Microstructural and Nondestructive testing analysis of Self-healing concrete with Pseudomonas fluorescens Bacteria, Calcium Lactate and M- Sand

Chiranjeevi Rahul Rollakanti[1], Kota Srinivasu[2]

[1]Research Scholar, Department of Civil Engineering, Dr.Y.S.R.A.N.U. College of Engineering & Technology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur Dist., Andhra Pradesh 522510, India

[2]Principal, NRI Institute of Technology, Guntur, Andhra Pradesh 522438, India

ABSTRACT

Bacterial concrete has emerged as a corrective measure for treating cracks in structures such as bridges, RCC buildings, RCC pipes, channel linings, flooring, etc. it cracks and reduces its resistance and also has an impact on the reinforcement once it has come into contact with water, carbon dioxide and various chemicals. To repair cracks in concrete, regular maintenance and special treatment is required, which can be extremely costly. In this paper, Pseudomonas fluorescens Bacteria with calcite lactate are used as part of different percentages, such as 5%, 10% and 15% of cement weight for M40 grade concrete. The effect of M- sand on the strength properties of bacterial concrete studied. SEM and X-Ray Diffraction examined on concrete. Bacteria produce $CaCO_3$ precious stones which hinders the smaller scale cracks and pores in the concrete subsequent to responding with calcium lactate. Up to 10%bacterial solution, the concrete strength increses. An empirical relation between flexural strength and compressive strength is proposed in the formula of ft = $0.866f_{ck}^{0.47}$.

Keywords: *Bacterial Concrete, Compressive strength, Split tensile strength, Flexural strength, Pseudomonas fluorescens*

DOI Number: 10.14704/nq.2022.20.6.NQ22859 **NeuroQuantology 2022; 20(6): 8680-8694**

INTRODUCTION

Concrete has become an essential building material in today's modern era of infrastructure projects around the world. Since this material is subject to cracking, it is unstable due to its fragility and is less resistant to deformation, which requires the use of steel reinforcements or reinforcements. Since the connection with steel bars, concrete becomes more effective in resisting stress than when it does not contain any reinforcement and the tensile strength of concrete is lower than the compressive strength. [1-3]. Bacterial concrete usually seeks to repair these defects in order to increase the useful life of any concrete structure. There is a material in the development of concrete self-healing, which will solve many of the problems generally associated with ordinary concrete. Bacillus subtilis bacteria, calcium lactate and nourishing broth mixed with cement [4-5]. This document will also explain, in depth, the method that extends in part behind the particular self-healing microorganism and can describe the various parts that partially extend to the process and then work individually and unite. This document will also become sensitive applications of this self-healing technique and, additionally, real integrations into current structures. Once the bacteria are open to the air and, therefore, to the "food", the microorganism exerts an action that causes them to harden and melt, filling the crack that has formed and adheres to the edges of the cracks to heal the cracks . The method to cure a crack will take very little [6].

Concrete constructions are currently designed to established standards that allow cracks to create up to 2 mm in width. Such small cracks are generally considered acceptable. While in the conventional samples have been produced remedies of 0.2 mm [7]. The basic idea behind our specific version of self-curing concrete is to use certain types of bacteria (in this case, Bacillus subtilis), then work to heal small cracks in the cement before they become larger and more resistant to handle the cracks . This method of bio-calcification involves many parts, to complete these tasks.Fashion techniques such as X-ray diffraction tests and SEM analysis are used to quantify the stages of deposition of trees on the surface and in fissures [8]. It is proven that the combination of calcium lactate and bacteria spores has healed the cracks of width 0.46 mm in control specimen. But in long term it has only healed cracks of 0.18mm wide which can be seen after 100 days of submersion in water [9]. Sporosarcinapaeteurii Zeolite has been used in fiber reinforced mortars and the results obtained showed that zeolite has great capacity of healing but the problem was the healing is good for the first four months and slowed down later on [10]. Sporosarcinapasteuri with 10^6 cell concentration provided good results when it is used along with light weight aggregate in concrete. There is a great reduction in the water absorption which is of about 10% and increase in compressive strength of about 20 % with the SEM analysis LWAC mixes shown good density [11].

Over investigation transmission of chloride on microbial concrete helps the reinforcement in the concrete against corrosion [10]. The abrasion resistance i.e surface abrasion loss and cantabro loss at various ages of concrete from 3 days to 90 days is influenced by flyash content and presence of M-Sand [12]. Field studies on microbial concrete where its solution is sprayed on the crack surface and on car parking concrete slab which showed an encouraging results which healed the cracks in the field successfully [13]. When microbial concrete is checked for the crack healing ability in colder areas by permeability measurements, a sea water immersed sample at 8^0C with 0.4mm crack was healed up to 95% and with 0.6mm crack was healed up to 93% both the samples were cured for 56 days [14].Experiment on usage of sporosarcinapaeteuriin cement mortar increased compressive strength up to 33% after 28 days [15]. In studying the effect of three bacterial solution in microbial surface treatment it is proved that in the presence of sulphate concrete degradation is controlled which in turn increased the durability [16]. Bacillus megaterium, Bacillus licheniformis BSKNAU, Bacillus megaterium BSKAU inclusion in concrete were studied for its self healing capacity and strength and the results were promising [17]. Permeability characteristics of bacterial concrete was studied and results showed that after 7 days there was 84 % healing and after 28 days there was 96 % healing in both the cases the water permeability decreased by 41% and 60% respectively[18]. After experimental investigation on optimum cell concentration of bacteria in concrete, cell concentration of $30x10^5$cfu/ml showed good results [19]. Abrasion tests such as Cantabro test, surface abrasion test, load wheel abrasion on pervious concrete were done and evaluated which resulted in weight loss for all the mixes [20].

2. MATERIALS AND TESTING METHODS

2.1 Cement

Ordinary Portland cement (OPC) of 53 grade used in this experimental work. This OPC was tested as per IS 4031-1996 and the physical properties shown in below Table.1

Table 1. Physical properties of Portland cement (53 grade)

Property	Blaine's fineness	Specific gravity	Soundness (Le-Chatlier Exp.)	7 days Compressive strength	28 days Compressive strength
Test Value	285 m^2/kilogram	3.01	2 mm	41 MPa	56MPa

2.2 Fine Aggregate

The particle size distribution curve of fine aggregate is shown in below figure.1 and the specific gravity of fine aggregate used was 2.68

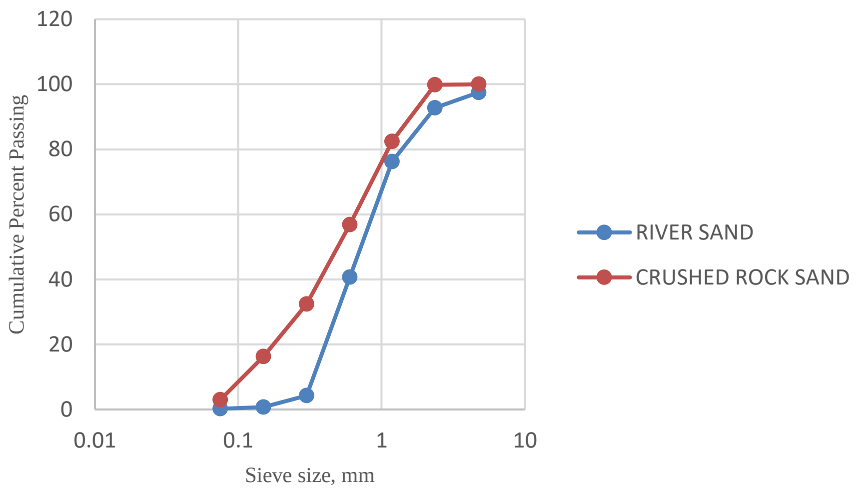


Figure. 1 Particle Size Distribution Curve of Fine Aggregate

2.3 Coarse Aggregate

The particle size distribution curves of coarse aggregates were shown in below figure.2 and properties of coarse aggregates were shown in below table 2.

Table 2. Properties of Coarse aggregate

Test Property	Specific gravity	Water absorption	AIV	ACV
Test Value	2.71	0.5%	21.5%	20.4%

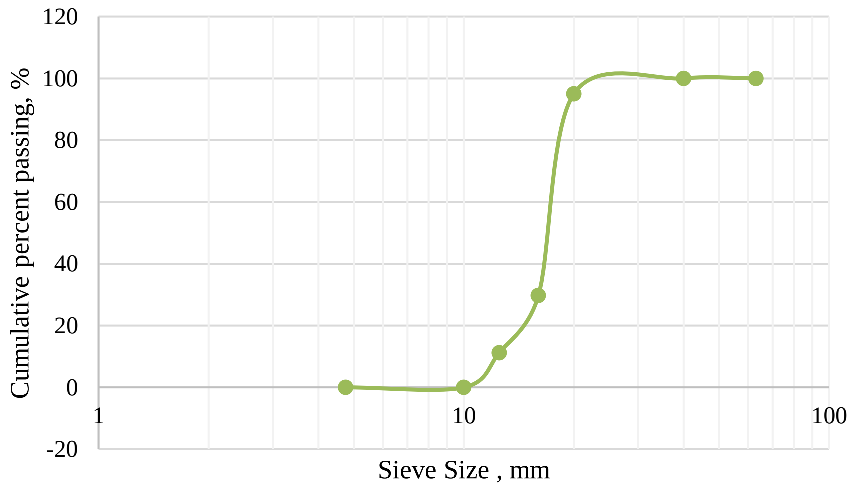


Figure 2 Particle Size Distribution Curve of Coarse Aggregate

2.4 Water

Locally available potable drinking water used in the experimental work for all mixes.

2.5 Microorganisms

Pseudomonas fluorescens bacteria used in this experimental work which is cultured at DVS Bio life Pvt Ltd Laboratory.

Figure.3 Pseudomonas fluorescens bacteria

2.5.2 Calcium lactate

Calcium lactate ($C_6H_{10}CaO_6$) used for this experimental work along with Pseudomonas fluorescens Bacteria.

Figure.4 Calcium Lactate

2.6 Compressive strength

The compressive strength test was carry out on 15cm x 15cm x 15cm cubes as per IS: 516-1959 specifications. Compressive strength of specimens was measured at 7, 14,28,60,90 days of curing age as per IS 516-1959.

2.7 Tensile strength

The split tensile strength test was conducted on 150mm diameter and 300mm long cylinder. Casting and testing was carried out in accordance with IS: 5816-1999.

2.8 Flexural strength

Flexural strength test was on 100 mm x 100 mm x 500 mm. all the specimens casting was carried out as per IS: 516-1959 specifications.

2.9 Mix Design

The mix proportions for M40 grade concrete are designed using IS: 10262-2009 . Materials required for 1 cubic meter of concrete is presented in table 3.

Table 3.Mix proportions for M40 grade concrete

Mixture No	**SHCR 00**	**SHCR 05**	**SHCR 10**	**SHCR 15**	**SHCM 00**	**SHCM 05**	**SHCM 10**	**SHCM 15**
Cement(kg/m^3)	395	395	395	395	395	395	395	395
River Sand(kg/m^3)	638	638	638	638	--	--	--	--
M-Sand(kg/m^3)	--	--	--	--	638	638	638	638
Coarse Aggregate (kg/m^3)	1270	1270	1270	1270	1270	1270	1270	1270
w/c ratio	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Admixture (kg/m^3) (ECMAS)	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9
Bacterial Cells (cfu/ml)	--	10^5	10^5	10^5	--	10^5	10^5	10^5
Percent of bacterial solution	00	05	10	15	00	05	10	15

3. RESULTS AND DISCUSSIONS

3.1 Compressive strength of bacterial concrete with curing age

The variation of compressive strength of bacterial concrete with curing age for M40 grade concrete graphically presented in fig. 5

As expected, the compressive strength of bacterial concrete for M40 grade increases with increasing curing age. From the below it was seen that the gain in compressive strength at 60 days and 90 days is more than the compressive strength at 28 days due to the fact that contribution of Pseudomonas fluorescens Bacteria along with calcium lactate to compressive strength is prominent at ages more than 28 days.

It was observed that as the percentage of bacterial concrete increased from 0% to 10% the compressive strength also increased, but at 15% the compressive strength is reduced, this is due to the fact that the hydration products are saturated at 10% bacterial solution, with further increase in bacterial solution does not contribute to strength and hence there is reduction in strength.

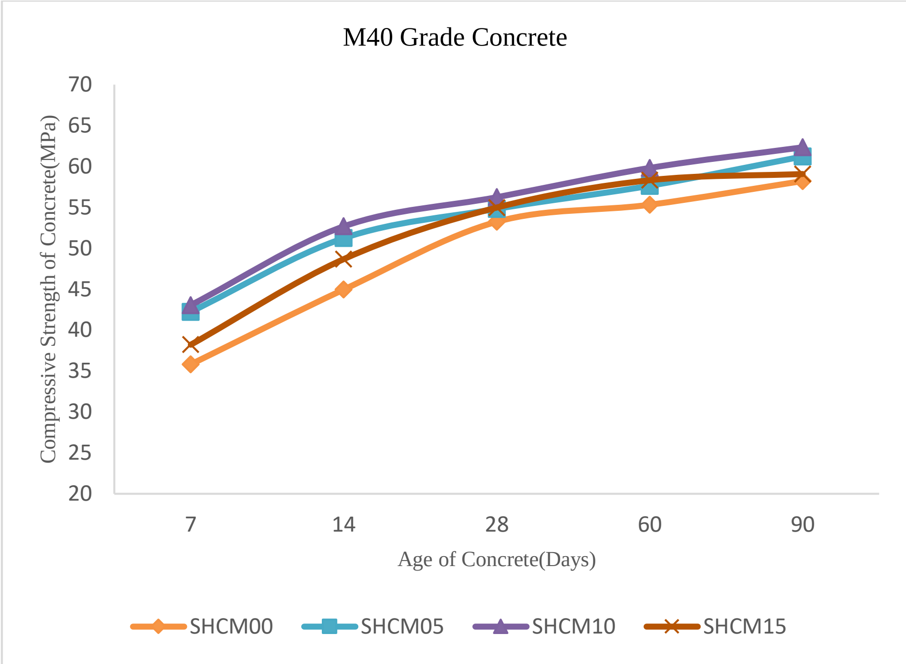


Figure.5 Variation of compressive strength with curing age

3.2 Split tensile strength of bacterial concrete with curing age

The variation of split tensile strength of bacterial concrete with curing age for M40 grade concrete graphically presented in fig. 6.

the split tensile strength of bacterial concrete for M40 grade increases with increasing curing age.

From the below it was seen that the gain in split tensile strength at 60 days and 90 days is more than the split tensile strength at 28 days due to the fact that contribution of Pseudomonas fluorescens Bacteria along with calcium lactate to split tensile strength is prominent at ages more than 28 days.

It was observed that as the percentage of bacterial concrete increased from 0% to 10% the split tensile strength also increased, but at 15% the split tensile is reduced, this is due to the fact that the hydration products are saturated at 10% bacterial solution, with further increase in bacterial solution does not contribute to strength and hence there is reduction in strength

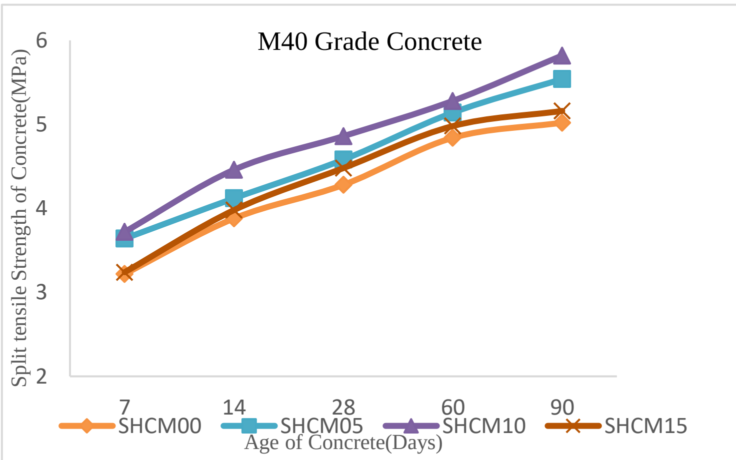


Figure.6 Variation of split tensile strength with curing age

3.3 Flexural strength of bacterial concrete with curing age

The variation of flexural strength of bacterial concrete with curing age for M40 grade concrete graphically presented in fig. 7 The flexural strength of bacterial concrete for M40 grade increases with increasing curing age.

From the above it was seen that the gain in flexural strength at 60 days and 90 days is more than the flexural strength at 28 days due to the fact that contribution of Pseudomonas fluorescens Bacteria along with calcium lactate to flexural strength is prominent at ages more than 28 days. It was observed that as the percentage of bacterial concrete increased from 0% to 10% the flexural strength also increased, but at 15% the flexural strength is reduced, this is due to the fact that the hydration products are saturated at 10% bacterial solution, with further increase in bacterial solution does not contribute to strength and hence there is reduction in strength.

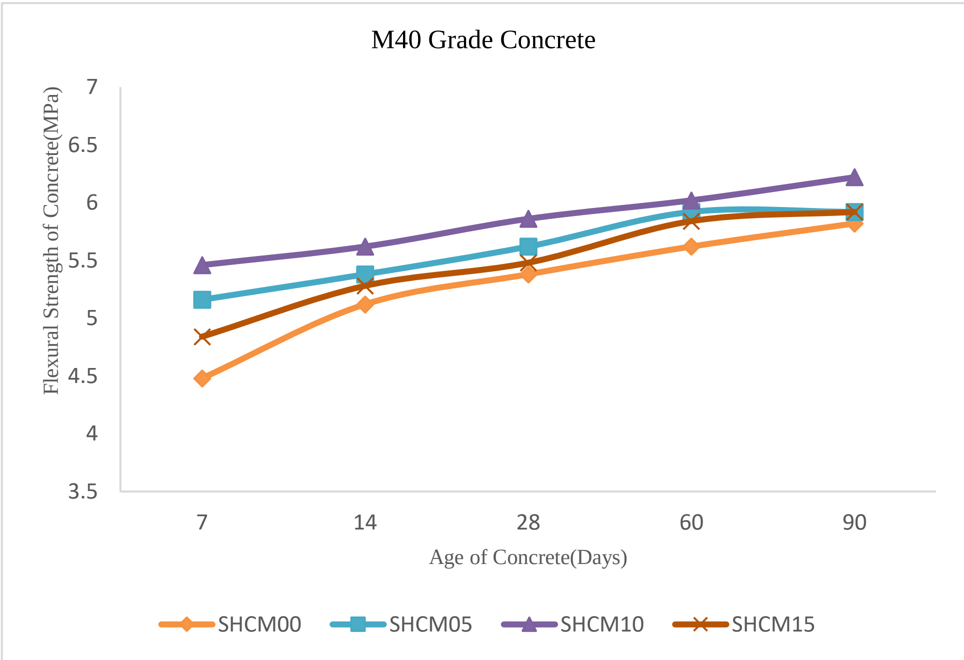


Figure.7 Variation of flexural strength with curing age

3.4 Proposed empirical relation between compressive strength and flexural strength of bacterial concrete:

Proposed empirical relation between compressive strength and flexural strength of bacterial concrete $f_t = f_t = 0.866\, f_{ck}^{\,0.47}$ M40 grade concrete Eq. (8)

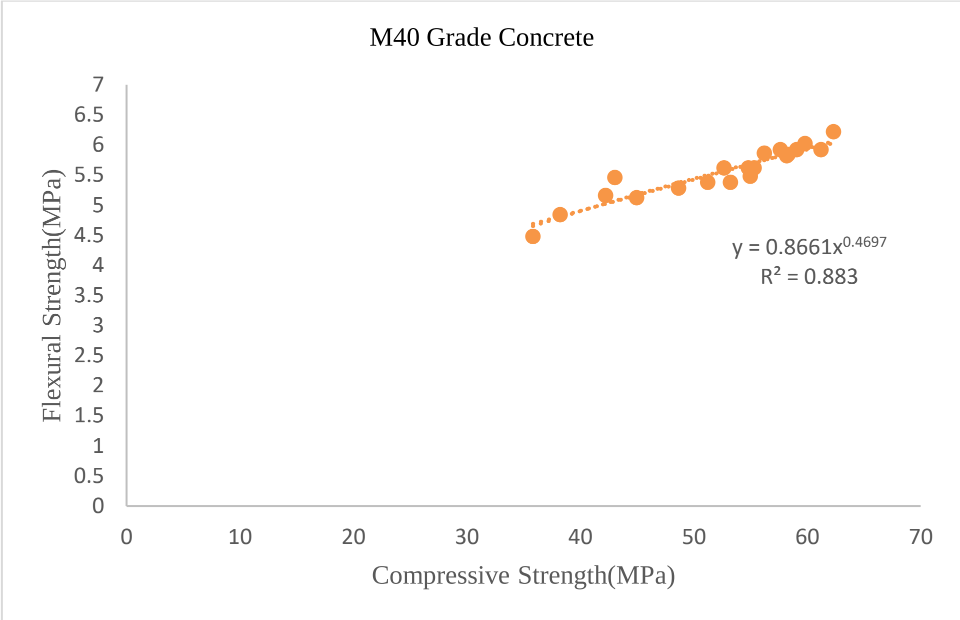
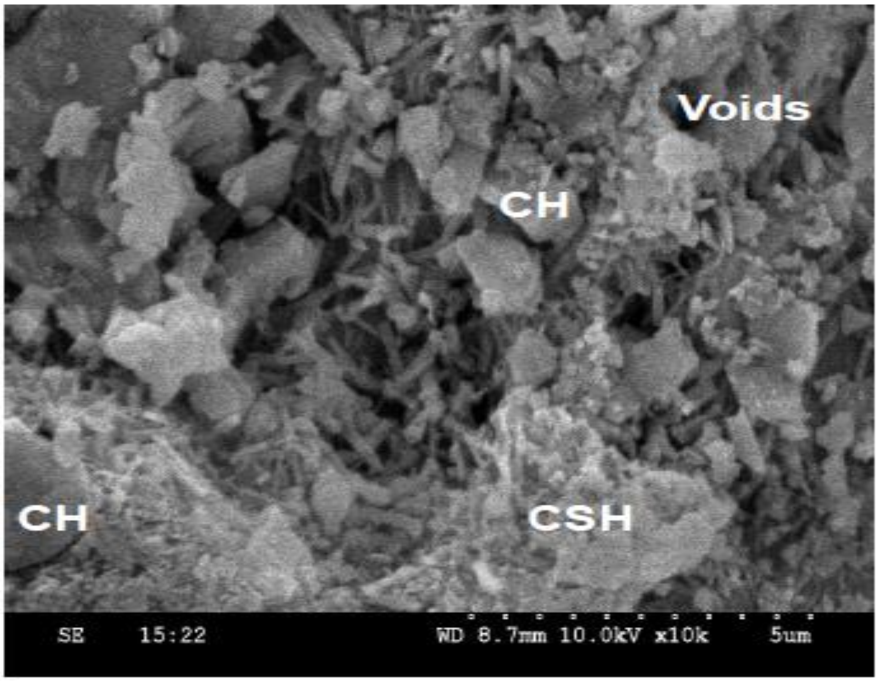


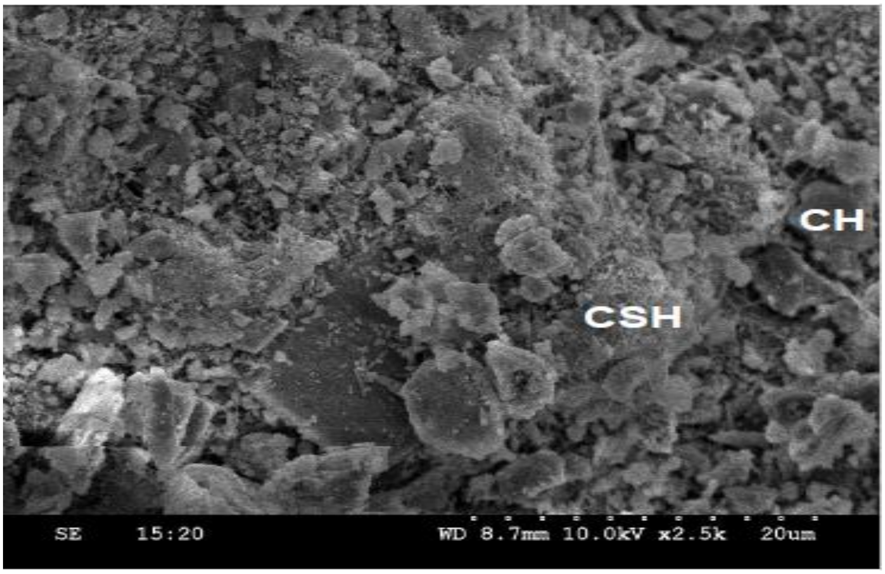
Figure.8 Empirical relation between compressive strength and flexural strength

3.5 Scanning Electron Microcopy (SEM) Analysis

The SEM analysis image for control concrete with a magnification of 10000 and 2500, Sub acceleration voltage 10000 volts, Pixel sizes of 9.921875 and 39.6875. Fig.9 shows the micro structure of the conventional concrete. The SEM analysis image for Bacterial concrete concrete with a magnification of 10000, 20000 and 2500, Sub acceleration voltage 10000 volts, Pixel sizes of 9.921875, 4.960938 and 39.6875.Fig.10 shows the micro structure of the Bacterial concrete. The SEM analysis images shows the occurrence of calcite precipitation in the bacterial concrete. CSH, CH and pores occurred in all samples. Calcite observed in pores, in case of bacterial concrete. It clearly shows that the porosity decreases and gain in strength and the cracks also healed due to calcite formation.

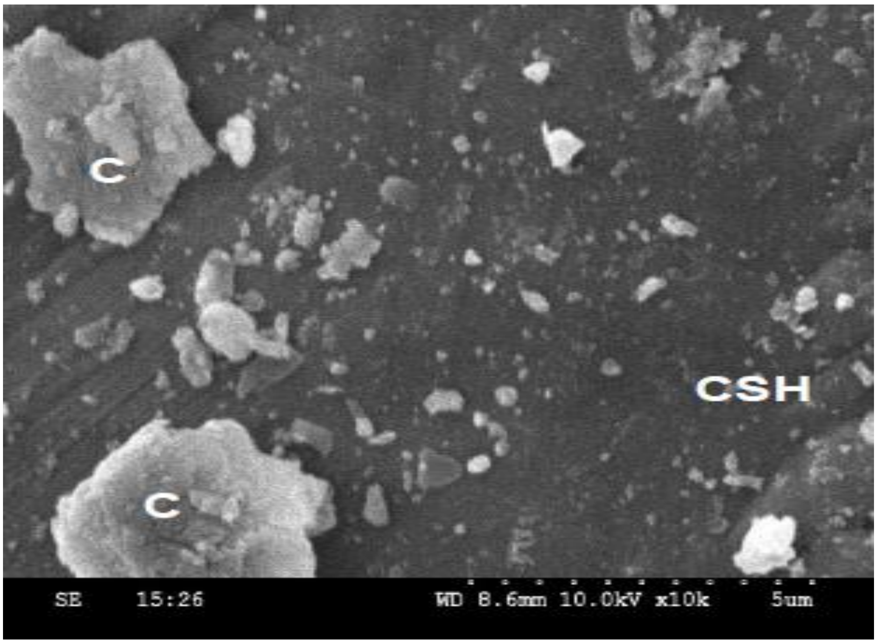


(a)

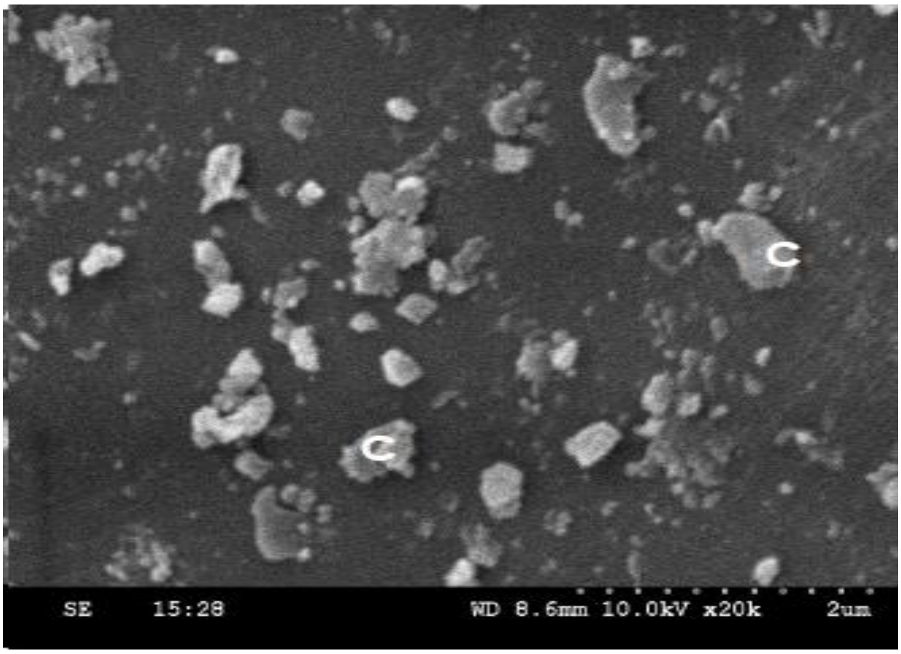


(b)

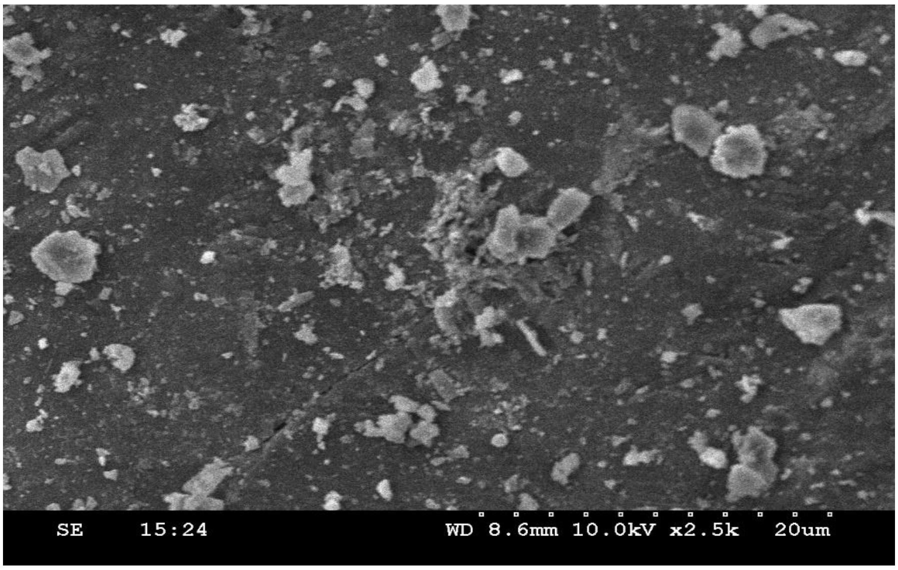
Fig. 9. SEM Micrograph of control concrete (a) magnification at 10000 (b) magnification at 2500



(a)



(b)



(c)

Fig. 10. SEM Micrograph of Bacterialconcrete (a) magnification at 10000 (b) magnification at 20000 (c) magnification at 2500

3.6 X-Ray Diffraction Analysis

Fig 11-12 shows peaks of quartz, calcium silicate hydrate, Larnite, calcite and ettringite . Calcite composition is increased expressively in bacterial concrete compared to control concrete. In Fig.12, from 28 to 33 specify the existence of unclear content along with calcite, Portlandite and larnite

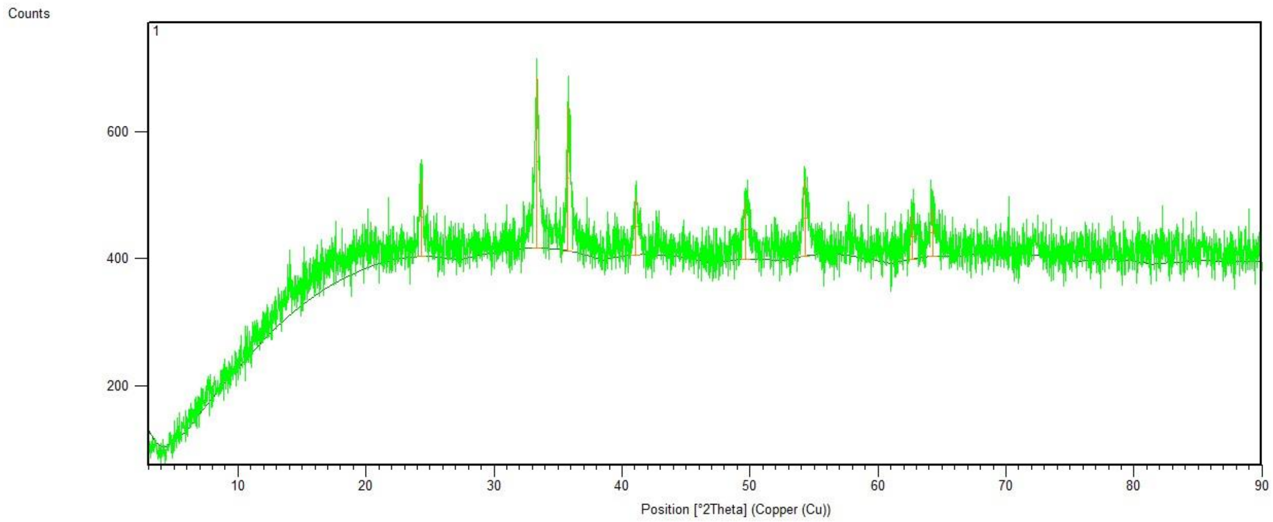


Fig. 11. XRD Diffractogram of control concrete

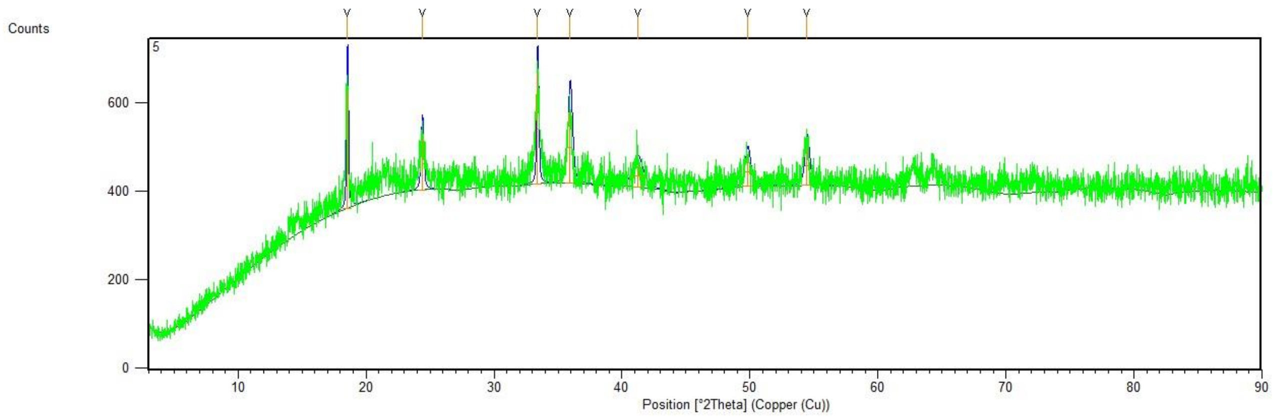


Fig. 12. XRD Diffractogram of Bacterial concrete

3.7 Ultrasonic Puluse velocity test

Ultrasonic Pulse Velocity (UPV) technique is the most useful and non-destructive technique for testing the quality of the concrete and also for checking the cracks and voids in the concrete. The frequencies of the ultrasonic wave are 20 kHz to150 kHz, which is higher than the upper audible limit (20 kHz) and the main reason for taking this frequency range is its path length from 1500 mm to 500 mm.This method consists of one ultrasonic pulse generator, two transducers and CRO and a digital time display unit. The transducer is a device which can transform any physical quantity like temperature, pressure, sound etc into electrical quantity voltage or current waves and vice versa. There are different types of transducers available like temperature transducer, pressure transducer, ultrasonic transducers etc. At one end of the concrete ultrasonic pulse generator and transducer and at the opposite end, second transducer is connected. The both transducers are connected to the concrete using jelly or grease. The first transducer or transmitting end transducer receives the ultrasonic pulse and converts it into electrical waves and this signal is induced into concrete. These waves travel in three types through concrete. Some of the waves travel long distance through concrete and reach the receiving transducer. These are called longitudinal waves. These longitudinal waves are important in our measurement of wave velocity. Some waves travel perpendicular to the longitudinal waves (wave propagation) and these are called transverse or shear waves. Surface waves or Rayleigh waves travel at the surface of the concrete at a depth of one wavelength. These waves travel in elliptical orbit.

The digital time display device is linked to the first transducer and the second transducer output probes. The Digital Time Display Unit shows the time taken to move through the concrete by the ultrasonic pulse. Use the CRO screen (cathode ray oscilloscope) to obtain the same outcome. The first transducer's output is linked to the CRO's x-channel and the receiver transducer's output is linked to CRO's y-channel. The time between the two leading corners of these pulses provides the time between the pulses of Tx and Rx. If the pulse speed is high, it shows the concrete's highest quality. UPV is not adequate to assess the compressive strength of concrete, as it requires a big amount of parameters such as materials and their blended proportions, environmental conditions etc.

The experimental progression of UPV of conventional concrete and Bacterial concrete mixes with the age was shown in below tables for river sand and M- sand mixes.

The ultrasonic pulse velocity increases with increase in curing ages of Bacterial concrete for all mixes are obtained. The UPV of Bacterial concrete mixes was found higher at 10% bacterial solution mixes for M40 grade concrete.

The ultrasonic pulse velocity of Bacterial concrete increases with increase in curing age for river sand and crushed stone sand mixes are observed

The UPV of M- sand mixes was found higher than the conventional concrete for all replacement levels of bacterial solution at all ages. The increase in UPV from 3 days to 7 days is at slower rate, but beyond 7 days to 90 days the UPV increases rapidly. This is due to the fact that the hydration rate is slow at initial ages with Bcateria solution and calcium lactate and faster at later ages.

Table 4 Bacterial Concrete with river sand

Age	**SHCR00**	**SHCR05**	**SHCR10**	**SHCR15**
7 Days	3.09	3.12	3.44	2.33
14 Days	3.24	3.35	3.77	2.66
28 Days	3.54	3.72	3.89	2.62
60 Days	3.91	4.25	4.32	3.12
90 Days	4.22	4.30	4.66	3.39

Table 5 Effect of bacteria on quality of Bacterial Concrete with river sand

Age	**Quality of Concrete**			
	SHCR00	**SHCR05**	**SHCR10**	**SHCR15**
7 Days	M	M	M	D
14 Days	M	M	G	D
28 Days	G	G	G	D
60 Days	G	G	G	M
90 Days	G	G	E	M

Table 6 Bacterial Concrete with M- Sand

Age	SHCM 00	SHCM 05	SHCM 10	SHCM 15
7 Days	3.22	3.28	3.82	2.76
14 Days	3.36	3.38	3.78	2.70
28 Days	3.82	3.88	3.94	2.88
60 Days	4.32	4.38	4.52	3.24
90 Days	4.50	4.58	4.72	3.62

Table 7 Effect of bacteria on quality of Bacterial Concrete with M- Sand

Age	Quality of Concrete			
	SHCM 00	SHCM 05	SHCM 10	SHCM 15
7 Days	M	M	G	D
14 Days	M	M	G	D
28 Days	G	G	G	D
60 Days	G	G	E	M
90 Days	G	E	E	G

4.CONCLUSIONS

Following are the conclusions drawn from the experimental work.

1. M- sand mixes showed higher compressive, flexural and split tension values than river sand mixes, due to the fact that crushed stone sand has cubical particles and more harshness in the mixes. Therefore, crushed stone sand can be used in the production of bacterial concrete for structural application.
2. Microstructure analysis demonstrated formation of calcite in the form of calcium carbonate after 28 days in all bacterial concrete mixes.
3. XRD and SEM analysis reveals the increased formation of CSH gel and nonexpansive ettringite formation which supports the increased compressive strength at 10% bacterial solution.
4. There is increase in compressive strength at 10% bacterial solution for M40 grade concrete at 28 days of curing in comparison with control mix concrete.
5. Based on test results, the optimum dosage of bacterial solution to improve strength at any age has been 10% by weight of cement.

References:

1. Schlangen, H.E.J.G., Jonkers, H.M., Qian, S. and Garcia, A., 2010. Recent advances on self healing of concrete. In *FraMCos-7: Proceedings of the 7th International Conference on Fracture Mechanics of Concrete and Concrete Structures, Jeju Island, Korea, 23-28 May 2010*.

2. Kumar, A., 2018. RSA Comparison of Fresh and Hardened Properties of Normal, Self Compacting and Smart Dynamic Concrete. *Int. J. Technol*, *9*, pp.707-714

3. Dong, Q., Wu, H., Huang, B., Shu, X. and Wang, K., 2010. Development of a simple and fast test method for measuring the

durability of Portland cement Pervious Concrete. *ASCE Journal of Materials in Civil Engineering*.

4. Ramadhansyah, P.J., Abu Bakar, B.H., Megat Azmi, M.J. and Wan Ibrahim, M.H., 2011. Engineering properties of normal concrete grade 40 containing Rice husk ash at different grinding times. *International Journal of Technology*, *2*(1), pp.10-19.

5. Ramakrishnan, V., Ramesh, K.P. and Bang, S.S., 2001, April. Bacterial concrete. In *Smart Materials* (Vol. 4234, pp. 168-176). International Society for Optics and Photonics.

6. Bhaskar, Sini., Khandaker M.A.H., Mohamed Lachemi and Marthinus Otini Kroukamp, 2017. Effect of self-healing on strength and durability of zeolite-immobilized bacterial cementitious mortar composites. *Cem. Concr. Compos*., 82,pp.23-33.

7. Dong, Q., abrasion test methods for pervious concrete. *Journal of Materials in Civil Engineering*, *25*(7), pp.886-892.

8. Zhang, Y., Guo, H.X. and Cheng, X.H., 2015. Role of calcium sources in the strength and microstructure of microbial mortar. *Construction and Building Materials*, *77*, pp.160-167.

9. V. Ramakrishnan, R.K. Panchalan, S.S. Bang, Bacterial concrete,2001 in: *In Proceedings of SPIE – International Society of Optical Engineering*, vol. 4234, pp. 168–176.

10. Dick, J., De Windt, W., De Graef, B., Saveyn, H., Van der Meeren, P., De Belie, N. and Verstraete, W., 2006. Bio-deposition of a calcium carbonate layer on degraded limestone by Bacillus species. *Biodegradation*, *17*(4), pp.357-367.

11. Dhami, N.K., Reddy, M.S. and Mukherjee, A., 2012. Improvement in strength properties of ash bricks by bacterial calcite. *Ecological Engineering*, *39*, pp.31-35.

12. Harrington, E.A., 1927. X-ray diffraction measurements on some of the pure compounds concerned in the study of Portland cement. *American journal of science*, (78), pp.467-479.

13. Siddique, R., Singh, K., Singh, M., Corinaldesi, V. and Rajor, A., 2016. Properties of bacterial rice husk ash concrete. *Construction and Building Materials*, *121*, pp.112-119.

14. Siddique, R., Jameel, A., Singh, M., Barnat-Hunek, D., Aït-Mokhtar, A., Belarbi, R. and Rajor, A., 2017. Effect of bacteria on strength, permeation characteristics and micro-structure of silica fume concrete. *Construction and Building Materials*, *142*, pp.92-100.

15. Achal, V., Mukherjee, A. and Reddy, M.S., 2010. Microbial concrete: way to enhance the durability of building structures. *Journal of materials in civil engineering*, *23*(6), pp.730-734.

16. Achal, V., Mukherjee, A., Basu, P.C. and Reddy, M.S., 2009. Lactose mother liquor as an alternative nutrient source for microbial concrete production by Sporosarcina pasteurii. *Journal of industrial microbiology & biotechnology*, *36*(3), pp.433-438.

17. Achal, V., Mukherjee, A., Basu, P.C. and Reddy, M.S., 2009. Strain improvement of Sporosarcina pasteurii for enhanced urease and calcite production. *Journal of*

industrial microbiology & biotechnology, *36*(7), pp.981-988.

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

19. Majumdar, S., Sarkar, M., Chowdhury, T., Chattopadhyay, B. and Mandal, S., 2012. Use of bacterial protein powder in commercial fly ash pozzolana cements for high performance construction materials. *Open Journal of Civil Engineering*, *2*(04), pp.218.

20. Ghosh, S., Biswas, M., Chattopadhyay, B.D. and Mandal, S., 2009. Microbial activity on the microstructure of bacteria modified mortar. *Cement and Concrete Composites*, *31*(2), pp.93-98.

21. Yogesh Hole et al 2019 J. Phys.: Conf. Ser. 1362 012121