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Synergistic Effects of CTP and *Bacillus megaterium* on Concrete Properties

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Abstract

Concrete deterioration is primarily caused by shrinkage, temperature changes, and reinforcement corrosion, which lead to crack formation. Continuous external loading can cause cracks to widen and form a network of cracks, enabling the infiltration of harmful gases, liquids and moisture, which weakens the strength and in turn the durability of concrete. Consequently, implementing measures to prevent degradation and to enhance the longevity by increasing durability of concrete is crucial. Continuous research efforts all around the globe have focused on enhancing concrete strength and durability using high-quality materials, additives including Supplementary Cementitious Materials (SCMs), and fibers. Ceramic Tiles Powder (CTP), fly ash, silica fume, and other materials have gained attention as SCMs, with CTP showing potential due to its high silicate and aluminum oxide content. The use of *Bacillus megaterium* in CTP-embedded concrete has scarcely been studied, prompting this research to explore its feasibility. This present research work investigates the strength and durability of M30 grade concrete with use of *Bacillus megaterium* at a concentration of 10⁵ cells/ml and with varying cement replacement percentages (5%, 10%, 15%, and 20%) using CTP. The test results indicate about 25% improvement in compressive strength of concrete with 10% cement replacement using CTP and *Bacillus megaterium* of concentration 10⁵ cells/ml. Petrographic analysis showed better surface composition, while SEM (Scanning Electron Microscope) images revealed increased calcite precipitation and reduced voids.

Keywords: Bacterial Concrete, *Bacillus megaterium*, Biomineralization, Ceramic Tiles Powder, Durable Concrete.

Introduction

Concrete is a widely used construction material, and its demand continues to grow at an annual rate of approximately 2.5% (1). The development of cracks caused by various factors increases the deterioration rate and hence hampers strength and durability to a great extent. Hence, it is imperative to develop techniques to reduce crack development and/or repair the cracks that have developed. Past research efforts have shown the successful implementation of bacteria to heal the developed cracks through calcite formation. Calcite crystals begin to precipitate when the surrounding solution reaches a certain level of supersaturation, with bacterial cell walls serving as nucleation sites. Further densification of calcite crystals fills the concrete's microcracks and voids, resulting in improved strength and durability. This process is known as Microbiologically Induced Mineral Precipitation (MIMP). Bacterial spores are dormant and cannot germinate or reproduce immediately in fresh concrete, but they can become active once the environment becomes

suitable.

Besides this, continuous research efforts are being employed to use SCMs. The presence of high silicate and aluminium oxide in CTP makes it a proper candidate to be employed as SCMs. The powder derived from ceramic tile waste contains reactive pozzolanic materials with a high silica content, which reacts with calcium hydroxide (Ca(OH)₂) to form additional calcium silicate hydrate (C-S-H) gel. Replacing cement with CTP utilizes tile waste, reduces cement usage, and enhances concrete strength and durability.

Extensive research confirms that cement production generates significant greenhouse gas emissions. Hence, continuous research efforts are being put into obtaining quality concrete with reduced cement content using SCMs. At the same time, it is equally important to achieve long-term performance from concrete, especially durability. Periodic maintenance/repair of concrete structures can sometimes be costly and timeconsuming. Hence, continuous research efforts are

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being carried out regarding the self-healing of concrete. The following sections outline the research efforts carried out in the selected domain of self-healing concrete and the use of SCMs as a partial replacement of cement in concrete.

Bacterial Concrete

Numerous experimental research efforts undertaken in the past century have enhanced the

quality of concrete because of the presence of bacteria. Figure 1 provides details of the microbes that researchers have used to improve the quality of concrete. In these research efforts, the optimal bacteria dosage was found to be between 10^5 cells/ml to 10^7 cells/ml. The extent of improvement in the quality of the concrete may vary based on the concentration of the bacteria.



Figure 1: Percentage of Usage of Microbes in Concrete (2)

The process and metabolic reactions responsible for calcite deposition in concrete can be found in previous research work (3). Application of various *Bacillus megaterium* groups of bacteria with specific proposed bacteria dosages of concentration has shown an increase in compressive strength of concrete in the range of 10% to 55% (4-16). The experimental results obtained by past researchers demonstrate that adding S. pasteurii or Bacillus pasteurii type bacteria in different dosages results in 10% to 30% improvement in compressive strength (17-28). Applications of Bacillus Halodurans, Bacillus Cohnil, Thermoanaerobacter, E. coli K12, etc., resulting in improvements in concrete properties, can be found in past research efforts (29-35).

Suitability of Bacteria for Concrete Applications

Bacterial characteristics are crucial for its use in the concrete. The bacteria must be strong enough to survive in extreme heat, pressure, and moisture conditions. Bacteria must be endospore-forming and withstand the high pH of concrete. Additionally, they must be grampositive, as gram-positive bacteria only produce endospores. Also, they must exhibit high urease activity to facilitate calcite formation. Most of the bacteria listed in Figure 1 possess these properties.

Ceramic Tiles Powder (CTP)

The ceramic tile manufacturing process requires high temperatures, typically achieved by burning various fuels, which significantly contribute to the carbon footprint. Also, the ceramic tile industry produces ceramic tile waste of about 2.5% of the total production capacity (2). Disposing ceramic tile waste on the open land is another critical environmental issue. From the EDS spectrum of the CTP presented in Figure 2 and the elemental composition shown in Table 1, it is evident that the CTP serves as a pozzolanic material, composed primarily of siliceous and aluminous compounds. CTP reacts with Ca(OH)₂ in presence of water to produce cementitious compounds. Hence, CTP can be a potential candidate for use in concrete as a material with partial cement replacement. Dedicated work in improving concrete properties using CTP can be found in research efforts of the past few decades (36).



Figure 2: EDS Spectrum for CTP Sample

Fable 1: Elemental Composition of C	TP Sample for Concrete	Application
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Element	Weight (%)	Atomic (%)
0 K	50.06	63.68
Na K	2.37	2.10
Mg K	0.69	0.58
Al K	8.92	6.73
Si K	35.51	25.73
S K	0.27	0.17
Ca K	1.48	0.75
Fe K	0.68	0.25

Materials and Methodology

A gap in research efforts in the existing literature volume in combining the use of SCMs, particularly CTP, and imparting self-healing properties using bacteria lead to the present research effort. The combination of Bacillus megaterium bacteria and Ceramic Tile Powder (CTP) has been chosen considering their complementary roles in enhancing concrete properties. Bacillus megaterium, known for its urease-producing ability that facilitates microbial induced calcite precipitation, which can improve durability and strength of concrete by filling voids in the concrete matrix. On the other hand, CTP, as a supplementary cementitious material, contributes to the concrete's strength through pozzolanic reactions. By combining these two additions, it is hypothesized that the microbial action of Bacillus megaterium will enhance the benefits of CTP, leading to a synergistic effect on the compressive strength and long-term durability of the concrete. Previous research efforts have highlighted the benefits of using bacterial agents like *Bacillus megaterium* and the effects of supplementary cementitious materials (other than CTP) on concrete performance (15, 17, 28, 31, 33). This research proposes the optimal percentage of cement replacement with CTP for the ideal dosage of the *Bacillus megaterium* strain MTCC 10086. The effects of incorporating bacteria and CTP into concrete are analyzed by comparing the compressive and tensile strengths of CTPembedded bacterial concrete with those of the control M30 grade concrete mix. The following subsections outline the properties of various ingredients used to produce CTP-embedded bacterial concrete.

Morphology of CTP Particles

The morphology and surface characteristics of the CTP particles were analyzed using the Energy Dispersive X-ray Spectroscopy (EDS) spectrum. Figure 2 shows the EDS Spectrum for the CTP sample. Table 1 presents the elemental composition of the CTP sample for concrete use, along with their respective percentages.

Microbial Culture Collections

In this present experimental investigation, *Bacillus megaterium* strain MTCC 10086 procured from MTCC-Chandigarh, India, is employed. The N-broth liquid medium is used to culture and promote the growth of bacteria. The growth media is prepared by combining 28 gm of N-broth with 1 litre of distilled water with heat to dissolve N-broth entirely in water. The medium is sterilized in an autoclave at 121° Celsius with 15 pounds of pressure for 15 minutes, refer to Figure 3. After sterilization, the medium is cooled and distributed into different containers, while maintaining the prescribed temperature. The cultural media are now ready for deployment.

Properties of Concrete Ingredient

Following the guidelines of BIS:10262 (2019) concrete mix design is carried out to obtain M30

grade concrete (37). Fresh and defect-free Ordinary Portland Cement (OPC) from Ultratech is used in all concrete mixes. Cement was tested to verify BIS:12269 (2013) code requirements (38). Following the BIS:383 (2016) guidelines, coarse aggregates are mixed in a ratio of 30% for 20 mm size and 70% for 10 mm size, based on the total aggregate weight (39). The properties of coarse aggregate and fine aggregate are mentioned in Table 2.

The water quality used for concrete mixing is assessed according to the guidelines outlined in BIS:456 (2000) (40). Ceramic tile waste is obtained from the various ceramic industries near Morbi City. The collected waste is then crushed into a fine powder with a particle size of less than 90 micros under controlled conditions at YOR Laboratory, Rajkot. Physicochemical characteristics of cement and CTP obtained following the ASTM-C1240 (41) and ASTM-C618 12a (42) are shown in Table 3.



Figure 3: Sterilization of Medium in Autoclave

Table 2: Prop	perties of	Aggregates
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	Fine Aggregates	Coarse Aggregates
Sp. Gravity	2.52	2.71
Zone	II	_
Water Absorption (%)	1.03	0.48

Table 3: Physicochemical Characteristics of Cement and CTP

	Cement	СТР
Colour	Gray	Creamy white
Sp. Gravity	3.15	1.217

Moisture content		0.19
Water absorption		19
Fineness	90 microns	75 microns
Chemical Composition by % of Mass		
SiO ₂	23.29	65.04
CaO	61.87	0.66
Al ₂ O ₃	6.14	22.56
Fe ₂ O ₃	4.49	2.12
MgO	4.51	2.88
SO ₃	3.13	0.10
$K_20 + Na_20$	1.04	-

Concrete Specimens

Concrete cube specimens of 150 mm size for the compression test were prepared and tested following the procedures outlined in BIS: 516 (1959) (43), while concrete cylindrical specimens of 150 mm diameter and 300 mm height for the split tensile test were prepared and tested according to the guidelines specified in BIS: 3085 (1965) (44). As part of the current experimental investigation, six distinct M30-grade concrete mixes are considered. The first series comprises a control mix (M1), 0% CTP and 0% bacteria. In all other bacterial concrete mixes, 10% of the water is substituted with *B. megaterium* with cell

Table 4: Ingredient Quantities for Concrete Mixes

BC1 to BC5 represent bacterial concrete mixes with 0% to 20% cement replacement by CTP, in 5% intervals. Water-Cement ratio of 0.48 is used for each concrete mix. The quantity of concrete ingredients in each concrete mix is shown in Table 4. Each concrete mix containing three specimens is cured in portable water at 27º ± 2º Celsius for a predetermined time as specified in BIS:516 (1959) (43) and subsequently tested to determine the compressive and tensile strengths. The compressive and tensile strength of the concrete for each mix are represented by the average values obtained from three cubes and three cylinders, respectively.

concentration of 10⁵ cells/ml each. The notations

Concrete Mix	Cement (kg/m ³)	CTP (kg/m³)	Coarse Agg. (kg/m³)	Fine Agg. (kg/m³)	Water (kg/m³)	Bacteria (10 ⁵ cells/ml) in ltr/m ³
M1	387.50	(0%) 00.00	1222.70	681.61	199.03	00.00
BC1	387.50	(0%) 00.00	1222.70	681.61	179.13	19.90
BC2	368.13	(5%) 19.37	1222.70	681.61	179.13	19.90
BC3	348.76	(10%) 38.75	1222.70	681.61	179.13	19.90
BC4	329.39	(15%) 58.12	1222.70	681.61	179.13	19.90
BC5	310.02	(20%) 77.50	1222.70	681.61	179.13	19.90

Results and Discussion

This section presents a detailed discussion of the results from compressive strength and tensile strength tests, along with the microstructural examination using SEM and EDS techniques. Figure 4 shows the compressive strength of each concrete mix at different specimen ages. A comparison between mix BC1 and the control mix M1 clearly demonstrates that the compressive strength of the concrete increases with the addition of bacteria, continuing to improve with age, reaching a maximum enhancement of 7.3% at 56 days. The increase in compressive strength can be attributed to the additional deposition of calcium carbonate

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(CaCO₃) within the concrete matrix pores, produced by *Bacillus megaterium*. The urease enzyme produced by the bacteria promotes calcium carbonate mineralization by raising the pH through ammonia production. This, in turn, facilitates the formation of carbonate ions, which combine with calcium to form CaCO₃. This process is a fundamental aspect of microbial-induced calcite precipitation. Similar observations are found in other research works (8, 11, 18). The compressive strength is observed to increase further with the partial replacement of cement by CTP. Similar to other supplementary cementitious materials (SCMs) like fly ash, CTP enhances the compressive strength of concrete by promoting the hydration process through pozzolanic reactions and filling voids within the mix. The maximum increase in strength at 7 days, 28 days, and 56 days age is 22.9%, 24.57%, and 25.58%, respectively, achieved for 10% cement replacement with CTP, i.e. concrete mix BC3.



Figure 5: Tensile Strength of Concrete Specimens

A comparable trend of strength improvement is also observed in the tensile strength of the concrete mixes. It can be seen from Figure 5 that the increase in tensile strength of concrete specimens with addition of bacteria and CTP is highest at an early age of 7 days for all mixes. Similar to compressive strength results, the maximum improvement in tensile strength is achieved for concrete mix having 10% cement replacement with CTP. The tensile strength improvement relative to the control mix (M1) is 23.39%, 23.38%, and 20.36% at concrete ages of 7, 28, and 56 days, respectively, for concrete mix BC3, which contains a 10% cement replacement with CTP. The increase in both compressive and tensile strength of concrete mix BC3 is attributed to pozzolanic reactions and the enhanced microstructure resulting from the pore-filling

ability of CTP, with 10% cement replacement being Interestingly, unlike optimal. compressive strength behaviour, the improvement in tensile strength diminishes at later ages, precisely at 56 days, compared to the values observed at 7 and 28 days across all specimens. Also, the tensile and compressive strength results showed minimal or no further improvement in both compressive and tensile strengths at 56 days compared to 28 days. This suggests that the beneficial effects of the bacterial agent on concrete strength are most pronounced during the early stages of curing, with little additional enhancement or no enhancement observed over time, likely due to the stabilization of microbial activity and the completion of the cement hydration process.

For SEM and EDS analysis, samples were collected from at least three different locations of each

tested concrete specimen at 28 and 56 days of age. A consistent trend was observed across all samples from the tested specimens, and therefore, representative results from the SEM and EDS analysis of different concrete mixes are included in this paper. Figure 6 shows the 10µm size SEM image of control concrete specimen (M1), the concrete specimen prepared by 10% replacement of cement with CTP (BC1), and the bacterial concrete specimen prepared by 10% replacement of cement with CTP (BC3). It is clearly observed that in concrete mix BC1, the voids in the concrete matrix are filled with crystalline calcite deposits resulting from microbial-induced precipitation, caused by the presence of Bacillus megaterium bacteria in the concrete matrix. The reduction of voids is further evident in the concrete specimen BC3, which demonstrates the influence of additional C-S-H gel produced and the pore-filling ability of CTP, as can be seen in Figure 6(c). Figure 7 displays the 5µm size SEM image of the representative sample obtained from failed concrete specimens tested at 56 days of age.

Elemental analysis or chemical characterization of materials can be performed using energydispersive X-ray spectroscopy, which relies on the interaction between an X-ray excitation source and the sample. The EDS spectrum displays the energy emitted by elements on the vertical axis, and the horizontal axis represents the atomic number of these elements. Analysis of the EDS spectra from two separate locations on the specimen reveals a consistent distribution of all elements. Hence, as stated before the representative results from the EDS analysis of different concrete mixes are included in this paper. Figure 8 illustrates the peaks found when EDX was performed on control concrete (M1) and bacterial concrete (BC3).

The calcite precipitation due to bacteria is an ongoing process within the concrete matrix. It can be observed from Figure 6 and Figure 7 that both the bacterial concrete specimens (BC3), prepared by replacing 10% of the cement with CTP, and BC1, prepared without cement replacement with CTP, exhibit a higher amount of calcite and fewer cavities compared to the control mix (M1). This observation is further supported by the EDS spectrum shown in Figure 8(D) for the 56-day specimen, where the intensity of the Ca peak is significantly higher than that seen for the 28-day specimen in Figure 8(C). The increase in the percentage of Ca, as shown in Table 5 for the 56day specimens compared to the 28-day specimens, indicates densification due to the precipitation of CaCO₃, which results from microbial-induced calcite precipitation.



Figure 6: 10μm size SEM Image at 28 days Age (A) Control Mix, M1 (B) Concrete Mix, BC1 (C) Concrete Mix, BC3



Figure 7: 5μm size SEM Image at 56 days Age (A) Control Mix, M1 (B) Concrete Mix, BC1 (C) Concrete Mix, BC3

Table 5: Elemental Composition of Concrete Mixes								
Element	Weight	Atomic	Weight	Atomic	Weight	Atomic	Weight	Atomic
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
	Concrete Mix (M1)		Concrete	crete Mix (M1) Concrete Mix		Mix (BC3)	BC3) Concrete Mix (BC3)	
	28 days age		56 da j	ys age	28 days age		56 days age	
СК	4.32	8.11	4	7.45	2.84	5.13	4.58	8.19
ОК	42.82	60.36	42.95	60.65	48.51	65.82	48.1	64.53
Al K	1.83	1.53	0.62	0.57	2.85	2.29	0.41	0.36
Si K	6.76	5.43	2.66	2.21	9.19	7.1	1.88	1.5
РК	0.13	0.09	9.71	7.74	0.12	0.08	6.27	4.79
S K	0.34	0.24	0.31	0.22	0.51	0.34	0.54	0.36
КК	0.31	0.18	0.67	0.39	0.29	0.16	0.11	0.06
Ca K	40.87	23	35.89	20.05	33.95	18.39	36.85	19.73
Fe K	2.62	1.06	3.19	1.28	1.75	0.68	1.26	0.49



Figure 8: EDS Spectrum for (A) Control Mix (M1) at 28 Days (B) Control Mix (M1) at 56 Days (C) Concrete Mix (BC3) at 28 Days (D) Concrete Mix (BC3) at 56 Days

Conclusions

In the present work, a parametric analysis is carried out to evaluate the impact of using CTP as a supplementary cementitious material (SCM) in conjunction with bacteria, specifically *Bacillus megaterium* strain MTCC 10086, at 10⁵ cells/ml. Various Concrete mixes with 5% to 20% cement replacement with CTP were designed following the guidelines stipulated in BIS 10262-2019 (37). Compared to the control mix specimens, the compressive and tensile strength improvements of about 25% are observed in CTP-embedded bacterial concrete specimens, BC3. The increase in strength of CTP-embedded bacterial concrete specimens of the densification of

voids due to the bacterial metabolic activity within the concrete mix as well as due to pozzolanic reactions and the enhanced microstructure, which results from the pore-filling capacity of CTP, with the optimal cement replacement being 10%. This phenomenon has also been observed in SEM images of concrete samples obtained from tested specimens. SEM images of the bacterial concrete specimen reveal more calcite precipitation and a lesser degree of void formation. The EDS spectrum verifies the elements contained in the concrete sample. The analysis of the test results from the present study suggests that CTP has the potential to serve as a sustainable building material, enhancing the overall quality of concrete and showing good compatibility with bacteria.

Abbreviations

Ca(OH)₂: Calcium Hydroxide, CSH: Calcium Silicate Hydrate, CTP: Ceramic Tiles Powder, EDS: Energy Dispersive Spectroscopy, EDXS: Energy Dispersive X-ray Spectroscopy, MIMP: Microbiologically Induced Mineral Precipitation, SCMs: Supplementary Cementitious Materials, SEM: Scanning Electron Microscope.

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Author Contributions

Dr. Kishor B. Vaghela: conducted all experimental work, analyzed the results and prepared the report, Dr. Jayeshkumar Pitroda: guidance for conducting this study, and contribution in analysing the results, and Dr. Tushar Bhoraniya: provided valuable insights and support in data interpretation, manuscript refinement, and overall research validation.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethics Approval

No ethical clearance certificate applies to the present study.

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