, *Sporosarcina pasteurii* and *Bacillus cibi*) self-healing method. Proceed was the bacterial strains were obtained from Ethiopia Biodiversity Institute and for each bacterial growth in nutrient broth solution for 24-72 hour. Secondly, according to the mixing design twenty seven concrete mixes for each day and for each bacterial at C25 grades of concrete using water cement ratio 0.58, 0.53 and 0.45 and cement, fine aggregate and course aggregate contents of 360 kg / m ^{3,} 800 kg / m ^{3} and 1087.5 kg / m ^{3} were prepared, respectively and tested in 7, 14 and 28 day. Significance of the process variables were analyzed using analysis of variance (ANOVA). Thus, the influence of all experimental variables, factors and interaction effects on the response was investigated. Dosage, time, water/cement ratio and in the case of bacteria *Bacillus cibi* use interaction between dosage and water cement ratio have significance effect on the compressive strength. As the result of RSM optimization, the best compressive strength were found on the 0.45 water cement ratio, 40 ml of dosage and the time after 14day. Also from the three bacterial *Sporosarcina pasteurii* selectable or give high compressive strength.

1. INTRODUCTION

1.1 Background

Concrete is an important and extensively used building material used for construction works. It is mainly used in heavy constructions because of its strength and durability. It is made up of two components, aggregates and paste. Aggregates are generally classified into two groups, fine and coarse, and occupy about 60 to 80 percent of the volume of concrete. The paste is composed of cement, water, and entrained air and ordinarily constitutes 20 to 40 percent of the total volume.

In construction engineering consumes a huge amount of energy and materials from non-renewable resources, which most of the materials contribute CO_2 emission to the air at their production or application stage (Ariyanti et al., 2012). Production of cement, which is a major component of concrete for construction material, is energy-consuming and environmentally-unfriendly. Energy represents 20–40 % of the total cost of cement production because temperature above 950 ºC is needed for transformation of limestone to cement clinker (Ivanov et al., 2015). New construction materials, such as microbial bio cement, can be produced from limestone, dolomite, or iron ore at temperature 20–60 ºC with less than 10 % of energy used for the manufacturing of conventional cement. Therefore, cost of microbial-based bio cementing can be lower than that of conventional cement, sustainability due to production from organic matter, low viscosity, and low risk of negative environmental consequences. Therefore, technology development related to the bio construction material and their production is necessary, in order to maintain the sustainability, save energy consumption and to reduce the production of CO_2 emission (Reddy et al., 2015). Moreover, the buildings and traditional structures constructed by conventional cement was get deteriorated after a given time. Therefore self-healing using bio cement is necessary to enhance the durability of building structures and the conservation of cultural heritage (Ivanov et al., 2015; Reddy et al., 2015; Ariyanti et al., 2012), as well as increases the workability of concrete, enhances the strength and improves its durability (Sato et al., 2003).

Xie et al (2012) confirmed these earlier finding that the compressive strength decreases with the increase in the maximum coarse aggregates size it have different particle sizes Viz.37.5 mm, 19 mm, 12.5 mm, 9.5 mm and 4.75 mm. Bhiskshma and Florence assessed the effect of size in higher grade concrete using high volume fly ash and found out that maximum size of coarse aggregates of 12.5 mm gave the highest compressive, splitting and flexural strengths; hence the aggregate sizes influence strength. Whereas, Zhou et al. (1995) concluded that, in contrast to other studies, increasing the coarse aggregate size from 10 mm (0.4 in.) to 20 mm (0.8 in.) increases the compressive strength of the concrete by about 10 percent.

No matter how carefully the concrete is mixed or reinforced it eventually ends up in cracking at some point and time. There are many factors that affect the durability and strength of concrete, such as natural, physical, chemical and biological factors, which cause cracks in concrete. Among these, natural factors, such as weathering, faults, earthquakes and human activities are the causes for cracks in concrete. Large cracks may affect the structural integrity while the small cracks reduce the durability of the structure. Cracks also increase the permeability of matrix there by increasing the chances of corrosion in reinforcement. Concrete is porous in nature due to which it easily absorbs and releases water. This absorbed water can freeze within the concrete and creating freeze-thaw conditions that are prone to spalling and cracking. In order to reduce the chances of crack formation a structure requires regular maintenance which can be costly and may further increase the maintenance cost of the structure. One way to reduce such costs and to increase the durability of structure is to use a concrete that has self-healing mechanism. This technique is based on bio mineralization of bacteria in concrete, a biological process commonly seen in few forms of microorganisms. The strength and durability can be increased by using these microorganisms as binders and fillers in concrete. Epoxy treatment is currently used for the repair works which is harmful to the environment and health as toxic fumes and gases evolved may cause serious skin and breathing problems. Hence the use of biological techniques should be focused. (Jasira B., Ifrah K., Aditya T. and Khushpreet S., 2016)

Self-healing bacterial concrete can be applicable for sectors such as tunnel-lining, structural basement walls, highway bridges, and concrete floors marine structures, as well as used for repairing of monuments constructed in limestone, healing of concrete cracks, used for construction of low cost durable roads, high strength building, river banks and low cost durable housing. Self-healing can be prepared in two ways by direct application and encapsulation in light weight concrete. In direct application, bacterial spores and calcium lactate are added directly while making the concrete and mixed. Here when the crack occurs in the concrete bacterial spores broke and bacteria come to life and feed on the calcium lactate and produced limestone which is filling the cracks. The encapsulation method, the bacteria and its food, calcium lactate, are placed inside treated clay pellets and concrete is made. About 6% of the clay pellets are added for making bacterial concrete. When concrete structures are made with bacterial concrete, then the crack occurs in the structure and clay pellets are broken and bacterial treatment occurs and hence the concrete is healed. Minor cracks about 0.5mm width can be treated by using bacterial concrete. (Gaurav A. and Rahul K., 2017)

Microbiologically Induced Carbonate Precipitation (MICP), resulting from metabolic activities of some specific microorganisms in concrete, is an alternative technique to overcome some of the shortcomings of conventional concrete such as poor tensile and impact strength, limited resistance to corrosion & acids, poor behavior under severe conditions and poor adhesion of fresh mortar or concrete to old concrete. The importance of selective cementation has been widely recognized in civil engineering, which is also related to calcite precipitation. It has been known that microorganisms play an important role in promoting calcite precipitation. Therefore bacterial induced calcium carbonate deposition has been proposed as an alternative and environment friendly technique. Also, microorganisms can be used as construction material which enhances the serviceability of infrastructures and protects the environment. (Dr. Asmita D., Parnnika and Harshal H., 2013). Microbial mineral precipitation resulting from metabolic activities of favorable microorganisms in concrete improved the overall behavior of concrete. The process can occur inside or outside the microbial cell or even some distance away within the concrete. Often bacterial activities simply trigger a change in solution chemistry that leads to over saturation and mineral precipitation. Use of these Bio mineralogy concepts in concrete leads to potential invention of new material called - Bacterial Concrete. (Akshaykumar A., Dr. Jayeshkumar P., Dr. Digvijaysinh R., 2017)

There are different types of bacteria which can survive in a high pH environment. Among strains of the bacteria genus Bacillus was found to thrive in this high-alkaline environment. It is found that the only group of bacteria that was able to survive is the ones that produced spores comparable to plant seeds. Such spores have extremely thick cell walls that enable them to remain intact for up to 200 years while waiting for a better environment to germinate. They would become activated when the concrete starts to crack, food is available, and water seeps into the structure. This process lowers the pH of the highly alkaline concrete to values in the range (pH 10 to 11.5) where the bacterial spores become activated (Vijeth N. and Radhakrishna, 2013).

Generally MICP is the process of the microbial urease hydrolyzes urea, to produce ammonia and CO_2 and ammonia released in surrounding subsequently the increasing of pH, leading to accumulations of insoluble calcium carbonate.

Bacteria constitute a large domain of prokaryotic microorganisms. Typically a few micrometers in length, bacteria have a number of shapes, ranging from spheres to rods and spirals. Bacteria were among the first life forms to appear on Earth, and are present in most of its habitats. Bacteria inhabit soil, water, acidic hot springs, radioactive waste, and the deep portions of Earth's crust. *Sporosarcina pasteurii* formerly known as *Bacillus pasteurii* is a bacterium with the ability to precipitate calcium carbonate in the presence of any carbonate source. This microorganism is well known for its resistive properties to resist the harsh conditions of sea water and very high temperatures which are generally found in shallow waters and in Sahara desert. *B. pasteurii* is a soil-borne microbe that is known to be non-toxigenic and non-pathogenic to the human species. Although it is not thought to be an indigenous inhabitant of the human intestine, it has been isolated from human feces and is considered a contaminated of the gut. Convert urea to ammonium carbonate more actively than any known bacterium. *Sporosarcina pasteurii* is a non-pathogenic, endospore producing soil bacteria that also produces urease and can tolerate highly basic environments. *Bacillus subtilis* is Gram – Positive bacterium, rod - shaped and catalase positive also can divide symmetrically to make two daughter cells (Binary Fission), or symmetrically producing a single endospore that can remain viable for decades and is resistant to unfavorable environmental condition such as drought, salinity, extreme pH, radiation. It is found in soil, water, air decomposing plant matter.

In most research work on bio concrete not optimize some factor on the strength of the concrete basically in Ethiopia generally not research work on this method which importee from some other foreign country .

The objective of this study was to investigate the effect of bio cement on the compressive strength through identify potent microbes; as well as to determine optimum dosage, time and water cement ratio on concert.

1.2. Statement of problem

Cement is a second most consumed material on the planet next to water, is an essential component of infrastructure development and most important input for construction industry, particularly in infrastructure and housing programs, which are necessary for the socioeconomic growth and development. The trend most Easter in Africa countries indicate that consumption of cement has shown drastic increase. In Ethiopia currently more than 16 cement plants are established and produce cement to fulfill the demand. In the production time each ton of cement production requires approximately 105 KWh of electricity and 60-130 Kg of fuel oil contingent upon the variety and process used, as per estimate by Global cement, release some greenhouse gases, As will as there were also some challenge like after it use for building there were need of water drinking the concrete at least for 7 day and in some building grow the plant on there this decrease the shelf life therefore the building easily damage in earthquake or other natural case so, in order to solve this kind of problem need some techniques that was cost effective and environmental friend techniques is self – healing mechanism. This mechanism use microbe that filling the crack and multiple treatments control the strength developed and some other advantage in some research work on it but not working on optimization and use different microbe mainly according to in Ethiopia completely not work on this mechanism in construction or in building.

1.3. Objective

1.3.1 General objective

The main objective of this research is Optimization of Parameters to Improve the Strength of Concrete Cube Using Bacteria Species

1.3.2 Specific object

- Identify the appropriate microbes (bacteria) from the three bacillus for bio cement production.
- To study the effect of isolated potent microbes on compressive strength
- To find optimum, dosage, time, and water cement ratio conditions for bio cement production.
- To developed optimized process for successful strength and reduce cracking.

1.4. Significance

The significance of this thesis is the use of micro-organisms in strength of concrete. The study has identified the amount of biomass (micro-organisms) on the compressive strength of Portland cement mortar cubes. Furthermore, finding the optimum time, microbe dosage and water cement ratio for solving the problem of concrete. Provide the basis for an alternative and high quality concrete sealant that is cost effective and environmentally safe.

2. LITERATURE REVIEW

A novel technique for the remediation of damaged structural formations has been employing a selective microbial plugging process in which microbial metabolic activities promote precipitation of calcium carbonate in the form of calcite. As a microbial sealant, $CaCO_3$ exhibited its positive potential to selectively consolidate simulated fractures and surface fissures in granites. Significance of microbial calcite precipitation is quantified by x-ray diffraction (XRD) analysis and visualized by scanning electron microscopy (SEM). In the authors' studies on sand consolidation, microbiologically –induced calcite constituted more than 30% of consolidated material in which microbial calcite plugging was affected by the porosity of the medium, the number of cells present, and the total volume of nutrient added. In crack remediation of granite, an average crack width of 2.7 mm (0.106 in.) and a mixture of silica fume (10%) and sand (90%) showed the highest compressive strength in microbial remediation of granite. Earlier, Kantzas et al. reported that sand consolidation by *B.pasteurii* reduced porosity by up to 50% and permeability by up to 90% in the areas where the cementation took place.

The concept of using microbial mineral precipitation in the remediation of cracks has been applied in microbial enhanced oil recovery (MEOR), which has benefited the oil industry by effectively utilizing all the mineral resources from a particular digging site. It is important to realize that microorganisms plug the pores of rock not only by acting as small particles but also by adhering to the available surfaces through extracellular organic compounds. Biotechnology of microbial crack remediation is therefore equally applicable to those of man-made structures, including concrete.

Cracking of concrete is a common phenomenon. Without immediate and proper treatment, cracks in concrete structures tend to expand further and eventually require costly repair. Even though it possible to reduce the extent of cracking by available modern technology. There are a large number of products available commercially for repairing cracks in concrete; structural epoxy, resins, epoxy mortar, and other synthetic mixtures. Currently, these of filler agent are extensively used in concrete crack repair. Because cracking in concrete in concrete structures continues over a long period of time, this type of one time, quick remedy should also be applied repeatedly as needed. (Santhosh K., V.Ramakrishnan and Sookle S.B., 2001)

Matrix capillary water of young concrete is typically characterized by pH values between 11 and 13. Bacteria added to the concrete mixture thus do not only have to resist mechanical stresses due to mixing but should also be able to withstand a high alkalinity for prolonged periods. Most promising bacterial agents for incorporation in the concrete matrix therefore appear to be alkaliphilic (alkali-resistant) spore-forming bacteria. As the concrete matrix is toxic due to ingress oxygen (diffusion through matrix capillaries) incorporated bacteria also need to be oxygen tolerant. Such aerobic alkaliphilic spore-forming bacteria occur within the genus Bacillus, and several representatives of these were therefore selected to test their applicability as healing agent in concrete.

Cement and water have a pH value of up to 13 when mixed together, usually a hostile environment for life most organisms die in an environment with a pH value of 10 or above. The search concentrated on microbes that thrive in alkaline environments which can be found in natural environments. Samples of endolithic bacteria (bacteria that can live inside stones) were collected along with bacteria found in sediments in the lakes. Strains of the bacteria genus Bacillus was found to thrive in this high-alkaline environment. *Bacillus pasteurii* had the growth in pH range of 7-9 and *Bacillus sphaericus* was 8-9. (Johnna H.K. and schlangen E. 2008).

Introducing the bacteria into the concrete makes it very beneficial it improves the property of the concrete which is more than the conventional concrete. Bacteria repair the cracks in concrete by producing the calcium carbonate crystal which block the cracks and repair it. Many researchers done their work on the self-healing nature of concrete and they had found the following result that bacteria improves the property of conventional concrete such as increase in 13.75% strength increased in 3 days, 14.28% in 7 days and 18.35% in 28 days. The development of calcium carbonate crystal Decreases the water permeability by decreasing the width of cracks from 0.5 mm to 0.35 mm. Compressive strength was increases by 30.76% in 3 days, 46.15% in 7 days and 32.21% in 28 days and in mathematical modal it was found that the bacterial concrete shows the better value of stress and strain as compared to controlled concrete for the high strength grade of concrete. (Rajaratnam S., Srinivasa R. and Sasikala Ch)

In below figure 2.1 shows self- healing process in bacterial concrete. In A, water inter from the left in to a micro crack activating the embedded bacterial spores. In B, the active bacterium seals the cracks with the production of limestone, protecting the embedded steel reinforcement (brown bar) from attack and erosion.

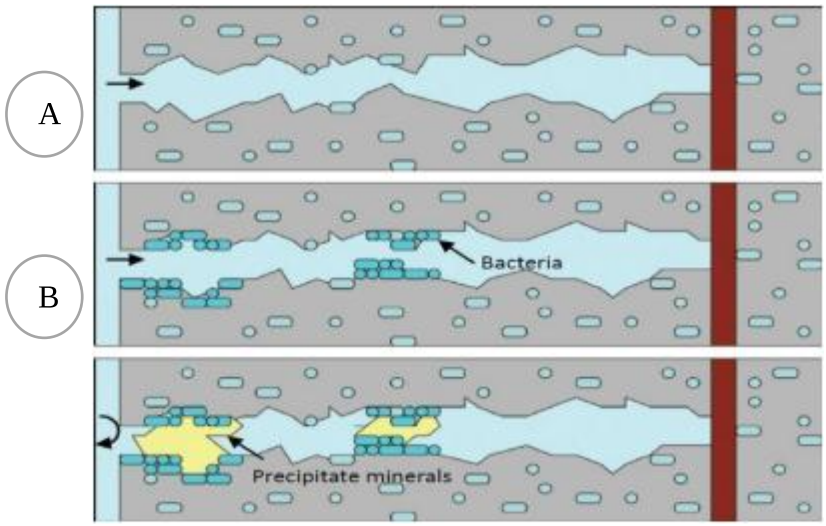


Figure 2.1 Schematic scenario of crack-healing by concrete immobilized bacteria (Suyog S. Pawar, Prof. S. R. Parekar, 2018)

Bacteria cell wall has negative charge and because of this reason, it is able to attract positively charged calcium ions (Ca^{2+}) to deposit on their cell wall surface (Eqn. 2.1). The Ca^{2+} ions then react with ions leading to the precipitation of calcium carbonate ($CaCO_3$) at the cell surface as shown in Eqn. (2.2). This precipitation serves as nucleation sit

$$Ca^{2+} + Cell \longrightarrow Cell - Ca^{2+} \qquad \text{Eqn. (2.1)}$$

$$Cell - Ca^{2+} + CO_3^{2-} \longrightarrow Ca\ CO_3 - Cell \downarrow \qquad \text{Eqn. (2.2)}$$

Bacillus is a type of bacteria that can produce $CaCO_3$ as a filler material and serve as a binding factor in concrete. $CaCO_3$ can reduce capillary pores of concrete and improve durability and compressive strength. This characteristic feature is noticed in some concrete structures by healing and sealing the micro cracks. Hence concrete permeability was significantly reduced according to Jonkers et al (2008).

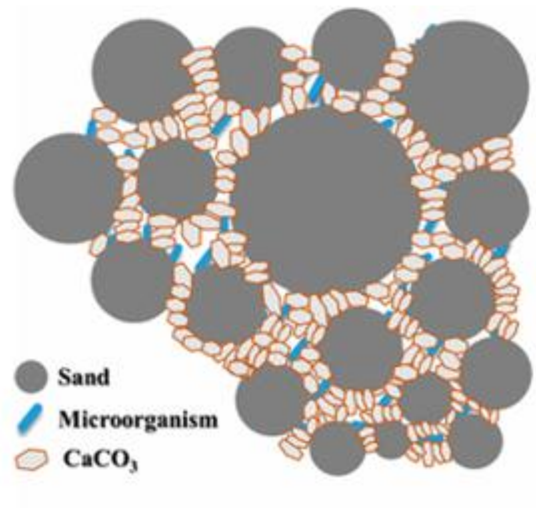


Figure 2.2 Biocementation formation based on $CaCO_3$ - precipitation

Following are the critical literature on various papers based on experimental research work on use of bacteria into the concrete.

G. Mohit and P. Krishna Chaitanya, this paper has presented that, experimental investigation on bacterial concrete by *B.pasteurii* bacterial species and also compare the results with the normal concrete within this cement is replacing by 30% of Ground Granulated Blast Furnace slag (GGBS) and fly ash in M25 concrete grade. Different tests have been conducted in this investigation tests like X- Ray diffraction test, compressive strength, flexural strength, slump flow test and split tensile strength for several sample of 40 ml, 50 ml, and 60 ml bacterial concentrations for each sample. The compressive strength improved by 30% in bio concrete and bacterial concrete with fly ash by 15% and GGBS by 20%. It was found that bio concrete of 40ml and 50ml bacterial solution is attains extreme flexural strength and split tensile strength but it's not true for 60ml bacterial solution after 14 days when they did flexural tensile strength. Also, the optimum dosage of bacterial solution used in concrete is 50 ml it's the point of maximum improvement of mechanical properties of bacterial concrete. Generally, more $CaCO_3$ is produce in concrete because of bacteria species which has enhancing durability of structure due to reducing of voids by 10%. (Mohit G., P. Krishna, 2015)

Abhishek T., A. Phogat, K. Singh (2016), This paper has presented the overview of several paper in the current years on the use of bio concrete for improving in the mechanical properties, durability and permeation features of normal concrete. They have been studies the analysis on bioconcrete by XRD and SEM tests and also several types of bacteria's, their isolation process, several methods used in the adding of bacterial species in concrete and their belongings on water absorption and compressive strength. Finally, they concluded the bacterial type such as *B. cereus* and *S. pasteurii* extreme rise in the compressive strength and the maximum reduce in water absorption for 28 days curing period of specimen respectively. The bacterial like *bacillus sphaericus*, *B. pasteurii*, and *Bacillus flexus* are not harm the human body and also, they have the potential to precipitate calcite but some other bacterial species is dangerous for human health. (Jasira B., Ifrah K., Khushpreet S. and Abhishek T., 2016)

Ghosh et al. (2006) said that compressive strength was increased in concrete (53 grade) and mortar (22.62% and 19%) by adding *Escherichia coli* in concrete at 105 concentrations per ml. by precipitation of inert filler matter. (Ghosh et al, 2007)

Muynck et al. (2007) stated that use of *Bacillus Sphaericusin* the concrete resulted in to greater resistant to corrosion and improved durability due to calcium carbonate ($CaCO_3$) precipitation. (Muynck et al., 2010)

Sung-Jin et al. (2010) studied that different calcite forming bacteria resulted into greater compressive strength and durability in cement mortar. (Sung-Jin et al., 2010)

Bang et al. (2010) identified that $CaCO_3$ precipitation could be increased by *Sporosarcina pasteurii* bacteria in the concrete and it results in increase of durability of concrete. (Bang et al.,2010)

Reddy et al. (2010) concluded that concrete strength, durability, density and microstructure can be improved by incorporating *bacillus subtilis* bacterias in to it. (Reddy et al., 2010)

Characteristics Examination Mix Proportion Determination below table. (Amirreza T., Mohanadoss P., Gholam R., Rosli M., Muhd Z. and Ali K., 2014)

Table 2.1 Colony morphology,cell morphology, gram stain reaction, and general properties of bacteria.

Bacteria genus	Colony morphology(from agar plates)					Cell Morrphology	Gram Reaction (+/-)	O_2 Use	Glucose use	Endospore (Y/N)
	Shape	Elevati on	Edge	Colour	Surface					
Bacillus	Circular	Flat	Entire	Cream	Smooth	Bacillus-rod	+	aerobe	No gas	Yes

The biotechnologically produced materials and construction biotechnologies have a lot of advantages in comparison with the conventional construction materials and processes so the practical implementations of the construction biotechnologies could give significant economic and environmental benefits. Figure 2.3 illustrates laboratory scale biocement production in 50ml fermenter. (V. Ivanov, J. Chu and V. Stabnikov, 2015)

Fig. 2.3 Cultivation of bacteria for biocement production in 50 L fermenter (photo taken at the Fermentation Facility, Iowa State University, USA)

2.1 Microbial Concrete and Various Microbes

The widely used microbial concrete for healing the cracks, surface treatment and as a water purifier along with microbes and a group of microbes with specific species for a particular operation used in constructional materials for Calcium Carbonate precipitation in addition to crystal, metabolism, nutrients and applications are tabulated in Table 2.2.

Table 2.2 Microbes used in concrete. (Pratyush, 2016)

No.	Name of the microbes	Types of microbial concrete
1	*i. Sporosarcina pasteurii* *ii. Bacillus pasteurii* *iii. Bacillus phaericus*	Crack healing microbial concrete
2	*i. Bacillus pseudifirmus* *ii. Bacillus cohnii*	Self-Healing microbial concrete
3	*i. Bacillus sphaericus* *ii. E.coli*	Microbial concrete for surface treatment
4	*i. Bacillus cereus* *ii. Bacillus pasteurii* *iii. Shewanella* *iv. Sporosarcina pasteurii* *v. Bacillus Sp. CT-5*	Concrete and cement mortar
5	*Bacillus phaericus, Bacillus subitilis, Thiobacillus, Bacillus thuringiensis*	Microbial concrete as water purifier

Scanning Electron Microscopy without microbe and with microbe shown in figure 2.4 respectively. The microbe mixed concrete not devlope pore. (Mr. Sudarshan K. and Tejas D.,2016)

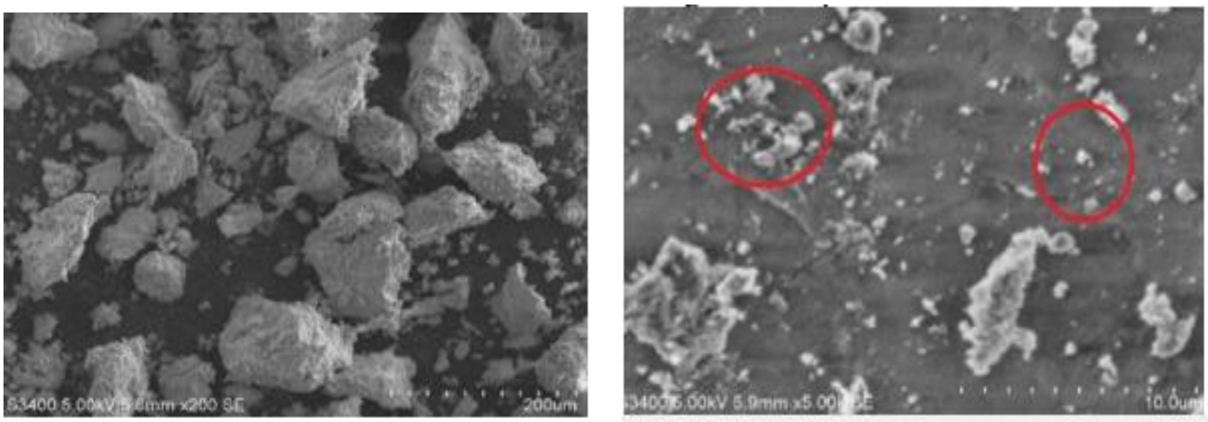


Figure 2.4 Scanning electron microscopy picture of normal concrete and bacterial concrete

Cements, are finely ground inorganic materials with adhesive and cohesive properties which make them capable of uniting or bonding together fragments or particles of solids matter into a compact whole. In engineering purpose cements are materials which form a paste which sets and harden by means of hydration reactions, and which after hardening retain its strength and stability even under water and can be molded or deformed but later it sets and hardens to a rigid mass.

2.2 Mechanism of Self-Healing Bacteria

The microbial organism used for manufacturing of bacterial concrete should be able to possess long-term effective crack sealing mechanism during its lifetime serviceability. The principle behind crack healing mechanism is that the bacteria should be able to transform soluble organic nutrients into insoluble inorganic calcite crystals, which seals the cracks. For effective crack healing, both bacteria and nutrients incorporated into concrete should not disturb the integrity of cement sand matrix pore-diameter and should not negatively affect other important fresh and hardened properties of concrete. In concrete cracks up to 0.2 mm wide are healed autogenously. Such micro cracks are acceptable as these do not directly influence the safety and strength of concrete. The in-built bacteria-based self-healing process was found to heal cracks completely up to 0.5 mm.

2.3 Biocement in civil engineering

Bio cementation is a process to produce binding material (bio cement) based on microbial induced carbonate precipitation (MICP) mechanism. This process can be applied in many fields such as construction, petroleum, erosion control, and environment. Application in construction field include wall and building coating method, soil strengthening and stabilizing, and sand stabilizing in earthquake prone zone . Primary role of microorganism in carbonate precipitation is mainly due to their ability to create an alkaline environment (high pH and DIC increase) through their various physiological activities. Three main groups of microorganism that can induce the carbonate precipitation are - (i) photosynthetic microorganism such as cyano-bacteria and microalgae; (ii) sulphate reducing bacteria; and (iii) some species of microorganism involved in nitrogen cycle. Photosynthetic microorganisms utilize urea using urease or urea amidolyase enzyme, based on which it is possible to use microalgae as media to produce bio cement through bio cementation. Mortar refers to ready to use binder material contained a binder, and sand or aggregate. Biological mortar consists of three main components such as limestone powder, nutrient and bacterial paste. Bio cementation applied in concrete rift remediation and the production of bacterial concrete has been investigated. Specimen of crack in concrete filled with bio cement shows the significant increment of strength and stiffness value compared with specimen without bio cement. (Sri R., A. Manjusha and M. Arun k., 2015)

2.4 Application of Bacteria in Construction Field

From enhancement in durability of cementations materials to improvement in sand properties, from repair of limestone monuments, sealing of concrete cracks to highly durable bricks, microbial concrete has been successful in all such cases. This new technology paves way for low cost and durable roads, high strength buildings with more bearing capacity, long lasting river banks, erosion prevention of loose sands and low cost durable housing. Another issue in conventional building materials is the high production of greenhouse gases and high energy consumed during production of these materials and these greenhouse gases leads to global warming. High construction cost of building materials is another drawback in such cases. These drawbacks have led to use of novel, eco-friendly, self-healing and energy efficient technology where microbes are used for remediation of building materials and enhancement in the durability characteristics (Mayur S. and Prof. Jayeshkumar P., 2013).

3. MATERIALS AND METHODS

In this research work, experimental studies on Optimization of parameters to improve the Strength of Concrete Cube Using Bacteria Species such as the dosage of microbes, type of microbes, time and water cement ratio which can influences on the compressive strength of 25 Mpa were tested. For each bacteria type 17 runs were done in triplicate and control were also made in concrete without microbes for each water cement ratio. The data obtained from the experimental procedures were analyzed and interpreted in comparison with control groups. Microbial growths were carried out at Addis Ababa institute of technology, School of Chemical and Bioengineering, biochemical laboratory whereas compressive strength tests were carried out at School of Civil and Environmental Engineering, construction materials laboratory. In addition, scan electronic microscope were done.

3.1 Materials

3.1.1 Apparatus/ Materials

Equipment's used for analysis: pH meter, mixer, electronics weighting balance, Oven (V 230, W 160, A 7, fuse 10, code DAS4200 Made of Italy), autoclave (Electrical Heated Vertical Steam Sterilizer Model LX-B50LC Digital , Made in China, working Ac 220 V,50 HZ, Power 2.5 kw, Des Temperature 129 ºC, Working Pressure 0.165 Mpa, No 16-006, Netwet.39 kg, Volume 0.05 m3), thermostatic Incubator shaker (Model. DH-500A, Temperature Range +5-65, Power. 0.4kw, Bio base Biodustry (Shandong) Co, Ltd, N0.51.South Gengye, and Road. Jinan City, China), cube mould (15 15 15 cm), Compression Load Control Machine and, Scanning Electron Microscopy (JSM-IT300, VERSION 1).

3.1.2 Reagents / Chemicals used

For pH adjust use HCl (Hydro chloric acid) and NaOH (sodium hydro oxide) and for media preparation such as Nutrient broth, Peptone, Yeast Extract, Urea, calcium chloride and agar.

3.1.3 Microbial Culture

Among the activities of this particular research were selected specific species of bacteria from a list of potential calcite induced bio-mineralizing microorganisms, used in different research works. After selection of three potent bacteria species, these bacteria species were kindly obtained from Ethiopia Biodiversity Institute, Addis Ababa that were extracted from different environmental conditions. These obtained three species of bacteria were grown in liquid broth of selective media for 24– 48 hrs. After 24-48 hrs checks the growth and the turbidity of media that showed the growth of microbes.

3.1.4 Cement

Ordinary Portland cement (OPC) produced as per CEM-I-32.5 grade contains 95% clinker and 5% gypsum produced by Dangote Cement PLC were used throughout the experiment to prepare pastes. OPC, were used for the preparation of all grades of concrete samples.

3.1.5 Aggregate

The Coarse Aggregates used to make up the concrete samples consist of crushed gravel passing sieve size of 19.0 mm to 4.75 mm to conform to the requirements of ASTM C 33 specification.

3.1.6 Water

Water is an important ingredient of concrete so all ingredients were mixed using tap water.

3.2 Methods

3.2. 1 Mix Proportions

The mixes were prepared using Dangote ordinary Portland cement (OPC) to test concrete strength using water to cement ratio of 0.58, 0.53 and 0.45 for each species of bacteria at different dosage. In addition, control treatments were done for each water cement ratio. Mixing design was calculated according to Ethiopian standard equations (Eqn 3.1 and 3.2)

………………………………………………………………...………….3.1

Where, f'ck = target average compressive strength at 28 days

fck = characteristic compressive strength at 28 days

S = Standard deviation

(Size of mold Size of mold Size of mold) m^3 number of concrete need………………..3.2

The mix proportion was adjusted namely: Cement = 360 kg/ m^3, Fine Aggregate = 800 kg/ m^3 and Coarse Aggregate = 1087.5 kg/ m^3.

Compressive strength of concrete is determined from tests on 150 mm cube sat the age of 28 days in accordance with Ethiopian Standards.

Table 3.1 Grades of Concrete and Characteristic Cylinder Compressive Strength fck (Negussie T., Asrat T., Bekele M., Mikyas A. and Shifferaw T., 1995)

Grades of Concrete	C15	C20	C25	C30	C40	C50	C60
f_{ck}	12	16	20	24	32	40	48

3.2.2. Mixing Design Stipulation

In Table 3.2 show the mixing design stipulation of concrete made like the grade of design, type of cement and some other mixing design which was used in this particular research.

Table 3.2 Mixing design stipulation

Grade designation	25
Type of cement	OPC 32.5
Maximum nominal size of aggregate	19mm
Water cement ratio	0.45, 0.53 and 0.58
Method of concrete placing	Manual
Proportion of cement: sand: aggregate	1:2:3

3.2.3 Inoculum preparation

Ethiopia Biodiversity Institute has kindly gifted three bacterial species, namely *Bacillus subtilis, Sporosarcina pasteurii and Bacillus cibi* for this particular study. These bacteria were grown in liquid media based on the following procedure.

3.2.3.1 *Bacillus subtilis*

Primarily 6.5g of Nutrient broth (media) was added to a 500ml conical flask containing distilled water, and then covered with a thick cotton plug and aluminum. It was then sterilized for 15 minutes in sterilizer at 121°C.

After media sterilization, the flasks were opened up and exactly 1ml of the bacterium from microbial bank were added to the sterilized flask and incubated at a temperature of 37°C and 125 rpm on orbital shaker for 24 -72 hr. (Anestraj S., Akila D., Arun S., PradeepKumar A. and Santhoshkumar A., 2015)

3.2.3.2 *Sporosarcina pasteurii*

This microorganism was known for its resistive properties to resist the harsh conditions of sea water and very high temperatures which are generally found in shallow waters and in Sahara desert. *Sporosarcina pasteurii* bacterium was used in the present work for checking the improvement in the compressive strength of concrete. This bacterium secretes calcium carbonate. 6.5g of Nutrient broth (media) was prepared and added to a 500 ml conical flask containing distilled water. Then covered with a thick cotton plug and aluminum and sterilized for 10-20 minutes in sterilizer at 121 ºC the solution became free from any contaminants and clear orange in color before the addition of the bacteria. The microbe sample (*Sporosarcina pasteurii*) was obtained from the microbial bank and added 1ml of bacterium to the sterilized flask and maintained at a temperature of 37 ºC and stirred at 120 rpm for 24 hours. (Rakesh C., Rahul N. and Smitha Y., 2014)

3.2.3.3 *Bacillus cibi*

The nitrite broth media for *Bacillus cibi* were prepared using similar techniques that were used for *Bacillus subtilis* and *Sporosarcina pasteurii*. Cells are aerobic rods that were optimally grown at temperature of 37 °C; and at pH range of 6.5–7.5. (Jung-Hoon Y., Choong-Hwan L. and Tae-Kwang Oh, 2005)

3.2.4 Serial Dilution

One ml of bacteria was taken from broth culture and added into 9ml of distilled water test tubes. The concentration of solution labeled as 10^{-1}, and then after well mixed, 1ml solution from this test tube were taken and added to the second test tube having 9ml distilled water. Finally, continued the serial dilution until 10^{-9}. Each serial dilution was done in triplicate for each bacterium.

Prepare agar media 14 g in 500 ml conical flask distilled water and cover the flask in cotton and aluminum then sterilized at 121 °C for 15min for remove any contamination. Spread the agar media in to Patrick plate. Take 1ml of serial diluted sample from test tube solution of concentration 10^{-4} to 10^{-9} on the Patrick plate with media in it. After 24-48 hrs incubation, check the type of colony formation in the Patrick plate and also made some more Patrick plates with the same media with different concentration.

3.2.4.1 Viable Cell Count

After streak the different type of colonies on different plates, and after 24-48 hr incubation period, the bacteria that form colony growth were directly counted and total number of cells were calculated based on the following calculation:-

Cells (Colony forming units) per milliliter of original sample = Plate count * Dilution factor……………………………………………………………………………………….3.3

3.3 Preparation of concrete specimens with and without bacteria species to determine compressive strength.

Mix microbe grows in broth with water that prepare according to mixing design. Add all ingredients (cement, fine aggregate, coarse aggregate and water) in to mixing machine that prepared according to mixing design for each amount of concrete. The hydrogen ion concentration of an organism’s environment has the maximum influence on bacterial growth, so that each bacterium has its own optimum pH and crucial to check the pH of mixed ingredients before adding the bacteria. Thus, after mixed well all ingredients, the pH was checked. Once the pH was adjusted to optimum level for each bacteria, fill the mixed ingredients into the mould having 15 15 15 cm size which was made to produce cement cube. The amount of each bacteria added into each mix of 15 15 15 cm mould size where calculated and described as shown in Table 3.3.

Table 3.3 Mix proportion summary for C-25 Concrete cubes for compressive strength test

ml of Bacteria	Number of specimen for			Mixing property		
	7 day	14 day	28 day	Cement (Kg / m^3)	Fine aggregate (Kg / m^3)	Coarse aggregate (Kg / m^3)
0 ml	27	27	27	360	800	1087.5
20 ml	27	27	27	360	800	1087.5
40 ml	27	27	27	360	800	1087.5
60 ml	27	27	27	360	800	1087.5

Proceed the concrete was poured in the mould and tempered properly after 24 hours removed the moulds**.**

3.4 Experimental design

Experimental data analysis was done using Design-Expert 6.0-8.0. software. The experimental design selected for this study was response surface methodology, three-factor quadratic model, Box-Behnken Design (BBD) and the response variable measured was the cement compressive strength. The three independent variables studied to determine cement cube compressive strength were bacteria dosage, time and water to cement ratio for each bacteria with their respective levels and the proposed BBD .

3.4.1 Optimization

According to BS EN 12390-3, different types of cubes were preparing for concrete mix with and without addition of microorganisms. The cube specimens have dimensions of 150 mm × 150 mm × 150 mm which was testing using Compression Load Control Machine. The specimen was testing at different time interval up to mature age of 28 days after curing.

The effects of microbial dosage, time and water/cement ratio parameters for each bacterium on the properties and strength of concrete cube were analyzed in comparison to the normal strength C-25.

3.4.1.1 Optimization of Bacterial Treatment.

Take the main three factors (Dosage of the liquid form microbes with different concentration, time and water cement ratio which have different ratio) that affect the strength of the concrete. The mixes were prepared using Dangote ordinary Portland cement (OPC) for concrete strength C25 using a water cement ratio of 0.53, 0.58 and 0.45 and cement, fine aggregate and coarse aggregate is 357.143 kg/m^3,800 kg/$m^3$and 1087.5 kg/m^3 content respectively.

All together a total of 34 mixes were prepared for each microbe (*B.subtilis*, *Sporosarcina pasteurii* and *Bacillus cibi*) and nine without microbe for control purpose. On the 150 150 150 mm cubes size and compressive strength test at 7th, 14th and 28th day were casted.

3.5 Compressive test

Compressive strength of structural concrete is specified according to design requirements of a structure. Compressive strength of the concrete is the capacity of the structure to resist the load acting on them. Normally, strengths specified will range from 21 to 35 MPa and less frequently up to 48 MPa or higher. (Sri R., A. Manjusha and M. Arun k., 2015)

Compressive strength of the cement cubes was tested at 7, 14 and 28 days using 0.58, 0.53 and 0.45 water to cement ratio using three different bacteria species. For comparison purpose, strength test was also done without the use of microbes as control for each treatment groups.

3.6 Characterization studies

In order to understand the effect of bacteria on the microstructure of concrete, Scanning Electron Microscopy (SEM) was used. Morphological analysis of the micro cracks of concrete was identified using SEM (JSM-IT300, VERSION 1).

4. RESULT AND DISCUSSION

4.1 Viable cell count

In order to count the cells number, 30 – 300 bacteria colonies must be needed. The calculation was done based eqn 3.3. In this case, dilution factor of 10^{-5} taking and which have 126 colonies. Therefore, there are 1.26 10^{7} cells per ml of original sample.

4.2 Strength test of cement cube treated with and without bacteria

The compressive strength of concrete cube treated with bacteria and without bacteria was described in table 4.1. The result indicated that using bacteria species (*Bacillus subtilis*, *Bacillus cibi* and *Sporosarcina pasteurii*) mix in the cement cube brought early strength gain and increased in the compressive strength of concrete. When we look the compressive strength in 14 day at 0.45 W/C ratio mixing the bacteria (*Bacillus subtilis*, *Bacillus cibi* and *Sporosarcina pasteurii*) has high compressive test 38.99, 29.73 and 38.89 MPa respectively compare to the control which was normal concrete (29.34 MPa).

Concrete cube made from mixed bacteria *Bacillus subtilis* species at 0.45 W/C ratio has got high compressive strength test at 14days whereas high compressive strength (46.89MPa) were recorded on the day of 28 from concrete cube prepared at 0.45 W/C ratio and mixed with the bacteria *Sporosarcina pasteurii.* Generally from the three bacterial species used, *Bacillus subtilis* species treated cube has early attain the required strength (at 7 days) at 0.53 water to cement ratio compared to the control whereas *Sporosarcina pasteurii* was selectable for giving higher strength at 28 day with small water to cement ratio (0.45) which means the use of these two species in bio construction can bailed building in short time without additional cost of water, cement and reduce the cost for panel, without compromising the strength. According to some research result like on the journals wrote by Akshaykumar Ardeshana1, Dr. Jayeshkumar Pitroda, and Dr. Digvijaysinh Rana on the title of “Improvement in compressive strength of concrete by inclusion of *Bacillious pesturi* bacteria”, Salmabanu Luha and Suthar Gourav “Areview paper on self-healing concrete” and some other research the result acceptable.

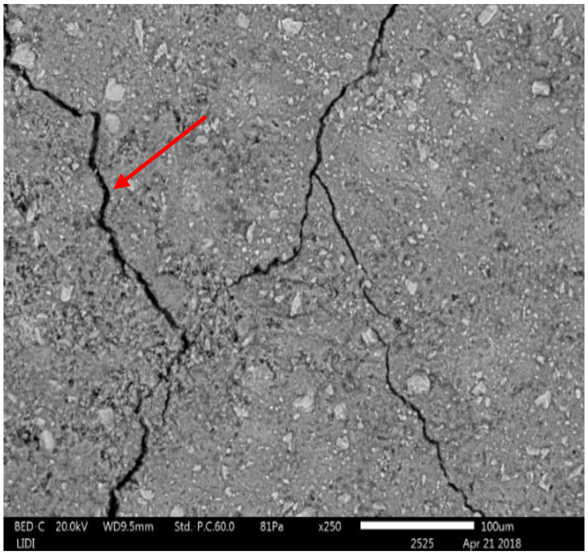
Table 4.1 Result of concrete compressive taste

Day	W/c ratio	Compressive taste without bacteria MPa	Dosage mL	Compressive taste with bacteria MPa		
				B. Subtilis	*S. pasteurii*	*B. Cibi*
14	0.53	23.08	40	34.66	27.9	30.89
28	0.53	24.17	60	24.92	27.62	24.17
28	**0.45**	**31.96**	**40**	**34.52**	**46.89**	**36.02**
7	0.45	25.22	40	32.54	31.92	26.81
14	0.58	17.72	20	17.46	19.15	25.47
28	0.58	19.96	40	21.53	25.67	20.07
7	0.53	20.24	20	25.26	25.9	21.28
14	0.53	23.08	40	29.66	26.74	32.04
28	0.53	24.17	20	24.74	28.09	26.24
14	**0.45**	**29.34**	**20**	**38.99**	**38.89**	**29.73**
14	0.53	23.08	40	35.28	27.69	32.89
14	0.53	23.08	40	35.73	28	32.11
14	0.58	17.72	60	19.15	25.47	15.13
7	0.58	14.83	40	14.2	14.71	15.09
7	0.53	20.24	60	25.19	25.77	19.07
14	0.45	29.34	60	30.02	37.97	30.8
14	0.53	23.08	40	31.92	28.11	30.43

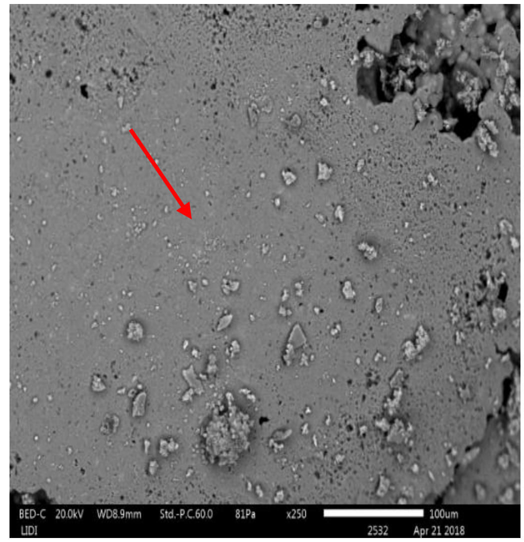
In the case of *Bacillus subtilis* result show as in table 4.1 gain high compressive strength of concrete in early time 14 day in the 0.45 water / cement ratio in the case of adding the dosage of microbe 20 ml in the mixing time this result also supporting in figure 4.1 (B) which show that the bacteria give the concrete smoothness on its surface area and which also indicate there is no pores on the micro vision of the inside part of the concrete this is make because of the presence of precipitated calcium carbonate in contact with bacteria and this result similarity with some other paper. (Meera C., 2016 and Adetifa O. A., 2013)

The table 4.1 also show the compressive strength of concrete using the bacteria *Sporosarcina pasteurii* the concrete gain it strength earlier but more increase in the time of 28 day or after that time compare to the normal concrete. Calcite precipitation in concrete was inspected by SEM analysis in figure 4.1 (c) indicate the SEM analysis of this bacteria mix concrete it show as the there is no pores in concrete compare to normal concrete shown in figure 4.1 (a) this is as result of precipitated calcite deposit was contact with spherical immersion of bacteria a result supporting with some paper. (Vijeth N., 2013 and Akshaykumer A., 2017)

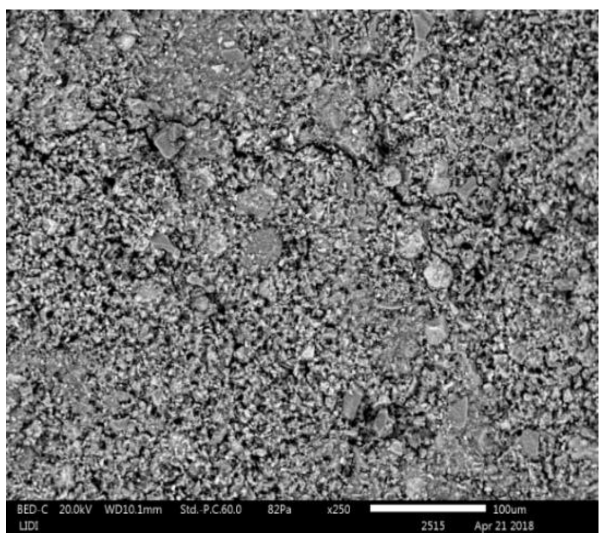
Figure 4.1 (a) seen pores form inside the concrete which indicate in the arrow.



(A)



(B)



(C)

Figure 4.1 Morphological analysis of bacterial treated and non-treated concrete with micro cracking a) Control b) *Bacillus subtilis* c) *Sporosarcina pasteriiu*

4.3 Optimization of Bacterial Treatment.

4.3.1 Optimization of *Bacillus subtilis*

Table 4.2 Optimization results of *Bacillus subtilis* on compressive test

Std	Run	Factor 1 A:Dosage mL	Factor 2 B: Time Day	Factor C:W/C	Response 1 Compressive test MPa
9	1	40	7:00	0.45	32.54
6	2	60	14:00	0.45	30.02
17	3	40	14:00	0.53	34.66
16	4	40	14:00	0.53	29.66
1	5	20	7:00	0.53	25.26
14	6	40	14:00	0.53	35.28
10	7	40	28:00	0.45	34.52
13	8	40	14:00	0.53	35.73
4	9	60	28:00	0.53	24.92
3	10	20	28:00	0.53	24.74
11	11	40	7:00	0.58	14.2
12	12	40	28:00	0.58	21.53
7	13	20	14:00	0.58	17.46
8	14	60	14:00	0.58	19.15
5	15	20	14:00	0.45	38.99
2	16	60	7:00	0.53	25.19
15	17	40	14:00	0.53	31.92

4.3.1.1 Analysis of variance (ANOVA)

In ANOVA analysis the model was significant when fisher's F test with a very low probability value. From table 4.3 observed that the Model F-value of 12.96 implies the model is significant. There is only a 0.14% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case C, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.The coefficent was linear effect of water cement ratio and dosage and time was least significant.

Table 4.3 Analysis of variance (ANOVA) for *Bacillus subtilis*

Source	**Sum of Squares**	**DF**	**Mean Square**	**F Value**	**Prob > F**	
Model	774.00	9	86.00	12.96	0.0014	Significant
A	6.43	1	6.43	0.97	0.3578	
B	9.07	1	9.07	1.37	0.2805	
C	507.69	1	507.69	76.53	< 0.0001	
A^2	62.65	1	62.65	9.44	0.0180	
B^2	87.74	1	87.74	13.23	0.0083	
C^2	42.78	1	42.78	6.45	0.0387	
AB	0.016	1	0.016	2.355E-0.03	0.9626	
AC	28.41	1	28.41	4.28	0.0773	
BC	7.16	1	7.16	1.08	0.3335	
Residual	46.44	7	6.63			

Lack of Fit	19.72	3	6.57	0.98	0.4844	Not significant
Pure Error	26.72	4	6.68			
Cor Total	820.44	16				

The “Lack of Fit F-value” of 0.98 implies the Lack of Fit not significant relative to the pure error. There is a 48.44% chance that a “Lack of Fit F-value” this large could due to noise. Non-significant lack of fit is good – we want the model to fit.

Std. Dev.	2.58	R-Squared	0.9434
Mean	27.99	Adj R-Squared	0.8706
C.V.	9.20	Pres R-Squared	0.5645
PRESS	357.28	Adeq Precision	11.442

The "Pred R-Squared" of 0.5645 is not as close to the "Adj R-Squared" of 0.8706 as one might normally expect. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 11.442 indicates an adequate signal. This model can be used to navigate the design space.

Equation

Model equations are given in terms of coded factors and actual factors. Coded factors indicate when the minimum and maximum values of the factors are represented by -1 and +1 respectively instead of their actual values.

Four important parameters, dosage (A), time (B) and water cement ratio (C) were considered. Consequently, A, B and C were chosen as the independent variables while the compressive test strength was selected as the response (dependent variable) of the study. The final central composite design obtained for percentage compressive strength with significant terms was quadratic as suggested by the software, and is given as:

Final Equation in Terms of Coded Factors:

Compressive test = $33.45 - 0.09\ A + 1.07\ B - 7.97\ C - 3.86\ A^2 - 4.54\ B^2 - 3.19\ C^2 + 0.062\ A\ B + 2.67\ A\ C + 1.34\ B\ C$

Final Equation in Terms of Actual Factors:

Compressive test = $-71.52144 - 0.33427\ \text{Dosage} + 0.52948\ \text{Time} + 538.21844\ \text{W/C} - 9.64375\text{E-}003\ \text{Dosage}^2 - 0.041406\ \text{Time}^2 - 754.43787\ \text{W/C}^2 + 2.97619\text{E-}005\ \text{Dosage}\ \text{Time} + 2.05000\ \text{Dosage}\ \text{W/C} + 1.95971\ \text{Time}\ \text{W/C}$

In above equation reveals how the individual variables (quadratic) or double interaction affected on compressive strength. The negative coefficient values indicate that individual or double interactions factors negatively affect compressive strength (i.e. compressive strength percentage decreases), whereas positive coefficient values mean that factors increase compressive strength in the tested range.

4.3.1.2 Diagnostic test for the responses

All diagnostic plots are also tested for all responses for adequacy of the models (normal plot of residuals, residuals vs predicted value, residuals vs factor, box cox plot, studentized residuals, leverage, etc.). From the plot as shown below, the normal probability plot indicates the residuals following by the normal % probability distribution, in the case of this experimental data the points in the plots shows fitted to the straight line in the figure 4.4, this shows that the quadratic polynomial model satisfies the assumptions analysis of variance (ANOVA) i.e. the error distribution is approximately normal.

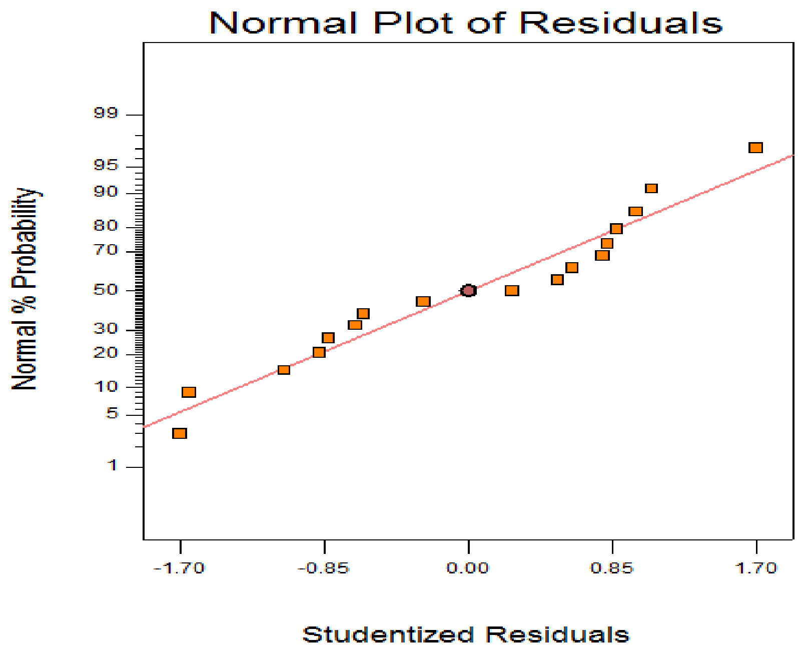


Figure 4.2 Normal plot of residual for *Bacillus Subtilis* Concrete

4.3.1.3 Response surface plot analysis

In order to analyze the regression equation of the model, three-dimensional surface and 2D contour plots were obtained by plotting the response (compressive strength) on the Z axis against any two variables while keeping the other variable at zero level. These plots are created to analyze the change in the response surface. Conical shape response surface plot indicates optimum operating conditions. On the below figure (4.3 to 4.4) show the effect of dosage, time and water/cement ratio on compressive strength of concrete mixing with the bacteria *Bacillus Subtilis*. Response surfaces were drawn for each response using design expert software 6.0.8.

From below figure 4.5 and 4.6 look the effect of Dosage and Time on the compressive strength test of concrete it show in the 40ml of dosage of microbe inter in to concrete mixing on the 14 day give high compressive strength of concrete.

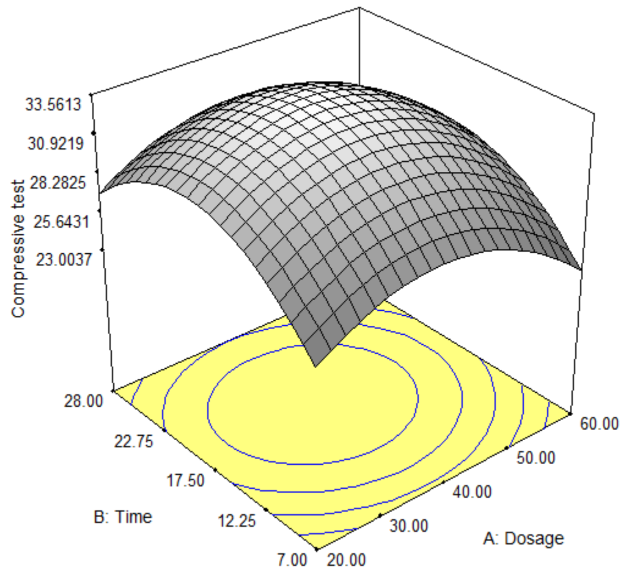


Figure 4.3. Response surface plots of the effect of Dosage and time on the compressive test constant water/cement ratio in the center For *Bacillus subtilis* concrete.

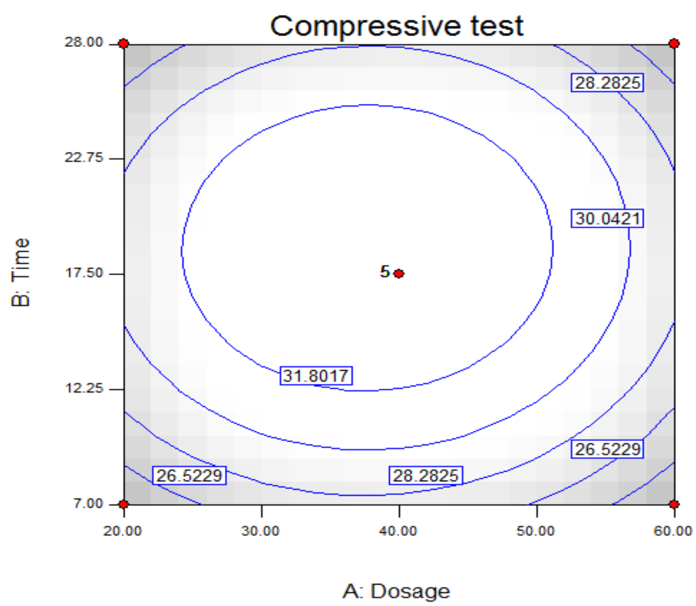


Figure 4.4 Contour plots of the effect of Dosage and time on the compressive test at fixed water/cement ratio For *Bacillus subtilis* concrete

To see the interaction of this to factor (Dosage and Time) on the bio concrete on the figure 4.5 there not have much interaction.

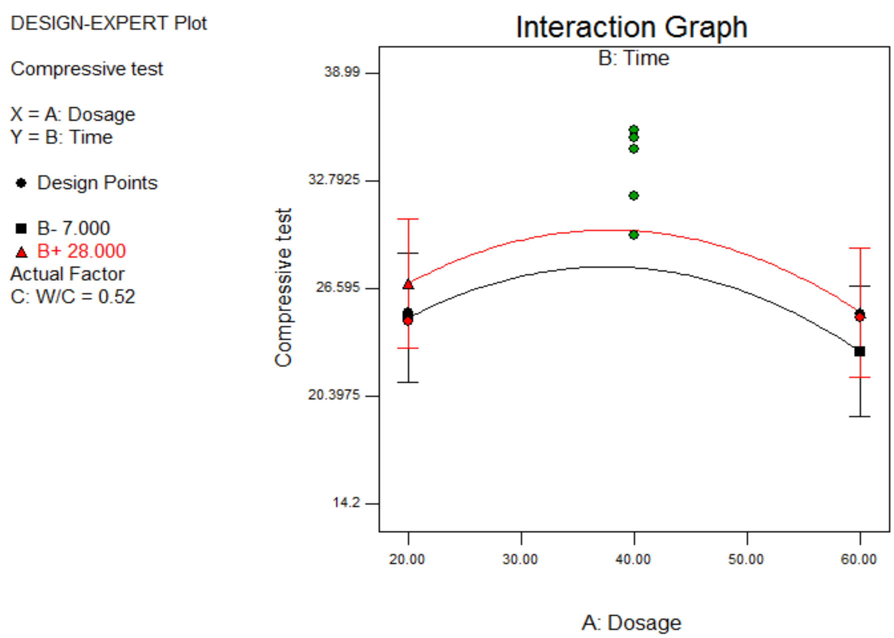


Figure 4.5. Effect and interaction of Dosage and time (fixed) on the compressive test at center of water/cement ratio For *Bacillus subtilis* concrete.

On the response surface and contour plot of the effect of Dosage and water/cement ratio at constant time in the center For *Bacillus subtilis* concrete show the in figure 4.6 and 4.7 respectively when the water / cement ratio gone increase the compressive strength test of concrete became decrease parallel it told that the dosage enter in to the concrete mixing have high compressive strength on the concrete middle (40ml) amount these result fit with some research. The interaction of the two factors also shown in figure 10 which show on the 40ml and 0.45 water / cement ratio the compressive strength was high which show there is no interaction of the two factor. At high level of dosage, the compressive strength low compared to the middle maximum point. This be due to when the increase of water content on the concrete mixing.

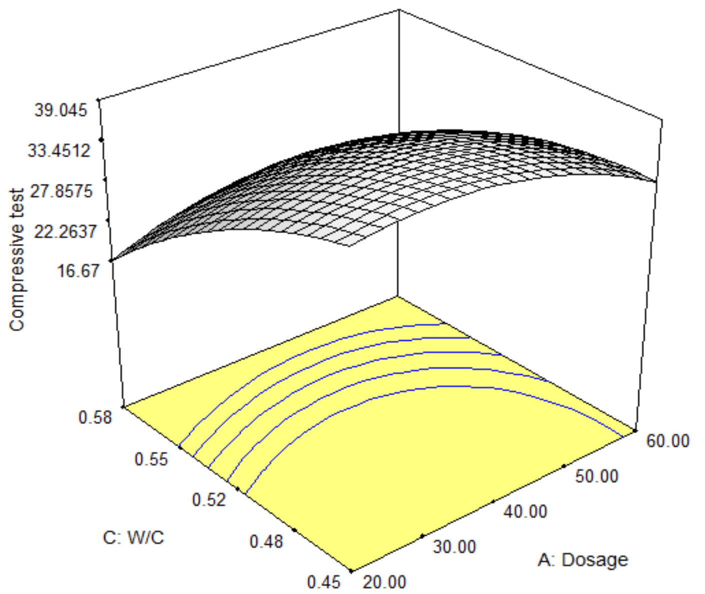


Figure 4.6. Response surface plot of the effect of Dosage and water/cement ratio at constant time in the center For *Bacillus subtilis* concrete.

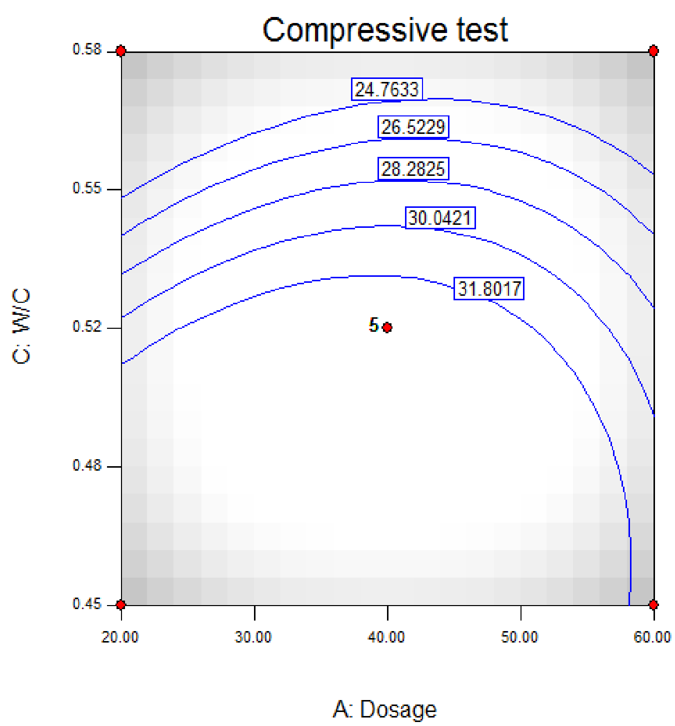


Figure 4.7 Contour plot of the effect of dosage and water/cement ratio at constant time in the center For *Bacillus subtilis* concrete.

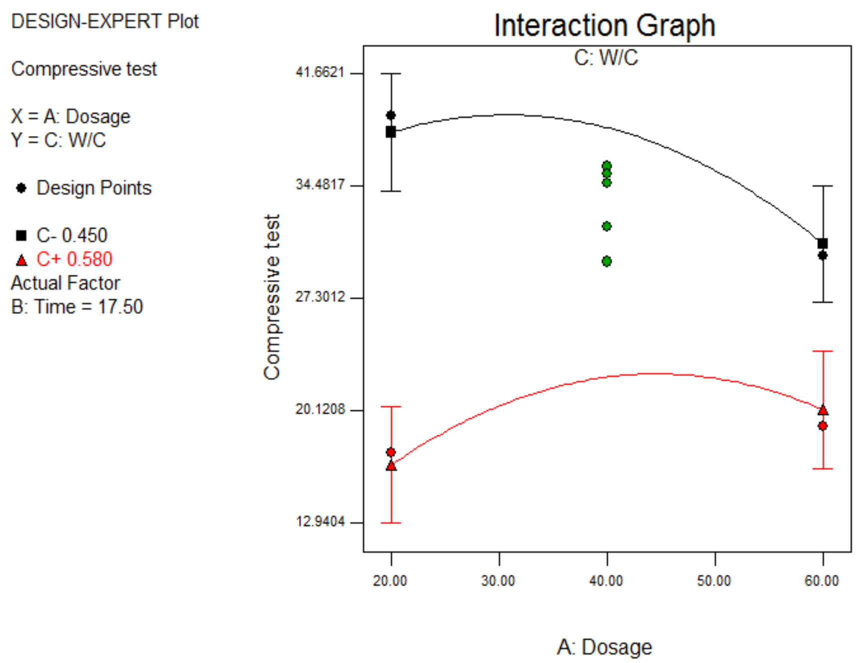


Figure 4.8. Effect and Interaction of dosage and water/cement ratio (fixed) at center of time For *Bacillus subtilis* concrete.

On the figure 4.9 to 4.10 show the effect and interaction of water / cement ratio and time on the compressive strength of concrete it show that this factor affect the compressive strength which generally when the water / cement ratio increase decreasing the compressive strength in 0.45 water / cement ratio have high strength and on the day 14 we can get effective strength on the concrete.

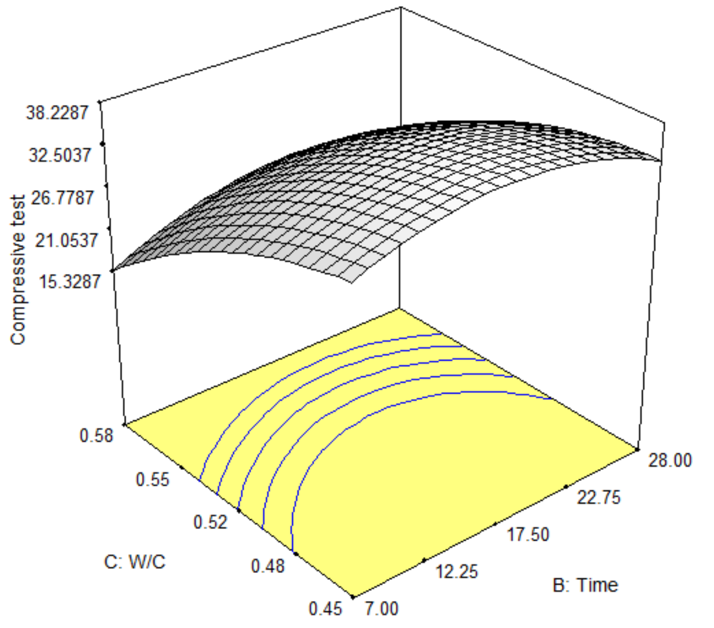


Figure 4.9. Response surface plot of the effect of time and water/cement ratio at constant dosage For *Bacillus subtilis* concrete.

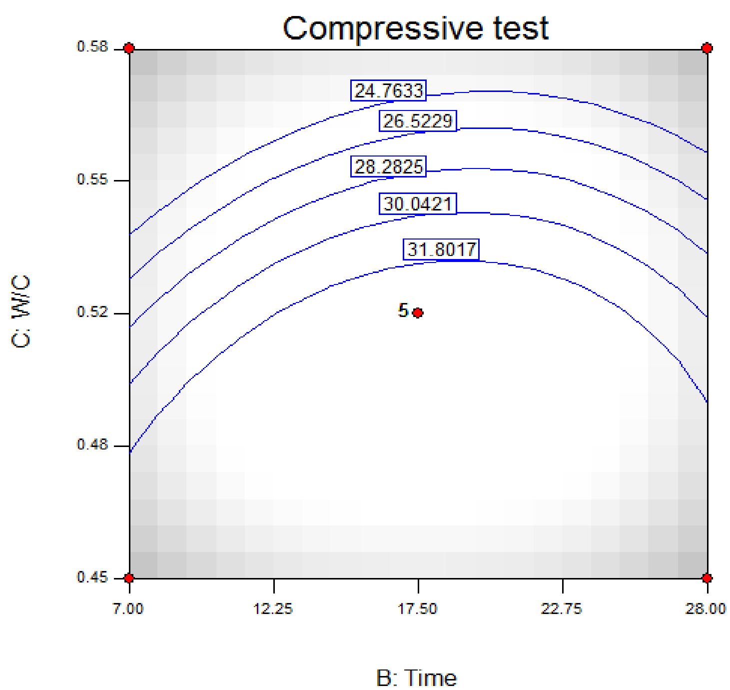


Figure 4.10 Contour plot of the effect of time and water/cement ratio at constant dosage For *Bacillus subtilis* concrete

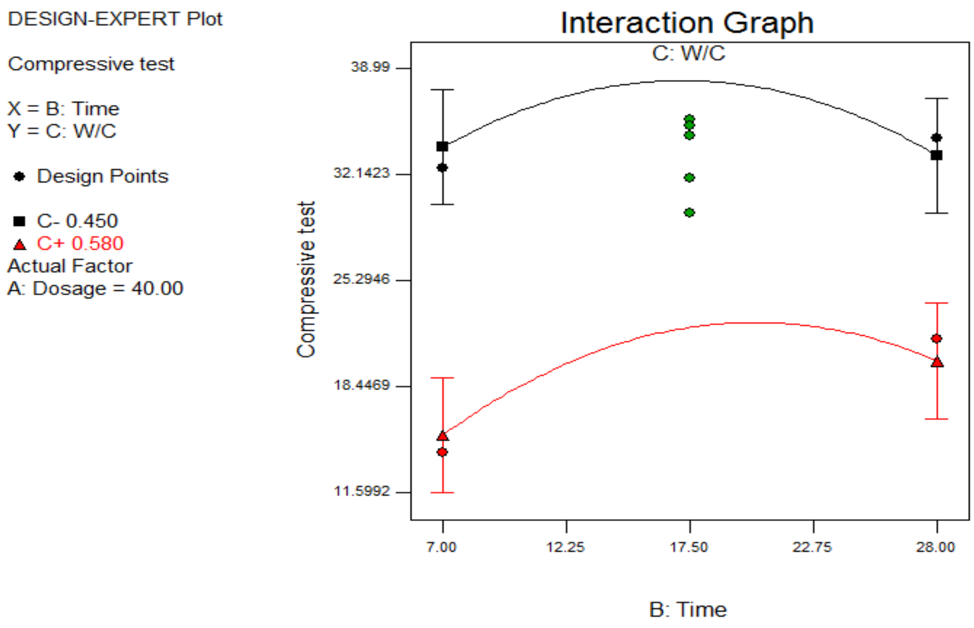


Figure 4.11. Effect of time and water/cement ratio (fixed) at center of dosage For *Bacillus subtilis* concrete.

4.3.1.4 Individual factor effect on the compressive strength

Effect of Dosage

From figure 4.12 understand the dosage of microbe inter into the concrete effect on strength. As show the amount around 40ml add into the concrete mixture increase it compressive strength

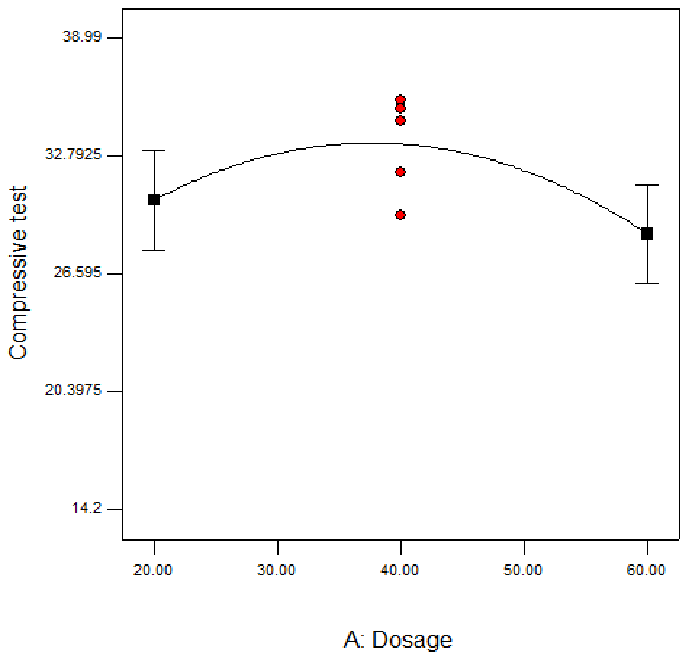


Figure.4.12. Effect of Dosage on compressive test for *Bacillus Subtilis* concrete.

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Effect of Time

From below fig look the effect of time on compressive strength of concrete it show as it high effect when the day past the strength increase as figure 4.13 the day after 14 day the compressive increase.

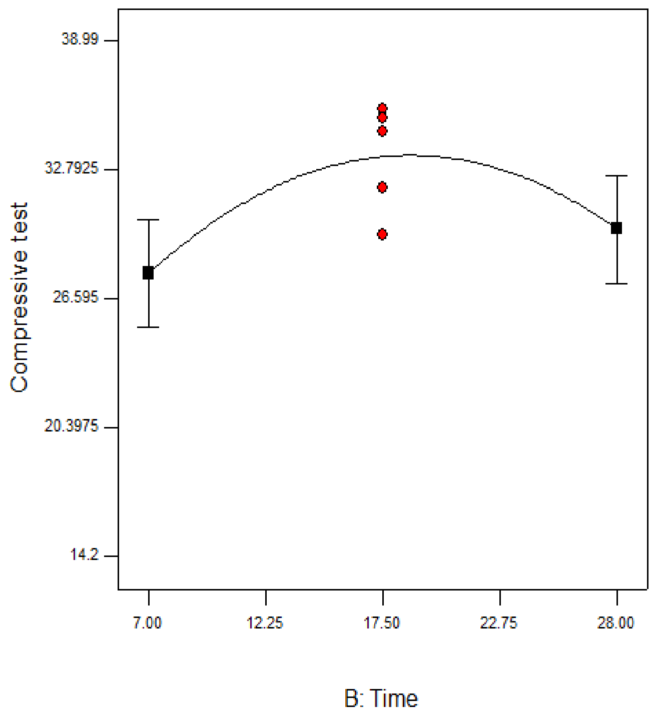

Figure 4.13. Effect of Time on compressive test for *Bacillus Subtilis* concrete.

Effect of W/C

According to (Bhosale M., 2017) show that the concrete got high concrete strength on 0.45 water / cement ratio so on figure 4.14 show approve it.

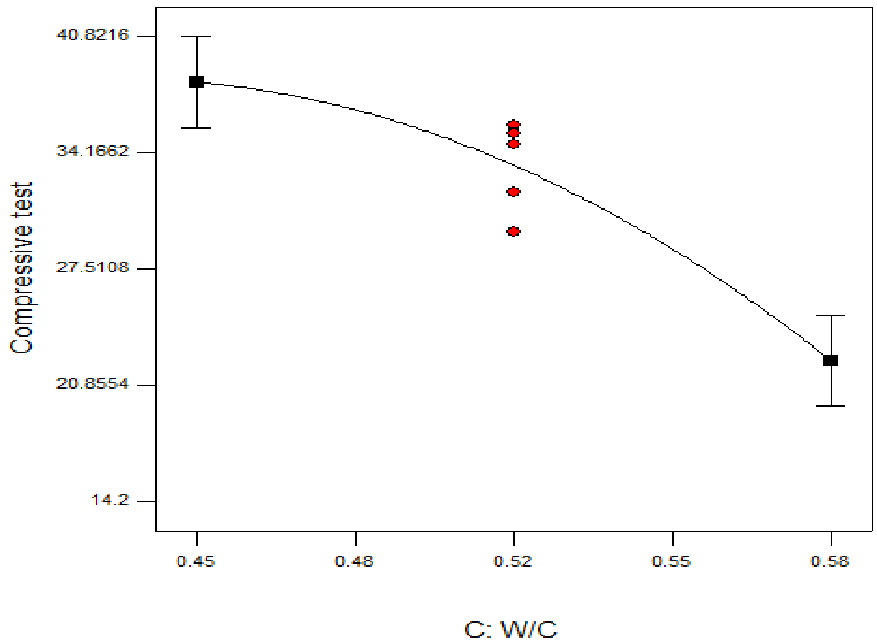

Figure 4.14 Effect of water/cement ratio on compressive test for *Bacillus Subtilis* concrete.

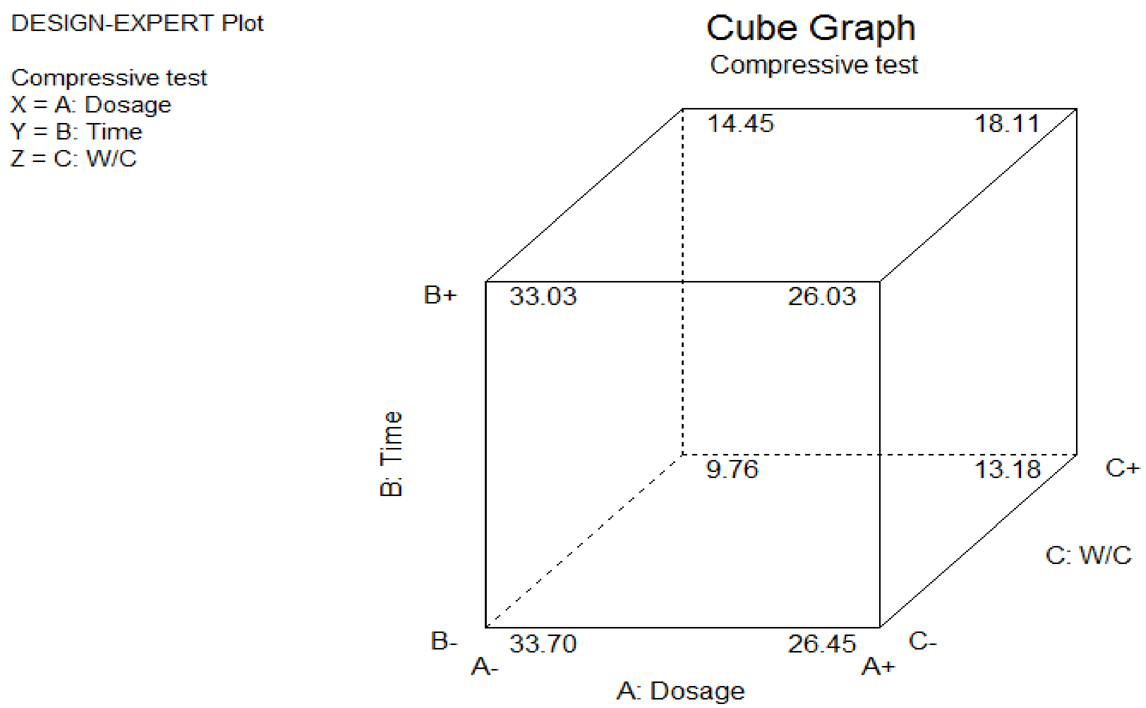

Fig 4.15 cubic graph for *Bacillus Subtilis* concrete

In above all figure looks the effect of water cement ratio effect on the compressive strength. It show the water cement ratio highly effect on the compressive strength of the concrete when increase of water cement ratio it also mean low amount of cement use for and high amount of water use for mixing it decrease the compressive strength of concrete. Generally it show as at 0.45 ratio have high compressive strength on the concrete.

4.3.2 Optimization of *Sporosarcina pasteurii*

Table 4.4 Optimization results of *Sporosarcina pasteurii* on compressive test

Std	Run	Factors			Responsible Variable Compressive test MPa
		Factor A: dosage mL	Factor B: time Day	Factor C: w/c ratio	
16	1	40	14	0.53	27.9
11	2	40	7	0.58	14.71
17	3	40	14	0.53	26.74
3	4	20	28	0.53	28.09
12	5	40	28	0.58	25.67
1	6	20	7	0.53	25.90
13	7	40	14	0.53	27.69
14	8	40	14	0.53	28
7	9	20	14	0.58	19.15
2	10	60	7	0.53	25.77
8	11	60	14	0.58	25.47
4	12	60	28	0.53	27.62
10	13	40	28	0.45	46.89
9	14	40	7	0.45	31.92

6	15	60	14	0.45	37.97
5	16	20	14	0.45	38.89
15	17	40	14	0.53	28.11

4.3.2.1 Analysis of variance (ANOVA)

In ANOVA analysis the model was significant when fisher's F test with a very low probability value. From Table observed that the Model F-value of 8.74 implies the model is significant. There is only a 0.46% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case B, C, are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The coefficient for the linear effect of W/C ratio and time was highly significant.

Table 4.5 Analysis of variance (ANOVA) for *Sporosarcina pasteurii*

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	791.33	9	87.93	8.74	0.0045	Significant
A	2.88	1	2.88	0.29	0.6091	
B	112.28	1	112.28	11.16	0.0124	
C	624.28	1	624.28	62.07	0.0001	
A^2	0.077	1	0.077	7.658E-003	0.9327	
B^2	2.11	1	2.11	0.021	0.6609	
C^2	33.42	1	33.42	3.32	0.1111	
AB	0.029	1	0.029	2.873E-003	0.9587	
AC	13.10	1	13.10	1.03	0.2912	

BC	4.02	1	4.02	0.40	0.5473	
Residual	70.41	7	10.06			
La1ck of Fit	69.19	3	23.06	75.67	0.0006	Significant
Pure Error	1.22	4	0.30			
Cor Total	861.74	16				

The "Lack of Fit F-value" of 75.67 implies the Lack of Fit significant. relative to the pure error. There is a 0.06% chance that a "Lack of Fit F-value" this large could due to noise. In this resulte we look the signifiacnte of the lack of Fit which show as most resulte data was similarty

Std. Dev.	3.17	R-Squared	0.9183
Mean	28.62	Adj R-Squared	0.8133
C.V.	11.08	Pres R-Squared	-0.2868
PRESS	1108.88	Adeq Precision	10.344

Equation

Four important parameters, dosage (A), time (B) and water cement ratio (C) were considered. Consequently, A, B and C were chosen as the independent variables while the compressive test strength was selected as the response (dependent variable) of the study. The final central composite design obtained for percentage compressive strength with significant terms was quadratic as suggested by the software, and is given as:

Final Equation in Terms of Coded Factors

Compressive test = $27.69 + 0.60\ A + 3.75\ B - 8.83\ C - 0.14\ A^2 - 0.71\ B^2 + 2.82\ C^2 - 0.085\ A\ B + 1.81\ A\ C - 1.00\ B\ C$

Final Equation in Terms of Actual Factors:

Compressive test = 249.74111 – 0.65291 Dosage + 1.35412 Time – 852.69990 W/C – 3.38125E - 004 Dosage^2 – 6.41950E-003 Time^2 + 666.80473 W/C^2 – 4.04762E-004 Dosage Time + 1.39231 Dosage W/C – 1.46886 Time W/C

In above equation reveals how the individual variables (quadratic) or double interaction affected on compressive strength. The negative coefficient values indicate that individual or double interactions factors negatively affect compressive strength (i.e. compressive strength percentage decreases), whereas positive coefficient values mean that factors increase compressive strength in the tested range

4.3.2.2 Diagnostic test for the responses

From the plot as shown figure 4.16, the normal probability plot indicates the residuals following by the normal % probability distribution, in the case of this experimental data the points in the plots shows fitted to the straight line in the figure. The data are also similarity each other.

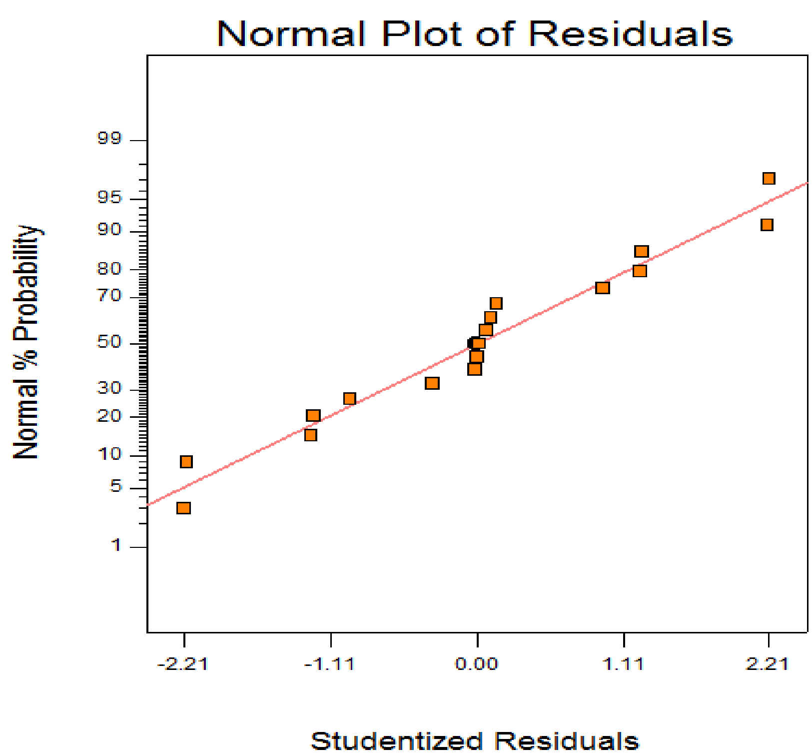

Figure 4.16. Normal plots of residuals using bacteria *Sporosarcina pasteurii*

4.3.2.3 Response surface plot analysis

On the below figure (17- 22) show the effect of dosage, time and water/cement ratio on compressive strength of concrete mixing with the bacteria *Sporosarcina pasteurii.*

On figure 4.17, 4.18 and 4.19 shown the effect and interaction of Dosage and Time on the compressive strength test which Generally show as increase of compressive strength with time increase and the interaction figure show no interaction of the two factor but which show as they effect parallel to the compressive strength test.

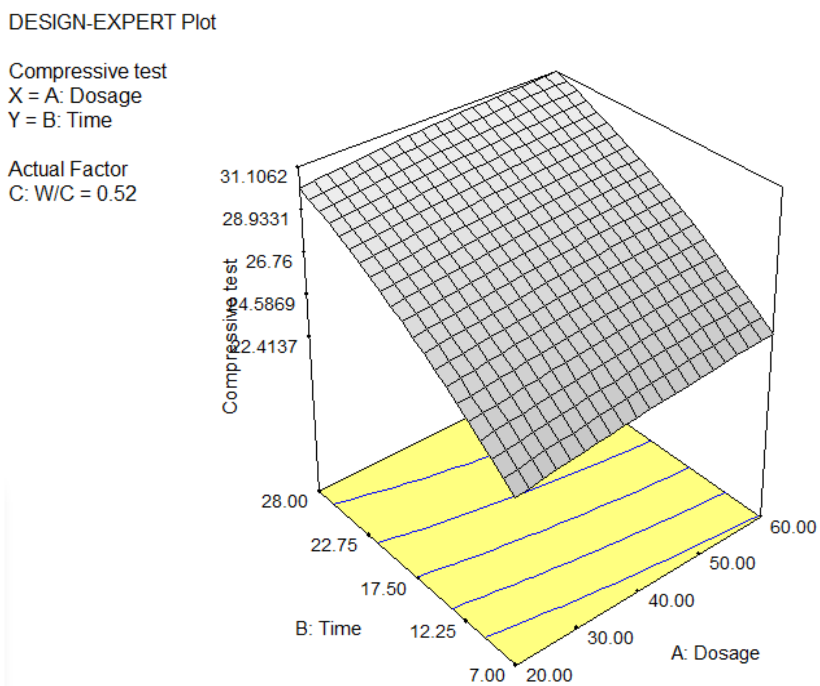

Figure 4.17 Response surface plots of the effect of Dosage and time on the compressive test constant water/cement ratio in the center For Sporosarcina pasteurii concrete

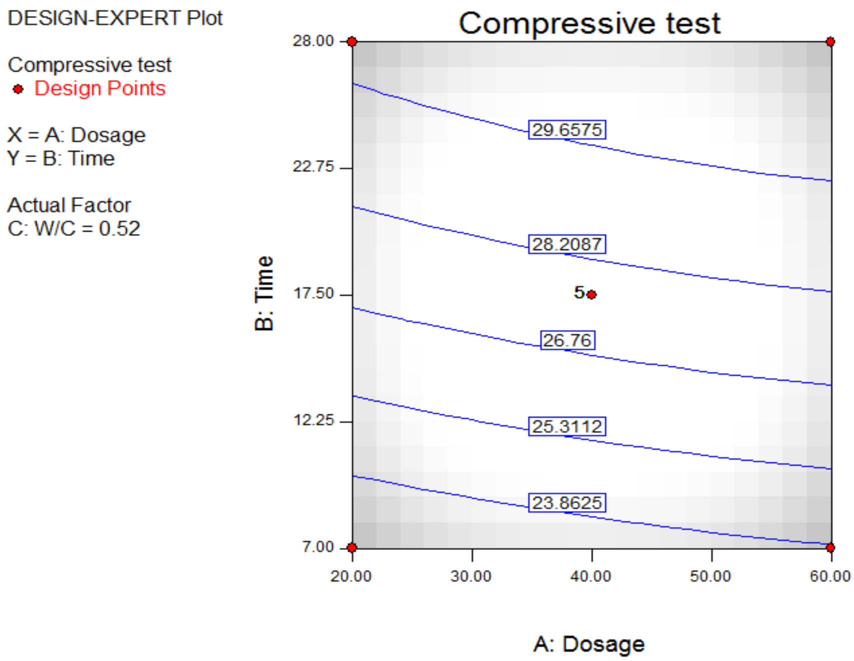

Figure 4.18 Contour plot of the effect of dosage and time at constant water/cement ratio For *Sporosarcina pasteurii* concrete

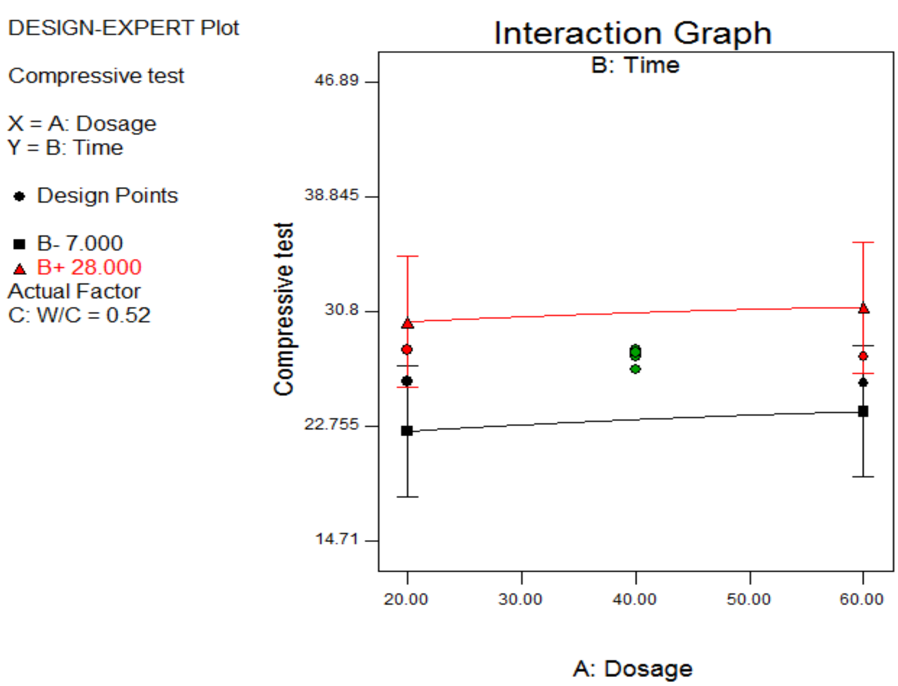

Figure 4.19. Effect of dosage and time (fixed) at center of water/cement ratio For *Sporosarcina pasteurii* concrete

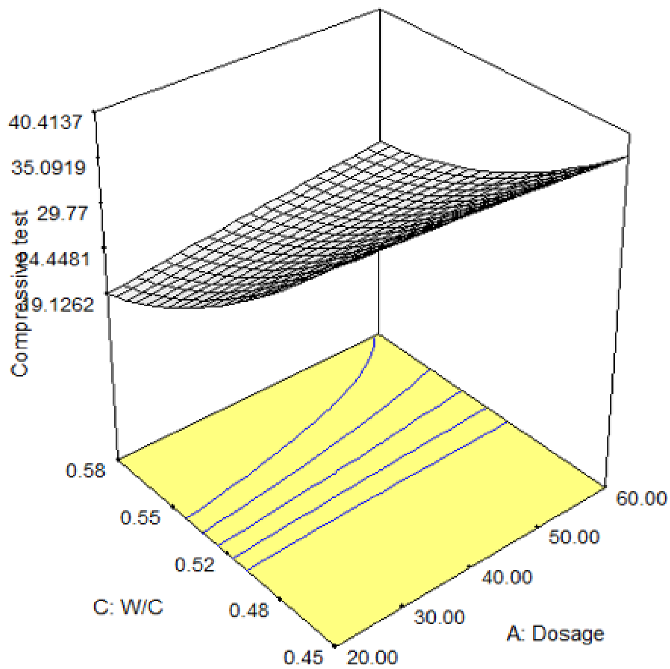

Figure 4.20 Response surface plots of the effect of Dosage and water / cement ratio on the compressive test constant time in the center For *Sporosarcina pasteurii* concrete

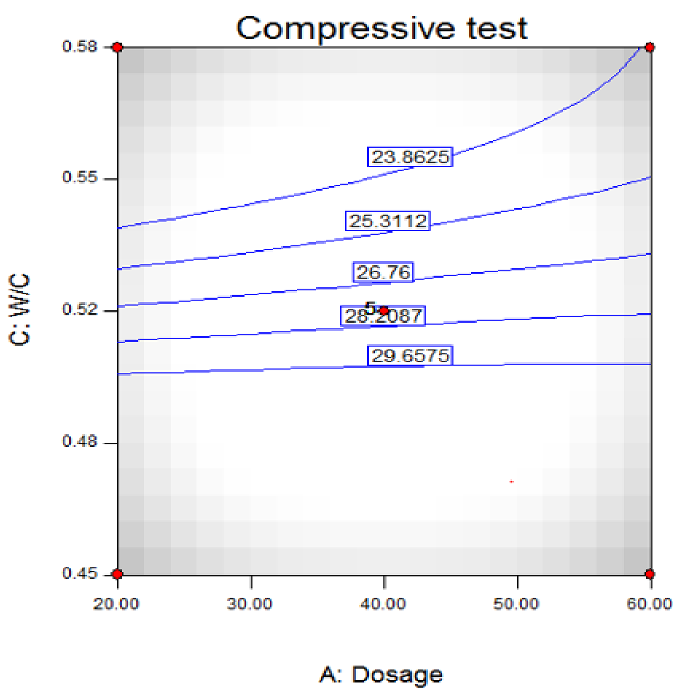

Figure 4.21 Contour plot of the effect of dosage and water / cement ratio at constant time For *Sporosarcina pasteurii* concrete

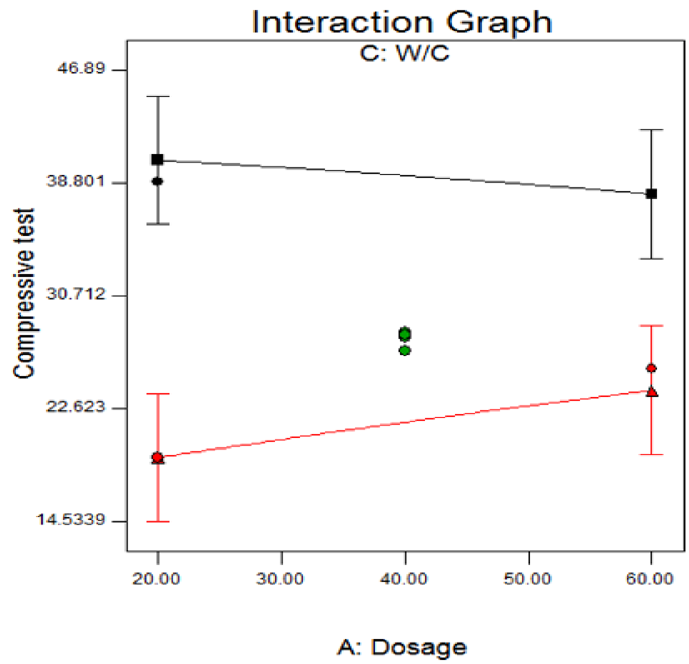

Figure 4.22. Effect of dosage and water / cement ratio (fixed) at center of time For *Sporosarcina pasteurii* concrete

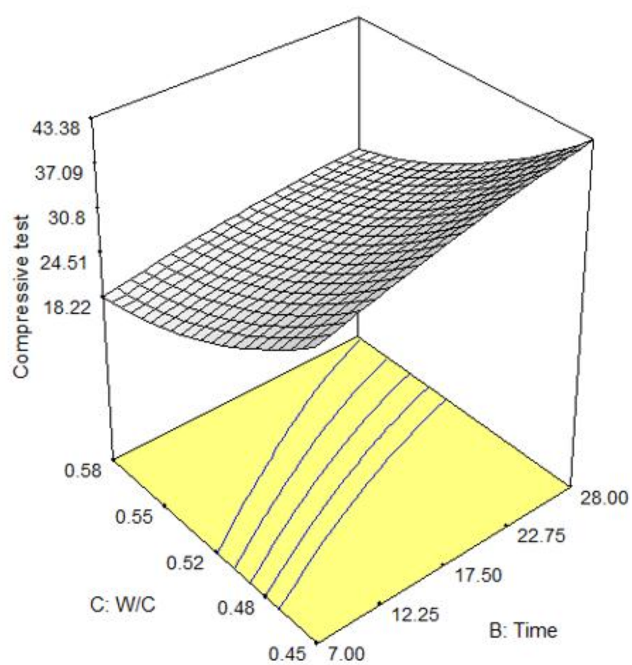

Figure 4.23 Response surface plots of the effect of time and water / cement ratio on the compressive test constant dosage in the center For *Sporosarcina pasteurii* concrete

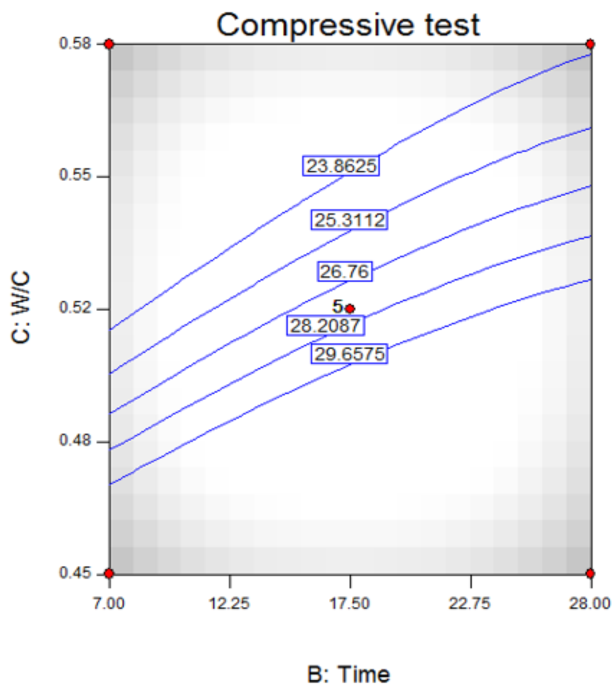

Figure 4.24 Contour plot of the effect of time and water / cement ratio at constant dosage For *Sporosarcina pasteurii* concrete

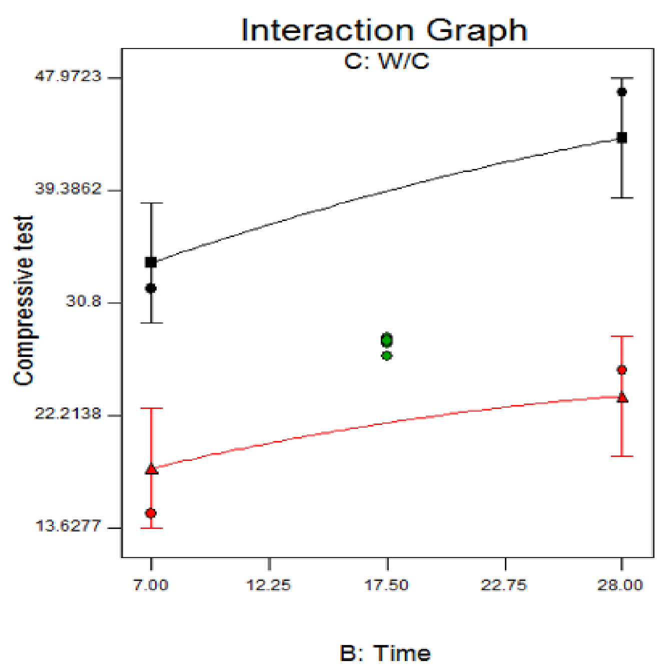

Figure 4.25 Effect of time and water / cement ratio (fixed) at center of dosage For *Sporosarcina pasteurii* concrete

4.3.3. Optimization of *Bacillus cibi*

Table 4.6 Optimization results of *Bacillus cibi* on compressive test

	Run	Factor 1 A:Dosage Ml	Factor 2 B: Time Day	Factor C:W/C	Response 1 Compressive test MPa
7	1	20	14:00	0.58	25.47
15	2	40	14:00	0.53	30.89
14	3	40	14:00	0.53	32.04
11	4	40	7:00	0.58	15.09
2	5	60	7:00	0.53	19.07
1	6	20	7:00	0.53	21.28
9	7	40	7:00	0.45	26.81
16	8	40	14:00	0.53	32.89
17	9	40	14:00	0.53	32.11
8	10	60	14:00	0.58	15.13
5	11	20	14:00	0.45	29.34
4	12	60	28:00	0.53	24.17
6	13	60	14:00	0.45	30.8
10	14	40	28:00	0.45	36.02
13	15	40	14:00	0.53	30.43

12	16	40	28:00	0.58	20.04
3	17	20	28:00	0.53	26.24

4.3.3.1 Analysis of variance (ANOVA)

In ANOVA analysis the model was significant when fisher's F test with a very low probability value. From Table pbserved that the Model F-value of 28.66 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, A^2, B^2, C^2, AC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The coefficient for the linear effect of Dosage, W/C ratio and time was highly significant. It was also observed that there is an interaction effect between Dosage and W/C ratio.

Table 4. 7 Analysis of variance (ANOVA) for *Bacillus cibi*

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	627.75	9	69.75	28.66	0.0001	Significant
A	21.65	1	21.65	8.90	0.0204	
B	73.33	1	73.33	30.13	0.0009	
C	278.95	1	278.95	114.63	< 0.0001	
A^2	72.29	1	72.29	29.71	0.0010	
B^2	98.57	1	98.57	40.51	0.0004	
C^2	23.12	1	23.12	9.50	0.0178	
AB	4.900E-003	1	4.900E-003	2.014E-003	0.9655	

AC	34.81	1	34.81	14.30	0.0069	
BC	4.54	1	4.54	1.86	0.2144	
Residual	17.03	7	2.43			
Lack of Fit	13.07	3	4.36	4.40	0.0933	Not significant
Pure Error	3.96	4	0.99			
Cor Total	644.78	16				

The “Lack of Fit F-value” of 4.40 implies the Lack of Fit not significant relative to the pure error. There is a 9.33% chance that a “Lack of Fit F-value” this large could due to noise. Non-significant lack of fit is bad – we want the model to fit.

Std. Dev.	1.56	R-Squared	0.9736
Mean	26.34	Adj R-Squared	0.9396
C.V.	5.92	Pres R-Squared	0.6661
PRESS	215.31	Adeq Precision	16.551

The "Pred R-Squared" of 0.6661 is not as close to the "Adj R-Squared" of 0.9396 as one might normally expect. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 16.551 indicates an adequate signal. This model can be used to navigate the design space.

Equation

Four important parameters, dosage (A), time (B) and water cement ratio (C) were considered. Consequently, A, B and C were chosen as the independent variables while the compressive test strength was selected as the response (dependent variable) of the study. The final central composite design obtained for percentage compressive strength with significant terms was quadratic as suggested by the software, and is given as:

Final Equation in Terms of Coded Factors:

$$\text{Compressive test} = 31.67 - 1.64\ A + 3.03\ B - 5.91\ C - 4.14\ A^2 - 4.84\ B^2 - 2.34\ C^2 + 0.035\ A\ B - 2.95\ A\ C - 1.07\ B\ C$$

Final Equation in Terms of Actual Factors:

$$\text{Compressive test} = -161.11885 + 1.91219\ \text{Dosage} + 2.62132\ \text{Time} + 598.54556\ W/C - 0.010359\ \text{Dosage}^2 - 0.043887\ \text{Time}^2 - 554.67456\ W/C^2 + 1.66667E\text{-}004\ \text{Dosage}\ \text{Time} - 2.26923\ \text{Dosage}\ W/C - 1.56044\ \text{Time}\ W/C$$

In above equation reveals how the individual variables (quadratic) or double interaction affected on compressive strength. The negative coefficient values indicate that individual or double interactions factors negatively affect compressive strength (i.e. compressive strength percentage decreases), whereas positive coefficient values mean that factors increase compressive strength in the tested range

4.3.3.2 Diagnostic test for the responses

All diagnostic plots are also tested for all responses for adequacy of the models (normal plot of residuals, residuals vs predicted value, residuals vs factor, box cox plot, studentized residuals, leverage, etc.). From the plot as shown below, the normal probability plot indicates the residuals following by the normal % probability distribution, in the case of this experimental data the points in the plots shows fitted to the straight line in the figure, this shows that the quadratic polynomial model satisfies the assumptions analysis of variance (ANOVA) i.e. the error distribution is approximately normal.

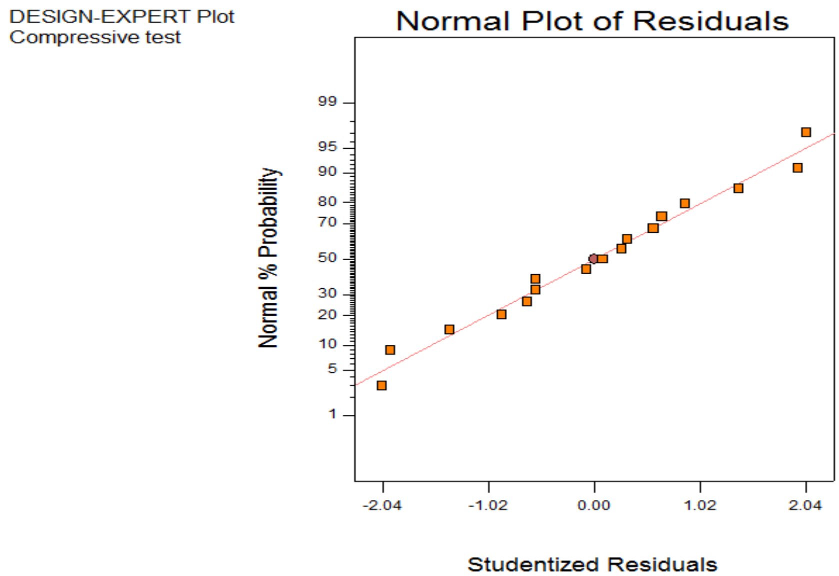

Figure 4.26. Normal plots of residuals using bacteria *Bacillus cibi*

4.3.3.3 Response surface plot analysis

On the below figure (4.27-4.29) show the effect of dosage, time and water/cement ratio on compressive strength of concrete mixing with the bacteria *Bacillus cibi*. Response surfaces were drawn for each response using design expert software 6.0.8.

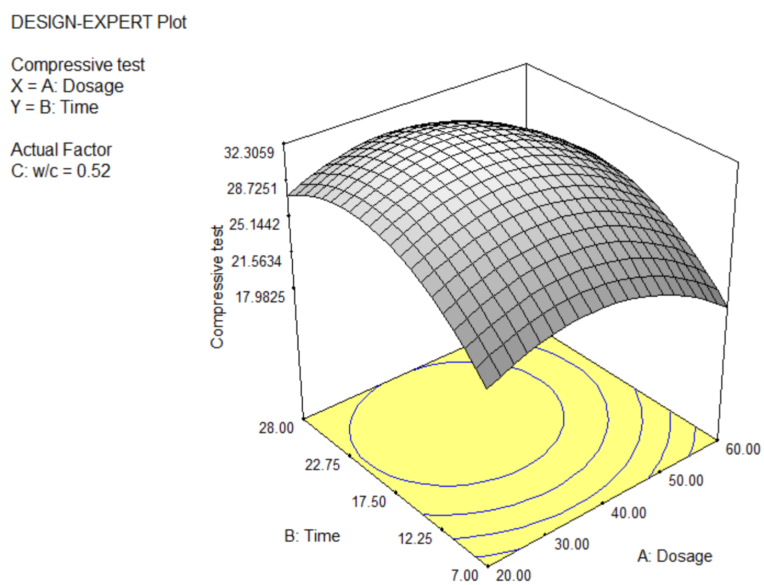

Figure 4.27. Response surface plots of the effect of Dosage and time on the compressive test constant water/cement ratio in the center using bacteria *Bacillus cibi*.

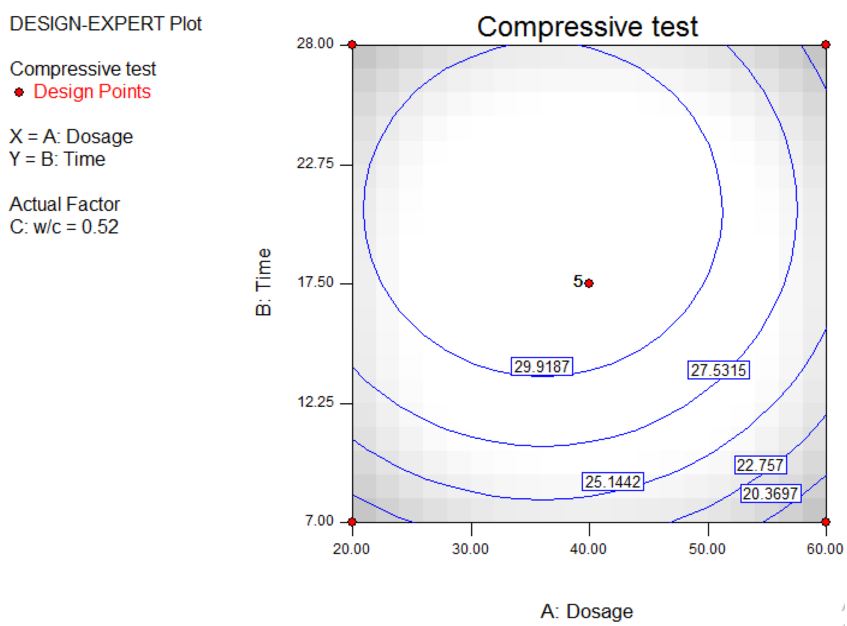

Figure 4.28 Contour plots of the effect of Dosage and time on the compressive test at fixed water/cement ratio using bacteria *Bacillus cibi*

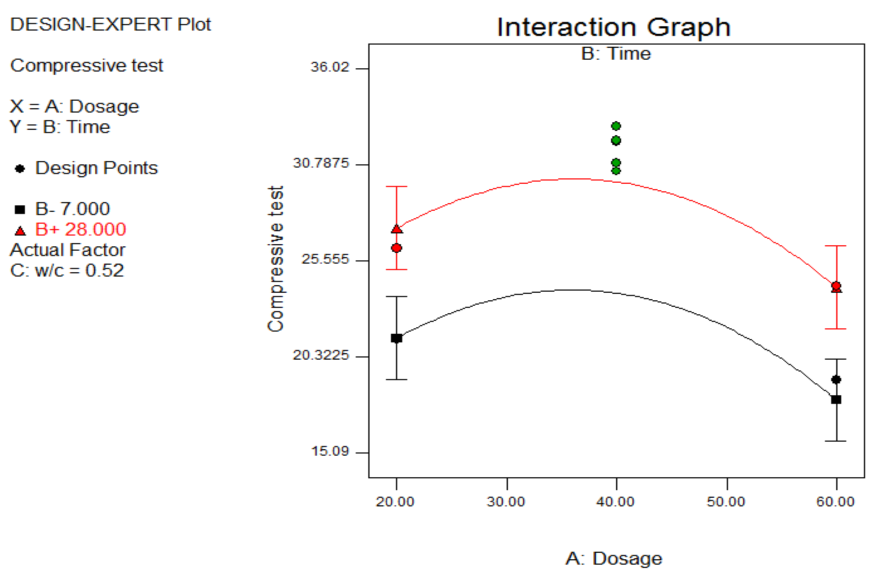

Figure 4.29. Effect and interaction of Dosage and time (fixed) on the compressive test at center of water/cement ratio using *Bacillus cibi*.

The other factor relation and individual effect on the compressive strength mixing the bacteria Bacillus Cibi in to concrete like effect of Dosage, Time and W/C ratio which show in Appendix (C).

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Concrete is an important and extensively used building material used for construction works. It is mainly used in heavy constructions because of its strength and durability. There are various factors such as natural, physical, chemical and biological factors, which cause cracks in concrete. So in order to solve this problem on this research use direct mixing the bacterial of bacillus such as *Bacillus subtilis*, *Sporosarcina pasteurii* and *Bacillus cibi* in to concrete and for control also made the normal concrete with the factors (water cement ratio, time and dosage) for each bacterial and optimized using response surface methodology. Significance of the process variables were analyzed using analysis of variance (ANOVA) and second order polynomial function was fitted to the experimental results. Thus, the influence of all experimental variables, factors and interaction effects on the response was investigated. Dosage, time, water/cement ratio and in the case of bacteria *Bacillus* cibi use interaction between dosage and water cement ratio have significance effect on the compressive strength. As the result of RSM optimization, the best compressive strength were found on the 0.45 water cement ratio, 40 ml of dosage and the time after 14 day. All points were located near to the Box – Behnken of the design. Also from the three bacterial *Sporosarcina pasteurii* selectable or give high compressive strength and strength it was also non- toxigenic for human species this result similarity with paper Mohit G in 2015.

In lab scale this bio concrete give effective strength for concrete or cost effectiveness and it reduce the cost expense of form work in the construction building time.

5.2 Recommendation

For further research recommended that bacterial concrete technology has proved to be better than many conventional technologies, because of its eco-friendly nature and very convent for usage also the application of microbial concrete to construction may also simplify some of the existing construction processes and revolutionize the ways of new construction process but still there has been much to explore in order to bring this environmentally safe, cost effective and convenient technology from lab to field scales.

On this paper identify selectable bacillus species with deferent dosage, water / cement ratio and time by testing the compressive strength and looking the microbial vision on the scanning Electron Microscopy (SEM) for addition strongly proven test using X-ray, diffraction test, flexural strength slump flow test, split testily strength and water absorption will be test. Additionally, to see huge effect of this bacteria it was selectable making the concrete cylindrical and on the mixing time adding some chemical like urea will be got more strength.

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APPENDIX A

A.1 Calculation for mix proportion of C25 (1:2:3) grade

A.1.1 Target mean strength of concrete

Where, f'ck = target average compressive strength at 28 days

fck = characteristic compressive strength at 28 days

S = Standard deviation

f'ck= 25+1.645 5 = **33.225 MPa**

For S refer New EBCS standard for cubic concrete and t at 90 % level confidence critical value 1.645.

A.1.2 Select of water cement ration

In this research also looking or optimize water cement ratio which effect on the strength of the concrete from maximum (0.58) to minimum (0.45) water cement ratio.

A.1.3 Mixing Design

Use random design method for this project which have c25 (1:2:3 ratio) compressive strength with different water cement ratio

Cement

1.5 = 0.25 m ^{3}

= where 0.035 m^{3}/bag is volume of cement bag

= 7.142 bag 50 kg / bag

= **357.15 kg / m ^{3} ≈ 360 kg / m^{3}**

Fine aggregate

1.5 = 0.5 m ^{3}

= 0.5 m ^{3} 1600 kg / m ^{3} = **800 kg /m^{3}**

Where 1600 kg / m ^{3} is density of light fine aggregate

Coarse aggregate

1.5 = 0.75 m^3

= 0.75 m^3 1450 kg / m^3 = **1087.5 kg/m^3**

Where 1450 kg / m^3 is density of coarse aggregate

Then calculate for 2, 3, 4 and 5 number of concrete as the size of 15 15 15 cm concrete size as follow form:-

(0.15 0.15 0.15) m^3 number of concrete need

Finally calculate need of cement, fine aggregate and coarse aggregate for number of 2, 3, 4 and 5 concrete as follow

Cement

For 2 concrete

(0.15 0.15 0.15) m^3 2 360 kg / m^3 = **2.43 kg**

Calculate for 3, 4 and 5 number of concrete just like above and get 3.645, 4.86 and 6.075 kg respectively.

Fine aggregate

For 3 concrete

(0.150.15 0.15) m^3 3 800 kg / m^3 = **8.1 kg**

Calculate for 2, 4 and 5 number of concrete just like above and get 5.4, 10.8 and 13.5 kg respectively.

Coarse aggregate

For 4 concrete

(0.15 0.15 0.15) m^3 4 1087.5 kg / m^3 = **14.68 kg**

Calculate for 2, 3 and 5 number of concrete just like above and get 7.34, 11.011 and 18.35 kg respectively.

Water

On this project done in different water cement ratio (0.45, 0.55 and 0.58) which affect the concrete strength so can calculate water content for all as follow:-

W / c = 0.45, 0.55 and 0.58 where w = water

C = cement

For 2 number of concrete those 0.45 water cement ratio

W / c = 0.45

W / 2.43 = 0.45

W = **1.09 kg** or liter water

For 3, 4 and 5 concrete on the ratio of 0.45 got 1.64, 2.187 and 2.734 kg or litter respectively.

For 2 number of concrete those 0.55 water cement ratio

W / c = 0.55

W / 2.43 = 0.55

W = **1.3365 kg** or liter water

For 3, 4 and 5 concrete on the ratio of 0.55 got 2, 2.673 and 3.34125 kg or litter respectively.

For 2 number of concrete those 0.58 water cement ratio

W / c = 0.58

W / 2.43 = 0.58

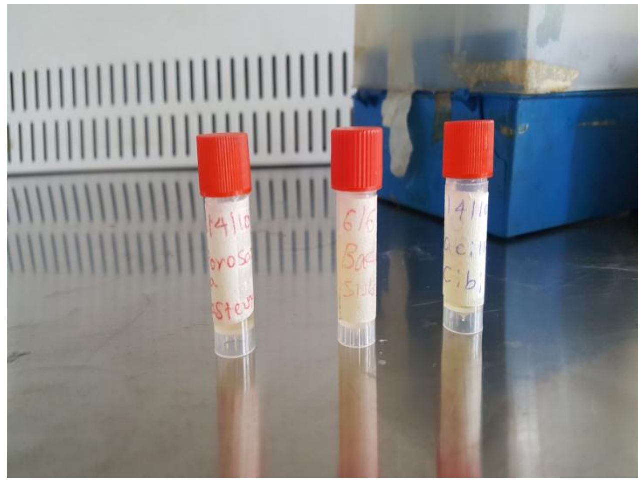
W = **1.41 kg** or liter water

For 3, 4 and 5 concrete on the ratio of 0.58 got 2.1141, 2.8188 and 3.5235 kg or litter respectively.

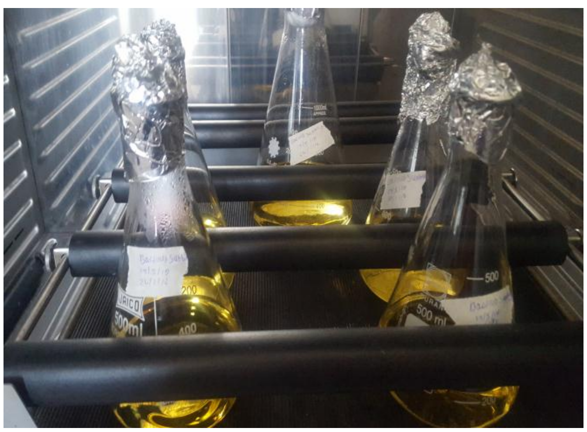
APPENDEX B

Sample Photo Gallery Taken During the Research

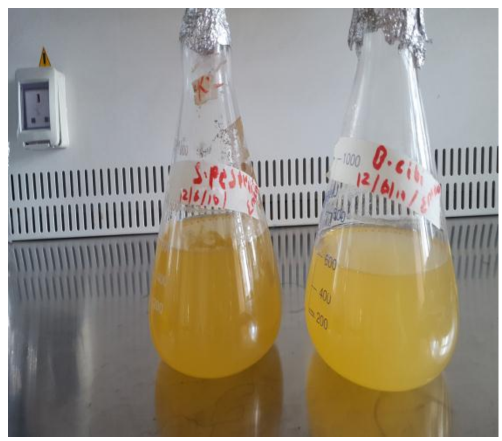
B.1 Photo: Nutrient broth (media)



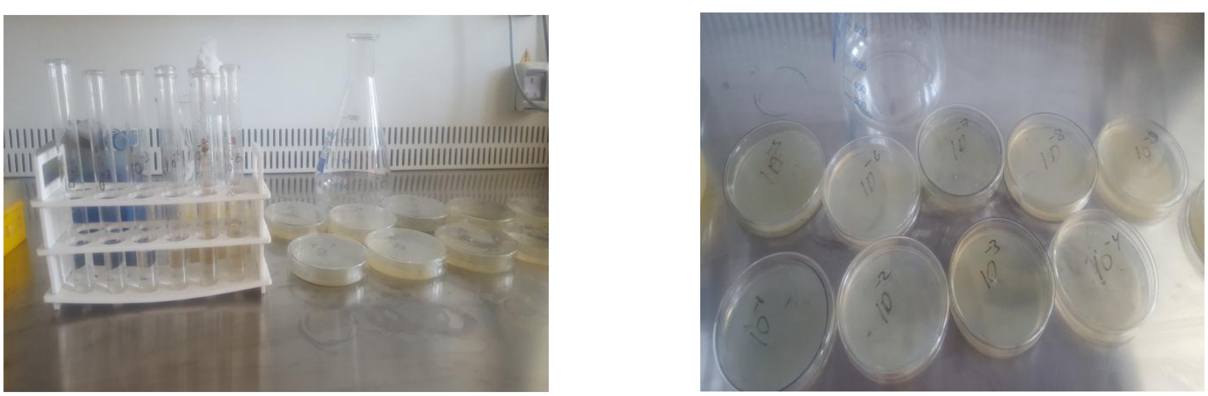
B.2 Photo: Bacteria from microbial bank



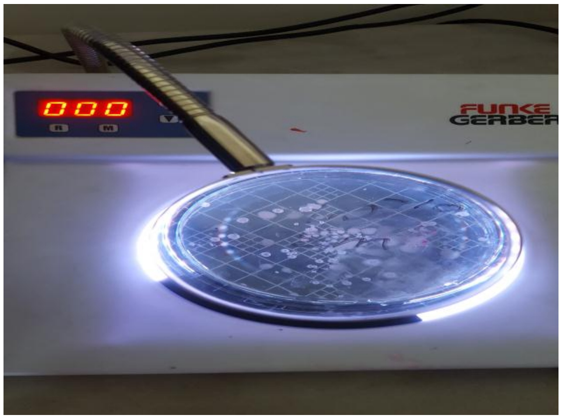
B.3 Photo: samples in shaker incubator



B.4 Photo: Grown microbe



B.5 Photo: Serial dilution

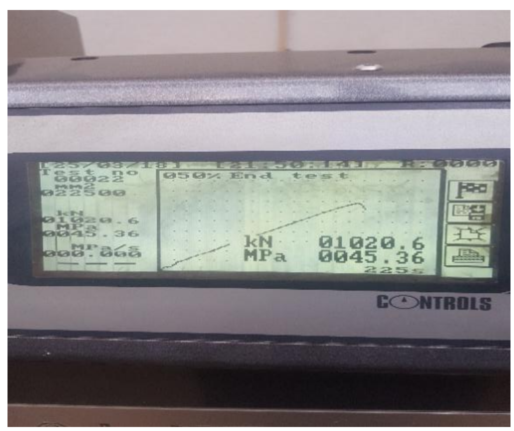


B.6 Photo: Count the microbes

B.7 Photo: Mechanical mixer

B.8 Photo: Sample of cube

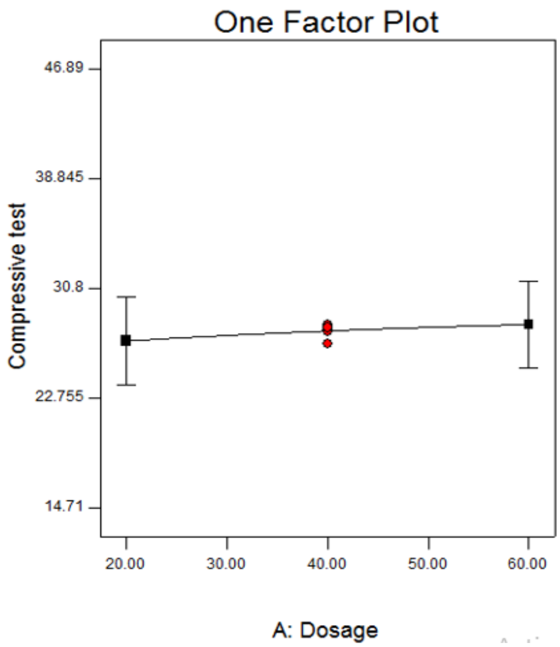
B.9 Photo: Compressive strength test machine



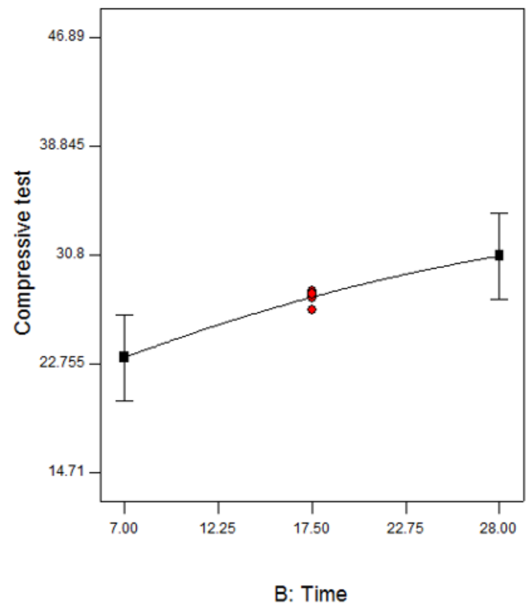
B.10 Photo: 28th day Compressive strength test result of C-25

APPENDEX C

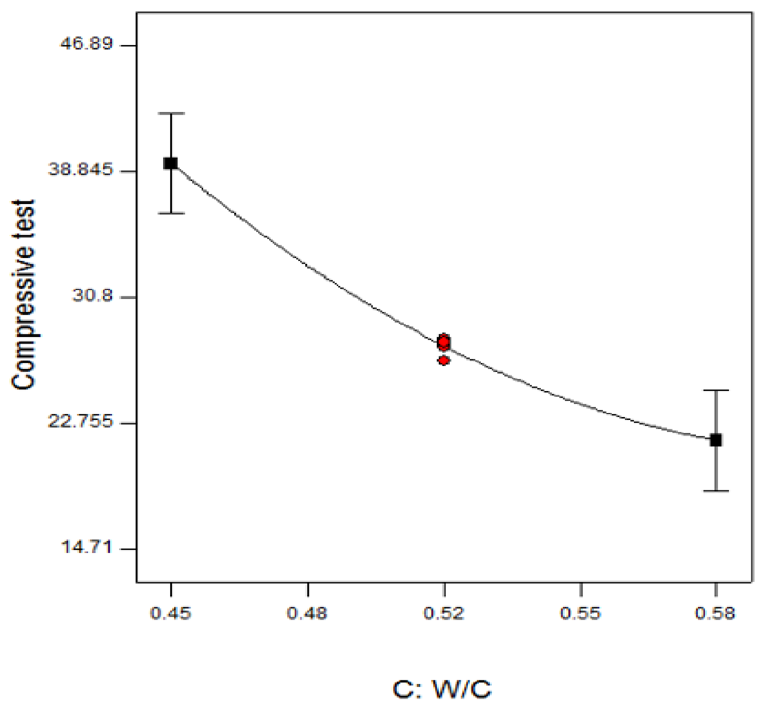
C.1 Individual factors (Dosage, Time and W/C ratio) effect on the compressive strength mixing the bacteria *Sporosarcina pasteurii* in concrete.



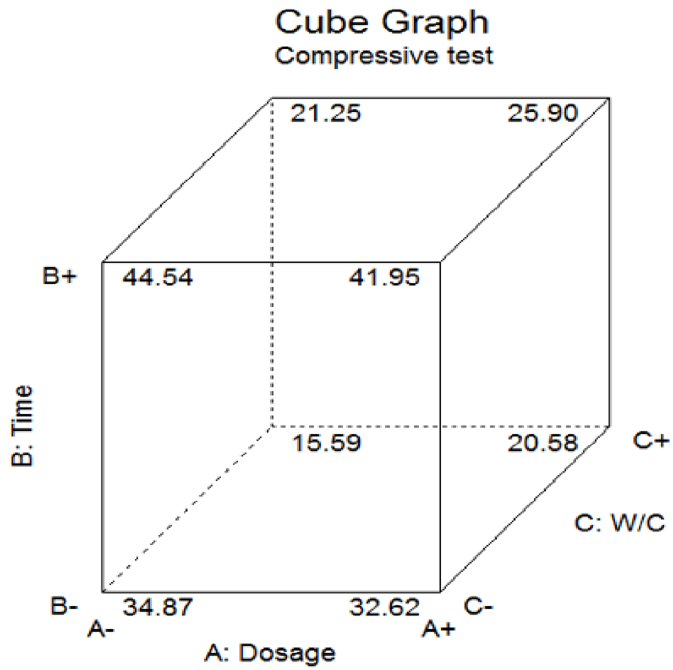
C.1 Photo: Effect of Dosage on compressive test



C.2 Photo: Effect of Time on compressive test

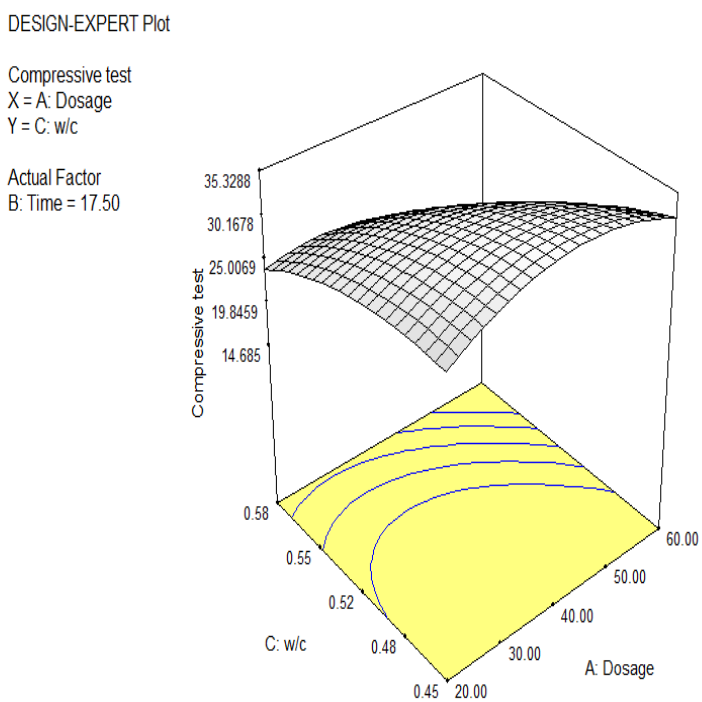


C.3 Photo: Effect of W/C ratio on compressive test

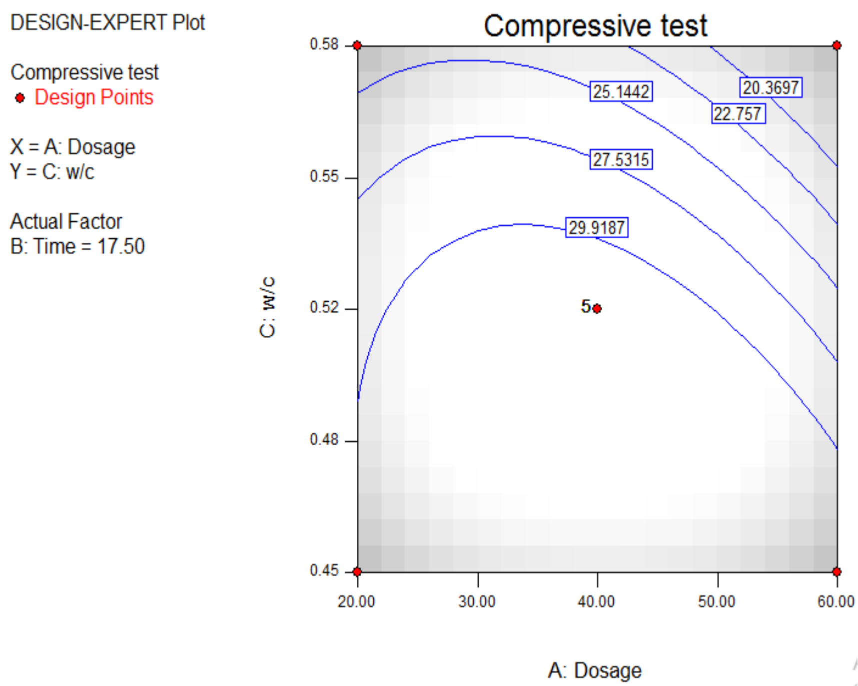


C.4 Photo: Cube graph of effect of concrete compressive strength

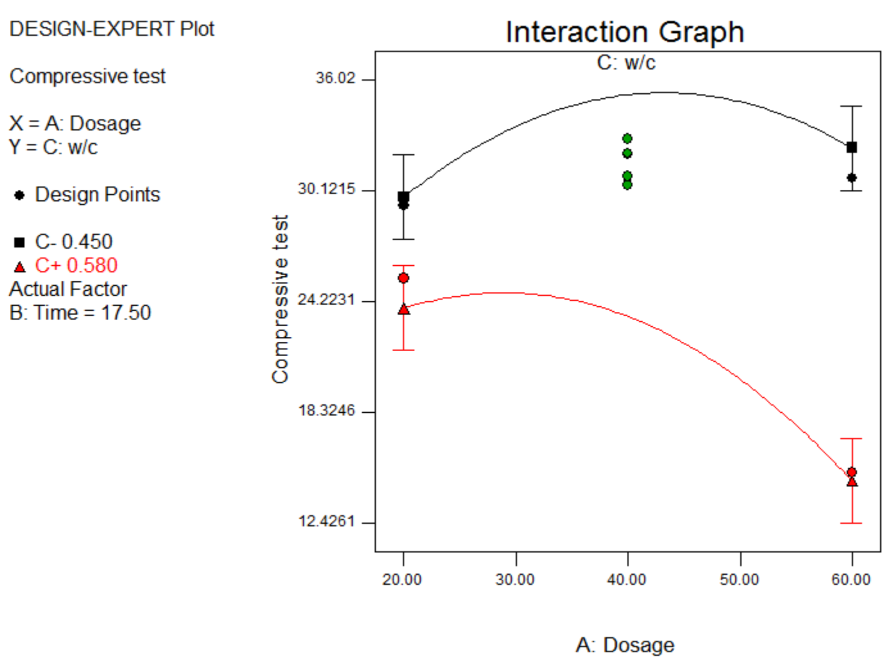
C.2 Factor (Dosage, Time and W/C ratio) effect on the compressive strength mixing the bacteria *B.Cibi* in concrete.



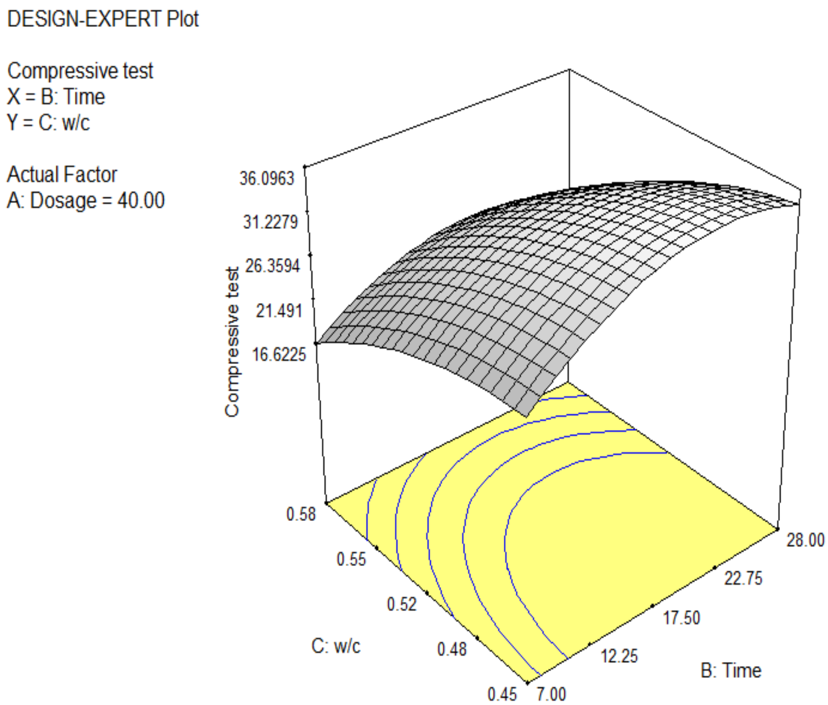
C.5 Photo: Response surface plot of the effect of Dosage and water/cement ratio at constant time in the center using bacteria *B.Cibi*.



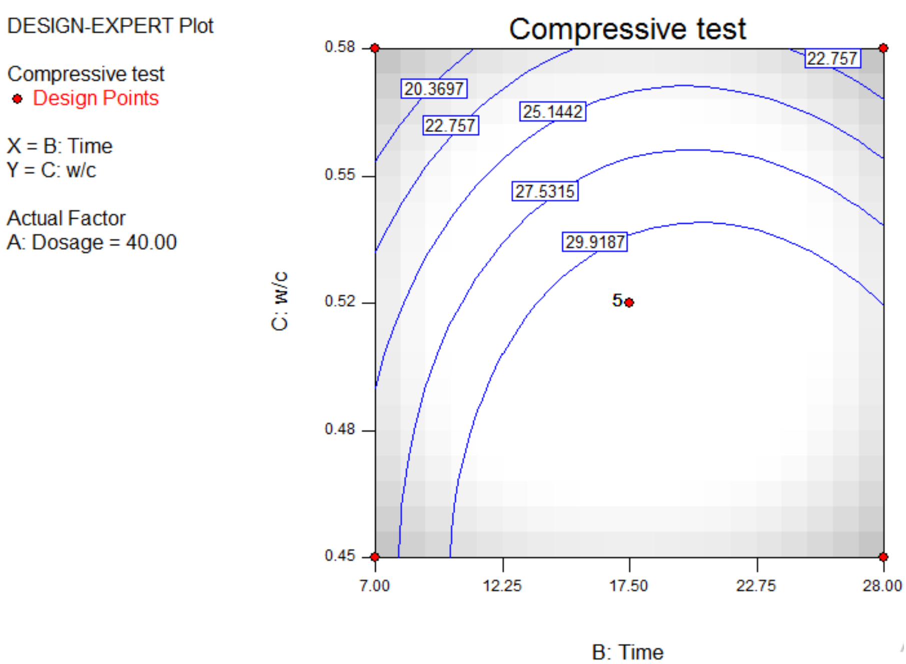
C.6 Photo: Contour plot of the effect of dosage and water/cement ratio at constant time in the center using bacteria *B.Cibi*.



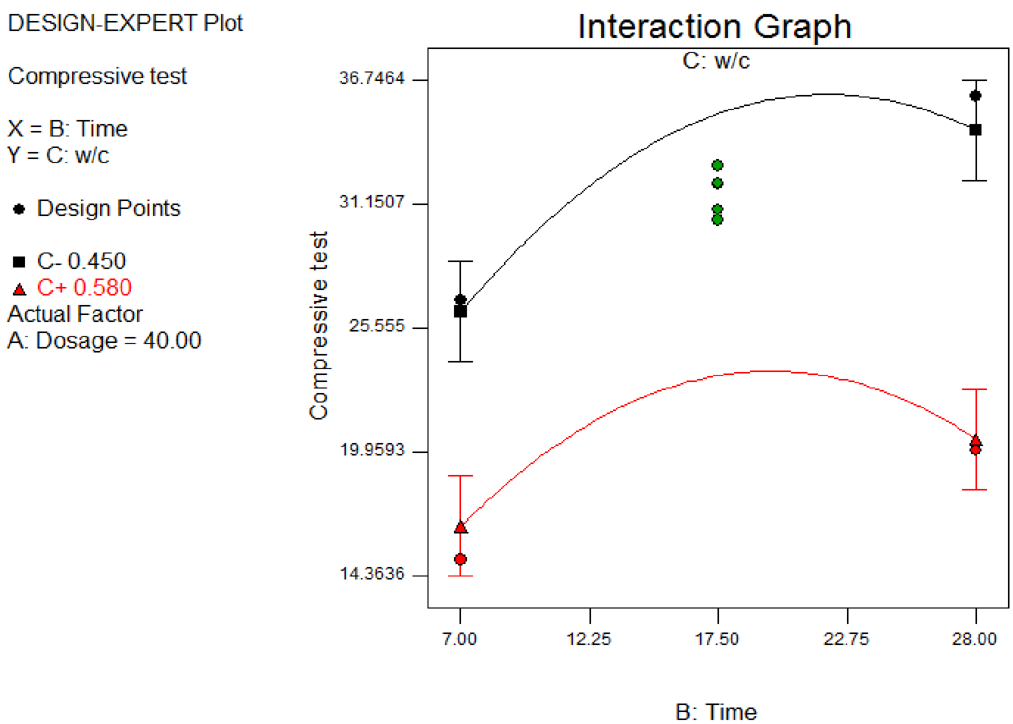
C.7 Photo: Effect and Interaction of dosage and water/cement ratio (fixed) at center of time using bacteria *B.Cibi.*



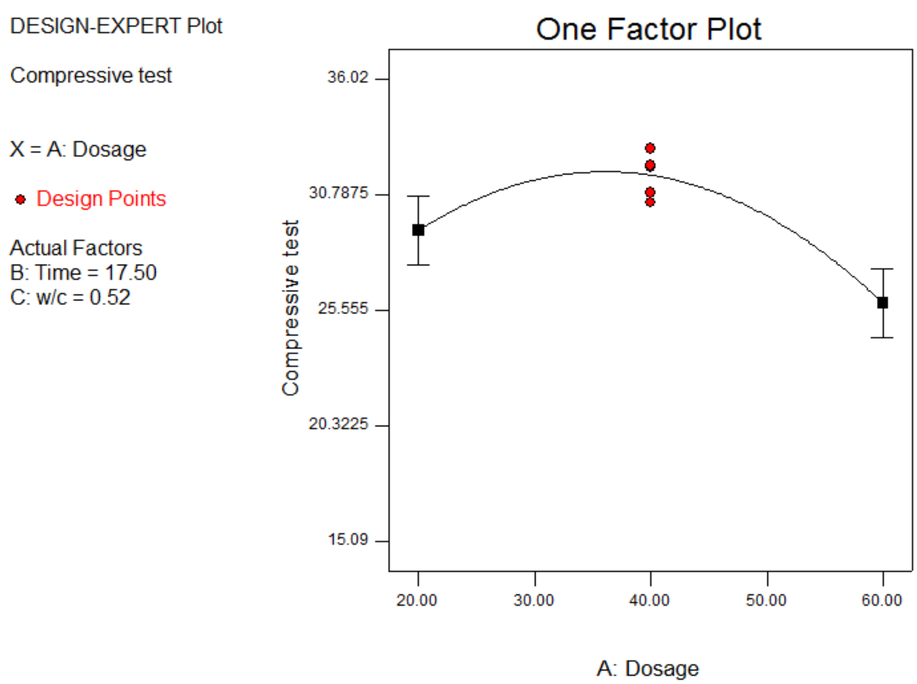
C.8 Photo: Response surface plot of the effect of time and water/cement ratio at constant dosage using bacteria *B.Cibi.*



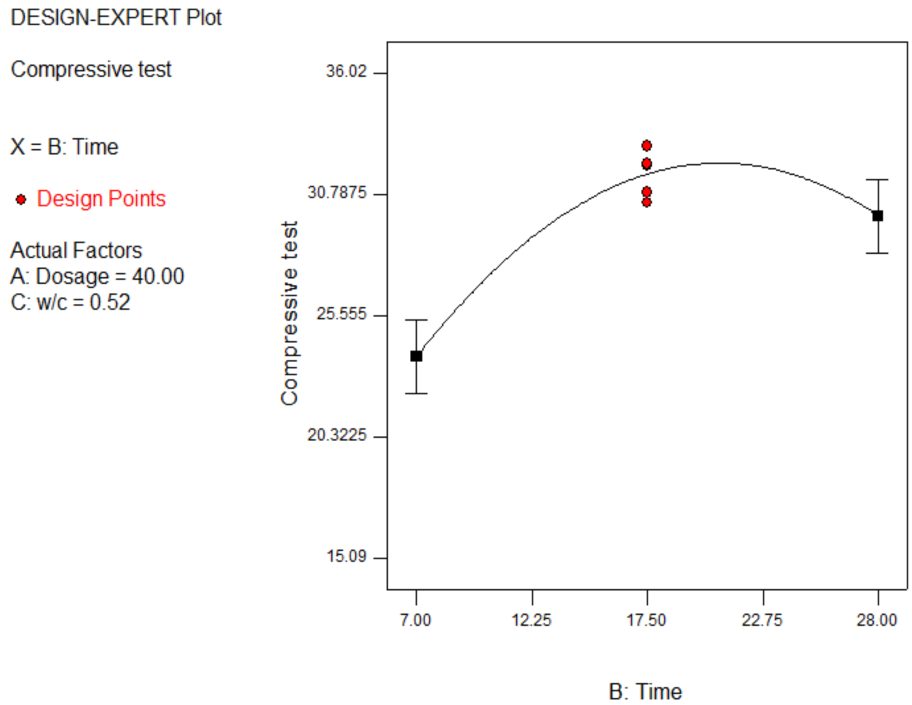
C.9 Photo: Contour plot of the effect of time and water/cement ratio at constant dosage using bacteria *B.Cibi*.



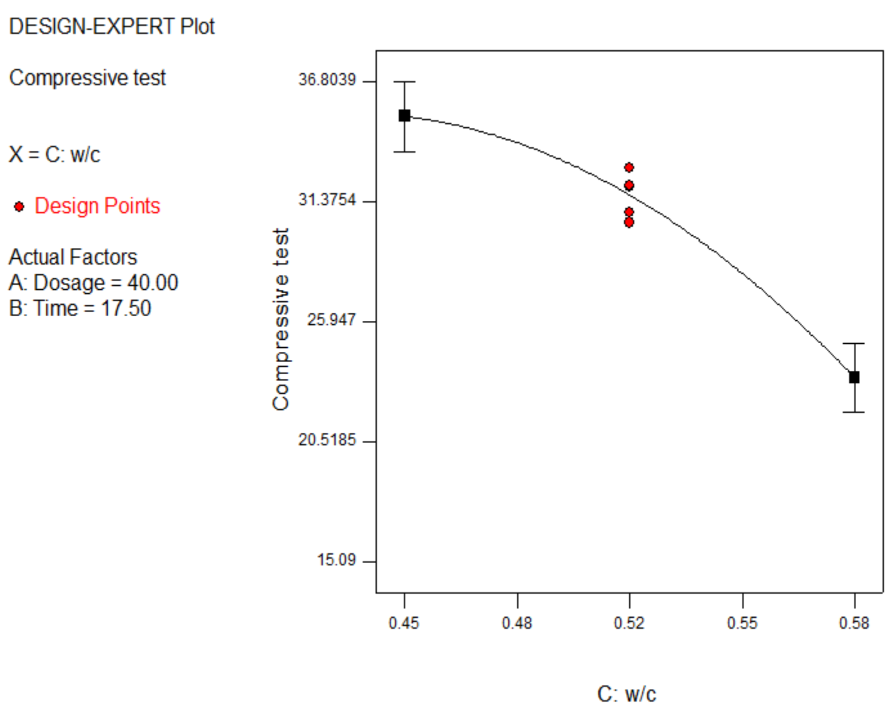
C.10. Photo: Effect of time and water/cement ratio (fixed) at center of dosage using bacteria *B.Cibi*.



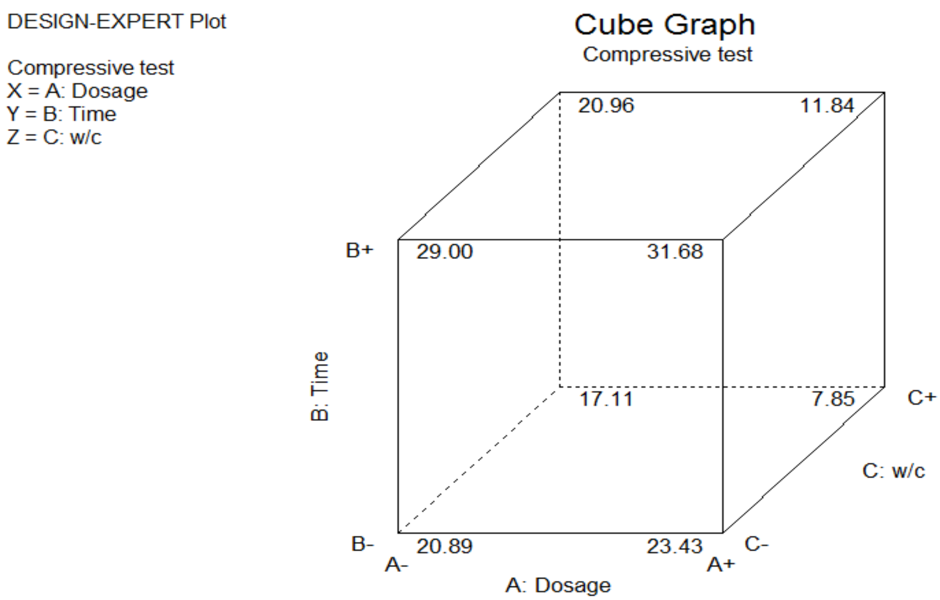
C.11 Photo: Effect of Dosage on compressive test



C.12 Photo: Effect of Time on compressive test



C.13 Photo: Effect of W/C ratio on compressive test



C.14 Photo: Cube graph of effect of concrete compressive strength using