USE OF SEWAGE EFFLUENT AS WATER REPLACEMENT IN MICROBIAL CONCRETE

A Thesis submitted in partial-fulfillment of the requirements for the award of degree of

MASTER OF ENGINEERING
IN
STRUCTURAL ENGINEERING

Submitted by

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DECLARATION

I, Samrat Goyal hereby declare that the work presented in this thesis entitled, "**USE OF SEWAGE EFFLUENT AS WATER REPLACEMENT IN MICROBIAL CONCRETE**", in partial fulfillment of the requirement for the award of degree of **Master of Engineering** in **Structural engineering** submitted at Department of Civil Engineering, Thapar Institute of Engineering and Technology (Deemed to be University), Patiala is an authentic record of my own work carried under the supervision of **Dr. Shweta Goyal, Associate Professor,** Department of Civil Engineering and **Dr. M. Sudhakara Reddy, Professor**, Department of Biotechnology, Thapar Institute of Engineering and Technology (Deemed to be University), Patiala from July, 2018 to July, 2019. The matter presented in this has not been submitted either in part or full to any other university or institute for the award of any other degree.

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CERTIFICATE

It is certified that the above statement made by student is correct to the best of my knowledge and belief.

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ABSTRACT

Microbial concrete is made by adding microbes to the standard material of the concrete. These microbes lead toward the formation of calcium carbonate crystals within the pores of the concrete specimen known as microbial induced calcite precipitation (MICP). This results in the formation of denser microstructure of concrete, having high compressive strength, lower initial surface absorption, lower rate of initial and secondary absorption and better corrosion resistance.

In this research work, sewage effluent was used in place of fresh water for casting and curing of the specimen. Microbes were mixed with sewage effluent along with nutrient media (NB and CSL) supplemented with calcium chloride and urea. Compressive strength test were performed after 3, 7, 28 and 90 days of curing while the durability tests were performed after 28 days of curing. From the experimental data it was observed that the performance of the microbial concrete produced by using sewage effluent was much better than the conventional concrete mix produced by using sewage effluent only. The relative increase in the 90 days compressive strength of the microbial concrete was 43.13% and 37.02% as compared to the control specimen (S-S) in case of microbial treated NB media and microbial treated CSL media, respectively. The durability properties of the microbial concrete were very much improved as compared to the control specimens. Also, microbial concrete is resistive to corrosion of the reinforcement bar. Corrosion of control specimen takes place after 6 days while the corrosion of microbial concrete takes place after 14 days and 10 days in case of microbial treated NB media and microbial treated CSL media, respectively.

Out of microbial treated NB media and microbial treated CSL media, microbial treated NB media is much better. This is because of the cost difference between them. CSL is an industrial by-product, while NB is an expensive laboratory nutrient medium. The experimental results regarding strength and durability properties of the concrete specimens were approximately same for both the nutrient medium. So for huge construction microbial treated CSL media should be economical.

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CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

In the construction field, main constituent is concrete. Concrete is tough in compression and delicate in tension, concrete has very poor resistivity to cracking because it has limited ductility. The characteristics and properties of concrete decide the life cycle cost and overall cost of building. The four main constituents of concrete are cement, fine aggregates, coarse aggregates and water. To increase concrete hardness, increase in cement amount is not a good option. Because by increasing cement amount, concrete hardness increases, but cost of production of concrete also increases and problems like shrinkage, creep also occurs. So researchers found many constituents like ground-granulated blast-furnace slag, flyash, silica fume etc, by using these constituents strength and durability of matrix will improve but production cost of concrete decreases.

One more type of concrete that is developed in the last decade is the microbial concrete, which was developed by using microbes into concrete. The strength and durability properties of microbial concrete are reported to be much better than conventional concrete having same quantity of the concrete ingredients. Mineral precipitation is the unique property of the microbial concrete in which mineral precipitates are formed in the pores of the concrete matrix and enhance strength properties and durability properties of matrix.

1.2 MICROBIAL CONCRETE

Microbial concrete is the type of concrete formed by adding bacterial culture to matrix. Addition of Bacterial culture was done during casting and curing of sample. This bacterial culture has an ability to form calcium carbonate precipitates, so the activity of $CaCO_3$ evolution is termed as MICP.

Cement, fine aggregates, coarse aggregates, water, bacterial cultures (*Bacillus megaterium SS3*) are the main constituents of microbial concrete. Along with the bacterial culture, NB (nutrient broth), urea, calcium chloride ($CaCl_2$) as calcium source are supplemented in microbial concrete. The basic constituents of microbial concrete are discussed briefly in the following sections:

1.2.1 Bacteria

Small unicellular organism having length varies from 0.5 µm to 5 µm are known as the bacteria. Bacteria are spherical, rod shaped or spiral in shape. "Millions of bacterial cells may available in one liter fresh water (Siddique et al. 2011). 40 millions of bacterial cells are available in 1 gm soil approximately". Microbial species are present in wide range in the natural environment that is responsible for the mineral carbonate (calcium carbonate) precipitation.

Microbes have a remarkable part in field of engineering and technology and science due to its readily availability in nature. Due to its advantages many researches and studies are done over these inhabitants. These are more popular in the field of medical and agriculture and now days due to its self healing nature it became more popular in the field of civil engineering.

1.2.1.1 Bacterial morphology

Bacterial cells mainly are of two types according to their shape i.e. cocci and bacilli. Cocci word taken from Greek word 'kokkos', which means grain and seed. While bacilli word taken from Latin word 'baculus', which means stick. Cocci are spherical bacterial species and bacilli are rod shaped species.

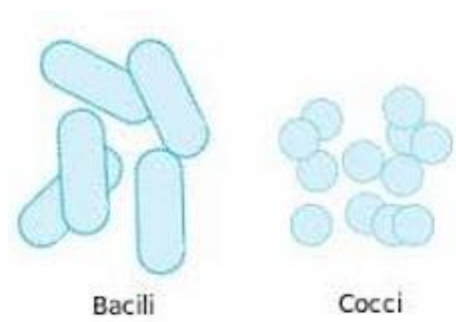


Figure 1.1: Shape of bacterial cells, bacilli and cocci

1.2.1.2 Bacterial reproduction

Reproduction of bacterial cells occurs by binary fission of cells. The process of reproduction of bacterial cell is very fast and it was studied that population of bacterial cells become approximately 2 times as compare to initial population just in 9.8 minutes (Siddique et al. 2011). Proper nutrients like NB (nutrient broth) and CSL (corn steep liquor) are provided for the bacterial cell growth. While in the natural environment, reproduction of bacterial cells are limited this is due to the limited availability of nutrients that is required for the proper growth of the bacterial cells.

1.2.1.3 Preference of bacteria for microbial concrete

The bacterial culture used in the microbial concrete should be alkaliphilic i.e. that the ideal growth of the culture occurs at pH of about 9 and no growth of the culture is observed at pH level below 6.5. The bacterial culture should be nonpathogenic and should have ability to deposit calcite homogeneously on the substratum. To increase the rate of MICP (Microbial Induced Calcite Precipitation) and ureolysis, bacterial culture should have ability to generate high volume of urease enzymes in the presence of ammonium in high concentration.

Ureolysis activity is a temperature dependent activity. The temperature in which ureolysis reaction is most effective ranges from $20^{o}C$ to $37^{o}C$. This temperature range is dependent on the bacterial cell concentration, environmental conditions and other materials present in system. Some researchers reported that the increase in the temperature leads toward the increase in the ureolysis reaction.

1.2.2 Nutrient broth

Nutrient broth is a basic media composed of a simple peptone and a beef extract. Peptone contributes organic nitrogen in the form of amino acids and long chained fatty acids. Beef extract provides additional vitamins, carbohydrates, salts and other organic nitrogen compounds. Nutrient broth media is responsible for the growth of the bacterial culture by providing proper nutrients to the microbes.

1.2.3 Urea and $CaCl_2$

The hydrolysis of urea by urease not only increases the pH, but also uses it as a nitrogen and energy source. It is possible that individual microorganisms can produce ammonia as a result of enzymatic hydrolysis of urea to create an alkaline micro-environment around the cell and increase the pH, subsequently inducing the $CaCO_3$ precipitation. Microbial cell surfaces have negatively charged and act as scavengers for cations (Ca^{2+}), in aquatic environments by binding them onto their cell surfaces. Therefore, the ideal calcium source and concentration is important for $CaCO_3$ precipitation. However, high concentrations of urea and $CaCl_2$ (above 0.5 M) decrease the efficiency of calcite precipitation (Okwadha and Li 2010) and increased efficiency was observed at low concentrations (0.05–0.25 M).

1.3 CALCIUM CARBONATE CRYSTAL PRECIPITATION BY BACTERIAL CELLS

From the last decade, the usage of bacterial cells in concrete became popular. This was because of the ability of formation of calcium carbonate crystals by the specific microbes in pores of the matrix. Concrete is extremely alkaline in nature having pH greater than 12 which may puts an adverse effect on the microorganisms but some species of bacillus were found which remain alive in highly alkaline conditions and are able to blocks the voids or pores of the concrete by accumulating calcium carbonate ($CaCO_3$) precipitation.

Different bacterial cells were reported by the researchers which have capability of formation of calcium carbonate crystals. Anaerobic microbes isolated from hot spring of Bakreshwar, India and develop in standard Luria Broth (LB) media were used for calcite precipitation (Ghosh et al. 2005); The bacterial culture called *S. pasteurii* which was produced by National Chemical Laboratory, Pune, India was used for calcium carbonate precipitation (Achal et al. 2010); *Bacillus sp. CT-5* isolated from cement was also taken for calcite precipitation (Achal et al. 2011); *Bacillus megaterium SS3* which was extracted from the calcareous soil accumulated from "Anantapur District, Andhra Pradesh, India" was taken for calcium carbonate precipitation (Dhami et al. 2013).

1.4 MECHANISM OF MICP (MICROBIAL INDUCED CALCITE PRECIPITATION) WITH UREA HYDROLYSIS

Most simple and straight forward mechanism for MICP is the $CaCO_3$ precipitation by bacterial culture using hydrolysis of urea with large amount of carbonate precipitation in shorter time. Hydrolysis of one mole of urea leads towards the formation of 1 mole of carbonate and 1 mole of NH_3 as shown in (Eq. 1.1) and this carbonate hydrolyzes and leads to the formation of one mole of NH_3 and H_2CO_3 spontaneously (Eq. 1.2). These products in the presence of water forms bicarbonate ion, 2 mole of hydroxide ion and 2 moles of ammonium (Eq. 1.3 and Eq. 1.4) and due to generation of $2OH^-$, pH of solution increases. This increase in pH of the solution leads toward the carbonate ions formation (Eq. 1.5) and in the presence of Ca^{2+}, these CO_3^{2-} leads toward the development of $CaCO_3$ precipitation as shown in (Eq. 1.6).

$$\underset{\text{Urea}}{CO(NH_2)_2} + \underset{\text{Water}}{H_2O} \longrightarrow \underset{\text{Carbonate}}{NH_2COOH} + \underset{\text{Ammonia}}{NH_3} \quad (1.1)$$

$$\underset{\text{Carbonate}}{NH_2COOH} + \underset{\text{Water}}{H_2O} \longrightarrow \underset{\text{Ammonia}}{NH_3} + \underset{\text{Carbonic Acid}}{H_2CO_3} \quad (1.2)$$

$$\underset{\text{Carbonic acid}}{H_2CO_3} \longrightarrow \underset{\text{Hydron Ions}}{H^+} + \underset{\text{Bicarbonate Ions}}{HCO_3^-} \quad (1.3)$$

$$\underset{\text{Ammonia}}{2NH_3} + \underset{\text{Water}}{2H_2O} \longrightarrow \underset{\text{Ammonium Ions}}{2NH^{4+}} + \underset{\text{Hydroxide Ions}}{2OH^-} \quad (1.4)$$

$$\underset{\text{Bicarbonate Ions}}{HCO_3^-} + \underset{\text{Hydron Ions}}{H^+} + \underset{\text{Hydroxide Ions}}{2OH^-} \longrightarrow \underset{\text{Carbonate Ions}}{CO_3^{2-}} + \underset{\text{Water}}{2H_2O} \quad (1.5)$$

$$\underset{\text{Calcium Ions}}{Ca^{2+}} + \underset{\text{Bicarbonate Ions}}{CO_3^{2-}} \longrightarrow \underset{\text{Calcium Carbonate}}{CaCO_3} \quad (1.6)$$

Bacterial surface is negatively charged and have an important part in calcium carbonate precipitation. Bacterial surface attracts Ca^{2+} ions from the solution and these Ca^{2+} ions attracts the CO_3^{2-} and HCO_3^- ions from the solution and form calcium carbonate precipitates over the bacterial cell surface and blocks the pores of the concrete and improves its mechanical and durability properties. The overall reaction of formation of $CaCO_3$ is represented in Figure 1.2.

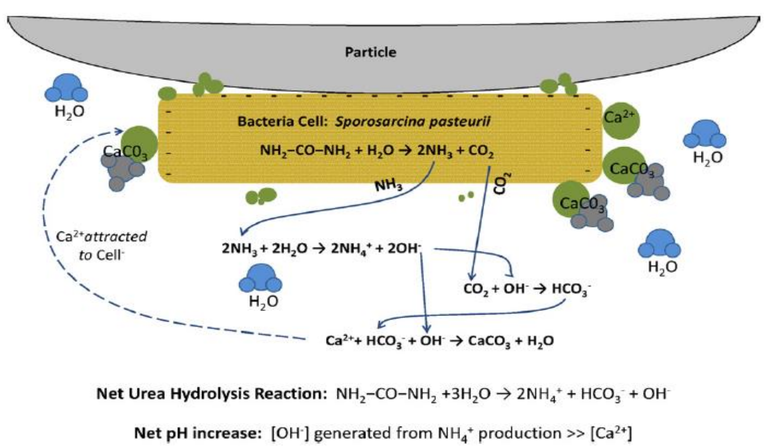


Figure 1.2: Overview of bio-mediated calcite precipitation using ureolysis by Dejong et al. (2010)

1.5 APPLICATION OF MICROBIAL CONCRETE IN CIVIL ENGINEERING

Microbial concrete enhance strength and the durability properties of matrix. Cell wall of bacterial culture is negatively charged and engage Ca^{2+} (cations) from the solution and these cations attracts the negatively charged carbonate ions (CO_3^{2-}) from the solution and forms the crystals of $CaCO_3$ in voids of matrix to make the matrix denser. This property of formation of $CaCO_3$ crystals in pores or cracks of matrix is known as the self healing property. If at any time cracks or voids are generated in the matrix, then such cracks should be filled by calcium carbonate crystals formed by the microbial concrete having self healing ability.

Various microorganisms were isolated by different researchers with different conditions and different culture media so it is very difficult to compare their results. The main application of the microbial concrete was in buildings and in wider construction, also microbial concrete is useful in the surface protection of concrete and mortar, soil stabilization, surface finishing and repairing of concrete structures.

In microbial concrete, pores are filled by the crystals of calcium carbonate which makes the structural element denser and destroy the pore continuity system of matrix. As the number of pores in the matrix decreases, water or any aggressive material from outside cannot enters into the structural element frequently and protect the steel bars to be rusted by the aggressive material.

1.6 USE OF ALTERNATIVE MATERIALS IN CONCRETE

Many researchers have been done research on microbial concrete in which they discussed about the alternatives of cement, fine aggregates, coarse aggregates, Nutrient broth media, But less research was found on the alternative of water. Corn steep liquor as an alternative to nutrient broth in microbial concrete (Achal et. al., 2010), replacement of cement with flyash (Achal et al. 2011), replacement of cement with flyash and rice husk ash (Dhami et al. 2012), addition of silica fume as binding agent and replacement of cement with ground-granulated blast furnance-slag (Depaa and kala, 2015), different bacterial cell concentration (Reddy et al. 2015), replacement of cement with flyash and ground-granulated blast furnance-slag (Goyal and Chaitanya, 2015), replacement of cement with calcium lactate (Babu and Siddiraju, 2016), replacement of cement with flyash (Bai and Varghese, 2016), replacement of nutrient broth with corn steep liquor (Joshi et al. 2018). So, the focus of this study is on the impact of waste effluent on the structural and durability properties of the microbial concrete.

1.7 IMPORTANCE OF WASTE EFFLUENT IN CONCRETE

Water is highly powerful resource over the earth, without which life is impossible. 70% of the earth surface is covered with water and out of this 70% only 1% is accessible for human use. Now a day due to increase in population, industrialization, urbanization, the percentage of fresh water going to decreases using the fresh water with more efficiently has become a necessary. In India in 1951, the water availability was 5177 cubic meter per capita while in 2009, it was reduced to 1650 cubic meter per capita (Swami et al. 2015).

Due to urbanization, industrialization and expansion in the lifestyle, the production of waste water is going to increase. The use of this treated waste effluent from the sewage treatment plant lead to utilization of effluent and reduce resource utilization. In the present study, utilization of sewage effluent in place of fresh water takes place. Sewage effluent was collected from the STP of TIET, Patiala, Punjab, India.

1.8 QUALITY OF WASTE WATER USED IN CONCRETE

It is commonly accepted that any potable water is good for the concrete production and if waste water is used in place of fresh water then it is desirable to check the chemical composition of the waste water before its usage in the concrete production. Highly alkaline or acidic waste water should not be used because it affects the concrete hardening but slightly acidic water is harmless. Sea water lower strength (long term) of matrix, this is because of chloride ions present on the sea water which cause the corrosion of the reinforcement (Younis et al. 2018). So, chemical configuration of waste water affects the setting properties, hardened properties and durability properties of matrix. Therefore before using the waste water in concrete, its chemical composition should be checked. Waste water should be reasonably free from organic matter, suspended solids and dissolved solids. pH of concrete mixing effluent should be between 6 and 8. According to ASTM C94 standards, waste water having 28 days compressive strength greater than 90% of conventional specimen is good for casting. For the concrete production either plain concrete or reinforced concrete, the permissible limits of the solids are as follows:

Table 1.1 Permissible limits of the solids in the waste effluent

Solid type	Permissible limit
Organic matter	200 mg/l
Inorganic matter	3000 mg/l
Sulphates (SO_4)	500 mg/l
Chlorides (Cl)	a) 1000 mg/l for RCC work b) 2000 mg/l for PCC work
Suspended matter	2000 mg/l

1.9 OBJECTIVE OF THE THESIS

The main purpose of investigation is the effect of sewage effluent as mixing agent on the matrix properties by adding microbes in sewage effluent. Concrete specimens were casted and cured with bacterial culture incorporated sewage effluent to enhance the matrix properties. Total five types of specimens were prepared; casting and curing with tap water; tap water casting and sewage effluent curing; sewage effluent casting and sewage effluent curing; specimens were casted with bacterial treated NB media where media was prepared by using sewage effluent and curing was done in same bacterial culture and specimens were prepared with bacterial treated CSL media where media was prepared by using sewage effluent and curing was done in same bacterial culture. These specimens were tested at defined age of curing for specific type of test. *Bacillus megaterium SS3*, which was extracted from the calcareous soil accumulated from "Anantapur District, Andhra Pradesh, India" in CORE lab of TIET, Patiala, Punjab, India. In this investigation, tests conducted were growth kinetics, pH, $CaCO_3$ precipitation, initial and final setting time, slump test, Compressive strength, ISAT, sorptivity test, corrosion and microstructure investigation of various specimens.

1.10 FLOW OF THE THESIS

Chapter 1: Introduction to the study, usage of waste water or sewage effluent in concrete by giving microbial treatment.

Chapter 2: Literature review, in this all investigation done by various researchers on waste water usage in concrete and microbial treatment is discussed.

Chapter 3: Discussion about the materials and procedures of the tests done during the entire investigation.

Chapter 4: Results and discussion of tests performed during investigation.

Chapter 5: Describe conclusions of the effect of using sewage effluent as mixing agent in concrete by giving microbial treatment

CHAPTER 2

LITERATURE REVIEW

This chapter reviewed literature in two aspects. First is the effect of using bacterial culture in concrete on the concrete properties. Second is the effect of using waste water or sewage effluent in concrete casting on matrix properties. The properties of matrix reviewed in this literature are fresh properties, strength properties and durability properties.

It was reviewed from the literature that the strength and the durability aspect of the hardened concrete are enhanced by addition of bacterial culture in the concrete matrix but use of waste water as casting water puts adverse consequence on strength and durability properties.

2.1 EFFECT OF INCORPORATION OF BACTERIAL CULTURE ON CONCRETE PROPERTIES

It was noticed from past studies that microbial matrix enhance strength and durability of the matrix. This was due to the mechanism called MICP. $CaCO_3$ precipitation in voids of the matrix makes concrete denser in comparison with conventional matrix. Microbial matrix has a good property of self healing so, microbial concrete can be used in the existing structures to improve the crack properties in the structure.

2.2 EFFECT OF BACTERIAL CULTURE AND WASTE WATER ON FRESH PROPERTIES OF CONCRETE

Noruzman et al. (2012) described use of treated effluent in place of fresh water in matrix. Treated effluents were taken by three places: heavy factory, domestic sewage and oil mill. Samples were made by using the effluents and fresh water. In this investigation, OPC, 10 mm coarse aggregates and 5 mm sand with fineness modulus 2.40 was used. All properties of treated effluent were under the tolerable limit as per the British Standards. The initial setting time and slump test was performed by preparing cement specimens by using portable water and three effluents. Initial setting time of the concrete made by using heavy industry effluent and domestic sewage effluent was lower as compare to the concrete made by using the palm-oil mill effluent. This may be due to the delay in hydration of OPC and CSH gel generation due to existence of oil.

Mixes were prepared using given effluents and showed the lower values of slumps in comparison with portable water. Slump values were within range of 25 mm – 50 mm as shown in Table 2.2. It was observed that the existence of particles in given effluent had influence on slump.

Table 2.1: Initial setting time by Noruzman et al. (2012)

Type of mixing water	Initial setting time (min)	Difference in setting time (min)
PW	117	0
TEHI	110	-7
TEDS	108	-9
TEPM	149	+32

Table 2.2: Workability test results by Noruzman et al. (2012)

Types of mixing water	Slump (mm)
PW	50
TEHI	35
TEDS	30
TEPM	25

Asadollahfardi et al. (2016) described the usage of treated domestic effluent in matrix. They produced concrete specimens by using potable water and treated domestic effluent. 54 triplicate cubic samples were made to check compressive strength of matrix.

Table 2.3: Concrete design of various concrete samples by Asadollahfardi et al. (2016)

Concrete specimen type	w/c	Cement (kg)	Free water (kg)	Coarse sand (kg)	Fine sand (kg)	Gravel (kg)
A300, B300, C300	0.6	300	180	797	212	900
A400, B400, C400	0.5	400	200	734	200	780

A indicates that the casting and curing of concrete specimen done by drinking water; B indicates that the casting of specimen done with potable water and curing done using treated waste water;

C indicates that the casting and curing of concrete specimen done by treated waste water, while 300 and 400 indicates the quantity of cement in kg/m^3.

Two mixes were prepared to check the workability of matrix made using potable water and domestic treated effluent and it was noticed that the workability of the concrete made with treated effluent not changed notably as compared to the concrete mix made using the potable water. Initial setting time of the matrix made with treated effluent increases in comparison with concrete made with potable water. The final setting time of the matrix made using potable water and treated effluent was 180 min. and 240 min, respectively.

Ghrair et al. (2018) describes the use of TGW and RGW in concrete. In the concrete mixtures two types of fine aggregates and two sizes of coarse aggregates were used. Pozzolanic Portland cement, 2 types fine aggregates and 2 sizes of coarse aggregates were taken to make concrete specimens. Water cement ratio increases slightly upto 0.4% and 0.2% for TGW and RGW, respectively. This was because of the TDS, TSS and other matter (present in water) which represent the more weight of water.

The initial setting time of cement puree form by using TGW and RGW were up to +25 minutes and +20 minutes as compared to control. This retard in initial setting time might be because of high content of dissolved solids in TGW and RGW. As per ASTM C94, if questionable water is used to made cement paste then the initial setting time will not be greater than 60 min. earlier nor greater than 90 min. later as compare to cement paste made by using same cement and distilled water.

Table 2.4: Concrete mixture proportions by Ghrair et al. (2018)

Mix contents	Quantity (kg/m^3)
Hemseyeh	514.7
Adaseyeh	370.3
Semsmeyeh	252.1
Swealeh Sand	502.7
Cement PC 42.5N	418.2
Total water	233.0
Superplasticizer	Zero

Matrix was prepared by using TGW, RGW and Distilled water. The result values showed that by using TGW and RGW, the slump values decreased by 30 mm and 35 mm, respectively in comparison with slump of concrete matrix made by using distilled water.

Joshi et al. (2018) described the replacement of nutrient broth (an expensive media) by CSL. In this study, Bacillus isolated from cement with high ability in $CaCO_3$ precipitation named as CT-5 was used. Concentration of CSL was 1.5%. Ordinary Portland cement, natural river sand of zone II with specific gravity 2.70, two types of coarse aggregates 20 mm and 10 mm was used. Cement standard consistency was 29.5%. The mix proportion used for the concrete mix was cement: fine aggregates: coarse aggregates: 1: 1.82: 3.24 (by wt.) and w/c was 0.5.

To determine cement setting time, five types of puree were prepared. (1) Control paste, (2) Corn Steep Liquor paste (1.5% CSL + 2% urea + 25 mM $CaCl_2$ + cement), (3) Nutrient Broth paste (1.3% NB + 2% urea + 25 mM $CaCl_2$ + cement), (4) CSL-CT5 paste (CT-5 bacterial grow in CSL media), (5) NB-CT5 paste (CT-5 bacterial grow in NB media). The setting time was 120 min (initial) and 240 min (final). In CSL paste, no delay was observed. Delay of 100 min (initial) and 250 min (final) was determined in NB puree in comparison with conventional puree. In CSL-CT5, delay of 20 min (initial) and 30 min (final) was determined. Delay of 40 min (initial) and 50 min (final) was determined in NB-CT5 in comparison with conventional puree. This might be because of organic matter presence.

Joshi et al. (2018) described the influence on structural and chemical properties of concrete by carbon and nitrogen amount of organic matter which is the nutrient compound of media. In the bacterial treated concrete specimens, the content of C and N were highly increased. In this study, bacillus with high ability of $CaCO_3$ precipitation named as CT-5 was used. NB media with 2% urea and 25mM $CaCl_2$ solution was used by giving 120 rpm at 37^{o}C. OPC-43, Zone II sand with 2.70 (specific gravity) and 1.8% (water absorption) was used for this investigation. While 10 mm coarse aggregates with 2.65 (specific gravity) and 1.4% (water absorption) and 20 mm coarse aggregates with 2.63 (specific gravity) and 1.38% (water absorption) was used. The mix proportion used for the concrete mix was cement: fine aggregates: coarse aggregates: 1: 1.82: 3.24 (by wt.) and w/c was 0.5.

To determine the setting time, 3 kinds of cement puree were made. In the first puree water added and known as the conventional mix puree. In the 2nd puree NB media was added. The 3rd puree was made using microbes develop in NB media. The control mix having setting time 120 min (initial) and 260 min (final). The cement paste in which only media was used, the initial setting time was delayed to 60 min, final setting time was delayed to 240 min in comparison with conventional puree. The third puree in which cement was mixed with bacterial culture, the initial setting time remain same while delay of 30 minutes was observed in the final setting time.

Meena et al. (2019) describes the use of waste effluent in matrix by checking its mechanical and durability properties. In this study, author used TTWW, STWW and TW for matrix casting by taking w/c as 0.47. In this study, OPC 43 with consistency 28.0 %, specific gravity 3.14 gm/cm^3 and fine aggregates has specific gravity (2.695 gm/cm^3), fine modulus (3.15) and grading zone 3.

Slump values for matrix form by using potable water, treated waste effluent and sludge waste effluent are 8 cm, 6 cm and 4 cm, resp. The reduction in the workability was due to existence of particles in treated waste effluent. Slump values of matrix reduces when the sludge content in the TWE increases.

2.3 IMPROVEMENT IN THE COMPRESSIVE STRENGTH BY GIVING MICROBIAL TREATMENT

Achal et al. (2010) described the use of Corn Steep Liquor as nutrient source. CSL was an industrial by product having very low cost. The bacterial culture called *S. pasteurii* which was produced by National Chemical Laboratory, Pune, India. Locally available dry, clean fine aggregates was taken for making mortar in ratio of cement: sand: 1 : 3. The mortar cubes of size 7.06 cm was taken to determine compressive strength after 3, 7 and 28 days curing.

28 days compressive strength of mortar cubes made with CSL – urea (31.2 N/mm^2) showed 35% improvement in comparison with conventional samples (23 N/mm^2). While in NB – urea and YE – urea, the compressive strength was 28 N/mm^2 and 27 N/mm^2, respectively. This enhancement in compressive strength was due to settlement of $CaCO_3$ within pores of mortar.

Achal et al. (2011) described approach of bacterial $CaCO_3$ precipitation in flyash matrix. Study described the effect of bacterial culture (*Bacillus Megaterium*) on the water absorption, water impermeability and compressive strength of flyash included mortar and concrete. OPC, fine aggregates (fine modulus 2.89) were used in this study. The fineness of the flyash was 28.8%. The proportion of the OPC: fine aggregates were 1: 3 with w/c ratio 0.47. Flyash was used as a replacement of cement with the concentration of 10%, 20% and 40%.

The mortar cube of side 7.06 cm was used. All the specimens were cured in NBU medium for an interval of 3, 7 and 28 days. Higher flyash amount, lower was strength in compression of specimens. But if bacterial cells were introduced in the mortar cubes then the compressive strength was increased irrespective of the percentage of flyash concentration. The enhancement in strength was 21% (without flyash) at 28 days (28 MPa) if mixing of cement and sand was done by bacterial cells as compared to conventional specimens (23.2 N/mm^2). Enhancement in strength of the mortar by using bacterial cells was 19% (having 10% flyash) (27.6 N/mm^2) in comparison with conventional sample (23.1 N/mm^2). If 20% cement was replaced by flyash, the enhancement in strength was 14% by microbes in comparison with conventional sample. Lastly, if the 40% cement was replaced by flyash, the enhancement in strength was 10% by microbes in comparison with conventional sample. This enhancement in compressive strength was due to settlement of $CaCO_3$ within pores of mortar.

Achal et al. (2011) described effect of "*Bacillus sp. CT-5"* on compression strength of matrix. OPC, fine aggregates (fineness modulus 2.89) were used in this study. Nutrient – Broth – urea media was used for the growth of the isolate.

To determine compression strength using "*Bacillus sp. CT-5"*, mortar cube of side 7.06 cm was form with cement: fine aggregates: 1:3 (by wt.). The w/c ratio or culture to OPC ratio was 0.47. After casting and demoulding of mortar cubes, cubes were cured in respective media for the interval at which compression strength were measured i.e. 3, 7 and 28 days. Control specimens also made using water and NBU medium. The 28 days compression strength of samples cast using microbes was 31 N/mm^2 (36% enhancement) while compression strength of specimens cast using H_2O and NBU medium was 23 N/mm^2 and 24 N/mm^2, resp. The enhancement in the compression strength was because of the settlement of the $CaCO_3$ precipitates in voids of sample.

Dhami et al. (2012) described ability of "*Bacillus megaterium"* to generate calcium carbonate and improvement in characteristics of ash bricks. To determine properties of samples, three types of samples were prepared, 30% fine aggregates samples, 30% rice husk ash samples and conventional samples. Bacterial solution was made by OD_{600} of one in NB media and formed samples are submerged within 20 liter of the culture media (in a plastic tub) for 96 hours. Conventional specimens only submerged in NB media (without bacterial culture). The samples were taken off after 4 days and sprinkling curing done by NBU media for four (4) weeks. The test results showed that the compressive strength of bacterial treated rice husk ash bricks and bacterial treated flyash bricks was significantly high as compared to the untreated bricks after various age of curing. This increase in strength was because of the formation of calcite precipitates in between the voids of the matrix.

Joshi et al. (2018) described the replacement of nutrient broth (an expensive media) by CSL. In this study, Bacillus isolated from cement with high ability in $CaCO_3$ precipitation named as CT-5 was used. Concentration of CSL was 1.5%. Ordinary Portland cement, natural river sand of zone II with specific gravity 2.70, two types of coarse aggregates 2 cm and 1 cm was used. Cement standard consistency was 29.5%. The mix proportion used for the concrete mix was cement: fine aggregates: coarse aggregates: 1 : 1.82 : 3.24 (by wt.) and w/c was 0.5.

Cubes of side 15 cm used to determine compression strength of concrete matrix. CBAT samples showed 25% enhancement in compression strength in comparison with conventional samples while CBST samples showed 16% enhancement in compression strength in comparison with conventional samples. NBAT samples showed 29% enhancement in compression strength in comparison with conventional samples. While NBST samples showed 8% enhancement in compression strength in comparison with conventional samples. The compression strength of NB treated samples was reduced 19% in comparison with conventional samples. The drastic reduction in compression strength of NB (only) treated sample was because of organic matter addition. The delay in the setting time was also observed due to addition of NB medium only in cement. NB alone had a retardation effect on hydration.

Joshi et al. (2018) described the influence on structural and chemical properties of concrete by carbon and nitrogen amount of organic matter which is the nutrient compound of media. In the bacterial treated concrete specimens, the content of carbon and nitrogen were highly increased.

In this study, bacillus with high ability of $CaCO_3$ precipitation named as CT-5 was used. NB media with 2% urea and 25mM $CaCl_2$ solution was used by giving 120 rpm at 37°C. OPC-43, Zone II sand with 2.70 (specific gravity) and 1.8% (water absorption) was used for this investigation. While 1 cm coarse aggregates with 2.65 (specific gravity) and 1.4% (water absorption) and 20 mm coarse aggregates with 2.63 (specific gravity) and 1.38% (water absorption) was used. The mix proportion used for the concrete mix was cement: fine aggregates: coarse aggregates: 1 : 1.82 : 3.24 (by wt.) and w/c was 0.5.

The concrete cube of side 15 cm was cast to measure compression strength of matrix. BAT specimens showed that compression strength increased by 29% in comparison with conventional samples. BST concrete samples in which microbes were launch to matrix by spraying over concrete during curing process and the compressive strength was slightly increased by 8% in comparison with conventional samples. Compression strength in concrete samples in which casting was done with nutrient medium only was decreased by 15.5% in comparison with conventional samples.

Tripathi et al. (2019) described the various methods of introduction of microbes in matrix and the influence of microbes on matrix characteristics. Bacterial cells are introduced in matrix by two ways in casting or in curing. OPC, fine aggregates (fineness modulus 2.89) and coarse aggregates (max. size 20 mm) was used in respective investigation. Bacterial culture taken in this investigation was "*Bacillus sp. CT-5"*. The mix proportion used for the concrete mix was cement: fine aggregates: coarse aggregates: 1: 1.54: 2.86 (by wt.) and w/c was 0.47 or bacterial culture to cement ratio 0.47.

Compression strength of matrix was found by casting 15 cm cubes. Three days compression strength "MC1R0, MC0R1, MC1R1 and MC1R2" in comparison with MC0R0 was 60%, 67.44%, 68.60% and 65.69% higher, respectively. While 7 days compressive strength of microbial treated (either in casting or in curing) was 20% to 25% higher in comparison with conventional samples. The 28 day compression strength of "MC1R0, MC0R1, MC1R1 and MC1R2" in comparison with MC0R0 was 21.11%, 18.33%, 13.88% and 23.88% higher respectively. The abrupt enhancement in strength after three day was due to the availability of nutrients in abundance and ability of microbes to grow was higher and form calcite crystals in high amount.

Table 2.5: Mix designation of the concrete specimens by Tripathi et al. (2019)

Sr. No.	Mix designation	Mode of bacterial introduction
1.	MC0R0	Control specimens with no bacteria
2.	MC1R0	Bacterial culture used for casting + cured in water
3.	MC0R1	Water is used for casting + cured in bacterial culture
4.	MC1R1	Bacterial culture used for casting + cured in bacterial culture
5.	MC1R2	Bacterial culture used for casting + sprayed with bacterial culture after 24 h of casting

The best results were found at 3 days when bacterial culture was used for casting followed by bacterial curing (MC1R1). In this the increase in the compression strength was 68.60% in comparison with conventional samples. This enhancement in strength was due to uniform mixing of bacteria with concrete ingredients. According to the study, sprinkling curing was better, because in MC1R1 (68.60%) and MC1R2 (65.69%) the increase in compressive strength was almost same, but in MC1R2 requirement of bacterial culture reduces. So, the cost of treatment was reduced.

2.4 EFFECT OF WASTE WATER ON THE COMPRESSIVE STRENGTH OF MATRIX

Silva and Naik (2010) described use of sewage effluent in mortar production. Potable water and recycled water were used for mortar mix. The Portland cement to std. sand ratio was 1:2.75 with w/c or water effluent from secondary treatment to cement ratio was 0.485. Compression strength of the mix was determined after 1, 7, 14, 28, 56 and 91 days. Test values represent that the compressive strength of sample cast using sewage effluent showed enhancement in compression strength during 3 to 28 days. This was due to the organic content in the sewage treatment plant water, which may act as a good dispersing agent.

Al-Jabri et al. (2011) described waste water influence on high strength concrete properties. Total four types of water was collected for analysis like one potable water and three waste effluents. Chemical composition of waste water was checked and it was found that the chemical composition was in the acceptable limits for concrete mixtures. OPC was used with w/c ratio 0.35. 25% to 100% of potable water was replaced by effluents for matrix. The mix proportion was as follows; Cement (400 kg/m^3); Silica fume (44 kg/m^3); Sand (710 kg/m^3); 10 mm coarse aggregates (1190 kg/m^3); Water (140 kg/m^3); Super plasticizer (7.9 kg/m^3).

To examine compression strength of matrix, 6 cubes of side 15 cm were made (3 cubes) for 7 day and 28 day interval. Test values showed 7 day and 28 day compression strength of 25% and 100% waste water samples were slightly lesser as compared to conventional matrix while compression strength of 50% waste water samples was approx. same as that of conventional matrix.

Table 2.6: Avg. concrete strength after 7 day and 28 day by Al-Jabri et al. (2011)

Mix	**$(F_{cu})_{7\ days}$ (MPa)**	**$(F_{cu})_{28\ days}$ (MPa)**
Tap Water (100%)	66.0	77
Wastewater (25%)	64.3	70
Wastewater (50%)	66.8	75
Wastewater (100%)	63.8	72

Noruzman et al. (2012) described use of treated effluent in place of fresh water in matrix. Treated effluents were taken by three places: heavy factory, domestic sewage and oil mill. Samples were made by using the effluents and fresh water. In this investigation, OPC, 10 mm coarse aggregates and 5 mm sand with fineness modulus 2.40 was used. All properties of treated effluent were under the tolerable limit as per the British Standards.

The compression strength test was done on triplets of the 10 cm cubes of concrete after 7, 14, 28 and 60 days. The compression strength of the matrix made with the heavy industry effluent was better than compression strength of matrix form using palm-oil mill effluent and domestic sewage. After full curing 9.4% enhancement in compression strength of the matrix made using heavy industry effluent was noticed as compared the concrete mix made with the potable water. After 14 days curing compression strength of remaining two samples decreased by more than 10% of the control cubes strength.

Shekarchi et al. (2012) described usage of biologically treated waste effluent in matrix. In the treatment plant of domestic waste water, three kinds of water were obtained like Primary TW, Secondary TW and Tertiary TW. The effluent collected from the secondary sedimentation tank was the biologically treated waste water because the microorganisms are removed by this section. The pH values of all the tested water were between 6.9 and 8.2. So, these waters were not acidic.

To determine compression strength of matrix, cubes of side 15 cm were cast for 7 days, 28 days, 90 days and 180 days. The samples were place in tap water for curing to particular required time. The decrease in compression strength was noted at 7 and 28 day, when primary treated water was used and the reduction in compression strength more than 10% at 7 and 180 day, when STW was taken. While up to 7% enhancement in the compression strength was noted when TTW was taken for casting.

It was also noted that if all the casting done by tap water and curing done by given three waste water then in that case, secondary treated waste water specimens give the last results as compared to the others and tertiary treated waste water specimens give the high strength curves. The compressive strength of all the waste waters were comparable, so biologically treated waste water was also used as curing water except the secondary treated waste water. The reduction of 18% compression strength was noted in case of STW specimens in comparison with conventional samples.

Mahasneh (2014) described the use of treated water and waste water in concrete. Three types of specimens were cast, (a) Specimen cast by using tap water, (b) Specimen cast by using treated water and (c) Specimen cast by using waste water. The properties of ingredients used for the concrete production were given in Table 2.7. OPC 43 (specific gravity 3.15) was used. The water to cement ration used in this study was 0.5.

Table 2.7: Characteristics of aggregate by Mahasneh (2014)

Aggregate size	Bulk S.G (SSD)	Crushing value	Impact value	Value of unit weight(kg/m³)	Absorption (%)
Coarse	2.61	25.6%<30% (ok)	17.3%<30% (ok)	1466 kg/m³	2%
Medium	2.62			1513 kg/m³	1.92%
Fine	2.46			1703.9 kg/m³	1.63%

To found compression strength of matrix, cubes of size 15 cm were made. Average decrease in compression strength of specimens formed using treated water was 7.3% in comparison with samples cast with potable water. Average decrease in compression strength of specimens form using waste water was 23.2% in comparison with samples cast with potable water. The waste water converted into unstable compounds which finally dissolves and formed more voids in specimens which cause reduction in compression strength.

Swami et al. (2015) described utilizing domestic effluent in concrete as mixing water. The waste effluent was accumulated by STP in New Delhi. 2 kinds of mixing effluents i.e. Treated domestic effluent (TE) and Tap water (TW) were used for casting. OPC 43 was taken for this investigation. Zone II fine aggregates (fineness modulus 3.25 and specific gravity 2.64), coarse aggregates of 10 mm and 20 mm sizes were used fineness modulus 5.92 and 6.60, respectively and the corresponding specific gravities were 2.96 and 2.93. There was high alkalinity in the sewage sample and the rest of the properties (chemical composition) were in the approximate limit and suitable for concrete production.

Table 2.8: Mix proportion data by Swami et al. (2015)

w/c	Water	Cement	FA	CA		SP (%)	Slump (mm)
				10 mm	20 mm		
0.4	178	445	628	523	777	0.30	120
0.5	196	392	628	523	777	0.20	120
0.6	209	349	628	523	777	0.00	120

(All values are in kg/m^3)

Table 2.9: Average test values of compression strength (N/mm^2) by Swami et al. (2015)

Mixing water	w/c		
	0.4	0.5	0.6
TW	42.38	33.02	26.75
TE	45.02	35.74	28.38
% Gain	6.23	8.24	6.09

To found compression strength of mix, concrete cube of side 15 cm were made. The better performance was seen in concrete samples in which we used treated effluent. An average increase of 7% in compression strength was noticed in TE specimens following 28 day in comparison with TW samples. The suspended and dissolved solids present in the treated effluents filled the pores of samples. So, compression strength of treated effluent specimens was increased in comparison with tap water specimens.

Asadollahfardi et al. (2016) described the usage of treated domestic effluent in matrix. They produced concrete specimens by using potable water and treated domestic effluent. 54 triplicate cubic samples were made to check compression strength of matrix.

Table 2.10: Concrete mix design of various concrete samples by Asadollahfardi et al. (2016)

Concrete specimen type	w/c	Cement (kg)	Free water (kg)	Coarse sand (kg)	Fine sand (kg)	Gravel (kg)
A300, B300, C300	0.6	300	180	797	212	900
A400, B400, C400	0.5	400	200	734	200	780

'A' indicates that the casting and curing of concrete specimen done by drinking water; 'B' indicates that the casting of specimen done with potable water and curing done using waste water; 'C' indicates that the casting and curing of concrete specimen done by treated waste water, while '300' and '400' indicates the quantity of cement in kg/m^3. Sample cast using drinking water and cured in treated waste water didn't influence compression strength of specimens, when it was compared with specimens cast and cured by using potable water.

Ghrair et al. (2018) describes the use of TGW and RGW in concrete. In the concrete mixtures two types of fine aggregates and two sizes of coarse aggregates were used. Pozzolanic Portland cement, 2 types fine aggregates and 2 sizes of coarse aggregates were taken to make concrete specimens. Water cement ratio increases slightly upto 0.4% and 0.2% for TGW and RGW, respectively. This was because of the TDS, TSS and other matter (present in water) which represent the more weight of water.

Compression strength of concrete was determined using cube of side 15 cm. A total 12 control concrete cubes were made (3 each for 7, 28, 120 and 180 day) to found compression strength of matrix. Now, the potable water was changed with 2 selected waste effluents (RGW and TGW) at various %ages (100, 75, and 50) in different matrixes. Slight increment in the compression strength of the concrete made by using TGW and RGW as compare to distilled water after 7 day. and no noticeable change in compression strength of the matrix form with distilled H_2O and

TGW after 28 and 120 day. While the compression strength of sample reduced to 7.7% and 13.9% after 28 and 120 day, respectively. 50% dilution represent small increase in compression strength up to 120 days and significant increase in compression strength after 200 day curing.

Meena et al. (2019) describes the use of waste effluent in matrix by checking its mechanical and durability properties. In this study, author used TTWW, STWW and TW for matrix casting by taking w/c as 0.47. In this study, OPC 43 with consistency 28.0 %, specific gravity 3.14 gm/cm^3 and fine aggregate has specific gravity (2.695 gm/cm^3), fineness modulus (3.15) and grading zone 3. The cubes of side 10 cm were made to measure compression strength of the concrete made by using various mixing effluents and various curing water. Mix proportion was cement: fine aggregates: coarse aggregates were 1: 1.597: 2.935.

From the experimental data it was found that, Replacement of 100% of TW with TTWW and curing in TW, compression strength of samples were decreased to 14.7 % after 7 day, 14.32% after 28 day and 6.41% after 90 day. Replacement of 100% of TW by TTWW and cured with TTWW, compression strength of the concrete samples were reduced to 28.44% after 7 day, 20.63% after 28 day and 14.48% after 90 day. Replacement of 100% of TW by STWW and cured with TW, compression strength of the concrete samples were reduced to 32.52% after 7 day, 27.98% after 28 day and 15.94% after 90 day and replacement of 100% of TW by STWW and cured with STWW, compression strength of the concrete samples were reduced to 32.52% after 7 day, 29.24% after 28 day and 16.03% after 90 day.

At the end it was noticed that compression strength depends on quality of waste effluent. Specimen form by using STWW has lower compression strength in comparison with TTWW. So, concrete made by using TTWW have higher compressive strength as compare to the STWW.

2.5 INFLUENCE OF BACTERIAL CULTURE AND WASTE WATER ON DURABILITY PROPERTIES OF CONCRETE

Al-Jabri et al. (2011) described waste water influence on high strength concrete properties. Total four types of water was collected for analysis like one potable water and three waste effluents. Chemical composition of waste water was checked and it was found that the chemical composition was in the acceptable limits for concrete mixtures. OPC was used with w/c ratio

0.35. 25% to 100% of potable water was replaced by effluents for matrix. The mix proportion was as follows; Cement (400 kg/m^3); Silica fume (44 kg/m^3); Sand (710 kg/m^3); 10mm coarse aggregates (1190 kg/m^3); Water (140 kg/m^3); Super plasticizer (7.9 kg/m^3).

Durability of high strength concrete was determined by ISAT. For ISAT, cube of side 15 cm were cast. The result represents that mixes follows a similar pattern of reduction of water absorption with time. During first 30 minutes the decrease was rapid, which later decreased at slower rate up to 120 minutes. The flow rate values ranges b/w 0.05 ml/(m^2.s) to 3.6 ml/(m^2.s) in 1^{st} ten minutes, which was in the specified limits.

Achal et. al., (2011) described approach of bacterial $CaCO_3$ precipitation in flyash matrix. Study described the effect of bacterial culture (*Bacillus megaterium*) on the water absorption, water impermeability and compressive strength of flyash included mortar and concrete. OPC, fine aggregates (fineness modulus 2.89) were used in this study. The fineness of the flyash was 28.8%. The proportion of the OPC: fine aggregates were 1: 3 with w/c ratio 0.47. Flyash was used as a replacement of cement with the concentration of 10%, 20% and 40%.

Concrete cube of side 15 cm was made to conduct impermeability test. The proportions of cement: fine aggregates: coarse aggregates were 1: 1.54: 2.86 with w/c or microbes to cement ratio 0.47. After the test, the samples were broken in 2 parts to check water penetration height. In the case of bioremediated cubes, there were lower penetration depths and this was due to the densification of concrete cubes by the generation of $CaCO_3$ precipitates in voids of specimen.

Table 2.11: Water penetration in fly ash matrix by Achal et al. (2011)

Fly ash (%)	Top penetration (mm)		Side penetration (mm)	
	Conventional	Microbes treated	Conventional	Microbes treated
0	33.14	9.32	41.44	18.67
10	36.52	9.73	43.18	19.78
20	39.32	10.48	46.72	21.72
40	41.56	20.88	57.81	33.72

Sorptivity test (on mortar cubes) was performed to determine the resistance of specimen towards the water penetration. The mortar samples were covered by the 2 coatings of the polysiloxane followed by the 1 coating of the silicon paint. To ensure that absorption was unidirectional, the above coating was done over 4 sides correspond to treated face. After that the sample was placed in oven for drying at 45°C. The specimen was located in such a way that the treated face facing down, ensuring that the water quantity was approx. 2 mm high from base of sample. Firstly note the mass of the sample at its dry condition and after that note the mass of the sample when it was in contact with water at the interval of (15 minutes, 30 minutes, 1, 1.5, 3, 5, 8, 24, 72, 96, 120, 144 and 168 hours.

In the period of 7 days, specimens formed using flyash (0%, 10% and 20%) by using microbes soak up approximately 3.5 time less H_2O in comparison with control specimen and in 40% fly ash mortar (with microbes) soak up approx. 2 time less H_2O in comparison with conventional sample.

Achal et al. (2011) described effect of "*Bacillus sp. CT-5"* on compression strength of matrix. OPC, fine aggregates (fineness modulus 2.89) were used in this study. Nutrient – Broth – urea media was used for the growth of the isolate.

Sorptivity test (on mortar cubes) was performed to determine the resistance of specimen towards the water penetration. The mortar samples were covered by the 2 coatings of the polysiloxane followed by the 1 coating of the silicon paint. To ensure that absorption was unidirectional, the above coating was done over 4 sides correspond to treated face. After that the sample was placed in oven for drying at 45°C. The specimen was located in such a way that the treated face facing down, ensuring that the water quantity was approx. 2 mm high from base of sample. Firstly note the mass of the sample at its dry condition and after that note the mass of the sample when it was in contact with water at the interval of (15 minutes, 30 minutes, 1, 1.5, 3, 5, 8, 24, 72, 96, 120, 144 and 168 hours.

The bacterial treated specimens absorbed nearly six times less water in comparison with conventional specimens. Presence of $CaCO_3$ precipitates along with bacterial cells (in the pores of the mortar matrix) caused the noticeable decrease in water absorption in comparison with conventional samples.

Noruzman et al. (2012) described use of treated effluent in place of fresh water in matrix. Treated effluents were taken by three places: heavy factory, domestic sewage and oil mill. Samples were made by using the effluents and fresh water. In this investigation, OPC, 10 mm coarse aggregates and 5 mm sand with fineness modulus 2.40 was used. All properties of treated effluent were under the tolerable limit as per the British Standards.

An ISAT was conducted to check permeability of matrix. Observations of ISAT were conducted within 10 min. to 120 min. at 7, 28, 60 days and results of the ISAT were expressed in ml/m^2/sec. The matrix generated by taking the portable water and treated effluent from heavy industry were less permeable as compared to the other two effluents. After the compaction process some of the air bubbles were there over surface of compacted concrete samples made by using domestic sewage effluent and palm oil mill effluent. This might lead toward the void content of the hardened concrete specimen.

Achal et al. (2012) described the effectiveness of microbial induced calcite precipitation in reinforcement corrosion reduction. The bacterial culture taken in this investigation was "*Bacillus sp. CT-5*". These microbes had the probability to produce urease and calcite precipitation. 2 kinds of media were taken in this investigation like NB (Nutrient Broth) and CSL (Corn Steep Liquor). OPC, fine aggregates and coarse aggregates of max. size 20 mm were taken. Mix proportion of concrete, cement: fine aggregates: coarse aggregates: 1: 1.54: 2.86 with W/C 0.47. Concrete samples (f_{ck} = 20 MPa) with size 20*20*10 cm were made and one reinforcement bar (Fe 415) of diameter 25 mm and length 300 mm was placed at the center of the specimen. The same bacterial culture or water was used for the curing of specimen for 28 days.

The corrosion of the reinforced bar was started by giving a constant anodic potential of 40 V and a constant positive potential was applied to the reinforcement bar. The samples were immersed in NaCl solution (3.5 %). A SS (Stainless Steel) mesh was wrapped over the sample. The positive side of DC supply was attached to exposed reinforcing bar and negative terminal of DC supply was connected to the SS mesh. The specimens were inspected daily for cracks. Linear Polarization Resistance (LPR) and the Tafel Plot (LP) techniques of non destructive tests were carried out for corrosion monitoring.

The positively charged reinforced bar attracted negatively charged Cl^- from NaCl mixture. When Cl^- came near reinforced bar, corrosion of steel starts. After the corrosion starts, the formation of expensive products was started. These expensive products put tensile stresses over matrix. When tensile stresses exceeded tensile strength of matrix, cracks in concrete was generated. After the formation of cracks there was increase in current, which resulted in movement of more chloride ions through the cracks and rate of corrosion increased.

In the control specimen, there was rapid increase in current from 17.5 mA to 40 mA during first two days and after 7 days high current nearly 180 mA was measured, which lead toward the formation of wider crack. While in the case of microbial treated samples, the current intensity was lower approximately 55 mA until 4 days in both NB and CSL media and after 7 days, the current intensity was reached approximately 85 mA in both the media. This lower current intensity as compare to the control specimens showed the higher electrical resistivity by MICP. The control samples had higher corrosion current density (I_{corr}) value (60.83 mA/cm^2). While nutrient media and CSL media had lower corrosion current density values, 14.78 mA/cm^2 and 20.03 mA/cm^2, respectively. The mass loss in case of control specimen was 5.94%. While in case of nutrient media and CSL media, mass loss was 2.7% and 3.65% respectively. The MICP reduced the mass loss by 53%.

Achal et al. (2013) described bacteria *Bacillus sp.* part on durability properties of mortar. Bacterial strain "*Bacillus sp. CT-5*" taken for lowering of porosity of cement based material. The lowering in porosity was because of ability of the bacterial strain to generate $CaCO_3$ ppt. in pores of the matrix and make it denser. The cubes of side 7.06 cm was prepared with cement: sand ratio 1:3. The w/c ratio or bacterial culture to cement ratio was 0.47.

To determine the total porosity, the mortar cubes were submerged in tap water for a day. Cubes were in contact with water and there was excess water in the utensil so that, all the specimen can absorb the water according to its absorption capacity. When samples prepared using "*Bacillus sp. CT-5*", there was more than 50% reduction in the porosity of specimen. This was because of formation of calcium carbonate ppt. in voids of samples. It also results in the generation of $CaCO_3$ layer over the surface of matrix, which leads toward reduction in water permeability of sample and make sample denser. The porosity percentage of control specimen was nearly 35% while porosity percentage of bacterial sample was about 17% to 18%.

Swami et al. (2015) described utilizing domestic effluent in concrete as mixing water. The waste effluent was accumulated by STP in New Delhi. 2 kinds of mixing effluents i.e. Treated domestic effluent (TE) and Tap water (TW) were used for casting. OPC 43 was taken for this investigation. Zone II fine aggregates (fineness modulus 3.25 and specific gravity 2.64), coarse aggregates of 10 mm and 20 mm sizes were used fineness modulus 5.92 and 6.60, respectively and the corresponding specific gravities were 2.96 and 2.93.

To determine the initial surface absorption cubes of side 15 cm were used. The absorption of water through the concrete surface was influenced by the constant water head of 200 mm. The measurements of uniaxial water penetration were taken after 10, 30, and 60 minutes. Treated effluent concrete specimens had a good refusal of water absorption in comparison with the tap water matrix specimens as shown in Table 2.12. This was because of the blocking of the pores of concrete mix by the suspended and dissolved solids of treated effluent.

Table 2.12: Initial Surface Absorption Test (ISAT) Results by Swami et al. (2015)

ISAT-10 (ml/mm^2s)			**ISAT-30 (ml/mm^2s)**			**ISAT-60 (ml/mm^2s)**		
w/c			**w/c**			**w/c**		
0.4	**0.5**	**0.6**	**0.4**	**0.5**	**0.6**	**0.4**	**0.5**	**0.6**
0.16	0.58	1.13	0.07	0.34	0.66	0.06	0.27	0.40
0.17	0.48	0.95	0.07	0.34	0.61	0.06	0.26	0.38
6.25	17.24	15.93	-	-	7.58	-	3.70	5.00

Sorptivity was done on a cylindrical specimen of dia. 10 cm and thickness 5 cm. Apply epoxy on the circumference of specimen and note the initial weight of the specimen. Test was performed acc. to ASTM C1585-04 guidelines. There was abrupt reduction in the secondary sorptivity values which showed that the volume of fine pores was reduced and hence a dense gel structure was formed. Table 2.13 shows the sorptivity test results of the concrete specimens.

Table 2.13: Sorptivity Test Results by Swami et al. (2015)

Initial sorptivity (10^{-2} mm/$s^{0.5}$)			**Secondary sorptivity (10^{-2} mm/$s^{0.5}$)**		
w/c			**w/c**		
0.4	0.5	0.6	0.4	0.5	0.6
1.89	3.02	4.19	0.09	0.10	0.10
1.76	2.82	3.46	0.07	0.07	0.07
6.88	6.62	17.42	22.22	30.00	30.00

Joshi et al. (2018) described the replacement of nutrient broth (an expensive media) by CSL. In this study, Bacillus isolated from cement with high ability in $CaCO_3$ precipitation named as CT-5 was used. Concentration of CSL was 1.5%. Ordinary Portland cement, natural river sand of zone II with specific gravity 2.70, two types of coarse aggregates 2 cm and 1 cm was used. Cement standard consistency was 29.5%. The mix proportion used for the concrete mix was cement: fine aggregates: coarse aggregates: 1: 1.82: 3.24 (by wt.) and w/c was 0.5.

This test was depending on absorption water in one direction by matrix because of capillary. The concrete cylinders of 10 cm diameter and 5 cm height were made to conduct sorptivity. Apply epoxy on the circumference of specimen and note the initial weight of the specimen. Single surface of cylinder was in contact with water and increment in weight of the sample was noted at various times i.e. 60 s, 5, 10, 20, 30, 60 minutes, 2, 3, 4, 5, 6 hours. A graph was drawn b/w the √time and V_{water} soaked up. The value of sorptivity of specimen was determined by getting the slope of the graph. Control mix had highest sorptivity coefficient as compared to the other mixes. Bacterial treated CSL specimen CBAT or CBST had the less sorptvity coefficient lead by bacterial induced NB specimens (NBAT and NBST). Samples made with only media had the lower sorptvity coefficient in comparison with conventional mixture but higher sorptivity coefficient as compared to the bacterial treated specimens.

Table 2.14: Sorptivity test results of concrete samples by Joshi et al. (2018)

Specimen	**Sorptivity coefficient**
Control	0.020
CSL treated (CT)	0.014
NB treated (NT)	0.014
CSL-bacterial admixed treatment (CBAT)	0.005
NB-bacterial admixed treatment (NBAT)	0.008
CSL-bacterial spray treatment (CBST)	0.005
NB-bacterial spray treatment (NBST)	0.007

Joshi et al. (2018) described the influence on structural and chemical properties of concrete by carbon and nitrogen amount of organic matter which is the nutrient compound of media. In the bacterial treated concrete specimens, the content of carbon and nitrogen were highly increased.

In this study, bacillus with high ability of $CaCO_3$ precipitation named as CT-5 was used. NB media with 2% urea and 25mM $CaCl_2$ solution was used by giving 120 rpm at 37°C. OPC-43, Zone II sand with 2.70 (specific gravity) and 1.8% (water absorption) was used for this investigation. While 1 cm coarse aggregates with 2.65 (specific gravity) and 1.4% (water absorption) and 2 cm coarse aggregates with 2.63 (specific gravity) and 1.38% (water absorption) was used. The mix proportion used for the concrete mix was cement: fine aggregates: coarse aggregates: 1: 1.82: 3.24 (by wt.) and w/c was 0.5.

This test was depending on absorption water in one direction by matrix because of capillary. The concrete cylinders of 10 cm diameter and 5 cm height were made to conduct sorptivity. Apply epoxy on the circumference of specimen and note the initial weight of the specimen. Single surface of cylinder was in contact with water and increment in weight of the sample was noted at various times i.e. 60 s, 5, 10, 20, 30, 60 minutes, 2, 3, 4, 5, 6 hours. A graph was drawn b/w the √time and V_{water} soaked up. The value of sorptivity of specimen was determined by getting the slope of the graph. Maximum absorption was observed by the control specimen and absorption rate decreases in bacterial culture induced specimens because of the generation of the $CaCO_3$ in voids of matrix called MICP.

Table 2.15: Sorptivity coefficient of various specimens by Joshi et al. (2018)

Specimen type	Coefficient of sorptivity (k)
Control	0.0191
MT specimens	0.0110
BAT specimens	0.0071
BST specimens	0.0075

Tripathi et al. (2019) described the various methods of introduction of microbes in matrix and the influence of microbes on matrix characteristics. Bacterial cells are introduced in matrix by two ways in casting or in curing. OPC, fine aggregates (fineness modulus 2.89) and coarse aggregates (max. size 2 cm) was used in respective investigation. Bacterial culture taken in this investigation was "*Bacillus sp. CT-5*". The mix proportion used for the concrete mix was cement: fine aggregates: coarse aggregates: 1: 1.54: 2.86 (by wt.) and w/c was 0.47 or bacterial culture to cement ratio 0.47.

Water permeability test conducted for check water penetration resistance of specimen. This test was conducted after 28 day interval on 15 cm cube sample. The test was continued for 72 hours. To check the water penetration depth, sample was cut in 2 parts after 72 hours. Permeability of bacterial treated concrete specimens was very less in comparison with conventional samples. This was because of generation of $CaCO_3$ crystals in voids of concrete samples by bacterial culture. When the bacterial specimens were compared with each other, there was no significant difference between them. Because if only curing was done by bacterial culture, then $CaCO_3$ film was formed over the surface of specimen which did not allow the water to penetrate within the specimen.

Table 2.16: Depth of penetration of samples with microbes by Tripathi et al. (2019)

Sample	Depth of penetration (mm)
MC0R0	33.24
MC1R0	8.87
MC0R1	9.94
MC1R1	6.15
MC1R2	6.64

2.6 EFFECT OF BACTERIAL CULTURE AND WASTE WATER ON MICROSTRUCTURE OF CONCRETE MATRIX

Achal et al. (2011) described approach of bacterial $CaCO_3$ precipitation in flyash matrix. Study described the effect of bacterial culture (*Bacillus Megaterium*) on the water absorption, water impermeability and compressive strength of flyash included mortar and concrete. OPC, fine aggregates (fineness modulus 2.89) were used in this study. The fineness of the flyash was 28.8%. The proportion of the OPC: fine aggregates was 1: 3 with w/c ratio 0.47. Flyash was used as a replacement of cement with the concentration of 10%, 20% and 40%. To study the role of MICP, flyash concrete and mortar specimens were analyzed by SEM. By SEM analysis, calcite precipitation within concrete and mortar sample was visualized. Rod structured bacterial cells were found in touch with $CaCO_3$ crystals as shown in Figure 2.1. By using the bacterial cells, the specimens became denser by forming calcium carbonate crystals.

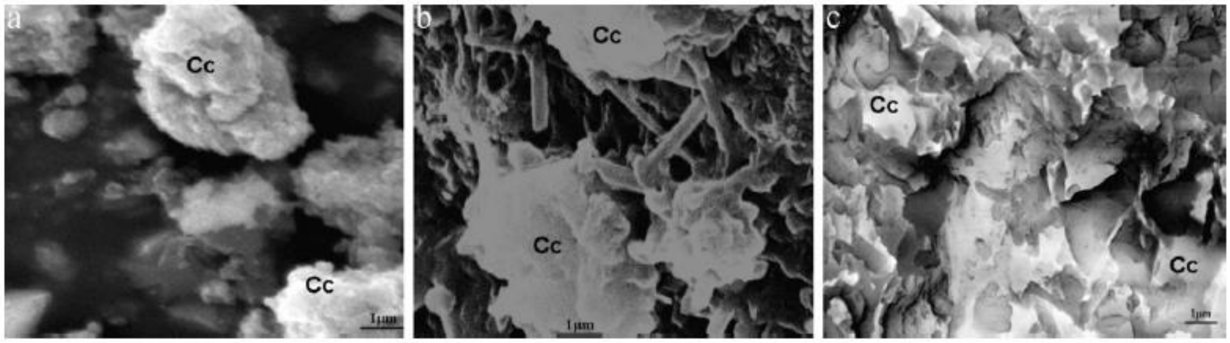


Figure 2.1: SEM of (a), (b) flyash admixed mortar (c) flyash admixed concrete; showing MICP by Achal et al. (2011)

Achal et al. (2011) described effect of "*Bacillus sp. CT-5"* on compression strength of matrix. OPC, fine aggregates (fineness modulus 2.89) were used in this study. Nutrient – Broth – urea media was used for the growth of the isolate. To determine compression strength using "*Bacillus sp. CT-5"*, mortar cube of side 7.06 cm was form with cement: fine aggregates: 1:3 (by wt.). The w/c ratio or culture to OPC ratio was 0.47. After casting and demoulding of mortar cubes, cubes were cured in respective media for the interval at which compression strength were measured i.e. 3, 7 and 28 days. Control specimens also made using water and NBU medium. The 28 days compression strength of samples cast using microbes was 31 N/mm^2 (36% enhancement) due to $CaCO_3$ settlement in voids of matrix.

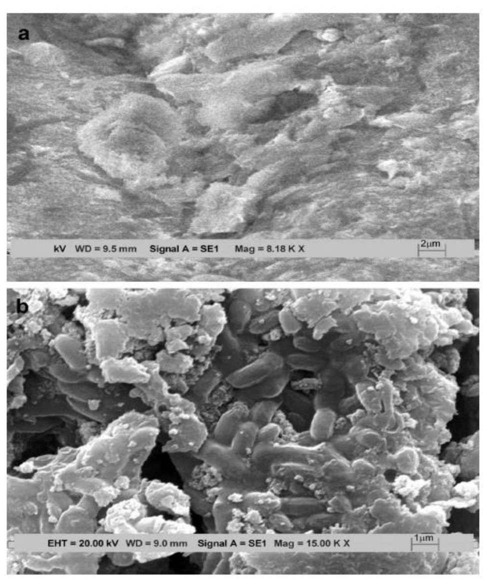


Figure 2.2: SEM of cement mortar samples, (a) without; (b) with, bacterial cells by Achal et al. (2011)

Dhami et al. (2012) described ability of "*Bacillus megaterium"* to generate calcium carbonate and improvement in characteristics of ash bricks. To determine properties of samples, three types of samples were prepared, 30% fine aggregates samples, 30% rice husk ash samples and conventional samples. Bacterial solution was made by OD_{600} of one in NB media and formed samples are submerged within 20 liter of the culture media (in a plastic tub) for 96 hours. Conventional specimens only submerged in NB media (without bacterial culture). The samples were taken off after 4 days and sprinkling curing done by NBU media for four (4) weeks.

By the SEM analysis, it was clearly indicated that there was involvement of bacteria cell in carbonate crystallization. Bacteria cell was in rod shape and it was observed that they were in close contact with calcite crystal as shown in Figure 2.3. By EDX, it was recorded that the amount of calcium in case of B-RHAB was 13.45% while in the case of conventional red brick; the amount of calcium was 3.01%. It was also revealed that the maximum of carbonate deposits were in the form of calcite crystals.

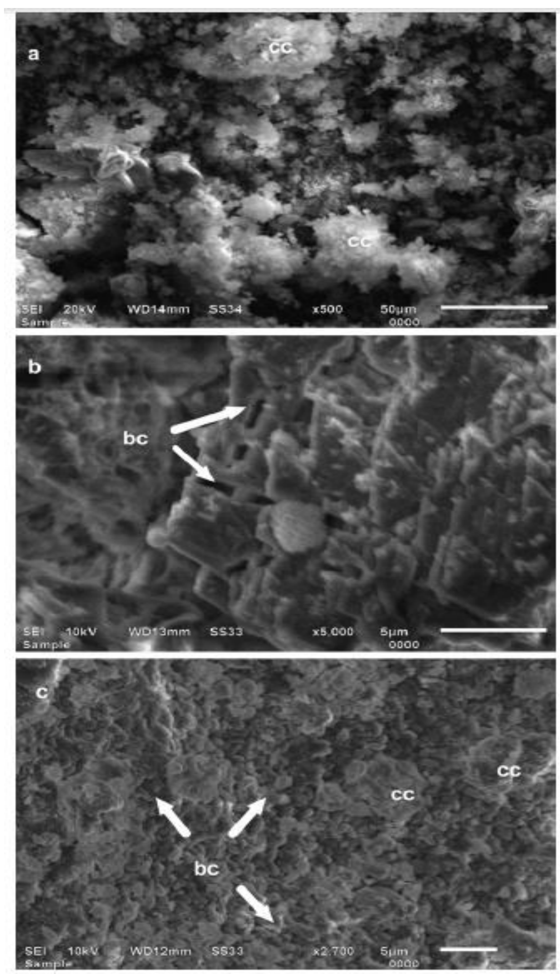


Figure 2.3: SEM of MICP in flyash bricks by Dhami et al. (2012)

Joshi et al. (2018) described the replacement of nutrient broth (an expensive media) by CSL. In this study, Bacillus isolated from cement with high ability in $CaCO_3$ precipitation named as CT-5 was used. Concentration of CSL was 1.5%. Ordinary Portland cement, natural river sand of zone II with specific gravity 2.70, two types of coarse aggregates 20 mm and 10 mm was used. Cement standard consistency was 29.5%. The mix proportion used for the concrete mix was cement: fine aggregates: coarse aggregates: 1: 1.82: 3.24 (by wt.) and w/c was 0.5.

In case of CBAT specimens, rhombohedral $CaCO_3$ crystals and spheroid vaterite crystals were found. The EDX spectrum shows presence of calcite molecules by showing the peaks of calcium and carbon. In CBST, closely bound rhombohedral calcium carbonate crystals were found. While conventional specimens had no crystals of $CaCO_3$. XRD spectrum shows maximum $CaCO_3$ crystals were calcite and vaterite in CBAT and CBST. While XRD analysis of control and CT specimens showed that the maximum present part was quartz, CAS, coesite and vaterite. Figure 2.4 represents the SEM-EDX images which represent the calcium carbonate crystals along with bacterial cells.

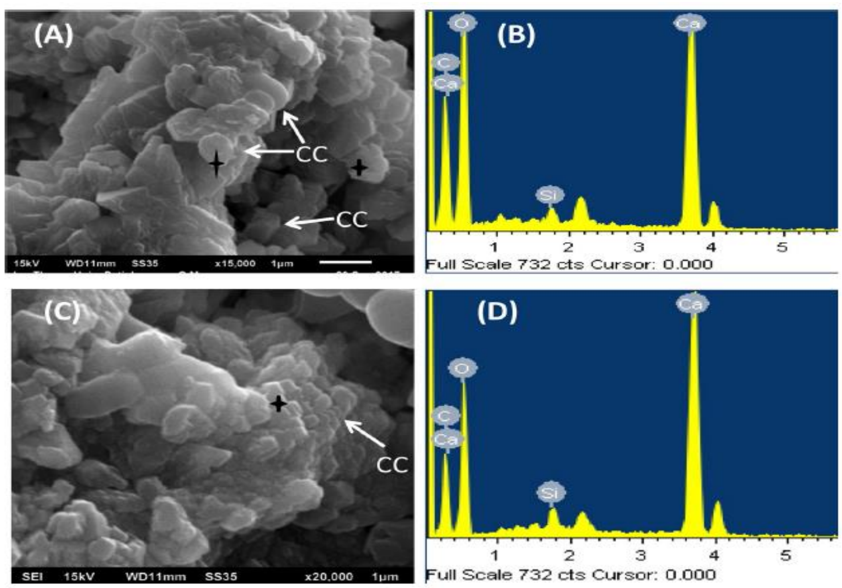


Figure 2.4: SEM-EDX images represent the CC ($CaCO_3$ crystals) by Joshi et al. (2018)

Joshi et al. (2018) described the influence on structural and chemical properties of concrete by carbon and nitrogen amount of organic matter which is the nutrient compound of media. In the bacterial treated concrete specimens, the content of carbon and nitrogen were highly increased. In this study, bacillus with high ability of $CaCO_3$ precipitation named as CT-5 was used. NB media with 2% urea and 25mM $CaCl_2$ solution was used by giving 120 rpm at 37°C. OPC-43, Zone II sand with 2.70 (specific gravity) and 1.8% (water absorption) was used for this investigation. While 10 mm coarse aggregates with 2.65 (specific gravity) and 1.4% (water absorption) and 20 mm coarse aggregates with 2.63 (specific gravity) and 1.38% (water absorption) was used. The mix proportion used for the concrete mix was cement: fine aggregates: coarse aggregates: 1: 1.82: 3.24 (by wt.) and w/c was 0.5.

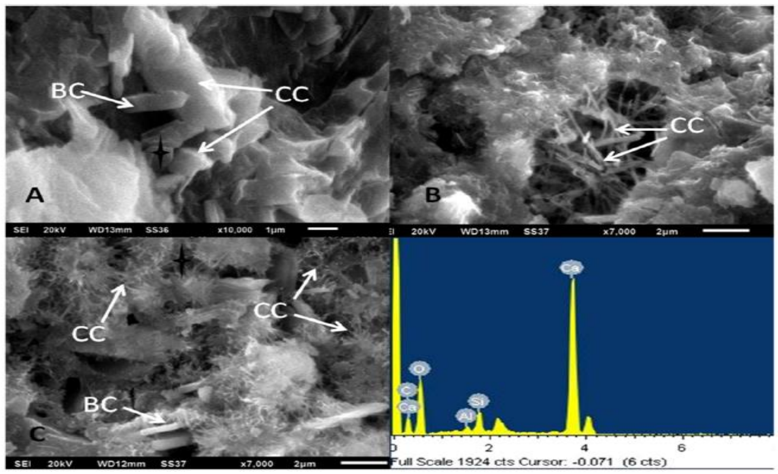


Figure 2.5: SEM imaging of BAT specimen with EDX analysis by Joshi et al. (2018)

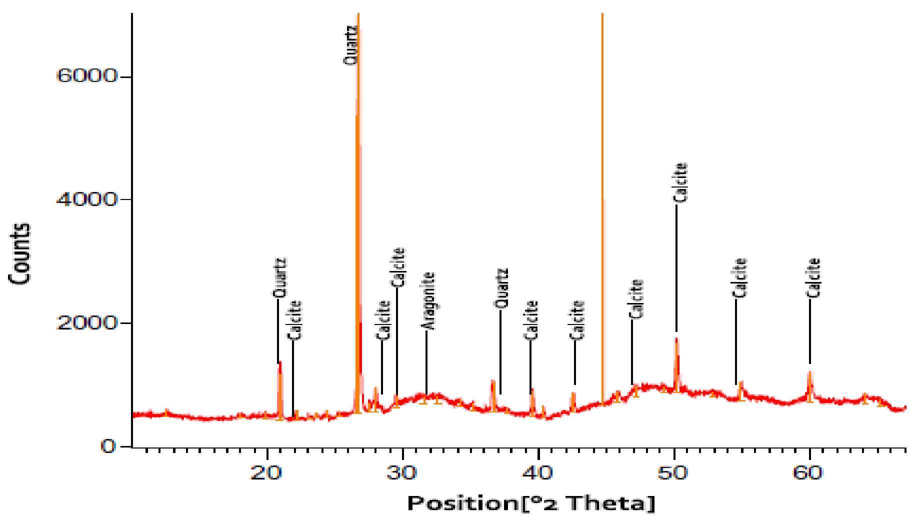


Figure 2.6: XRD pattern of BAT specimen by Joshi et al. (2018)

The SEM images of BAT and BST showed the presence of calcite crystals incorporated with bacterial cells. In BAT specimen, rhombohedral $CaCO_3$ crystals and needle shaped aragonite crystals found as shown in Figure 2.5. The peaks in EDX analysis show excessive mass of calcium and carbon as shown in Figure 2.6. Also in BST specimens, calcium carbonate precipitation and bacterial cells were identified. There were also high peaks of calcium and carbon in BST specimen identified by EDX.

Tripathi et al. (2019) described the various methods of introduction of microbes in matrix and the influence of microbes on matrix characteristics. Bacterial cells are introduced in matrix by two ways in casting or in curing. OPC, fine aggregates (fineness modulus 2.89) and coarse aggregates (max. size 2 cm) was used in respective investigation. Bacterial culture taken in this investigation was "*Bacillus sp. CT-5"*. The mix proportion used for the concrete mix was cement: fine aggregates: coarse aggregates: 1: 1.54: 2.86 (by wt.) and w/c was 0.47 or bacterial culture to cement ratio 0.47.

To discover presence of calcite precipitation by bacterial treatment, SEM of the control specimen and bacterial treated specimens were done. SEM of the samples were analyzed under high vòltage ranges 30 to 35 kV and XRD investigated at 2θ (diffraction angle) ranging from 10° to 80°. XRD of the microbial sample represents peaks of calcium carbonate (calcite) crystals as shown in Figure 2.7. SEM of the microbial sample represents existence of calcium carbonate ($CaCO_3$) along with bacterial cells which showed the enhanced mechanical properties of the bacterial treated specimens at different ages as shown in Figure 2.8. SEM showed that the $CaCO_3$ was in the form of needle shaped aragonite crystals, lamellar rhombohedral calcite crystal along with rod shaped bacterial cells.

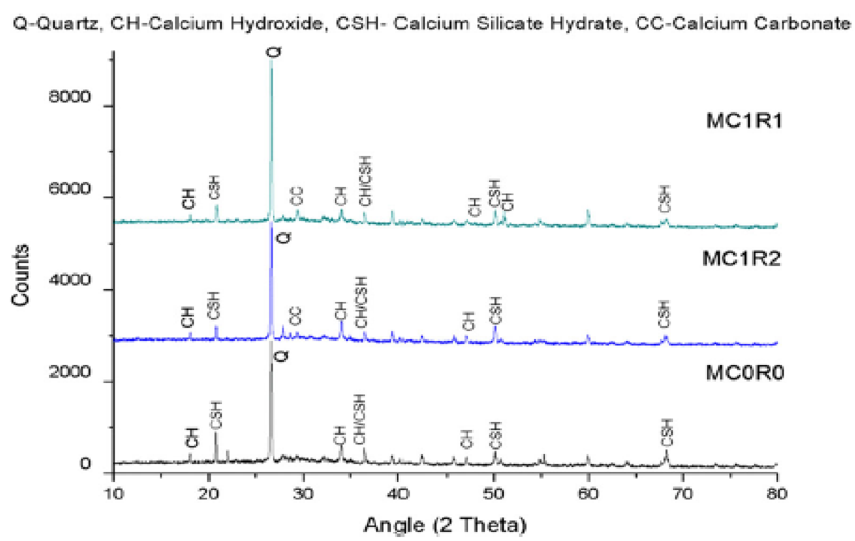


Figure 2.7: XRD patterns of MC1R1, MC0R0 and MC1R2 by Tripathi et al. (2019)

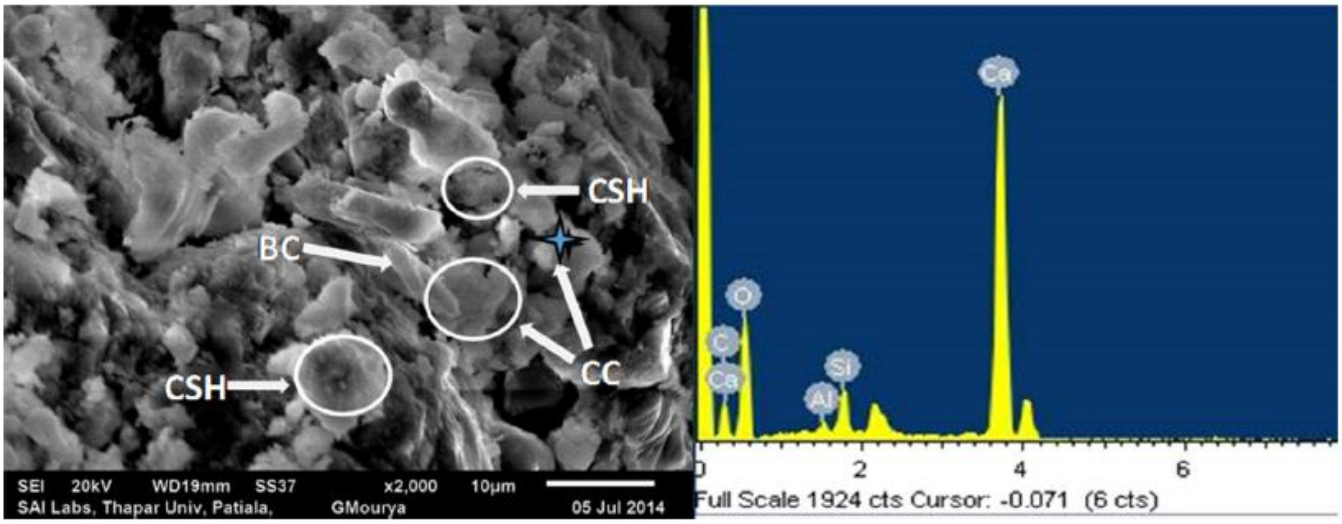


Figure 2.8: Existence of $CaCO_3$ precipitates in microbial concrete along bacteria cells by Tripathi et al. (2019)

CHAPTER 3

EXPERIMENTAL PROGRAM

This chapter represents the effectiveness of using bacterial culture in concrete structure by using sewage effluent as water to form media. The effectiveness of using bacterial culture is monitored by strength, durability and microstructure analysis.

3.1 MATERIAL TESTING

Cement, fine aggregates, coarse aggregates (10 and 20 mm), reinforcing bar, tap water, sewage effluent, *bacillus megaterium SS3* are the basic material considered in the study to made concrete specimens.

3.1.1 Cement

OPC 43 as per IS: 8112-2013 guidelines were taken in experimental investigation. The chemical and physical properties of cement are reported in Table 3.1 and Table 3.2, respectively.

Table 3.1: Chemical composition of ordinary portland cement 43

Sr. no.	Element	Compound (%)
1.	CO_2	6.50
2.	Na_2O	1.42
3.	MgO	0.58
4.	Al_2O_3	1.17
5.	SiO_2	21.74
6.	SO_3	6.11
7.	K_2O	2.85
8.	CaO	59.24
9.	FeO	0.39

Table 3.2: Physical Properties of ordinary portland cement 43

Sr. no.	Characteristic	Obtained Result	Standard Values
1.	Fineness	3.5 %	Less than 10 %
2.	Specific Gravity	3.143	-
3.	Normal Consistency	30 %	-
4.	Initial Setting Time	150 min.	Should not be less than 30 min.
5.	Final Setting Time	385 min.	Should not be more than 600 min.

3.1.2 Fine aggregates

Locally available, well graded, clean, dry sand of second zone conforming to "IS 383-1870" was taken as fine aggregates in present investigation. The sieving set up of fine aggregates is shown in Figure 3.1. To remove the large particles from the fine aggregates, the fine aggregates was sieved by 4.75 mm and then washed to detach the dust molecules.

Figure 3.1: Sieve shaker for sieve analysis

Table 3.3 and Table 3.4 represent the sieve analysis with zone distribution and physical properties of the fine aggregates, respectively.

Table 3.3: Sieve analysis of fine aggreagtes

IS sieve	Wt. retained (gm)	Cum. Wt. retained (gm)	Cum. % retained	Cum. % Passing	Zone			
					I	II	III	IV
10 mm	0	0	0	100	100	100	100	100
4.75 mm	28.0	28.0	2.80	97.20	90-100	90-100	90-100	90-100
2.36 mm	186.50	214.50	21.45	78.55	60-95	75-100	85-100	95-100
1.18 mm	177.5	392.0	39.20	60.80	30-70	55-90	75-100	90-100
600 µm	130.0	522.0	52.20	47.80	15-34	35-59	60-79	80-100
300 µm	291.0	813.0	81.30	18.70	5-20	8-30	12-40	15-50
150 µm	138.0	951.0	95.10	4.90	0-10	0-10	0-10	0-15
PAN	49.0	1000.0	ΣC = 292.05					

Fineness Modulus of F.A = (Summation of Cumulative % age retained upto 150 µ) / 100

Fineness Modulus of F.A = (292.05 / 100) = 2.92 (Approx.)

Zone = II

Table 3.4: Physical Properties of fine aggregates

Sr. no.	Characteristic	Value
1.	Specific Gravity	2.63
2.	Fineness Modulus	2.92
3.	Water Absorption	1.83 %
4.	Grading Zone	Zone II
5.	Bulk Density	Loose = 1.65 g/cc Compacted = 1.72 g/cc

3.1.3 Coarse aggregates

Crushed stones having size 20 and 10 mm were taken as coarse aggregates for this investigation as per IS: 383-1970. Ratio of 20 mm to 10 mm coarse aggregates used for mix design was 60/40 (by weight). Sieve analysis of 20 mm coarse aggregates, 10 mm coarse aggregates and physical properties of the coarse aggregates (10 mm and 20 mm) are represented as follows:

Table 3.5: Fineness Modulus of coarse aggregates (20 mm)

IS sieve (mm)	Wt. retained (gm)	% age Wt. retained	Cumulative Wt. retained (gm)	Cumulative % age retained
20 mm	160	3.20	160	3.20
10 mm	4410	88.20	4570	91.40
4.75 mm	360	7.20	4930	98.60
2.36 mm	70	1.40	5000	100
1.18 mm	0	0	5000	100
600 µm	0	0	5000	100
300 µm	0	0	5000	100
150 µm	0	0	5000	100
PAN	0	0	5000	ΣC = 693.20

Fineness Modulus of C.A (20 mm) = (Summation of Cumulative % age retained) / 100

Fineness Modulus of C.A (20 mm) = (693.20 / 100) = 6.932

Table 3.6 Fineness Modulus of coarse aggregates (10 mm)

IS sieve (mm)	Wt. retained (gm)	% age Wt. retained	Cumulative Wt. retained (gm)	Cumulative % age retained
20 mm	0	0	0	0
10 mm	379	18.95	379	18.950
4.75 mm	1442.50	72.125	1821.50	91.075
2.36 mm	178.50	8.925	2000	100
1.18 mm	0	0	2000	100
600 µm	0	0	2000	100
300 µm	0	0	2000	100
150 µm	0	0	2000	100
PAN	0	0	2000	ΣC = 610.025

Fineness Modulus of C.A (10 mm) = (Summation of Cumulative % age retained) / 100

Fineness Modulus of C.A (10 mm) = (610.025 / 100) = 6.1 (Approx.)

Table 3.7 Physical Properties of coarse aggregates

Sr. no.	Characteristic	Values of 20 mm	Values of 10 mm
1.	Type	Crushed	Crushed
2.	Specific Gravity	2.705	2.695
3.	Total Water Absorption	0.33 %	0.57 %
4.	Fineness Modulus	6.932	6.10

3.1.4 Reinforcing bar

HYSD steel bars of grade Fe-500 of 12 mm diameter were used. These reinforcing bars are used in corrosion test to evaluate the corrosion intensity of various mixes.

3.1.5 Water

Water in concrete act as a binding agent when added to the mix. On addition of water cement undergoes the hydration reaction which is responsible for strength of cement. In this study, casting of control specimens was done using clean and fresh tap water. As per IS 3025-1984, the used potable water should free from any type of heavy metal and other material that puts an unfavorable effect on the quality of concrete.

3.1.6 Sewage effluent

In this study, effect of sewage effluent as mixing water was studied. So, casting and curing was done by using sewage effluent after knowing its chemical composition. The chemical composition of the sewage effluent was in the acceptable limit as per ASTM C94. The density of the sewage effluent was 1.01 g/cc which were approximately equal to the tap water. Characteristics of sewage effluent are given in Table 3.8. The pH of the sewage effluent was 8, which is in acceptable limit (6 to 8) and total solids were 720 mg/l (< 50,000 mg/l).

Table 3.8: Characteristics of the sewage effluent

Compound	Unit	Test methods	Results	Acceptable limit
Zinc (Zn)	mg/l	APHA 23rd Edn. 3120 B	< 0.05	500
Copper (Cu)	mg/l	APHA 23rd Edn. 3120 B	< 0.05	500
Lead (Pb)	mg/l	APHA 23rd Edn. 3120 B	0.11	500
Cadmium (Cd)	mg/l	APHA 23rd Edn. 3120 B	< 0.01	-
Nickel (Ni)	mg/l	APHA 23rd Edn. 3120 B	< 0.05	-
Chromium (Cr)	mg/l	APHA 23rd Edn. 3120 B	< 0.05	-
Titanium (Ti)	mg/l	APHA 23rd Edn. 3120 B	0.08	-
Magnesium (Mg)	mg/l	APHA 23rd Edn. 3120 B	30.3	-
Barium (Ba)	mg/l	APHA 23rd Edn. 3120 B	2.36	-
Aluminium (Al)	mg/l	APHA 23rd Edn. 3120 B	0.31	-
Sodium (Na)	mg/l	APHA 23rd Edn. 3120 B	106	-
Calcium (Ca)	mg/l	APHA 23rd Edn. 3120 B	47.7	< 2000
Iron (Fe)	mg/l	APHA 23rd Edn. 3120 B	0.25	-
Manganese (Mn)	mg/l	APHA 23rd Edn. 3120 B	0.06	500

3.1.7 CSL and NB

Corn steep liquor is an industrial waste, used as nutrient sources for bacterial cells in microbial concrete. Where NB stands for nutrient broth. NB is an expensive laboratory nutrient medium. In this investigation, result of both the CSL and NB on the fresh, structural, durability and microstructure properties was studied. CSL is less expensive medium for urease activity and calcium carbonate production by *Bacillus megaterium SS3*.

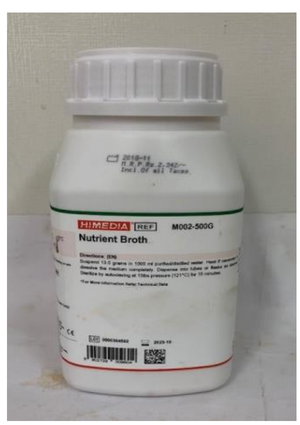


Figure 3.2: Nutrient broth (NB)

Table 3.9 and Table 3.10 show the characteristics of corn steep liuor and Composition of Nutrient Broth, respectively.

Table 3.9: Characteristics of corn steep liquor

Component	Quantity
pH	4.0
Total carbohydrates (%)	5.80
Proteins (%)	24.0
Fats (%)	1.0
Minerals (%)	8.2

Table 3.10: Composition of Nutrient Broth

Component	Quantity
Peptone	10 g/L
Yeast extract	10 g/L
Sodium chloride	5 g/L

3.1.8 Bacterial culture

In this study, *Bacillus megaterium SS3* which was extracted from the calcareous soil "collected from Anantapur District, Andhra Pradesh, India" was used. These bacteria can survive upto pH 11. This bacterial culture is able to produce calcium carbonate precipitates by the enzymatic hydrolysis of urea and produced ammonia increases pH of solution.

Bacterial culture has negative charge on its cell wall and attracts the positively charged Ca^{2+} ions towards it and deposits over its cell wall. Ca^{2+} ions react with CO_3^{2-} ions and form $CaCO_3$ (calcium carbonate) precipitates at the cell surface.

$$\underset{\text{Urea}}{CO(NH_2)_2} + \underset{\text{Water}}{H_2O} \longrightarrow \underset{\text{Carbonate}}{NH_2COOH} + \underset{\text{Ammonia}}{NH_3} \qquad (3.1)$$

$$\underset{\text{Carbonate}}{NH_2COOH} + \underset{\text{Water}}{H_2O} \longrightarrow \underset{\text{Ammonia}}{NH_3} + \underset{\text{Carbonic Acid}}{H_2CO_3} \qquad (3.2)$$

$$H_2CO_3 \longrightarrow H^+ + HCO_3^- \quad (3.3)$$

Carbonic acid → Hydron Ions + Bicarbonate Ions

$$2NH_3 + 2H_2O \longrightarrow 2NH^{4+} + 2OH^- \quad (3.4)$$

Ammonia + Water → Ammonium Ions + Hydroxide Ions

$$HCO_3^- + H^+ + 2OH^- \longrightarrow CO_3^{2-} + 2H_2O \quad (3.5)$$

Bicarbonate Ions + Hydron Ions + Hydroxide Ions → Carbonate Ions + Water

$$Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3 \quad (3.6)$$

Calcium Ions + Bicarbonate Ions → Calcium Carbonate

<u>Overall reaction</u>

$$CO(NH_2)_2 + 2H_2O + Ca^{2+} \longrightarrow 2NH^{4+} + CaCO_3 \quad (3.7)$$

Urea + Water + Calcium Ions → Ammonium Ions + Calcium Carbonate

3.2 STEPS TO PREPARE BACTERIAL CULTURE

Following steps were followed to prepare the bacterial culture

Step1: Take 100 ml of distilled water and add 1.3 gm of nutrient broth and autoclave the formed solution for 15 minutes at 115^{o}C as shown in Figure 3.3.

Figure 3.3: Autoclave

Step 2: Cool the autoclaved solution by keeping it in normal environment and then add one colony of *Bacillus megaterium SS3* into the autoclaved solution from NA (nutrient agar) plate with great precautions.

Step 3: Now place that bacterial culture solution on shaker for overnight at 120 rpm at 37^{o}C for proper growth of bacillus as shown in Figure 3.4 and this was the base solution which was used for concrete specimen casting in bulk.

Figure 3.4: Shaker rotating at 120 rpm at 37^{o}C

Step 4: The quantity of the bacterial culture required for the concrete specimen casting was prepared by applying 1% inoculum of Bacillus megaterium SS3 (base solution) to the autoclaved CSL or NB solution (made by using sewage effluent) and then placed in shaker at 120 rpm at 37^{o}C for shaking till the optimum density (OD600) of the bacterial culture becomes 0.5 as shown in Figure 3.5.

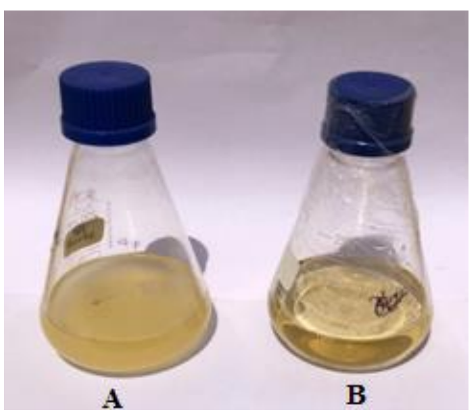


Figure 3.5: Normal NB solution (A) with Bacterial culture growth (B) without Bacterial culture growth

Step 5: After the full growth of the culture add 25 mM $CaCl_2$ and 2% urea in the culture i.e add 20 gm of urea in 1000 ml of water and 3.67 gm of $CaCl_2$ in 1000 ml of water.

3.3 MIX DESIGN

Design of M30 grade of concrete mix was done acc. to IS: 10262-2009 with w/c ratio kept as 0.48. The proportion of the matrix cement: Fine aggregate: Coarse aggregate is 1: 1.62: 2.77. The target strength of the mix is 38.25 MPa. The coarse aggregates used in the mix proportion were of two sizes i.e. 10 mm and 20 mm. The ratio of 10 mm coarse aggregates to 20 mm coarse aggregates used in this mix design were 0.67 (by weight). Quantity of mixing ingredients of concrete is shown in Table 3.11.

Table 3.11: Quantity of mixing ingredients of concrete

Material	Quantity (Kg/m^3)
Cement	410.4
Fine aggregates	665.5
Coarse aggregates (10 mm)	454.4
Coarse aggregates (20 mm)	681.6
Water, Sewage effluent, Bacterial culture	197

3.4 DESIGNATION OF MIXES

The specimens were divided into three (3) main categories; control specimens, sewage effluent mixes and bacterial treated specimens. Further in tap casting specimen, two type of curing was given; tap water curing and sewage effluent curing and in bacterial treated specimens two types of nutrient source was given i.e. nutrient broth and corn steep liquor.

Table 3.12: Designation of concrete mixes

Sr. no.	Specification of mix	Designation
1.	Samples were cast and cured in tap water;	T-T
2.	Samples were cast in tap water and cured in sewage effluent;	T-S
3.	Samples were cast with sewage effluent and cured in sewage effluent;	S-S
4.	Samples were prepared with bacterial treated NB media, where media was prepared by using sewage effluent and curing was done in same bacterial culture;	NSB-NSB
5.	Samples were prepared with bacterial treated CSL media, where media was prepared by using sewage effluent and curing was done in same bacterial culture;	CSB-CSB

N1-N2 where N1 stands for casting medium and N2 stands for curing medium; T stands for tap water, S stands for sewage effluent, NSB stands for bacterial culture grown in nutrient broth media where sewage effluent was used in place of tap water to form media and CSB stands for bacterial culture grown in corn steep liquor media where sewage effluent was used in place of tap water to form media.

3.5 EXPERIMENTAL PROCEDURE

The objective of investigation is to evaluate consequences of sewage effluent over matrix properties and if bacterial culture incorporated in the sewage effluent, then what is the consequence on matrix properties. Experimental procedure of sample preparation follows the following steps:-

1) Collect all the required material as per Indian standards like cement, fine aggregates, coarse aggregates, sewage effluent.
2) Perform all the basic tests over all the used material as per Indian standards, to collect the material data.
3) Design mix proportions of matrix as per IS: 10262-2009 guidelines by defining the water or bacterial culture or sewage effluent to cement ratio.
4) Casting of cubes (side 15 cm) and cylinders (diameter 10 cm and height 20 cm) by taking various mixing agents like potable water, sewage effluent, bacterial culture grown in NB media and CSL media. Moulds were cleansed, tightened and lubricated properly. Mix cement, F.A and C.A in dry state for 2 minutes according to their mix proportion. After that add water, sewage effluent or bacterial culture in the dry mix and mix it thoroughly. Fill the mould with this matrix in 3 parts and tamper each part using tamping rod. After that place the concrete filled mould over vibrator for proper compaction of concrete mix. Remove the concrete specimen from mould after 1 day.
5) Putting samples in respective curing agent for curing after 24 hours from casting for defined curing age.
6) After the completion of curing, the samples were getting off from water tank and perform respective test over it. The whole process of casting and curing is presented in Figure 3.6.

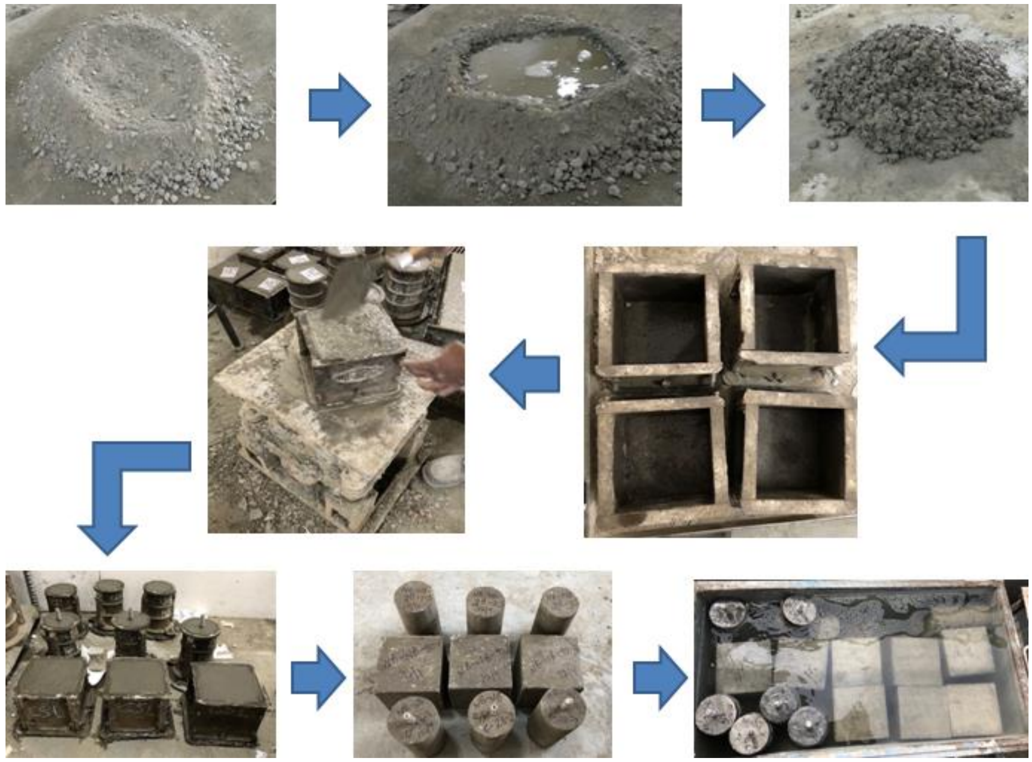


Figure 3.6: Casting and Curing process of samples

3.6 TEST PROCEDURE

Following tests were conducted on concrete specimens to check its fresh, strength, durability and microstructure properties.

3.6.1 Microbiological analysis

3.6.1.1 Growth kinetics

To study the growth kinetics of bacterial culture in media, *bacillus megaterium SS3* isolated from the calcareous soil selected from "Anantapur District, Andhra Pradesh, India" was used. The microbes were grown in two mediums i.e. nutrient broth (NB) media made by using sewage effluent and corn steep liquor (CSL) media made by using sewage effluent. To study the growth kinetics, optimum density (OD_{600}) was measured at 600 nm wavelength by using spectrophotometer shown in Figure 3.7.

Media for the bacterial culture was prepared by using NB (13 g in 1L) and CSL (2% v/v) nutrients in the sewage effluent. Take two 250 ml flasks and put 100 ml sewage effluent in both the flask. In one flask put 2 ml CSL while in other put 1.3 g NB and put both the flask in autoclave to kill all the bacteria. After that cool down the flask and apply 1% inoculum of *Bacillus megaterium SS3* in both the flasks and to set the zero on the spectrophotometer, make two more control flasks in which no bacterial culture was added. Now put the flask for incubation at 37^{o}C in the rotating shaker at 120 rpm and take the reading of optimum density (OD_{600}) after every two hours for 24 hours.

Figure 3.7: Spectrophotometer to get optimum density (OD_{600})

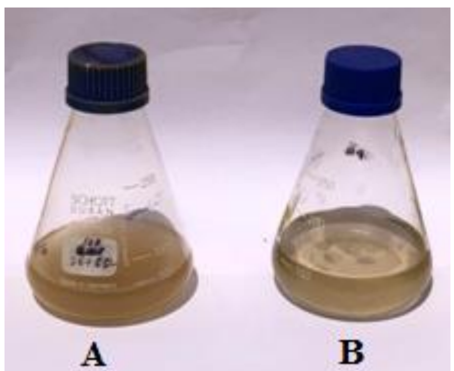


Figure 3.8: Normal CSL solution (A) with Bacterial culture growth (B) without Bacterial culture growth

3.6.1.2 pH

This test was directed to investigate pH of bacterial culture made with the sewage effluent. From the literature it was found that *Bacillus megaterium SS3* culture survives up to pH 11. The pH of the bacterial culture was found after every two hours by using pH meter shown in Figure 3.9. Media for the bacterial culture growth was made by putting NB (1.3%) and CSL (2%) in the sewage effluent and then the flasks were put for autoclave to sterilize the media. Now apply 1%

inoculum of *Bacillus megaterium SS3* culture to the media and supplement the bacterial culture with 25 mM $CaCl_2$ and filter sterilized 2% urea solution. Now put the flask for incubation at 37ºC in the rotating shaker at 120 rpm and take the reading of the pH after 0, 2, 4, 6, 24, 48, 72, 96, 120 hours. In general the pH of the concrete mix is about 11, so this bacterial culture can easily survive in concrete matrix.

Figure 3.9: pH meter

3.6.1.3 $CaCO_3$ precipitation

Bacterial cultures are added in the concrete mixes, because they have potential of generation of $CaCO_3$ crystals in voids of matrix and make matrix denser. Media for the bacterial culture growth was made by putting NB (1.3%) and CSL (2%) in the sewage effluent and then the flasks were put for autoclave to sterilize the media. Now apply 1% inoculum of *Bacillus megaterium SS3* culture to the media and supplement the bacterial culture with 25 mM $CaCl_2$ and filter sterilized 2% urea solution. Now put the flask for incubation at 37ºC in the rotating shaker at 120 rpm and leave the flask on shaker for 3 days. After 3 days, the calcium carbonate precipitates were filtered through autoclaved Cellulose nitrate membrane having pore size 0.45 micron (μ) by using vacuum pump. Now, wash the filter paper by using ethanol solution (20%, 30%, 40%, 50%, 70%, 80%, 90%) and dry the filter papers at 37ºC for one day so that all the moisture escape out and we get the clear image by SEM. By SEM, morphology of the calcium carbonate crystal was evaluated and the bacterial cell shape was also evaluated. Figure 3.10 shows the filter paper of NB solution and CSL solution for SEM imaging.

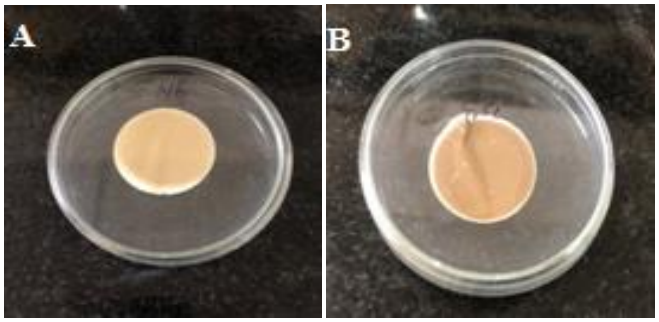


Figure 3.10: Filter paper (A) NB solution (B) CSL solution

3.6.2 Fresh properties

3.6.2.1 Initial setting time and Final setting time

Test was conducted to evaluate consequence of inclusion of sewage effluent and bacterial culture on the setting time of the cement as per BIS 4031: 1988 (part 5) by using Vicat apparatus (shown in Figure 3.11). Firstly the standard consistency of the cement was found acc. to IS 4031: 1988 (part 4). To find cement paste's setting properties, 0.85 times the water require for std. consistency was added to cement. The given cement has std. consistency 30%, but to evaluate setting properties 25.5% (0.85*30) of the water was added.

Total four kinds of the cement puree were made; control puree was prepared by using the tap water, one cement paste was prepared by using the sewage effluent, CSB puree and NSB puree were made by growing microbes in corn steep liquor media and NB media with 2% urea and 25mM $CaCl_2$.

Table 3.13: Cement puree mixes components

Cement mixes	Cement (g)	Water (g)	Sewage effluent (g)	Bacterial culture in NB media (g)	Bacterial culture in CSL media (g)
Control	400	102	-	-	-
S.E.	400	-	102	-	-
NSB	400	-	-	102	-
CSB	400	-	-	-	102

Figure 3.11: Vicat apparatus

3.6.2.2 Slump test

Concrete workability is checked by slump test by using slump cone as per BIS 1199: 1959. An internal diameter of the slump cone at top is 10 cm and at bottom it is 20 cm with height 30 cm. Slump cone was rested over hard platform and fill cone in four parts with matrix and temper each part with 25 blows. The radius and length of tamping rod was 8 mm and 600 mm, respectively. The slump cone was lifted up carefully so that slump cone will not put effect on slumped matrix. Slump of matrix is the vertical difference b/w head level of slump cone and head of slumped matrix.

Figure 3.12: Slump test apparatus

3.6.3 Strength properties

3.6.3.1 Compression test

Concrete cubes having side 15cm were prepared for determining the strength of the matrix. The consequence of microbes develops in nutrient media (made by using sewage effluent) during casting and curing on strength properties of the specimens were evaluated at curing of 3, 7, 28 and 90 days acc. to BIS 516: 1959 using CTM. Also, cubes were cast using the tap water (control) and sewage effluent to compare its strength with the bacterial incorporated specimens.

After casing and curing of concrete cubes in respective medium, the cubes were taken out and water from surface of cube shall be wiped off. The surface of CTM should be sponge clean, before sample placement in CTM, also remove any unattached sand or other matter from the surface of concrete. In case of cubes, the load shall not be applied over top and bottom surface of cubes as cast. The sample axis and applied load axis shall be in proper alignment. The load increased continuously at a rate 140 kg/cm^2/minutes (approximately) without any impact or shock. The maximum value of load applied to the sample before its failure shall be noted. The total 60 concrete cubes were prepared, 3 cubes for each mix for each curing age. The mean of three test results will give compressive strength of particular mix at particular curing age.

Figure 3.13: Compression testing machine of cube testing

3.6.4 Durability properties

3.6.4.1 Initial surface absorption test (ISAT)

ISAT was conducted to check permeability of specimens as per recommendations given in BS 1881: part 208:1996. An oven dried concrete cube of side 150 mm was used to evaluate initial water absòrption after 28 days curing. The observations of the ISAT were taken at 10, 30, 60 and 120 minutes and results of the ISAT were expressed in (ml/m^2.s). The set up of the ISAT was as per given in Figure 3.14. Fill the reservoir in such a way that a constant head of water of 18 cm to 22 cm was maintained at top surfacė of specimen. Flexible tubes were used to connect the inlet of the cap to the reservoir. So that it was used to remove the bubble from the pipe by pinching the flexible tube. Fill the reservoir up to proper head by closing the tap. Now, open the tap and water was allowed to flow to the cap through the flexible tube and note the time. Remove the air from tube by pinching flexible pipe. To prevent the overflow from the capillary tube, outer end of the capillary tube was raised. To ensure that the reservoir is not empty fill it time to time.

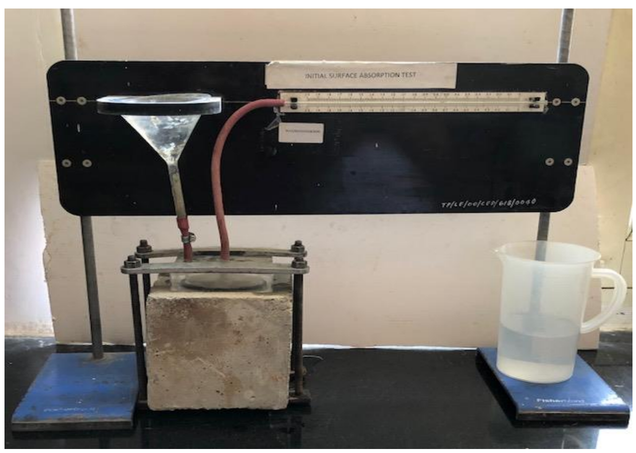


Figure 3.14: Initial surface absorption test arrangement

The surface absorption rate goes on decreasing as the test proceeded. Because the pores present on the surface of concrete become filled with water and moisture in specimen increases. Before test interval, keep capillary tube in horizontal position so that water completely fills the capillary tube. At the time of testing, close the tap and water from the capillary tube flow back. Start stop watch for five seconds to notice divisions that meniscus has moved. If the number of divisions are less than 3 then record the movement of the meniscus for 2 minutes; if the number of divisions are from 3 to 9 then record the movement of the meniscus for 1 minute; while if the divisions are from 10 to 30 then record the movement for 30 seconds; if the divisions are greater than 30 then the absorption is greater than 3.60 ml/(m^2.s). if 10 minutes observation is less than 0.05 ml/(m^2.s), then the matrix is very stiff and stop the test. While if the 10 minutes reading is greater than 3.60 ml/(m^2.s), then matrix is very permeable.

3.6.4.2 Sorptivity test

The water absorption rate by concrete specimen is measured by increase in the mass of the concrete specimen w.r.t time when single surface is disclosed to water. ASTM C 1585-04 was given to evaluate sorptivity of concrete sample. The concrete cylindrical specimens of dia. 10 cm and height 5 cm were used for sorptivity test. Measure the diameter of the concrete specimen from at least four sides and take its average to calculate nearly accurate diameter. Apply epoxy on the circumference of specimen and note the initial weight of the specimen. Place supports below the sample and put the water in the pan in such a way that level of water was approximately 1 mm to 3 mm overhead the support. The change in mass of the sample was recorded at 1, 5, 10, 20, 30 minutes, 1, 2, 3, 4, 5, 6 hours, 1, 2, 3, 5, 6, 7 and 8 days.

A graph was drawn b/w the √time and V_{water} soaked up. The value of initial and secondary absorption was given by the slope of the best fit line to initial and secondary curves, respectively. While the constant of the best fit line equation gives the sorptivity coefficient. The value of initial and secondary absorption was given by the slope of the best fit line to initial and secondary curves, respectively. While the constant of the best fit line equation gives the sorptivity coefficient. To evaluate slope of curve, least - square linear regression was used. If the correlation coefficient (R) is less than 0.98 (linear relationship is not followed by the curve), then the water absorption rate cannot be determined.

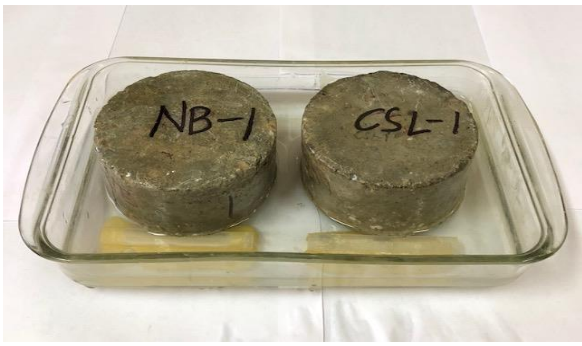


Figure 3.15: Sorptivity test apparatus

3.6.4.3 Corrosion

In this test, the effect of microbial concrete on the corrosion rate was studies. Bacterial culture forms calcium carbonate crystals and fills them in the pores of the concrete mix and makes the specimen denser and corrosion rate goes on decreasing. For corrosion test, cylinders of dia. 10 cm and thickness 20 cm were cast. A steel bar (Fe 500) of diameter 12 mm and length 230 mm was immersed at the centre of sample with 18 cm bar inside cylinder and 5 cm bar was outside the concrete cylinder. The outer 50 mm length of the steel bar was covered with epoxy to protect that part of the bar from corrosion. Now only the 18 cm bar was prone to corrosion. After the casting, cylinders were kept for curing in the respective medium for 28 days.

After curing, corrosion of the reinforced bas was started by giving a constant anodic potential of 5V and a constant positive potential was applied to the reinforcement bar. The samples were immersed in NaCl solution (3.5 %). A SS (Stainless Steel) mesh was wrapped over the sample. The positive side of DC supply was attached to exposed reinforcing bar and negative terminal of DC supply was connected to the SS mesh as shown in Figure 3.16. The specimens were inspected daily for cracks. Linear Polarization Resistance (LPR) and the Tafel Plot (LP) techniques of non destructive tests were carried out for corrosion monitoring.

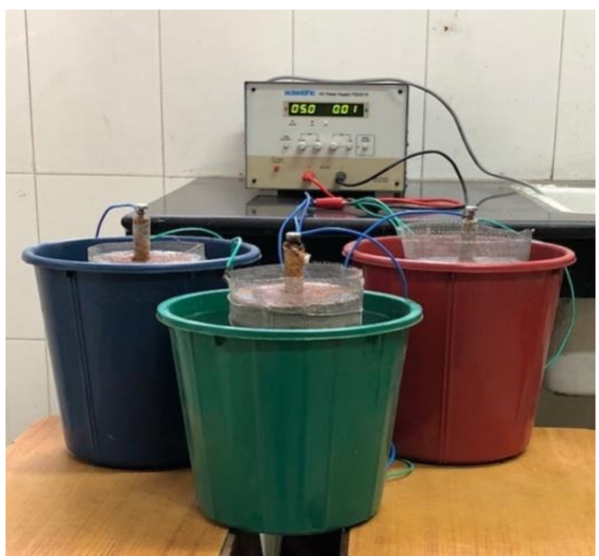


Figure 3.16: Corrosion test apparatus with DC supply

The positively charged reinforced bar attracted negatively charged Cl^- within NaCl mixture. When Cl^- came near the steel, the corrosion of steel started. After the corrosion starts, the formation of expensive products was started. These expensive products put tensile stresses over matrix. When tensile stresses exceeded tensile strength of matrix, cracks in concrete was generated. After the formation of cracks there was increase in current, which resulted in movement of more chloride ions through the cracks and rate of corrosion increased. Figure 3.17 represent the (A) damaged specimens after corrosion and (B) corroded bars.

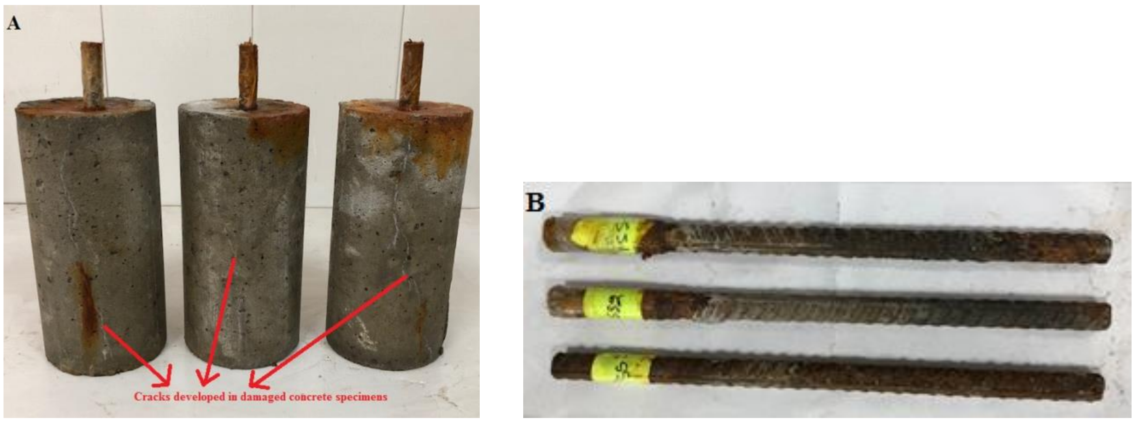


Figure 3.17: (A) Damaged specimens after corrosion, (B) Corroded bars

As shown in Table 3.14, from the corrosion current density value, the rate of the corrosion will be determined.

Table 3.14: Corrosion rate of reinforced bar in matrix

Rate of Corrosion	**Corrosion Current Density, I_{corr} ($\mu A/cm^2$)**
High	10-100
Medium	1-10
Low	0.1-1
Passive	<0.1

3.6.5 Microstructure analysis

3.6.5.1 SEM-EDS / XRD

SEM analyze the compositional variation in observed specimen. Tiny pieces of concrete were accumulated after 28 days compressive strength for SEM-EDS analysis and gold coating of the collected specimen was done with a sputter coating to improve the sample imaging as shown in

Figure 3.18. Also, by coating a conductive layer of fluid was formed on the sample which reduces the thermal damage.

In this study, calcium carbonate crystal formation in bacterial specimen was seen by SEM and densification of the sewage effluent concrete mix was also judged by evaluating the pores in the specimen in comparison with conventional mix. These test evaluate the changes in microstructure of sample. The micro structural crystal's elemental composition and chemical characterization was evaluated by EDS. An examination was done on the spectrum, which was selected on the specific features that was observed in SEM image.

Powdered sample sieved from 90 µm sieve was used for X-ray diffraction. X'Pert PRO diffractometer was taken to obtain XRD spectra with diffraction angle (2θ) ranging from 10^o to 80^o. XRD provides data about the crystallographic structure, physical and chemical configuration of matter.

Figure 3.18: Coated sample for investigation under SEM

CHAPTER 4

RESULTS AND DISCUSSION

In the respective study different tests were performed, test outcomes from test investigation are shown in this chapter. As discussed in the experimental program, specimens were cast and tested after specific curing age i.e. 3, 7, 28, 90 days.

4.1 MICROBIOLOGICAL ANALYSIS

4.1.1 Growth kinetics

To study the growth kinetics, optimum density (OD_{600}) was measured at 600 nm wavelength by using spectrophotometer. Both bacterial treated NB media (NSB) and bacterial treated CSL media (CSB) follow the same trend, but bacterial growth in CSB is slightly less as compared to the NSB. Optimum density of the bacterial culture was checked after every 2 hours up to one day (24 hours) and proper growth was seen in both the NSB and CSB after 24 hours shaking at 120 rpm at 37^{o}C. Bacterial culture can be used to make concrete mix if its optimum density (OD_{600}) becomes greater than 0.5. So, in NSB after 6 hours of inoculums and in CSB after 8 hours of inoculums, bacterial culture can be used to make concrete mix.

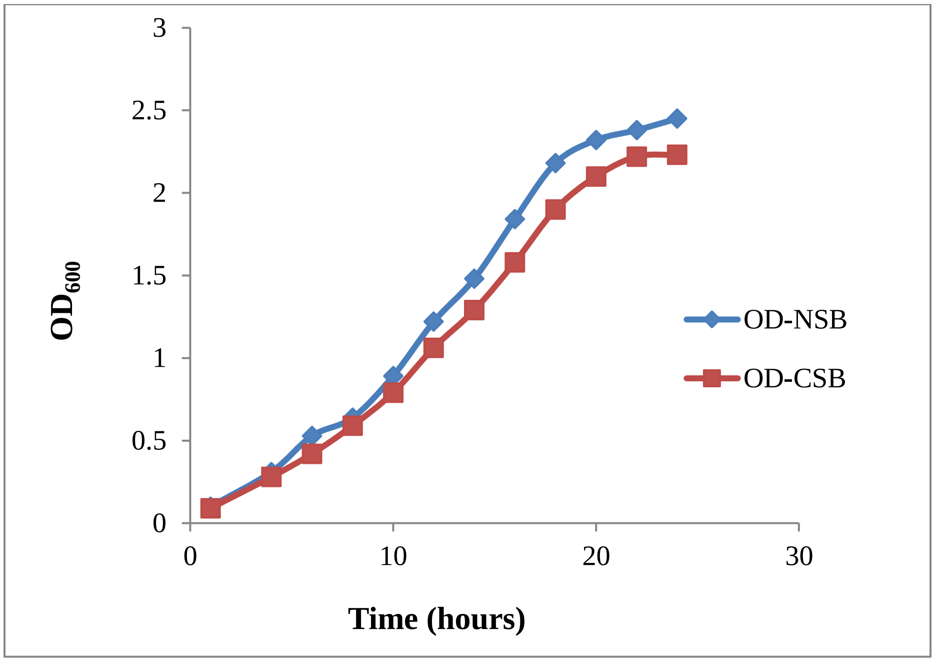


Figure 4.1: Optimum density of NSB and CSB w.r.t time

Table 4.1: Optimum density (OD_{600}) of NSB and CSB

Time (hours)	OD_{600} – NSB	OD_{600} – CSB
1	0.1	0.09
4	0.31	0.28
6	0.528	0.42
8	0.638	0.59
10	0.891	0.79
12	1.22	1.062
14	1.48	1.29
16	1.841	1.58
18	2.18	1.9
20	2.32	2.1
22	2.38	2.22
24	2.45	2.231

4.1.2 pH

The following test was used to investigate pH of culture made with sewage effluent of pH 7 to 8. From the literature it was found that *Bacillus megaterium SS3* culture survives up to pH 11. The pH of the bacterial culture was found after 0, 2, 4, 6, 24, 48, 72, 96, 120 hours by using pH meter. From the experimental data it was found that pH of the bacterial culture goes on increasing and becomes almost stable after 4 days. Corn steep liquor (CSL) is acidic in nature (pH 4), so the pH of the bacterial culture formed with the CSL is less as compared to the bacterial culture in the NB media as shown in Table 4.2. As the pH of the bacterial culture increases, the precipitation of $CaCO_3$ starts. $CaCO_3$ precipitation relies on amount of Ca^{2+} and CO_3^{2-} in the mixture, which is based on the alkalinity of the mixture. More the alkalinity of mixture more will be the concentration of CO_3^{2-} ions abundant of carbonate (CO_3^{2-}) and calcium (Ca^{2+}) ions (Qian et al. 2010).

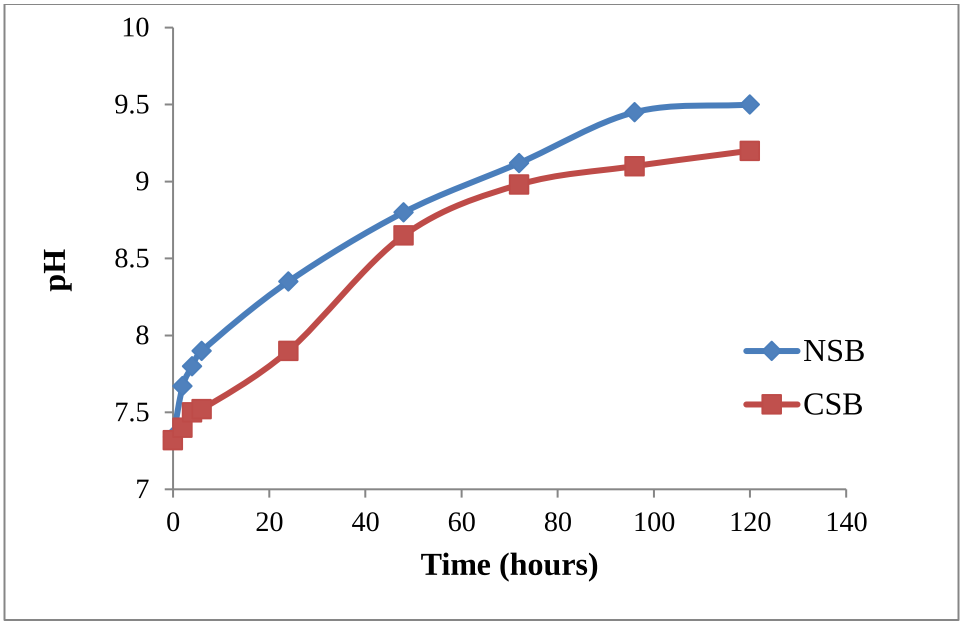


Figure 4.2: Curves showing the pH of the bacterial culture (NSB and CSB)

Table 4.2: pH of NSB and CSB mixtures at various time intervals

Time (hours)	NSB	CSB
0	7.34	7.32
2	7.67	7.4
4	7.8	7.5
6	7.9	7.52
24	8.35	7.9
48	8.8	8.65
72	9.12	8.98
96	9.45	9.1
120	9.5	9.2

4.1.3 $CaCO_3$ precipitation

Incubation of bacterial culture was done for 3 days in NB and CSL media at 37°C supplemented with 25 mM CaCl2 and 2% urea. The calcium carbonate precipitates collected over Cellulose nitrate membrane having pore size 0.45 micron (µ) by using vacuum pump. Now, wash the filter paper by using ethanol solution and dry the filter papers at 37°C for 24 hours

The calcium carbonate precipitates were analyzed using SEM (scanning electron microscopy) equipped with EDS as shown in Figure 4.3, 4.4 and 4.5. For the SEM imaging it was found that the calcium carbonate precipitates are in the form of lumps and there is no specific shape of the crystals also the rod shaped bacillus was seen along with $CaCO_3$. EDS confirms the elemental composition of the formed lumps i.e. presence of carbon and calcium confirms the presence of calcium carbonate ($CaCO_3$). The size of the bacterial cells found varies from 1.50 μm to 2.00 μm in longer direction; this confirms the presence of bacilli.

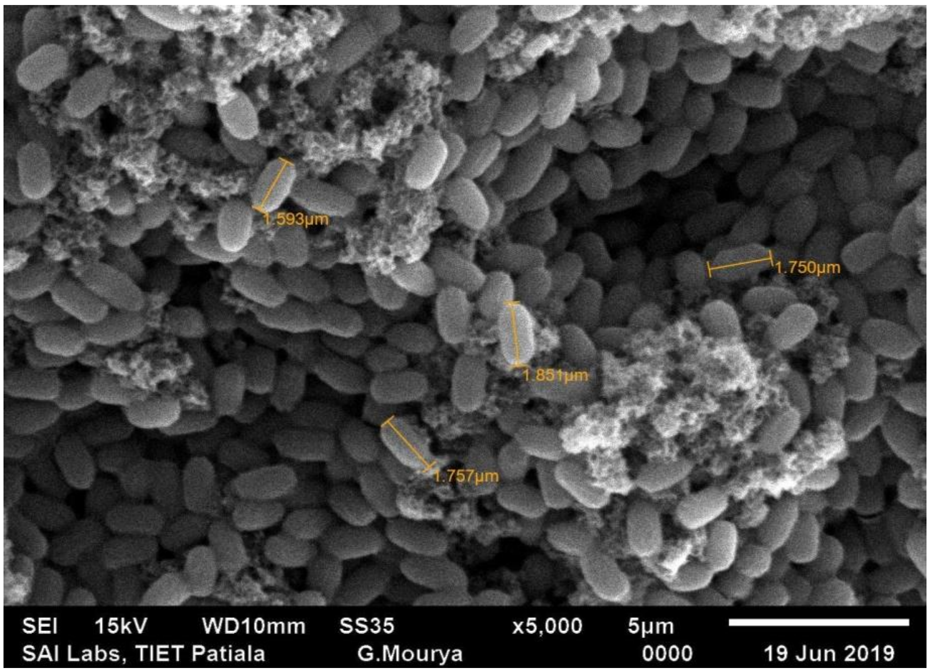


Figure 4.3: SEM imaging of the bacterial cells from filter paper confirming the bacilli size

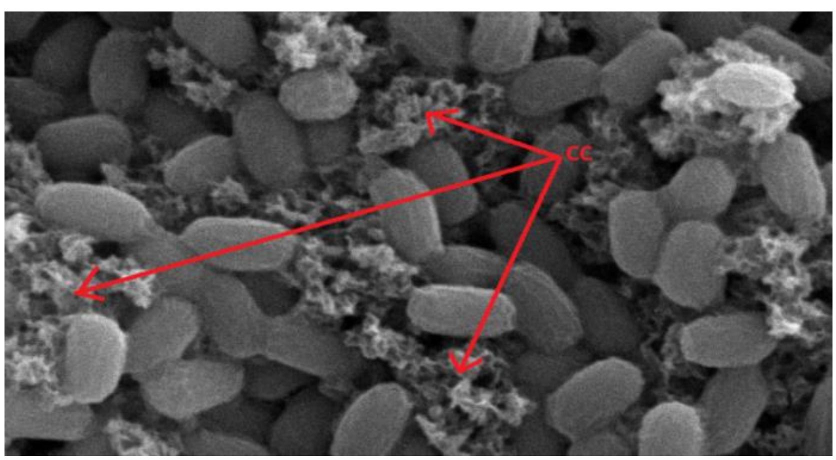


Figure 4.4: SEM imaging using EDS analysis confirms calcium carbonate (cc) lumps incorporated with bacteria

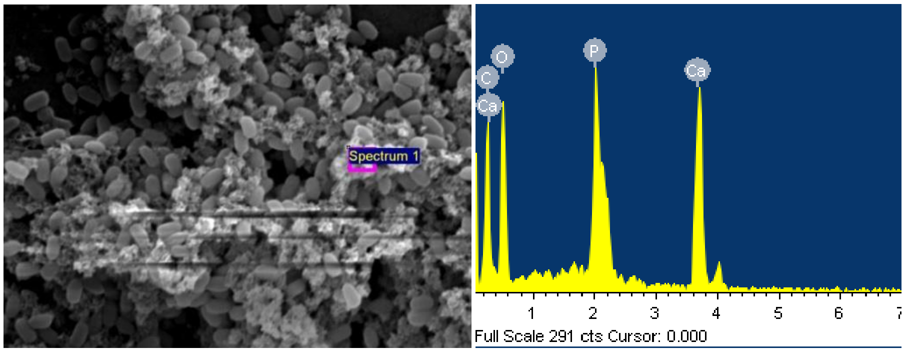


Figure 4.5: EDS analysis of specific spectrum

4.2 FRESH PROPERTIES

4.2.1 Initial setting time and Final setting time

Initial and final setting time of the cement puree prepared with tap water was 150 and 385 minutes, respectively. The increase in the initial and final setting time of the cement puree made with sewage effluent was 21 and 30 minutes, respectively. As per ASTM C94, if questionable water is used to made cement paste then the initial setting time will not more than 60 min. earlier nor greater than 90 min. later as compare to cement puree form by same cement and distilled water. The retard in the initial and final setting time of cement puree made by using sewage effluent may be because of the delay in the hydration of cement with the delay in generation of CSH gel due to presence of impurities (dissolved solids) in the sewage effluent.

The delay in the setting time of CSB (bacterial treated CSL media) was 15 (initial) and 35 (final) min. While in case of NSB (bacterial treated NB media) delay in setting time was 27 (initial) and 46 (final) min. as compared to setting times of cement puree prepared with sewage effluent. This delay in setting time may be because of organic content existence in cement puree (prepared with CSB and NSB) puts an unfavorable effect on chemical properties of puree of cement, which cause delay in hydration of cement (Joshi et al. 2018).

Table 4.3: Setting time of different mixes

Water type	Initial	Final
Tap Water	150 min.	385 min.
Sewage Effluent	171 min.	415 min.
NSB	198 min.	461 min.
CSB	186 min.	450 min.

4.2.2 Slump test

This test was done on a fresh concrete mix prepared by using various mixing agent i.e. tap water, sewage effluent, CSB, NSB. Test result data shows that slump value of the concrete prepared by using tap water and sewage water was almost same. This might be due to the small difference in density of tap water (1.00 g/cc) and sewage effluent (1.01 g/cc). So there was less quantity of suspended and dissolved particles in the sewage effluent which puts less influence on the slump value. While significant distinction in slump value of NSB and CSB w.r.t tap water concrete mix or sewage effluent concrete mix was observed as shown in Table 4.4. This might be due to the denser or viscous nutrients in the bacterial culture, but not proper evidence for the reduction in slump of the bacterial mix was given in literature. Further study was needed to improve the slump value of bacterial culture induced matrix.

Table 4.4: Values of slump of various matrix

Mixing agent	Slump value
Tap Water	78 mm
Sewage Effluent	71 mm
CSB	45 mm
NSB	38 mm

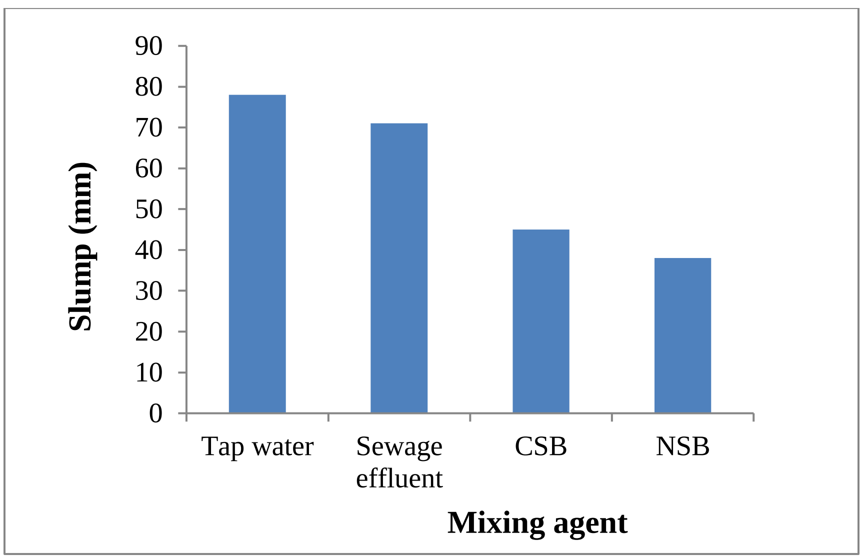


Figure 4.6: Comparison of slump values of different concrete mixes

4.3 STRENGTH PROPERTIES

4.3.1 Compressive strength

Control cubes, sewage effluent cubes and microbial cubes for five different mixes such as; (T-T) fresh water used for casting and curing; (T-S) fresh water used for casting and sewage effluent for curing; (S-S) sewage effluent used for casting and curing; (NSB-NSB) specimens prepared with bacterial culture where media was prepared by using sewage effluent and curing was done in bacterial culture; (CSB-CSB) other mix specimens prepared bacterial treated corn steep liquor (CSL) media, also in this sewage effluent was used for media preparation and curing was done in bacterial treated corn steep liquor media. Now after casing and curing in respective medium, the cubes were taken out to check the compressive strength at various intervals. The sample axis and applied load axis shall be in proper alignment. The load increased continuously at a rate 140 kg/cm^2/minutes (approximately) without any impact or shock. The maximum value of load applied to the sample before its failure shall be noted. The compressive strength readings were taken on each 3, 7, 28, 90 days.

The test data reveals that the increase in strength is maximum at 3 days, 52.11% and 66.19% in case of NSB and CSB specimens, respectively as compared to the S-S specimens. It shows that bacterial culture grows properly inside the concrete and mix up with the environment of the specimen. Because pH of the concrete is about 11 and the used bacterial culture can survive at this pH. As the bacterial culture grows in the concrete sample, formation of calcium carbonate precipitate starts and compressive strength increases. While the strength of S-S specimens decreases 29.70% on comparing with (T-T) conventional specimens, this may be due to impurities present in the sewage effluent. “In the previous studies it was investigated that the waste water converted into the unstable compounds (mono sulfate aluminates), which dissolves in matrix and lead the formation of additional pores in the matrix, also bond formed between cement and aggregates is weaker which results in decrease in the compressive strength of matrix cast with waste water (Mahasneh, 2014)”.

Calcium carbonate layers formed on concrete cube is termed as MICP. Maximum crystals of the calcite or vaterite or aragonite formed over concrete top, because culture grows only in aerobic environment. After 7 days, the compressive strength of the NSB and CSB specimens was 19.80% and 15.23% increased, respectively, on comparing with the S-S specimens. This densification of matrix cause the increase in strength by refinement of pore structure with $CaCO_3$

precipitates. While the compressive strength decreased by 18.30% in case of S-S specimens on comparing with conventional specimen.

After curing period of 28 days (when 99% of characteristic strength was achieved by specimen), the compressive strength of NSB and CSB specimens increased by 43.13% and 37.02%, respectively, on comparing with S-S specimens. While in case of S-S, the decrease in strength is 29.70% on comparing with conventional specimen. But according to ASTM C94 standards, waste water has 28 day strength greater than 90% of conventional specimen is good for casting. The 28 days strength in compression of concrete samples cast using sewage effluent is 70.30%, so this sewage water is not recommended for casting by strength point of view. It was seen that effective increase in compressive strength after 28 days was less in comparison with 3 day strength, which was because of blocking of pores that lead toward the anaerobic condition for bacterial cells and the bacterial activity decreases so, bacterial cells transform them into spores and effective increase in compressive strength decreases.

Table 4.5: Compressive strength values at various ages

Mixes	Compressive strength (MPa)								
	T-T Control	T-S	% relative increase w.r.t control	S-S	% relative increase w.r.t T-S	NSB-NSB	% relative increase w.r.t S-S	CSB-CSB	% relative increas e w.r.t S-S
3 days	19.80	20.20	2.02	14.20	-29.70	21.60	52.11	23.60	66.19
7 days	25.20	26.77	6.23	21.87	-18.30	26.20	19.80	25.20	15.23
28 days	35.80	36.67	2.43	25.53	-30.38	36.20	41.79	34.90	36.70
90 days	36.50	37.27	2.11	26.20	-29.70	37.50	43.13	35.90	37.02

After 90 days, the percentage increase in strength of T-S specimen (37.27 MPa) was 2.11% on comparing with the (T-T) specimens (36.50 MPa) and percentage decrease in the strength of the S-S sample (26.20 MPa) was 29.70% as compared to the T-S specimen. When we compare the 90 days compressive strength of NSB-NSB specimens (37.50 MPa) and CSB-CSB specimens (35.90 MPa) with S-S specimens, the percentage increase in the strength of the NSB-NSB specimens and CSB-CSB specimens was 43.13% and 37.02%, respectively as compared to the S-S specimens as shown in Table 4.5.

The study is further extended to observe the consequence of curing water over strength properties. The Compressive strength of T-T specimens and T-S specimens were compared. Relative increase in strength of T-S sample is negligible (upto 6%) on comparing with T-T samples at any age of curing.

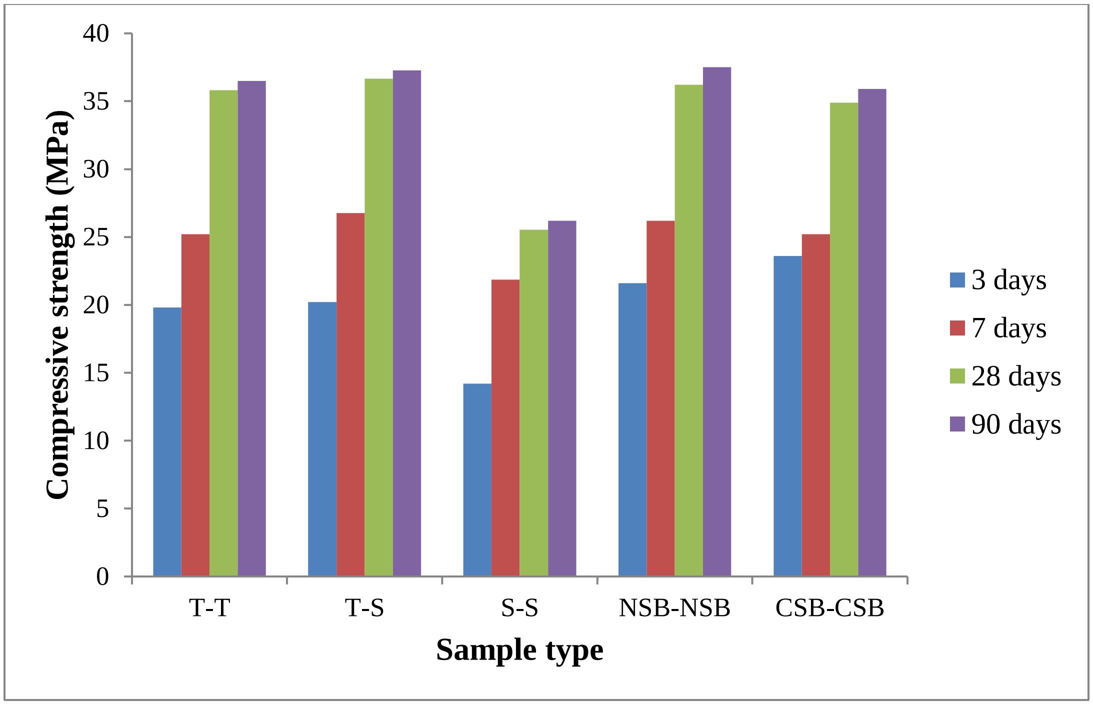


Figure 4.7: Compressive strength of various concrete mixes

4.4 DURABILITY PROPERTIES

4.4.1 Initial surface absorption test (ISAT)

ISAT was conducted to check permeability of the matrix. The observations of the ISAT were conducted at 10, 30, 60 and 120 minutes after 28 days and data of the ISAT was expressed in (ml/m².s). The flow values of T-S specimens are higher (15% to 40%) than the T-T specimens except at 30 minutes interval (approximately same). This is because of the formation of unstable compounds on sample surface, these compounds finally dissolve and pores are generated. The flow value of S-S specimens is greater as compared to the T-S specimens. When casting of concrete specimens done by using sewage water containing impurities, these impurities finally dissolves and causes the formation of additional pores in the matrix. These voids become water filled and cause increase in flow value.

Table 4.6: ISAT results of various mixes

Flow (ml/m².s)	T-T Control	T-S	% relative increase w.r.t control	S-S	% relative increase w.r.t T-S	NSB-NSB	% relative increase w.r.t S-S	CSB-CSB	% relative increase w.r.t S-S
10 min.	0.22	0.26	18.18	0.34	30.77	0.172	-49.41	0.203	-40.29
30 min.	0.1	0.097	-3.00	0.15	54.64	0.08	-46.67	0.09	-40.00
1 hour	0.045	0.055	22.22	0.08	45.45	0.032	-60.00	0.032	-60.00
2 hour	0.022	0.03	36.36	0.05	66.67	0.015	-70.00	0.017	-66.00

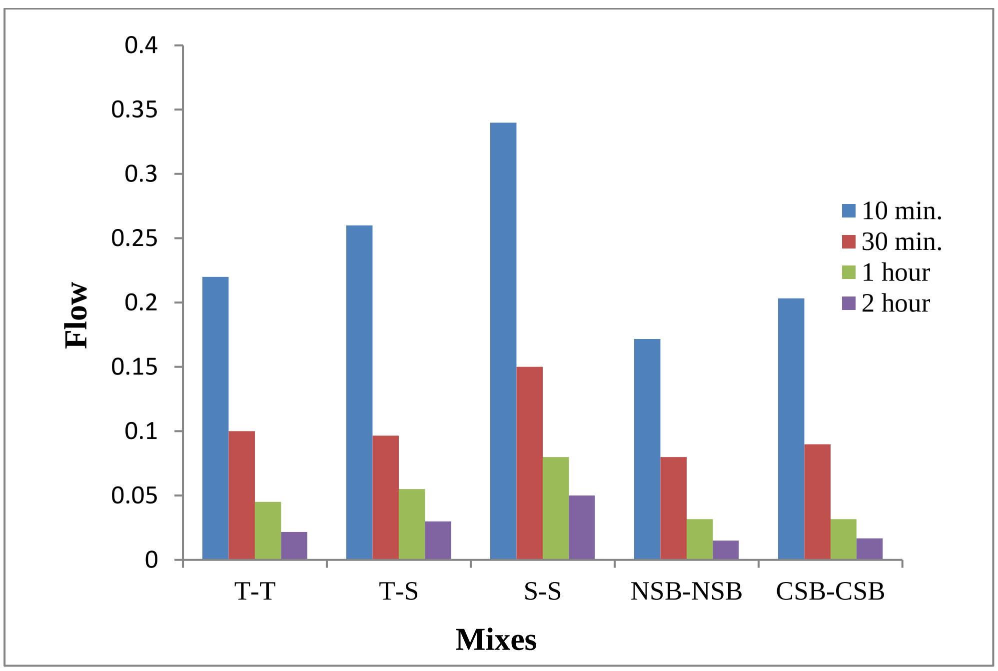


Figure 4.8: Surface water absorption of specimens for various interval

The flow value of the NSB-NSB and CSB-CSB specimens is very less as compared to the S-S specimens. Both NSB-NSB and CSB-CSB are bacterial admixed specimens, in which bacterial cells are incorporated in the NB and CSL media (by using sewage effluent) during casting and curing. These bacterial cells have potential of generation of $CaCO_3$ precipitates within voids of specimens, this calcium carbonate precipitates makes the concrete specimen less porous. It can be concluded that bacterial treated effluent concrete specimens had a good resistance to water immersion than sewage effluent specimens. The percentage relative decrease in the flow (ml/m².s) of bacterial treated specimens was 40% to 70% as compared to the S-S specimens at different time intervals. While percentage relative increase in the flow of S-S specimens was 30% to 70% as compared to T-T specimens at different time intervals as shown in Table 4.6.

4.4.2 Sorptivity test

Sorptivity test depends on absorption of water in uni-directional by specimen, because of the rise between pore systems of specimen. The change in mass of the specimen was recorded at 1, 5, 10, 20, 30 minutes, 1, 2, 3, 4, 5, 6 hours, 1, 2, 3, 5, 6, 7 and 8 days. A graph was drawn b/w the √time and V_{water} soak up. The value of initial and secondary absorption was given by the slope of the best fit line to initial and secondary curves, respectively. While the constant of the best fit line equation gives the sorptivity coefficient.

The sorptivity test results showed that the initial and secondary sorptivity coefficients of NSB-NSB specimens (0.065, 0.400) and CSB-CSB specimens (0.081, 0.419) were lower than that of S-S specimens (0.113, 0.538). This may be because of the impurities present in the sewage effluent. These impurities cause the formation of unstable compounds, which finally dissolves and results in generation of additional voids in the matrix. In NSB-NSB and CSB-CSB specimens, there was development of $CaCO_3$ precipitates in voids of matrix by bacterial activity of the *Bacillus megaterium SS3*, these $CaCO_3$ precipitates choke voids of specimen and reduce the water absorption capacity of the specimen. Linear relationship was not followed by initial sorptivity data (between 1 min. and 6 hours) of the T-S specimen. The correlation coefficient of the T-S specimen is lower than 0.98 (systematic curvature). That’s why initial sorptivity coefficient and initial rate of absorption of the T-S specimen cannot be determined. While the secondary sorptivity coefficient of the T-S specimen (0.352) is lower than that of the S-S specimen (0.538) and equivalent to the T-T specimen (0.388).

The initial and secondary rate of absorption of NSB-NSB specimens (28.72×10^{-4}, 5.24×10^{-4}) and CSB-CSB specimens (32.60×10^{-4}, 6.43×10^{-4}) were lower than that of S-S specimens (41.80×10^{-4}, 12.88×10^{-4}), because of the development of $CaCO_3$ precipitates in voids of matrix. While secondary rate of absorption of the T-S specimen (9.16 X 10^{-4}) is lower than that of the S-S specimen (12.88 X 10^{-4}) and higher than that of T-T specimen (7.66 X 10^{-4}). The curing of T-S specimen was done by sewage effluent, which may cause the formation of pores in the matrix at some extent (less than that of S-S specimen).

Table 4.7: Sorptivity test results of various matrix

Sample Type	Sorptivity coefficient		Absorption (10^{-4} mm/√s)	
	Initial	Secondary	Initial	Secondary
T-T	0.070	0.388	32.86	7.66
T-S	-	0.352	-	9.16
S-S	0.113	0.538	41.80	12.88
NSB-NSB	0.065	0.400	28.72	5.24
CSB-CSB	0.081	0.419	32.60	6.43

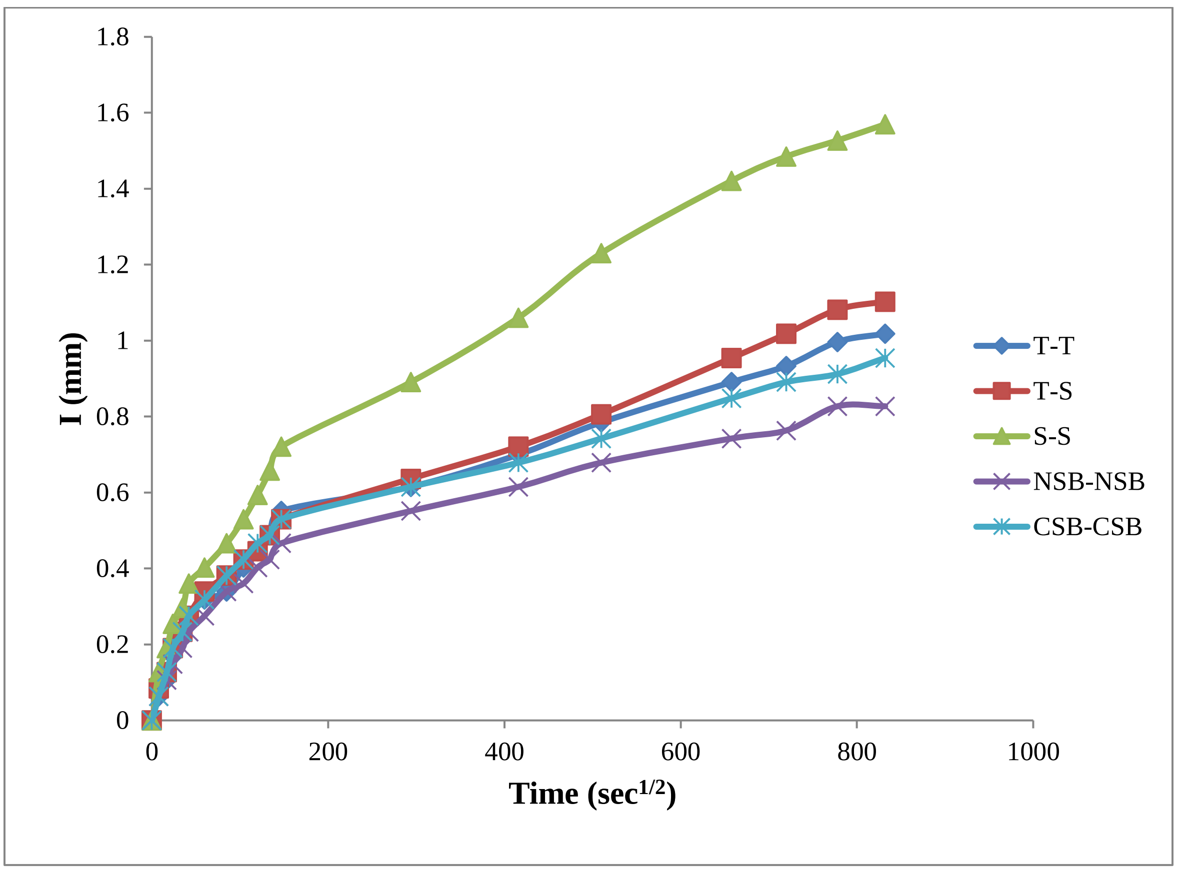


Figure 4.9: Sorptivity test results of various mixes

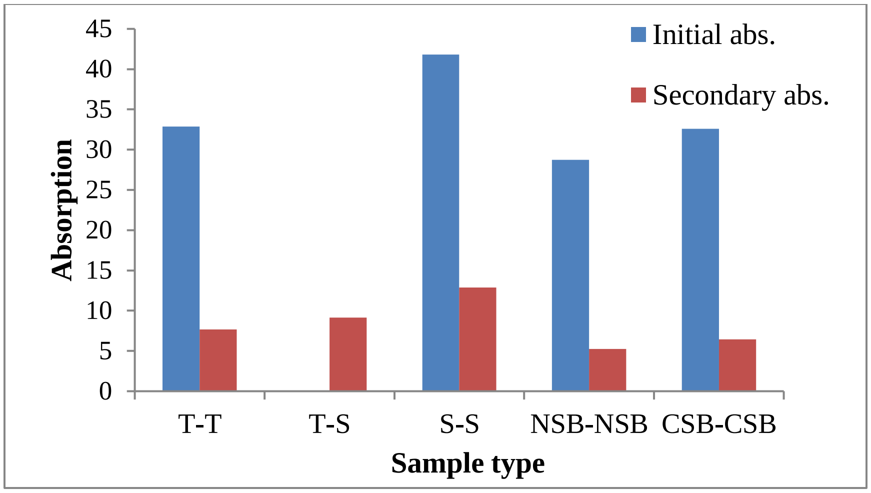


Figure 4.10: Comparison of initial and secondary absorption rate of various mixes

4.4.3 Corrosion

The corrosion of the reinforced bar was started by giving a constant anodic potential of 5.0 V and a constant positive potential was applied to the reinforcement bar. The samples were immersed in NaCl solution (3.5%). A SS (Stainless Steel) mesh was wrapped over the sample. The positive side of DC supply was connected to exposed reinforcing bar and negative side of DC supply was connected to the SS mesh. The specimens were inspected daily for cracks. Linear polarization resistance (LPR) and the Tafel plot (LP) techniques of non destructive tests were carried out for corrosion monitoring.

The positively charged reinforced bar attracted negatively charged Cl^- from NaCl mixture. When Cl^- came near reinforced bar, corrosion of steel starts. After the corrosion starts, the formation of expensive products was started. These expensive products put tensile stresses over matrix. When tensile stresses exceeded tensile strength of matrix, cracks in concrete was generated. After the formation of cracks there was increase in current, which resulted in movement of more chloride ions through the cracks and rate of corrosion increased.

The initiation of the reinforcement corrosion was determined by the current vs time curve by jump up in the current recorded. Initially the increase in current rate is lower due to oxide layer as shown in Figure 4.8. Which act as a protective shield for the reinforced steel (Achal et. al., 2012). In the middle stage, corrosion rate increases due to generation of cracks in concrete sample and chloride ions penetrate through that cracks. In the last stage resistivity to corrosion decreases and more chloride ions penetrate to the reinforced steel. When we compare the specimens with each other, it was found that in the S-S specimen, corrosion takes place with corrosion current density 0.1157 μA/cm^2 after 6 days while in T-T and T-S specimens, corrosion takes place at same day (after 14 days) with corrosion current density 0.185 μA/cm^2 and 0.1064 μA/cm^2, respectively. This shows that the type of curing water will not affect the corrosion rate.

When we compare the conventional concrete (S-S) with the microbial concrete, a huge difference in the corrosion rate was observed. In conventional concrete, corrosion takes place after 6 days while in NSB-NSB (0.13374 μA/cm^2) and CSB-CSB (0.10625 μA/cm^2), corrosion takes place after 14 days and 10 days, respectively. This may be due to the presence of more pores in the S-S specimens as compared to the microbial concrete. In microbial matrix, voids available in the matrix were charged by calcium carbonate precipitates formed by the bacterial culture present in the microbial concrete. More the number of voids in concrete sample, more will be the perforation of Cl^- to reinforced steel and originate the more corrosion to the reinforcement bar.

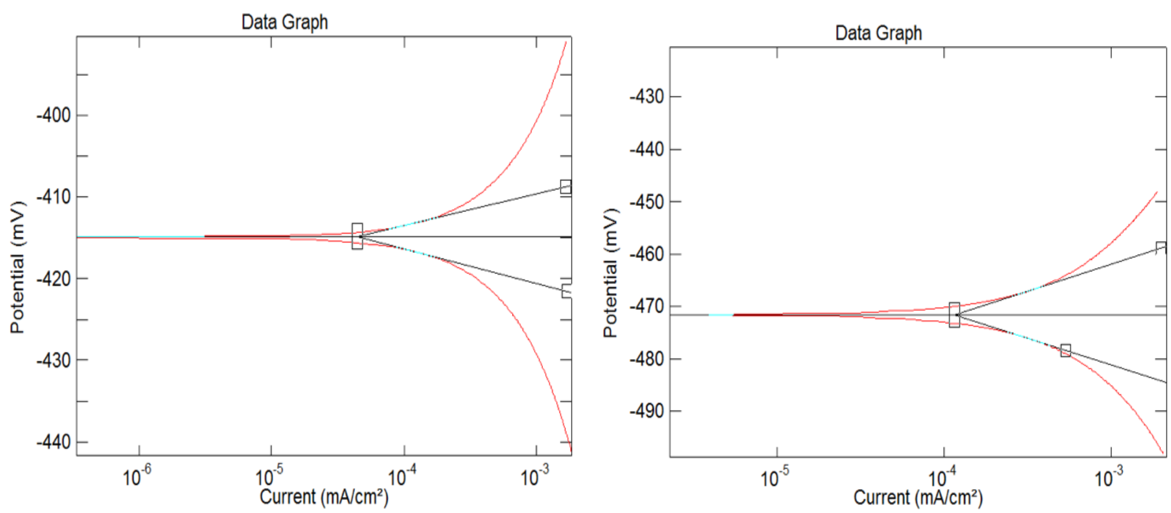


Figure 4.11: Tafel plot of initial (before DC supply) and the final (when corrosion occurs) corrosion current density of the S-S specimen

Rest potential or half cell potential also expands with time. As rest potential of sample increases with time i.e specimen became more prone to the corrosion. The half cell potential of the NSB-NSB specimen (-59.8 mV initial) is approximately half of the S-S specimen (-126.4 mV initial), it means specimen cast and cure in sewage effluent is highly susceptible to corrosion in comparison of the microbial matrix (NSB-NSB). Initially (0 day) the half cell potential of the S-S specimen and the CSB-CSB specimen is approximately same while with passage of time half cell potential of the S-S specimen becomes greater than CSB-CSB specimen. The main reason of the less corrosion in microbial concrete may be microbial induced calcite precipitation (MICP).

In Table 4.8, the corrosion current density and the half cell potential of all the five matrix at various time periods i.e. 0 day to 14 days are represented.

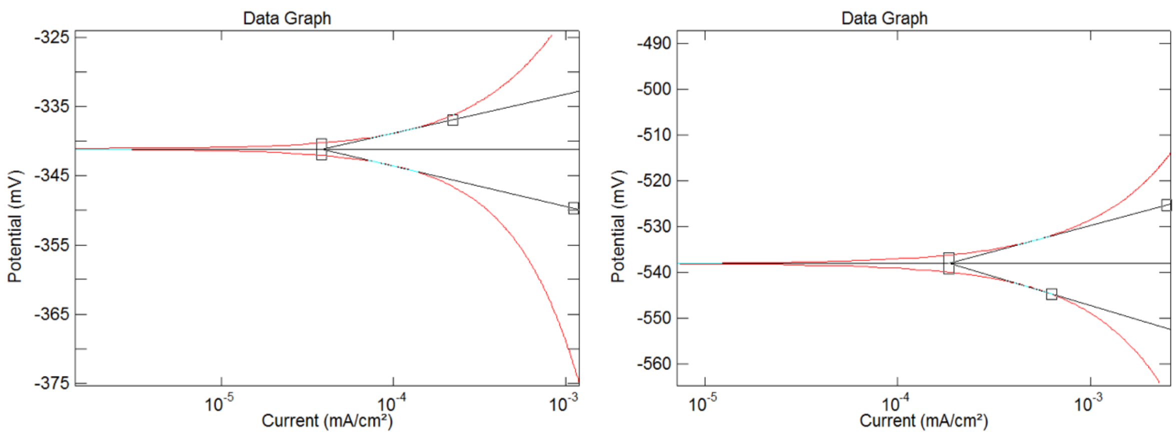


Figure 4.12: Tafel plot of initial (before DC supply) and the final (when corrosion occurs) corrosion current density of the NSB-NSB specimen.

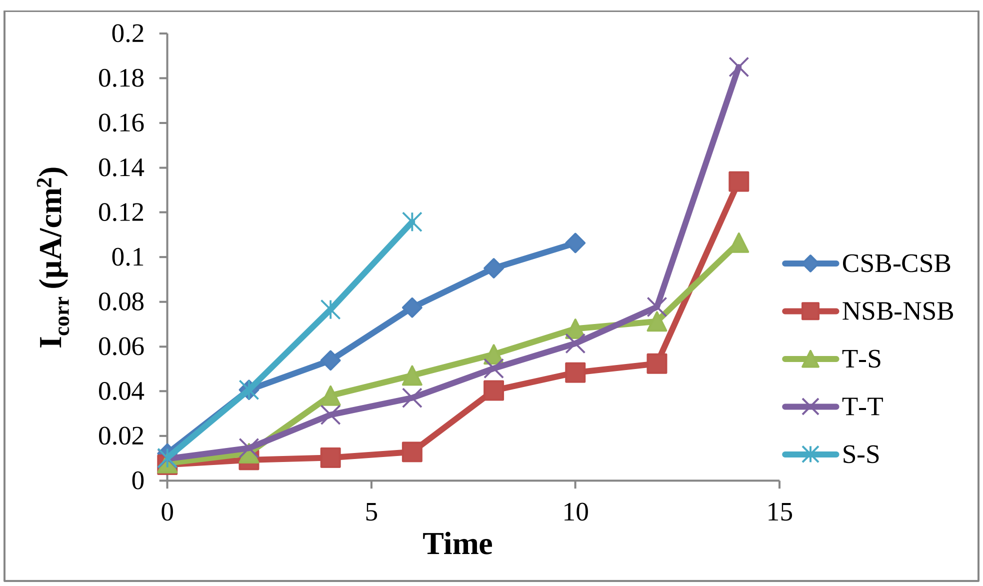


Figure 4.13: Relationship between I_{corr} (μA/cm^2) and time interval (days) of various specimens at constant reinforcing bar voltage

Table 4.8: I_{corr} ($\mu A/cm^2$) and R_p (mV) of the various specimens at different time intervals

Days	Values	T-T	T-S	S-S	NSB-NSB	CSB-CSB
0	I_{corr}	0.00977	0.00782	0.01017	0.00715	0.01216
	R_p	-136.4	-118.1	-126.4	-59.8	-130.8
2	I_{corr}	0.01455	0.01225	0.04058	0.00923	0.04055
	R_p	-212.2	-334.7	-325.2	-157.4	-225.3
4	I_{corr}	0.02946	0.03797	0.07649	0.0102	0.05377
	R_p	-277.2	-344.4	-433.7	-211.6	-362.0
6	I_{corr}	0.03701	0.04711	0.1157	0.01278	0.07742
	R_p	-333.4	-379.7	-473.1	-238.9	-420.3
8	I_{corr}	0.05022	0.05655	-	0.04025	0.09495
	R_p	-370.7	-393.6	-	-318.5	-506.3
10	I_{corr}	0.06041	0.06791	-	0.04831	0.10625
	R_p	-405.5	-405.6	-	-382.4	-548.1
12	I_{corr}	0.07762	0.07127	-	0.0524	-
	R_p	-445.4	-426.3	-	-423.8	-
14	I_{corr}	0.185	0.1064	-	0.13374	-
	R_p	-485.7	-455.5	-	-539.1	-

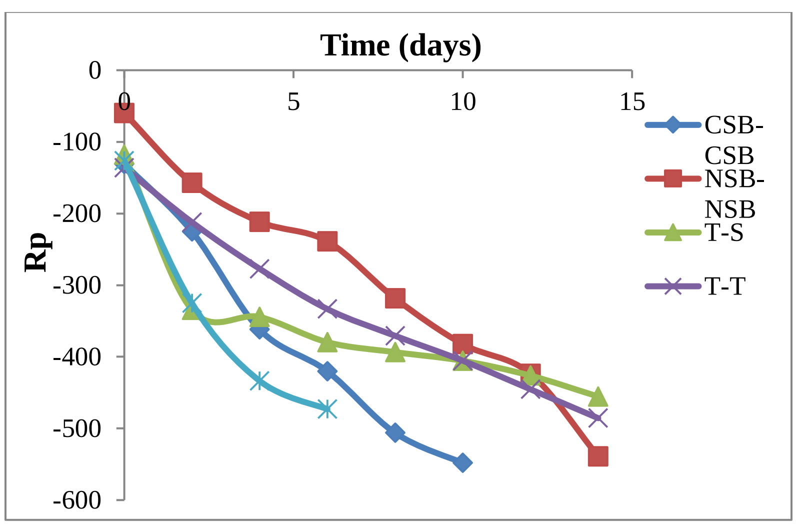


Figure 4.14: Relation b/w R_p (mV) and time interval (days) in various specimens at constant reinforcing bar voltage

4.5 MICROSTRUCTURE ANALYSIS

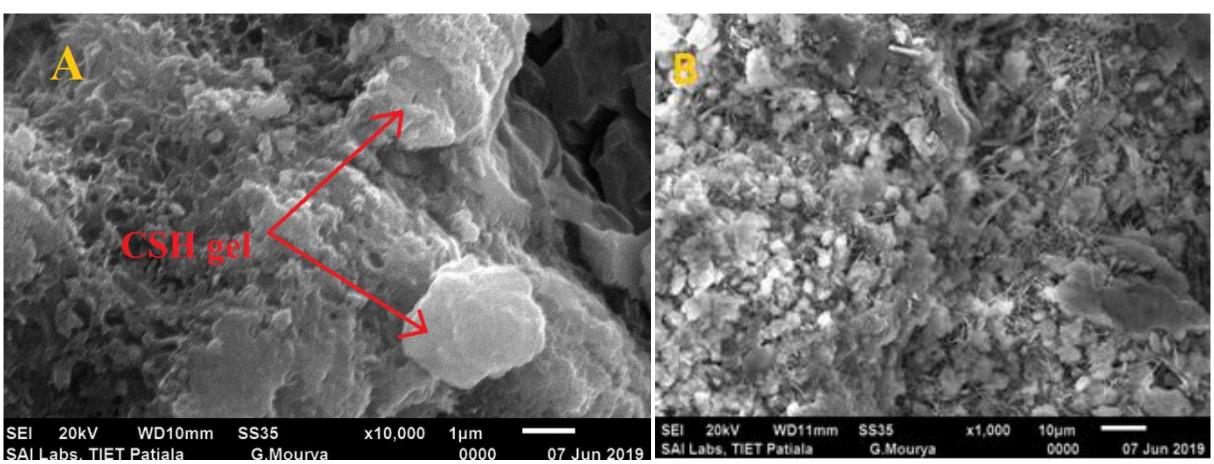
4.5.1 SEM-EDS

For this, a broken sample piece was selected from samples after 28 days from each concrete matrix. SEM was used to analyze compositional variation in observed specimen. The micro structural crystal's elemental composition and chemical characterization was governed by EDS.

Bacterial incorporated specimens show the maximum strength because of the formation of $CaCO_3$ in pores of concrete matrix. Figure 4.15, 4.16, 4.17, 4.18 and 4.19 show SEM-EDS results of all samples. From the results it is observed that no calcite crystals were found in the specimens without bacterial culture, while in the microbial concrete samples, rhombohedral crystals of calcite and aragonite crystals of calcium carbonate ($CaCO_3$) having needle shape were found. This shows that in the microbial concrete there was continuous formation of calcite and no crystals were observed in conventional concrete. Figure 4.19 (A) confirms the presence of calcium carbonate incorporated with bacterial cell.

On the other hand, less compressive strength was observed in S-S sample as compare to T-T sample and T-S sample. This may be due to the more porous concrete matrix of the S-S sample as compared to the others as shown in Figure 4.15 (B), 4.16 (B) and 4.17 (B). All these three images were taken at same magnification i.e. (x 1000) and it was observed that T-T sample is denser as compared to the T-S sample and T-S sample is denser as compared to the S-S sample.

In the EDS analysis, high peaks of the calcium (Ca) and carbon (C) governs existence of $CaCO_3$ crystals in microbial matrix. While in conventional concrete, peaks of calcium (Ca) were present but carbon (C) peaks were not observed.



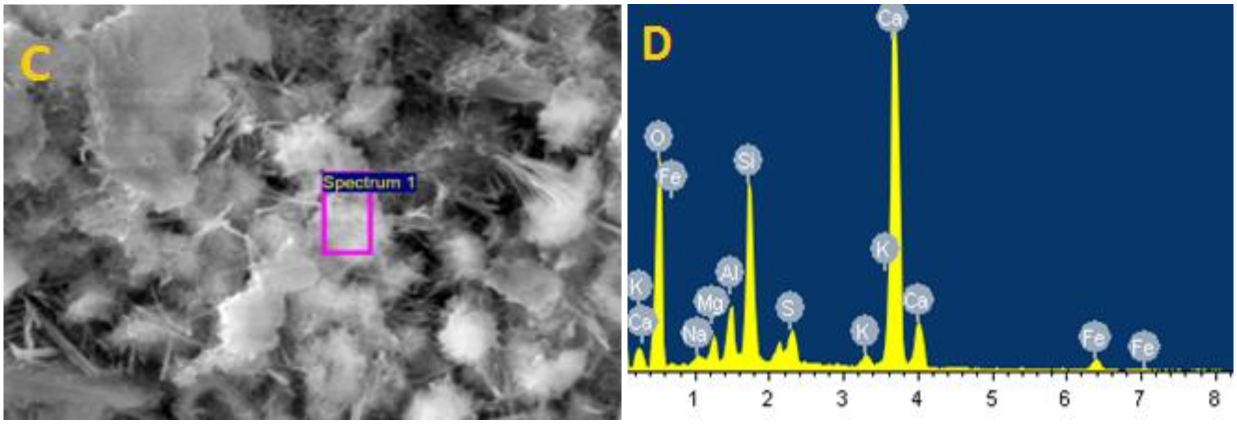


Figure 4.15: SEM-EDS of T-T specimen

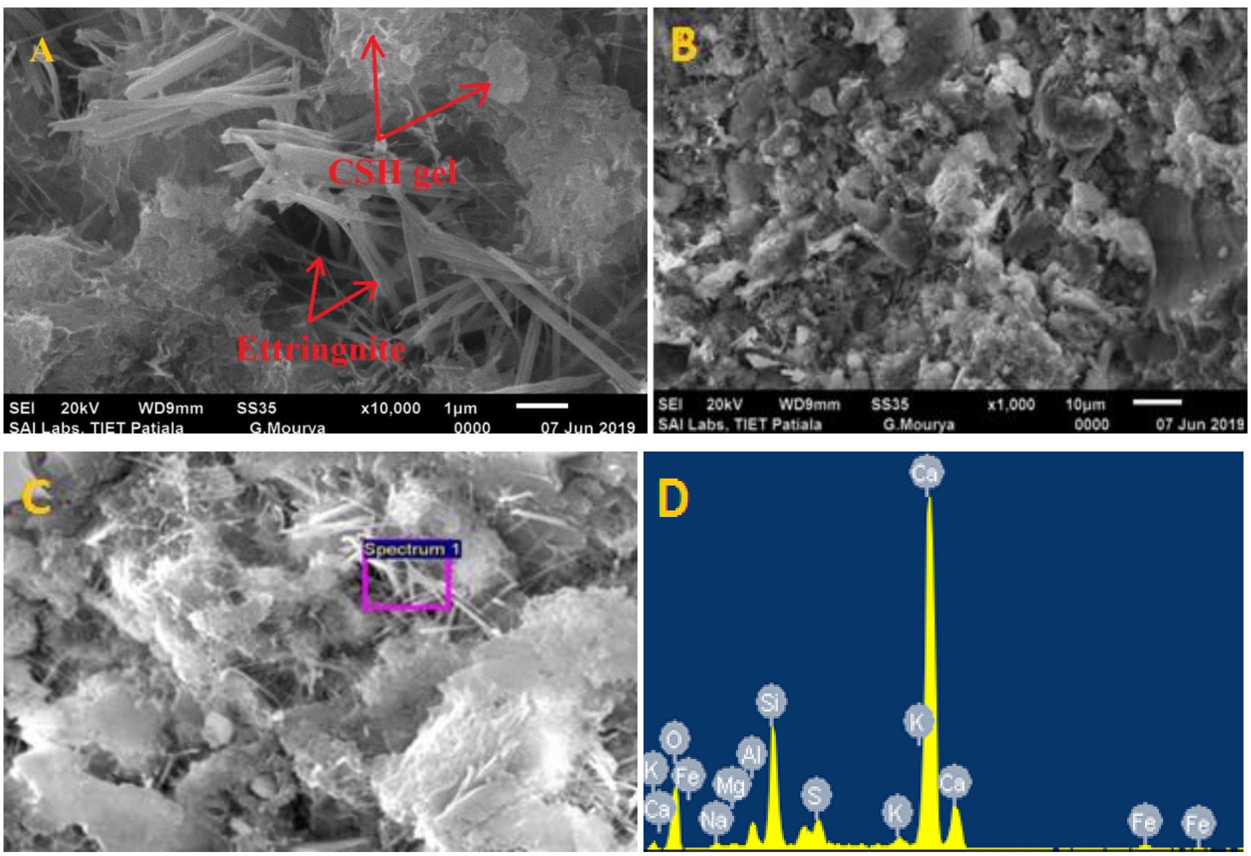


Figure 4.16: SEM-EDS of T-S specimen

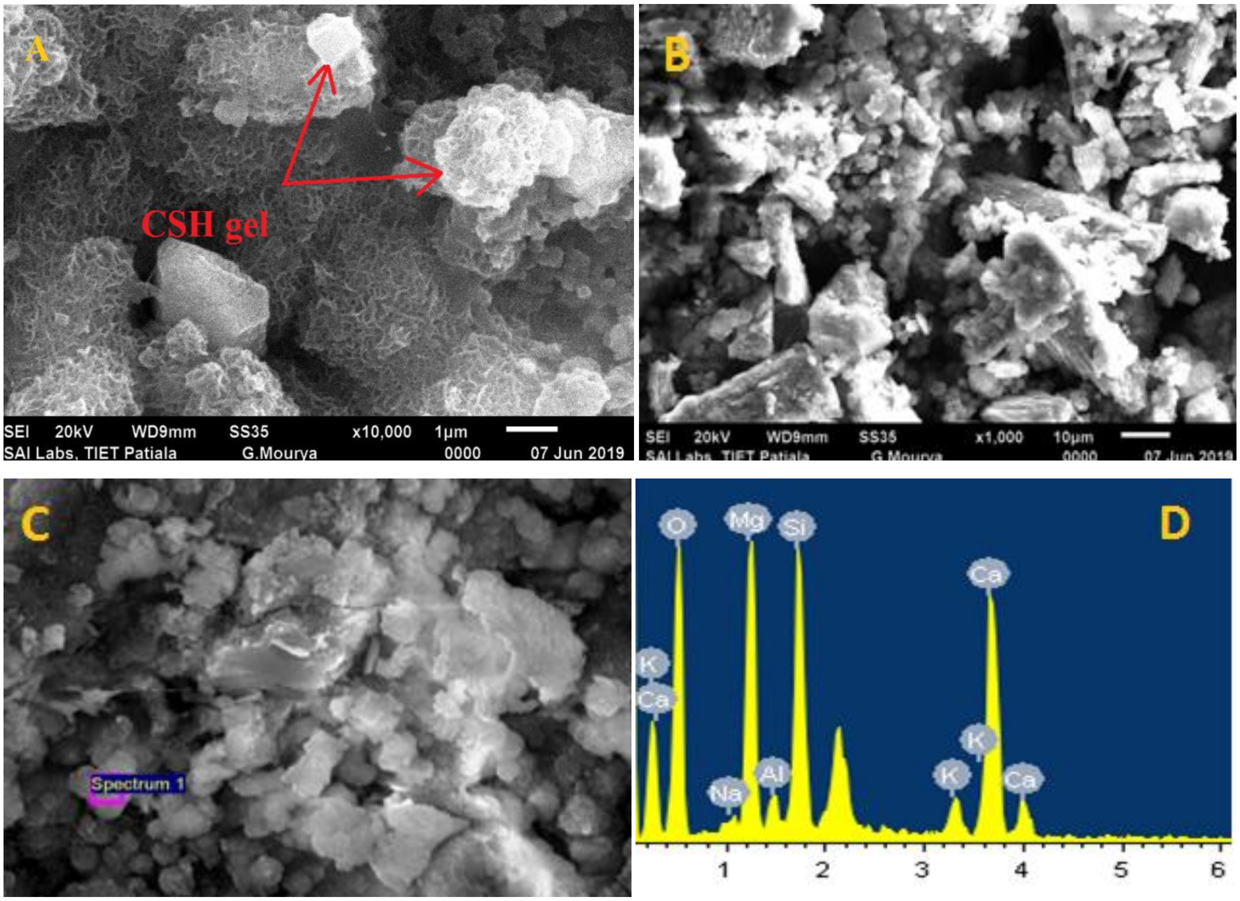
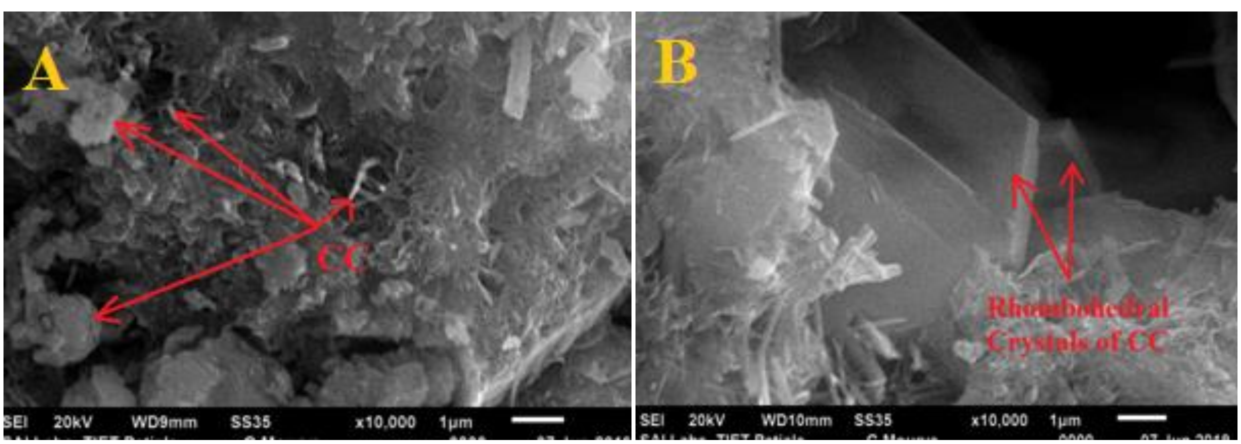


Figure 4.17: SEM-EDS of S-S specimen



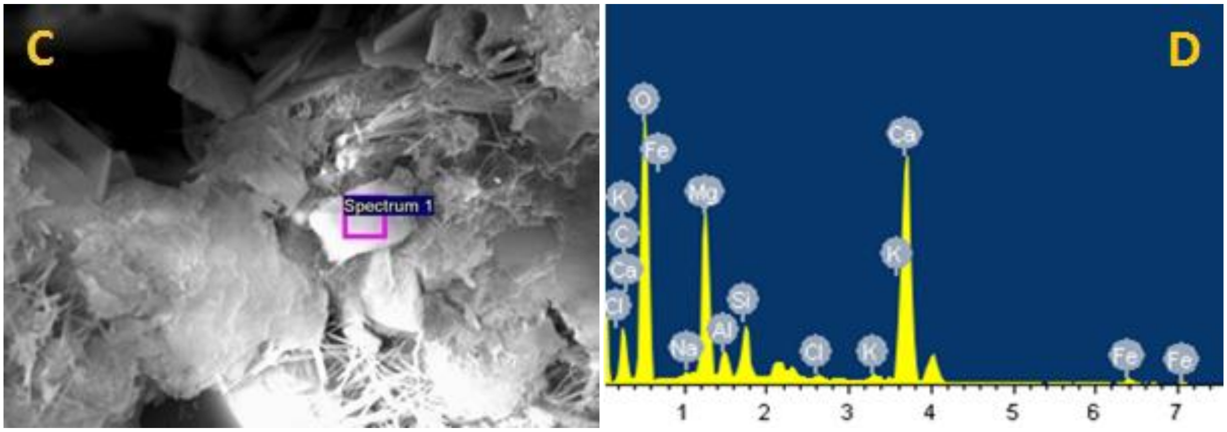


Figure 4.18: SEM-EDS of NSB-NSB specimen

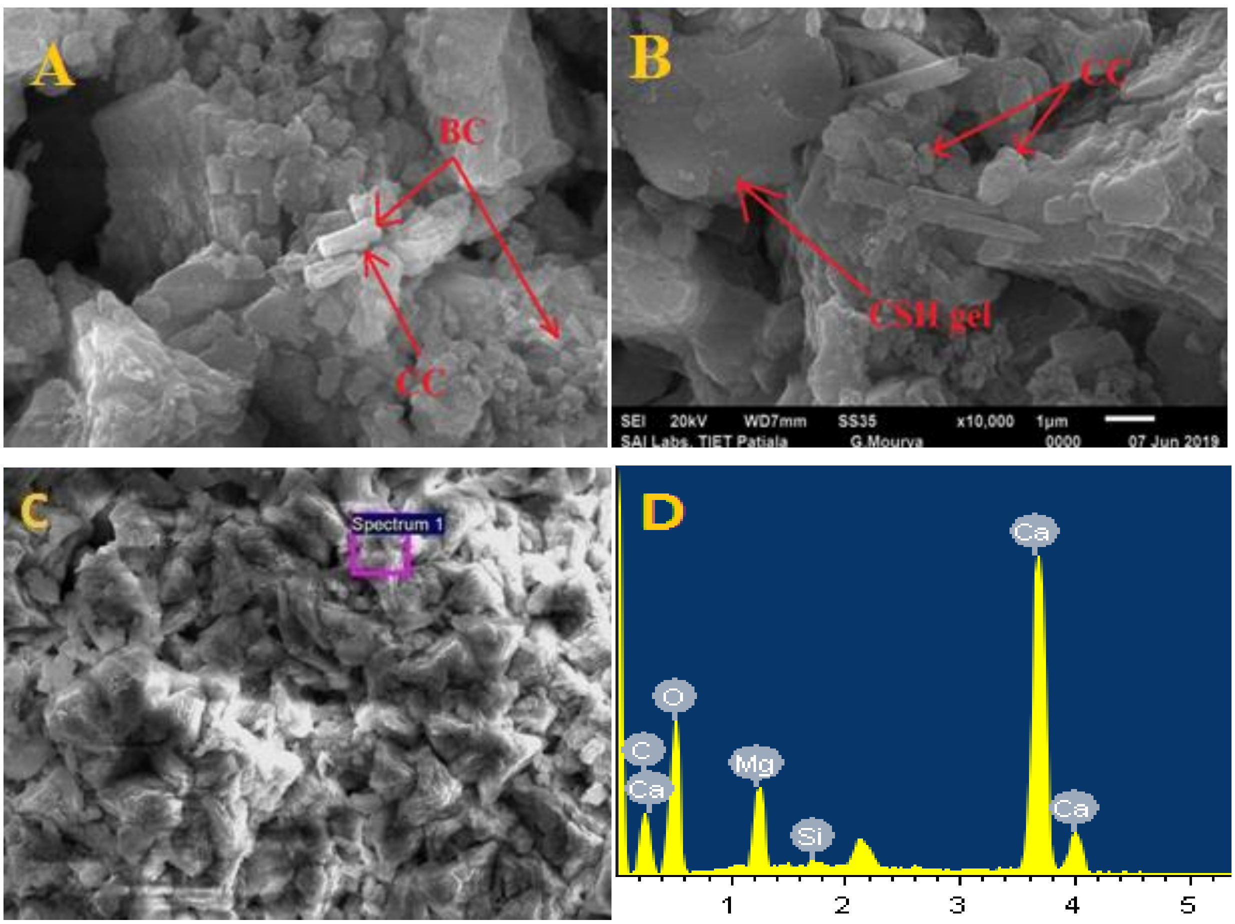


Figure 4.19: SEM-EDS of CSB-CSB specimen

4.5.2 XRD

The orientation and structure of even single grain or crystal was found by XRD. The morphology of crystal and internal stresses in the crystal can also be determined by the XRD. For the X-ray diffraction, the powdered concrete sample passing through 90μ was needed. Sample scanning was done at 10 degree to 80 degree to observe the XRD pattern. The presence of different elements in the matrix was shown by the peaks in the XRD pattern.

The XRD pattern of all the samples, with bacterial culture or without bacterial culture is shown in Figure 4.20, 4.21, 4.22, 4.23, 4.24. The XRD pattern shows the peaks of the different elements present in the matrix like quartz, calcite, vaterite, calcium aluminium silicate, aragonite, coesite etc. From the XRD pattern, it was observed that the control specimens (T-T, T-S, S-S) do not have any calcium carbonate, calcite, vaterite peak.

Calcite peaks present in the microbial concrete mix confirms the existence of $CaCO_3$ in microbial concrete. This shows that calcite crystals are formed by the bacterial cell in voids of sample and makes the concrete heavy; hence the compressive strength of microbial samples is remarkably greater than conventional concrete.

Q represents the quartz; CH represents the calcium hydroxide; CC represents the calcium carbonate; V represents the vaterite; A represents the aragonite; C represents the calcite; CAS represents the calcium aluminium silicate; CS represents the calcium silicate;

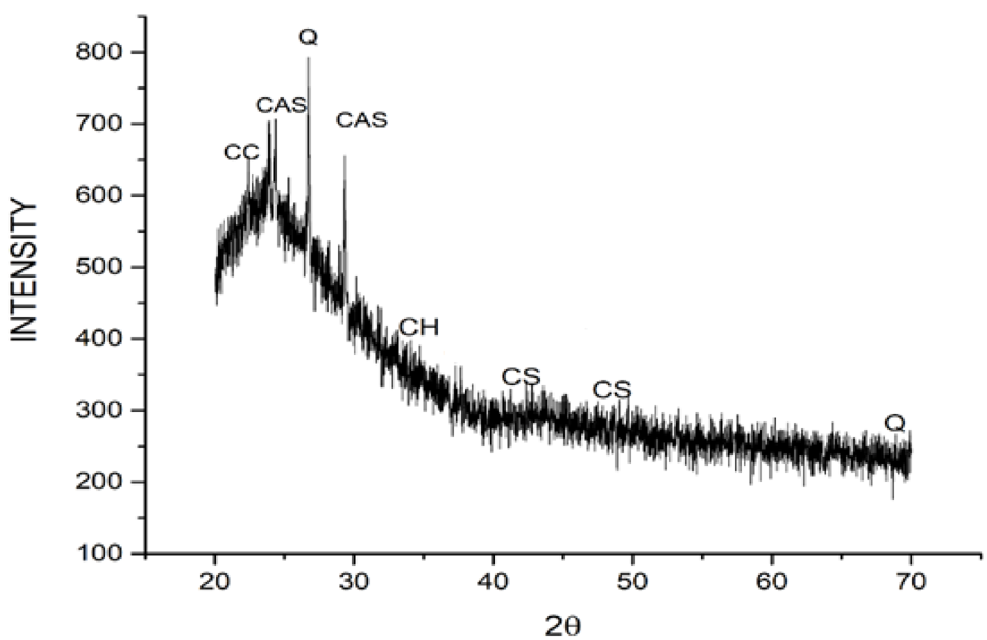


Figure 4.20: XRD pattern for T-T specimen

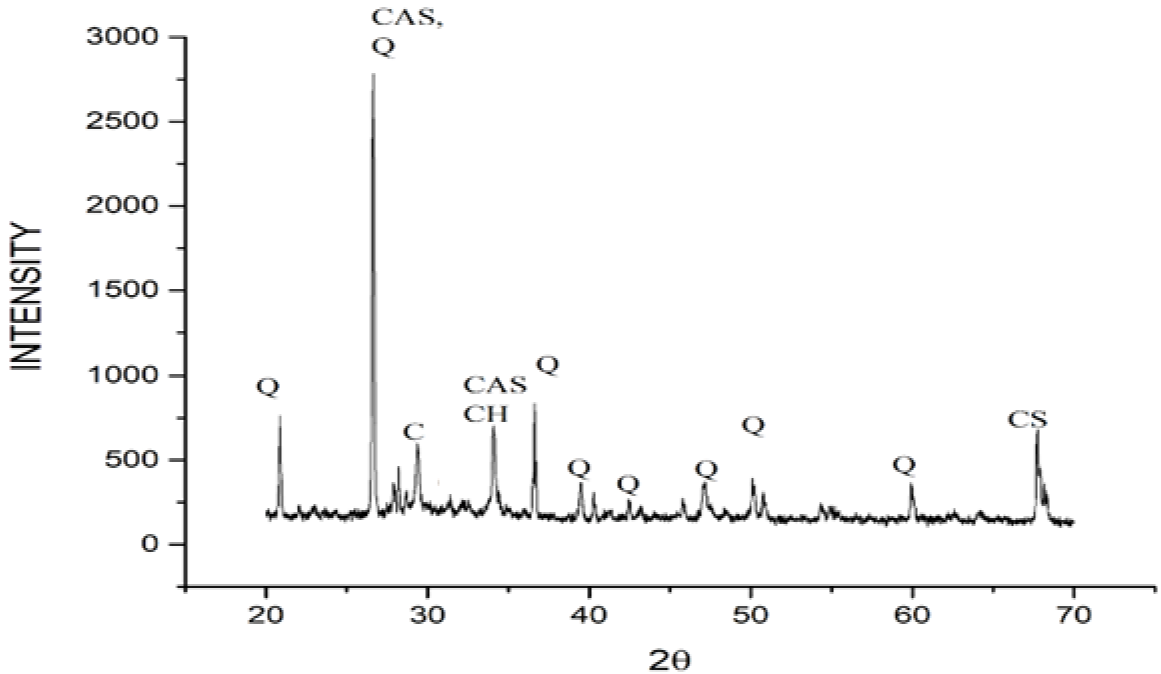


Figure 4.21: XRD pattern for T-S specimen

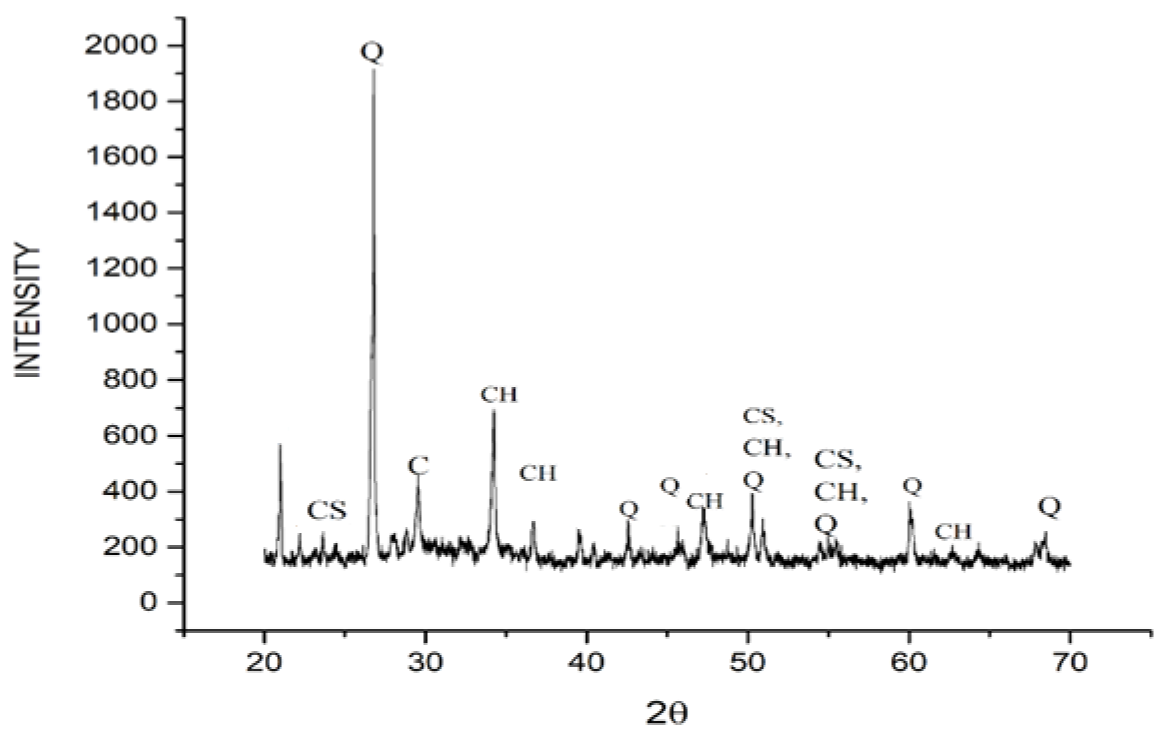


Figure 4.22: XRD pattern for S-S specimen

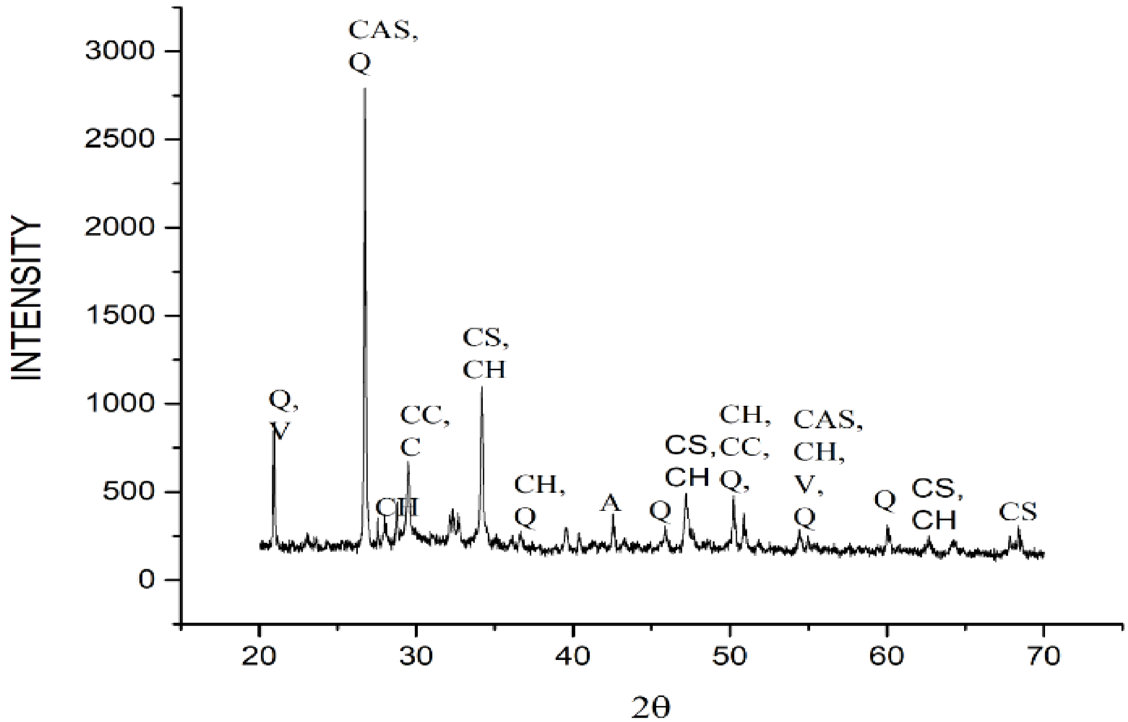


Figure 4.23: XRD pattern for NSB-NSB specimen

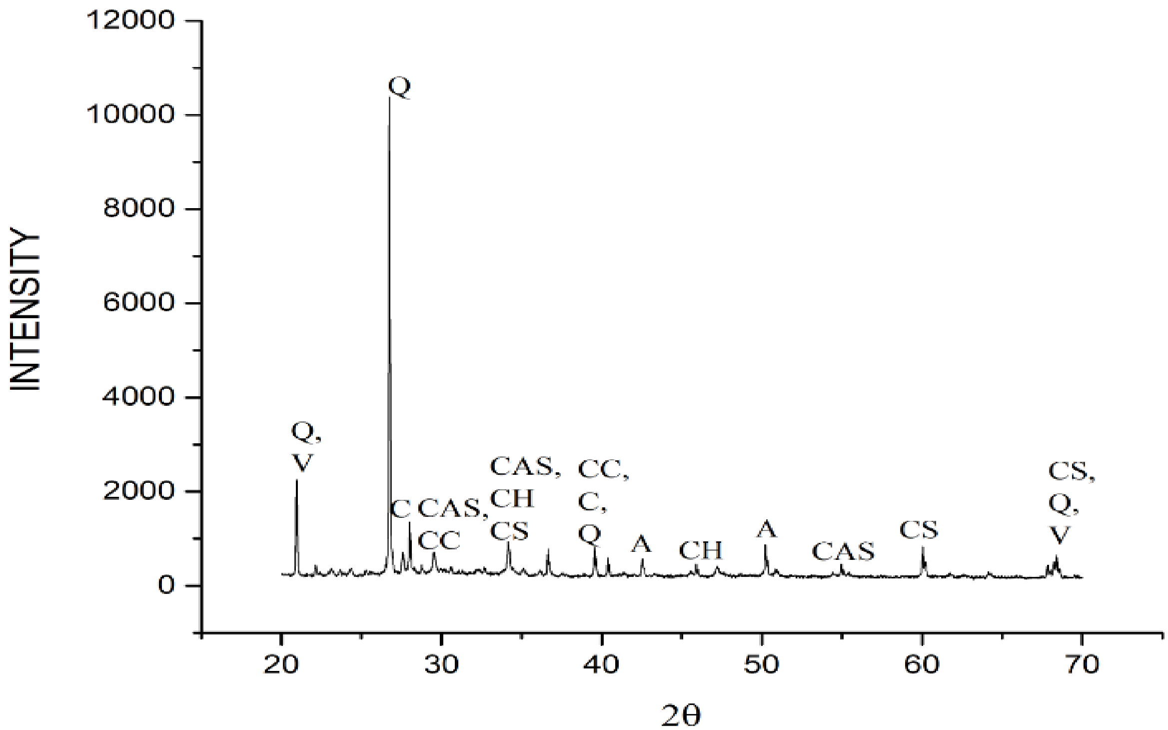


Figure 4.24: XRD pattern for CSB-CSB specimen

CHAPTER 5

CONCLUSIONS

In the respective chapter, all the experimental program result values are summarized and conclude that:

1. The initial setting time will not exceed 60 min. earlier nor greater than 90 min. later as compare to cement paste made by using same cement and distilled water. The initial and final setting time of the paste made with sewage effluent, bacterial treated nutrient broth media and bacterial treated corn steep liquor media, where media was prepared by using sewage effluent are in the acceptable limit.

2. The concrete slump made with fresh water is 78 mm and sewage effluent is 71 mm and the concrete slump made with bacterial treated nutrient broth media (38 mm) and bacterial treated corn steep liquor media (45 mm) is approximately half of the above values. This may be because of the denser or viscous nutrients in the bacterial culture.

3. Sewage effluent alone can't be used as a casting and curing water, because the 28 days relative percentage decrease in strength was greater than 10% in comparison with conventional matrix. However, the compressive strength of a microbial sample is higher when compared with control concrete. This is because of development of $CaCO_3$ precipitates in voids of the matrix which makes it denser.

4. The flow value of the specimens made with bacterial treated nutrient broth media (NSB-NSB) and bacterial treated corn steep liquor media (CSB-CSB) is very less as compared to the specimens cast and cured with sewage effluent (S-S). This is because of the generation of vaterite or calcite or aragonite crystals in voids of the specimen and specimen became denser. This relative percentage decrease in the flow ($ml/m^2.s$) of bacterial treated specimens was 40% to 70% as compared to the sewage effluent specimens at different time intervals. While percentage relative increase in the flow of sewage effluent specimens was 30% to 70% as compared to specimens cast and cured using tap water at various intervals.

5. Initial and secondary absorption rate of specimens cast and cured using bacterial treated nutrient broth media (NSB-NSB) and bacterial treated corn steep liquor media (CSB-CSB) were lower than that of specimens cast using sewage effluent, this is because of the generation of calcite or vaterite or aragonite precipitates in the voids of matrix. The secondary rate of absorption of the specimen cast using tap water and cured in sewage effluent (T-S) is lower than that of the sewage effluent specimen (S-S) and higher than that of specimen cast and cured in tap water (T-T). The curing of T-S specimen was done by sewage effluent, which may cause the formation of pores in the matrix at some extent (less than that of S-S specimen).

6. MICP plays an effective role in drastic reduction in corrosion current density in microbial concrete specimens. In sewage effluent specimens (S-S), corrosion takes place after 6 days while in microbial concrete (NSB-NSB and CSB-CSB) corrosion takes place after 14 days and 10 days, respectively. This may be due to the presence of more voids in the S-S specimens as compared to the microbial concrete.

7. The existence of calcium carbonate precipitates along with rod shaped bacterial cells was confirmed by the SEM analysis of microbial concrete. Precipitates of the calcium chloride were confirmed by the EDS analysis of the selected crystals. More number of pores was found in the SEM analysis of specimens made using sewage effluent as compared to T-S (cast with tap water and cured in sewage effluent) and T-T specimens (cast and cured using tap water), while the pores in the Microbial concrete was filled by the calcium carbonate precipitates. The peaks of calcite, vaterite, calcium carbonate and aragonite were shown by the XRD analysis in the microbial concrete.

REFERENCES

A.H. Noruzman, B. Muhammad, M. Ismail, Z. Abdul-Majid, "Characteristics of treated effluents and their potential applications for producing concrete", Journal of Environmental Management (2012) 110, 27-32.

A.M. Ghrair, O.A. Al-Mashaqbeh, M.K. Sarireh, N. Al-Kouz, M. Farfoura, S.B. Megdal, "Influence of grey water on physical and mechanical properties of mortar and concrete mixes", Ain Shams Engineering Journal (2018) 9, 1519–1525.

ASTM C94, standard specification for ready mix concrete, American Society for testing and materials.

B.Z. Mahasneh, "Assessment of Replacing Wastewater and treated water with Tap water in making Concrete Mix", Electronic Journal of Geotechnical Engineering (2014).

D. Swami, K. Sarkar, B. Bhattacharjee, "Use of treated domestic effluent as mixing water for concrete: Effect on strength and water penetration at 28 days", Indian Concrete Journal (2015) 89 (12), 0-0.

E. Tripathi, K. Anand, S. Goyal, M.S. Reddy, "Bacterial based admixed or spray treatment to improve properties of concrete", Sadhana (2019), 44:19.

G.D.O. Okwadha, J. Li, "Optimum conditions for microbial carbonate precipitation", Chemosphere (2010) 81, 1143–1148.

G. Asadollahfardi, M. Delnavaz, V. Rashnoiee, A. Fazeli, N. Gonabadi, "Dataset of producing and curing concrete using domestic treated wastewater", Data in Brief (2016) 6, 316–325.

IS: 10262, Indian Standard Guidelines for Concrete mix proportioning, (2009).

J.T. Dejong, B.M. Mortensen, B.C. Martinez, D.C. Nelson, "Bio-mediated soil improvement", Ecological Engineering (2010) 36, 197–210.

K.S. Al-Jabri, A.H. Al-Saidy, R. Taha, A.J. Al-Kemyani, "Effect of using Wastewater on the Properties of High Strength Concrete", Procedia Engineering (2011) 14, 370–376.

K. Meena, S. Luhar, “Effect of wastewater on properties of concrete”, Journal of Building Engineering (2019) 21, 106–112.

M. Silva, T.R. Naik, “Sustainable use of resources – recycling of sewage treatment plant water in concrete”, Second international conference on sustainable construction materials and technologies (SCMT) (2010).

M. Shekarchi, M. Yazdian, N. Mehrdadi, “Use of biologically treated domestic waste water in concrete”, Kuwait J. Sci. Eng. (2012) 39 (2B) pp, 97-111.

N.K. Dhami, M.S. Reddy, A. Mukherjee, “Improvement in strength properties of ash bricks by bacterial calcite”, Ecological Engineering (2012) 39, 31– 35.

N.K. Dhami, M.S. Reddy, A. Mukherjee, “Bacillus megaterium mediated mineralization of calcium carbonate as biogenic surface treatment of green building materials”, World J Microbiol Biotechnol (2013) 29, 2397–2406.

N.K. Dhami, M.S. Reddy, A. Mukherjee, “Biomineralization of Calcium Carbonate Polymorphs by the Bacterial Strains Isolated from Calcareous Sites”, J. Microbiol. Biotechnol. (2013) 23(5) 707–714.

P. Ghosh, S. Mandal, B.D. Chattopadhyay, S. Pal, “Use of microorganism to improve the strength of cement mortar”, Cement and Concrete Research (2005) 35, 1980 – 1983.

P. Anbu, C.H. Kang, Y.J. Shin, J.S. So, “Formations of calcium carbonate minerals by bacteria and its multiple applications”, Springer Plus (2016) 5, 250.

P. Magudeaswaran, “Bacterial concrete: a review”, International Journal of Civil Engineering and Technology (2017) 8(2), 588–594.

S. Joshi, S. Goyal, A. Mukherjee, M.S. Reddy, “Microbial healing of cracks in concrete: a review”, J Ind Microbiol Biotechnol (2017) 44(11), 1511-1525.

S. Joshi, S. Goyal, M.S. Reddy, “Corn steep liquor as a nutritional source for biocementation and its impact on concrete structural properties”, Journal of Industrial Microbiology and Biotechnology (2018) 45(8), 1-11.

S. Joshi, S. Goyal, M.S. Reddy, "Influence of nutrient components of media on structural properties of concrete during biocementation", Construction and Building Materials (2018) 158, 601–613.

V. Achal, A. Mukherjee, M.S. Reddy, "Biocalcification by sporosarcina pasteurii using corn steep liquor as the nutrient source", Industrial Biotechnology (2010) 6(3), 170-174.

V. Achal, A. Mukherjee, M.S. Reddy, "Effect of calcifying bacteria on permeation properties of concrete structures", J Ind Microbiol Biotechnol (2011) 38, 1229–1234.

V. Achal, X. Pan, N. Ozyurt, "Improved strength and durability of fly ash-amended concrete by microbial calcite precipitation", Ecological Engineering (2011) 37, 554–559.

V. Achal, A. Mukherjee, M.S. Reddy, "Microbial concrete: a way to enhance the durability of building structures", J. Mater. Civil Eng. (2011) 23(6), 730–734.

V. Achal, A. Mukherjee, S. Goyal, M.S. Reddy, "Corrosion Prevention of Reinforced Concrete with Microbial Calcite Precipitation", ACI Materials Journal (2012) 109(2), 157-164.

V. Achal, A. Mukerjee, M.S. Reddy, "Biogenic treatment improves the durability and remediates the cracks of concrete structures", Construction and Building Materials (2013) 48, 1–5.

W.D. Muynck, N.D. Belie, W. Verstraete, "Microbial carbonate precipitation in construction materials: A review", Ecological Engineering (2010) 36, 118–136.