PERFORMANCE ENHANCEMENT OF LIGHT WEIGHT CONCRETE USING MICROBIAL INDUCED CALCITE PRECIPITATION BY SPOROSARCINA PASTEURII

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ABSTRACT. This study presents the results of an experimental investigation carried out to evaluate the influence of bacteria Sporosarcina pasteurii with different cell concentrations ($10^{4,}$ 10^5,10^6, 10^7cells per ml) on the performance enhancement of light weight concrete developed using bacterially treated fly ash aggregate.The composite aggregates were prepared using cold bond technique in certain proportions of predominantly fly ash to cement using alkaline activators.The present study also investigates the effects of biotic factors such as growth medium, bacterial concentration and different buffers concerning the preparation of bacterial suspensions on the compressive strength of cement mortar.Microbiologically induced calcite precipitation (MICP)by Sporosarcina Pasteurii will plug the pores resulting in improved strength and durability properties of concrete. The influence of growth medium on calcification efficiency of S. pasteurii was insignificant. Substantial enhancement in the compressive as well as the tensile strength of concrete was observed. Permeation properties of light weight concrete have shown more pronounced decrease when treated with bacteria.

Keywords: Bacterial concrete, Durability, Fly ash aggregates, MICP, Permeation properties.

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INTRODUCTION

Microbially Induced Calcium Precipitation (MICP) is a process through which pores in the concrete can be sealed effectively using calcium carbonate crystals produced by microbial activity of the bacteria induced into the concrete during mixing stage.In this paper the effect of various biotic and abiotic elements on the process of bacterial activity in enhancing mechanical and durability properties are studied to understand the role of bacteria in improving the performance of concrete. Biotic elementconsidered is the bacterial cells and abiotic elements are nutrient broth (food for bacterial growth while incubation), calcium carbonate (calcium source added while incubation) and calcium lactate (as long term calcium supplement) for bio-mineral formation. The bacteria used in the present study is “Sporosarcina pasteurii” which belongs to class bacilli. Being alkaliphilic in nature it has an ability to survive in dormant state in concrete environment. It gets activated inside the cement-sand matrixto precipitate calcium carbonate crystals within the cracks or pores. In the present study light weight bacterially treated fly ash coarse aggregate is used to develop bacterial concrete of M20 grade.

Erhan Gunuyisi et al. [1] prepared artificial coarse aggregates by adding fly ash and cement as binderin pelletization process and the surface of fly ash concrete is treated with water glass and cement- silica slurry to improve surface texture, density, water absorption, and crushing strength. The authors also found that, artificial aggregates prepared with fly ash and cement in the ratio of 90:10 in the pelletization process pellets through coagulation process are treated with Na_2SiO_3 to have more strength and low water absorption value and specific gravity value. Past research reported that light weight concrete made with fly ash as coarse aggregate is 23% lighter than conventional concrete. In the present study artificial fly ash aggregate are prepared using cold bond technique.

LIGHT WEIGHT FLY ASH AGGREGATE

In the present study, Class ‘F’ fly ash is used along with alkaline activator solutions NaOH (optimum molarity) and Na_2SiO_3 for casting of mortar cubes of 70.6 mm x 70.6 mm x 70.6 mmsize and tested for 28 days compressive strength. The optimum molar NaOH and Na_2SiO_3proportion that yields the maximum strength when cured in oven for 24 hrs at 60°C after rest period of 24 hrs (Geo-polymerization), is utilized to make the fly ash aggregate based bacterial concrete. Alkaline activators are mixed before 30 minutes of concrete making. Geo-polymerization process involves reaction between alumina-silicate oxides under alkaline conditions yielding polymetric Si-O-Al-O bonds. The normal consistency is found to be35% for samples prepared with fly ash and cement in the ratio of 90:10, with various molarities of 8M, 10M, 12M, 14M and 16MNaOH and $Na_2SiO_3$with SiO_2/Na_2O ratio=2. The Na_2SiO_3/NaOH ratio is taken as 2.5 based on previous literature.Various mortar cubes are prepared with the above ingredients and found that14M NaOH is the optimum molar at which maximum strength is achieved.

To prepare fly ash aggregate, its dry mix quantities are calculates as shown below-
For 1 m^3 volume of paste mix, required quantities for the proportion of fly ash to cement as 90:10 with Na_2SiO_3 + NaOH to binder ratioas 0.35.

Specific gravity of fly ash and cement are 2.13 and 3.15. Density of water is 1 g/cm^3

1. Weight of fly ash = 0.90 x 2.13 g/cm^3x 10^3= 1917 kg/m^3
2. Weight of cement = 0.10 x 3.15 g/cm^3x 10^3= 315 kg/m^3

Total Binder = 1917+315 = 2232 kg/m^3

Alkaline activator solution = 0.35 x 2232= 781.2 kg/m^3

Na_2SiO_3/ NaOH = 2.5

i.e. Na_2SiO_3/ NaOH = 5/2

Na_2SiO_3 solution = 781.2 x 5/7 = 558 kg/m^3

NaOH solution = 781.2 x 2/7 = 223.2 kg/m^3

Calculation of NaOH flakes and water required for m^3-

Molecular weight of NaOH = 40 gm/mol. So for optimum 14M NaOH, flakes required are 14x40=560 gm for 1000ml of water (Solubility in water is 1110 gm/1000mlat 20°C)

So to prepare 1000ml of NaOH solution dissolve 560 gm of NaOH flakes in 1000ml of water.

NaOH flakes/ Water = 560/1000 = 0.56

Sodium hydroxide solution = 0.56 x water + water = 1.56 xWeight of Water.

223.2 = 1.56 xWeight of Water

Therefore, weight of water required is 143 kg/m^3 to dissolve NaOH flakes of 80.2 kg/m^3

So quantity of NaOH = 80.2 kg/m^3

Quantity of Na_2SiO_3 = 80.2x2.5 = 200.5 kg/m^3

For 1 m^3 volume of drymix, required quantities for the proportion of fly ash to cement is 90:10 is as follows:

Table 1 Quantities for 1 m^3 dry mix of Paste

MATERIAL	QUANTITY (KG/M^3)	PROPORTIONS
Flyash	1917	6.08
Cement	315	1
NaOH flakes	80.2	0.25
Na_2SiO_3	200.5	0.64

For the dry mix of fly ash and cement in the ratio of 90:10 as per weightand add alkaline activator solution and makepaste. Now that paste is transferred to tray and compacted to required thickness of aggregate. Cut the compacted paste into required square size pellets and place in oven for 24 hrs at 60°C. After 24 hrs the hardened aggregates are kept in the mixer and rotate for 3-5 minutes then we obtained cubically angular shaped aggregates.Due to its high water absorption capacity of fly ash aggregates, these aggregates are treated with bacterial solution before using in the concrete.

In the present method of preparation of fly ash aggregates, cubical with angular shaped aggregates were obtained using cold bond technique but if pelletizing process is adopted, rounded aggregates will be obtained. Thesefly ash aggregates are tested for its suitability by conducting tests as per appropriate IS: 2386.

Table 2 Properties of fly ash aggregates

TEST CONDUCTED	FLY ASH AGGREGATES	NATURAL AGGREGATES	PERMISSIBLE LIMITS		PROPERTY OF AGGREGATE
			FOR ROAD PAVEMENTS	FOR OTHER STRUCTURES	
Aggregate crushing value (IS 2386- Part 4)	35.23%	27.51%	≤30%	≤45%	Crushing strength
Aggregate impact value (IS 2386- Part 4)	37.14%	27.94%	≤30%	≤45%	Toughness
Aggregate abrasion value (IS 2386- Part 5)	30%	26%	≤30%	≤50%	Wear and Tear
Specific gravity (IS 2386- Part 3)	1.84	2.55	2.5 to 2.9	2.5 to 2.9	Specific gravity
Water absorption (IS 2386- Part 3)	17.84%	0.6%	0.5 to 1%	1.0 to 2.0%	Porosity

MICROBIOLOGICALLY INDUCED CALCIUM CARBONATE PRECIPITATION (MICP)

Microbiologically induced calcium carbonate precipitation (MICP) is a bio-chemical process that induces calcium carbonate precipitation within the soil matrix. In this process bio-minerals are formed through reaction of metabolic activities by microorganisms with the surrounding environment. Microbially induced carbonate precipitation is a natural process through which calcium carbonate minerals are formed from calcium and carbonate ions. By incorporating live bacteria into concrete, MICP has found to improve properties of concrete. Several mechanisms have been identified by which bacteria can induce the calcium carbonate precipitation, including urea hydrolysis, de-nitrification, sulphate production, and iron reduction. Calcium carbonate is one of the most suitable fillers for concrete due to high compatibility with cementitious compositions. This study enables us to understand the factors responsible for enhancement of strength properties in bacteria incorporated concrete. Bacteria precipitate minerals in the concrete during its metabolic activity by consuming nutrients and calcium supplements. Nutrients are used to culture bacteria into colonies or cells and calcium supplement is used to grow. During growth of bacteria calcium sources are consumed and calcite crystals are precipitated as a by-product of bio-mineralization.

OBJECTIVES OF THE PRESENT STUDY

The aim of the study is to understand the performance of ordinary (M20) bacteria incorporated concrete made with light weight fly ash based aggregate. The study is planned into two phases, they are -

1. Design and development of light weight fly ash aggregate
2. Development of fly ash based bacterial concrete and assess its performance.

Phase 1 is organized into 2 stages

Stage 1: Determining the dry mix quantities.
Stage 2: Preparation of fly ash aggregates using cold bond technique

Phase 2 can be organized into following stages-

Stage 1: Culture and growth of Bacteria
Stage 2: Effect of Bacteria and its nutrients in cement mortar specimens
Stage 3: Optimum cell concentration of bacterial cells
Stage 4: Strength studies
Stage 5: Water absorption and porosity studies

MATERIALS

Cement
Ordinary Portland cement of 53 Grade is used and tested for properties as per IS4031-1988.
Fine Aggregate
The locally existing river sand is used as fine aggregate in the current study.
Coarse Aggregate
Both artificial prepared bacteria treated fly ash aggregate and natural aggregate obtained crushed angular stone from local quarry are used as coarse aggregate
Water
Potable water has been used for casting normal concrete and for bacterial concrete cubes potable water is replaced by bacterial water prepared in laboratory.
Bacteria (Sporosarcina pasteurii)
Sporosarcina pasteurii, a laboratory cultured bacterium is used in this investigation. It is a gram-positive bacterium, with rod shaped cells. Pure culture of bacteria was obtained from NCIM-Pune, National Chemical Laboratory, in a slant cultured test tube, with nutrient agar.
Nutrient Broth
Nutrient broth consists of Beef extract, Yeast extract, Peptone and Sodium chloride (NaCl)
Calcium carbonate ($CaCO_3$)

It acts as short-term calcium supplement for bacterial growth and crack healing. This supplement is added during culturing which acts as food for bacteria.

Calcium lactate ($C_6H_{10}CaO_6$)

Based on past research, Calcium lactate of 5% weight of cement is added to mortar to provide long term calcium supplement for bacterial activity.

Figure 1 Slant culture of Sporosarcina pasteurii

Growth and Culture of Bacteria

Thirteen grams of nutrient broth is added to 1 litre of distilled water and stirred until a uniform mix is obtained. This solution (in properly plugged flasks) is kept in BOD incubator for 48 hours at a temperature of 37 degrees. Two types of solutions were made in this study:

i. Bacterial solution without calcium supplement

ii. Bacterial solution with calcium supplement (where 5 grams of CaCo3 is added along with nutrient broth)

Confirmation for bacterial growth is done if after incubation period the solution becomes turbid as shown below.

Figure 2 Solution without $CaCO_3$

Concrete Mix Proportions

The mix proportions for M20 grade concrete is designed using IS: 10262-2009.The mix proportions and materials required for one cubic meter of concrete for M20 is given below.

Mix proportion 1: 2.27: 3.45: 0.54

Cement: 320.4 kg

Fine aggregate: 727.3 kg

Coarse aggregate: 1105.4 kg
Water: 173 L

Two types of concrete mixes of M20 grade i.e. one without bacteria (control samples) and one with bacteria (bacterial specimens) are prepared in the current work. The concrete mixes have similar proportions of sand, coarse aggregates, cement and water. However, the major difference was in the water constituent used. Normal tap water is used for conventional concrete specimens without bacteria while "S pasteurii" suspended water is used for specimens with bacteria. In order to obtain a suspension of bacteria, "S pasteurii" bacteria are grown in micronutrient distilled water under sterilized conditions in biotechnology laboratory. The culture of bacteria in the suspension was added to concrete during the concrete mixing process.

METHODOLOGY

Culture and Growth of Bacteria Sporosarcina pasteurii

Bacteria used in this study are "Sporosarcina pasteurii" which was previously known as Bacillus pasteuriithat has a capability to precipitate calcite given a calcium supply through the process known as MICP (microbiologically induced calcite precipitate). Bacteria to culture is obtained from NCIM Pune National Chemical Laboratory, in a slant cultured test tube and maintained constantly on nutrient agar slants in the laboratory. To grow bacteria, a particular colony of the culture is inoculated or introduced into nutrient broth of 25 ml in a 100 ml conical flask and the growth environment are maintained at 37°C temperature and placed in 125 rpm orbital shaker. Then obtained bacterial water is used in concrete at mixing phase to study its effect.

Effect of Bacteria and its nutrients on cement mortar specimens

To determine the role of bacterial action in the performance improvement of bacteria induced cementitious material, six different types of cement mortar samples of size 70.6 mmx70.6mm x70.6mm are casted.

1. Type-1 cement mortar specimens are prepared with normal mixing water.
2. Type-2 cement mortar specimens are prepared with bacterial solution containing only nutrient broth to ascertain the consequence of bacterial activity.
3. Type-3 cement mortar specimens are prepared with bacterial solution containing nutrient broth and supplement calcium carbonate during incubation stage. Calcium carbonate supplied during this stage will help bacteria to grow and precipitate calcite minerals during the initial hydration period of cement mortar.
4. Type-4 cement mortar specimens are prepared with bacterial solution that contains nutrient broth and calcium carbonate in suspension and in addition calcium lactate is supplied in powder form during mixing. Calcium lactate will be used as food source for bacterial activity so that mineral precipitation can occur during later periods of hydration.
5. Type-5 cement mortar specimens are made with nutrient broth solution alone (no bacterial cells) to ascertain the effect of nutrient broth solution on properties of cement mortar.

6. Type-6 cement mortar specimens are made with water and calcium lactate (no bacterial cells). Calcium lactate is added in residue form to cement to last the bacterial activity for extended periods of hydration.

Optimum cell concentrations of bacterial cells

This study was mainly carried out to understand the effect of the concentration of bacterial cells on the quantity of calcium carbonate precipitation. Additional bacteria with sufficient nutrients will release extra calcite in the laboratory conditions, but in the cement-sand environment the precipitation of calcite from bacteria depends on its compatibility with the matrix of cement sand. Also bacteria introduced into the cement-sand should not influence physicochemical properties of cement-sand. The appropriate concentration of bacterial cells for maximum calcite crystals precipitation can be evaluated by determining the 28-day compressive strength of cement mortar samples. Standard cement-mortar cubes of size (70.6mm x 70.6mm x 70.6mm) are incorporated with soil bacteria "Sporosarcina pasteurii"of various cell concentrations were cast, and cured for 28 days then tested to study the compressive strength. Various bacterial cell concentrations such as10^3, 10^4, 10^5, 10^6 and10^7 cells/ml of mixing water are used to prepare mortar cubes. Control specimens (without bacteria) are also casted for comparison. The mortarsample whose compressive strength is highest at 28 days determines the optimal cell concentration to be used for further studies.

Compressive strength and split tensile strength

The study is carried out to assess the compressive and split tensile strength of cement concrete. Cubeswith and without bacteria of optimum concentrationof 100mm x100mmx100mm sizewere cast and tested to study the compressive strength at 3, 7 and 28 days.Concrete cylinders of size 100 mm x 300 mm are also casted and tested for split tensile strength at 28 days.

Water absorption and porosity studies

To study water absorption and porosity of concrete specimens incorporated with bacteria, mortar samples of size 100mm x100mmx100mm were casted and cured for 28 days for testingas per ASTM C642 to evaluate the water absorption capability and porosity of bacterial concrete specimens

$$\text{Water Absorption Capacity (WAC)} = [(M_b - M_a)/ M_a] \times 100$$

Where: M_a= mass of oven-dried sample in air, kg; M_b= mass of surface-dry sample in air after immersion, kg;

Porosity 'P' or percentage of interconnected pore space was calculated using the formula

$$\text{Total porosity} = (V_v/V) = (M_b - M_a) / \rho_w V$$

Where, V_v = volume of voids in cc= M_b - $M_a$in grams; V = total volume of specimen in cc = 7.06 x 7.06 x7.06 cm^3 where $\rho_w$the unit mass of water = 1 g/cc

TEST RESULTS AND DISCUSSION

Results of experimental investigations are presented in tables below -

Optimum cell concentration of bacterial cells

For microbial cell concentration of 10^5 cells per milliliter of water, 28 days of compressive strength of bacteria incorporated cement mortar matrix is highest as shown in Figure 3. This improvement in the compressive strength is primarily due to the metabolic precipitation of $CaCO_3$ in cavities or pores in the cement-sand matrix modifying the pore structure of the bacteria. It is understood that for cement mortar matrix specimens made with S pasteurii cell concentration of 10^7 cells per ml the compressive strength has decreased drastically. Maximum strength was observed at 10^5 cells / ml of water hence it is considered as an optimal cell concentration for further investigation to investigate the effects of bacteria on concrete properties.

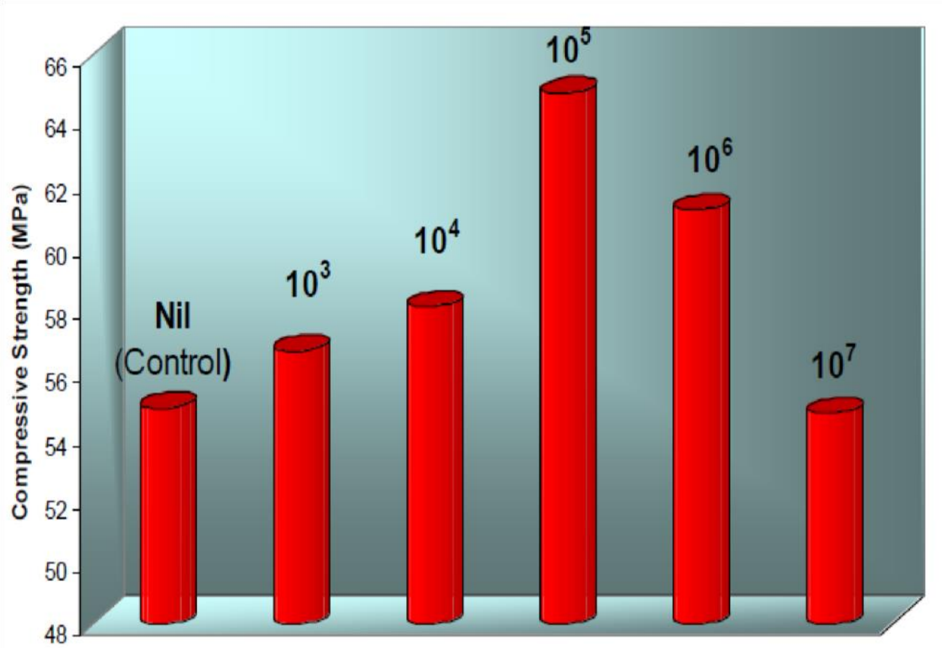

Figure 3 Effect of bacteria cell concentration on compressive strength

Effect of biotic and abiotic factors on the compressive strength of cementitious materials

Compressive strengths of various types of cement mortar specimens are assessed todetermine the role of bacteria and nutrients action in the strength improvement of cementitious materials.

Table 1 Compressive strength of various types of cement mortar specimens

SAMPLE COMPOSITION	COMPRESSIVE STRENGTH (MPa)			
	3 DAYS	7 DAYS	28 DAYS	% INCREASE
Type-1 Cement mortar specimens prepared with normal mixing water.	27.2	38.2	54.3	-
Type -2 Cement mortar specimens prepared with bacterial solution containing only nutrient broth	30.1	40.8	56.2	3.5
Type -3 Cement mortar specimens prepared with bacterial solution containing nutrient broth and	34.3	44.8	65.3	20.3

supplement calcium carbonate				
Type -4 Cement mortar specimens prepared with bacterial solution containing nutrient broth and calcium carbonate in suspension and in addition calcium lactate is added in powder form during mixing	35.9	45.8	68.4	25.9
Type -5 Cement mortar specimens made with nutrient broth solution alone (no bacterial cells)	27.6	38.9	55.1	1.5
Type -6 Cement mortar specimens made with water and calcium lactate (no bacterial cells)	32.4	44.2	60.3	11.1

Studies on compressive and split tensile strength

The results of the compressive and split-tensile strength for ordinary grade M20 normal and bacteria induced concrete at 28 daysare given in Table 2

Table 2 Effect of the S pasteurii bacteria on compressive and split-tensile strength

TYPE OF CONCRETE	COMPRESSIVE STRENGTH (MPa)	SPLIT-TENSILE STRENGTH (MPa)
M20 Conventional	28.18	3.26
M20 Bacterial	32.74	3.73
% Increase	16.18	14.42

It is seen that in M20 grade concrete, with the addition of bacteria, the compressive and split-tensile strength of concrete showed a significant increase of 16.18 % and 14.42 % respectivelydue to persistent calcium carbonate mineral precipitation by Bacillus pasteurii that fills the pores in the concrete, making the concrete structure denserby modifying concrete microstructure.

Water Absorption Studies

The water absorption capacity test is to determine the amount of water absorbed relating to the porosity. Absorption is the ability of samples to retain water, while the capillary rise is the degree to which the water fills.

Table 3 Water Absorption at different time intervals of M20 grade Controlled and Bacteria incorporated concretes

MEASUREMENT INTERVALS T_I (MIN)	CONTROLLED CONCRETE		BACTERIAL CONCRETE	
	m_o= 2.49 kg		m_o= 2.51 kg	
	m_i(kg)	M_i(%)	m_i(kg)	M_i(%)
0	2.49	0.00	2.51	0.00
15	2.51	0.80	2.52	0.40
30	2.59	4.02	2.54	1.20
60	2.60	4.42	2.56	1.99
90	2.61	4.82	2.58	2.79
180	2.62	5.22	2.58	2.79

480	2.62	5.22	2.58	2.79
1440	2.63	5.62	2.58	2.79
2880	2.63	5.62	2.58	2.79

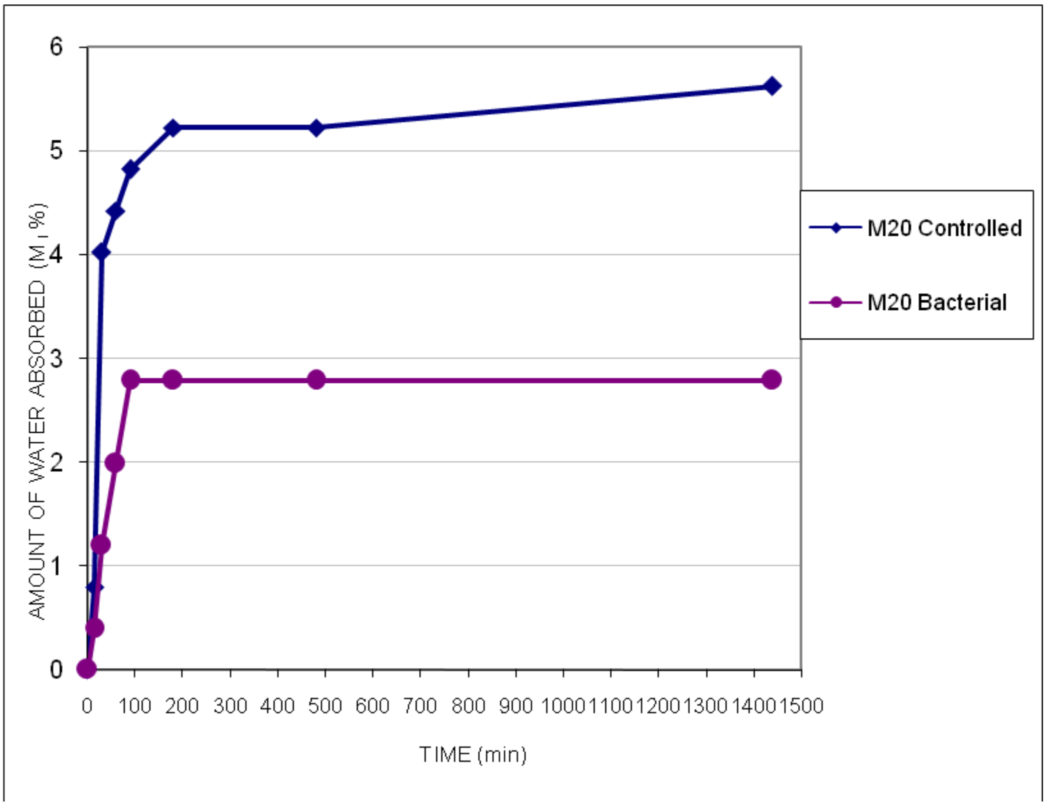

Figure 4 Plot showing amount of water absorption with time for M20 Grade Controlled and bacterial specimens

Table 4 Durability Classification as per ASTM C 642

DURABILITY CLASSIFICATION	WATER ABSORPTION CAPACITY (% BY WEIGHT)
Excellent	<5
Good	5-6
Normal	6-7
Marginal	7-8
Bad	>8

Table 5 Water Absorption Capacity (WAC) and Porosity of M20 grade controlled and bacteria incorporated concrete

	CONTROLLED CONCRETE	BACTERIA INCORPORATED CONCRETE
M_a	2.49	2.51
M_b	2.63	2.58

Water Absorption Capacity (WAC) (%)	5.62	2.79
Porosity, P	0.14	0.04
Decrease in Porosity	-	72%

CONCLUSIONS

Following are the conclusions drawn from the present work:

1. Fly ash added with cement in the ratio of 90:10 with 14M $NaOH+Na_2SiO_3$/ binder ratio 0.35 gave the maximum compressive strength for mortar cubes.
2. The fly ash aggregates prepared with the above proportion of paste will have aggregate properties within the permissible limits as per IS specification. Based on the obtained results, flyash aggregates can be used as light weight non-structural elements as well as sub-base course in the pavements.
3. Adding the S pasteurii bacteria improves the strength of cement mortar. The cement mortar cube specimens which contained cell concentration of 10^5 bacterial cells/ml of mixing water were found to attain higher compressive strength compared to the controlled model. The mineral precipitation in cement mortar reduces the connection of the concrete pore structure by reducing the size of pore size (pores refinement) which is directly related to durability.
4. In M20 grade, concrete induced with bacteria has increased compressive strength and split tensile strength by16.18% and14.42% respectively at 28 days due to continuous calcium carbonate mineral precipitation by S Pasteurii.
5. The water absorption capacity (WAC) of bacteria incorporated in concrete samples is reduced by 50 % for M20 grade compared to WAC of controlled concrete specimens, and the reduction of concrete porosity is nearly 35% with incorporation of bacteria into concrete.

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