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Research Article

Bioimmobilized Limestone Powder for Autonomous Healing of Cementitious Systems: A Feasibility Study

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For preserving concrete structures and hindering ingress of chemicals through cracks and fissures, repair is inevitable. Microbial calcite precipitation is an intrinsic approach for crack rectification and emulating way of sustainability for reducing anthropogenic greenhouse gases (GHGs) along with conserving the natural resources. In this study, *Bacillus subtilis* strain is applied for intrinsic repair of concrete's cracks because of its high pH endurance and capability of sporulation. For prolonged survival of microorganisms, immobilization technique was employed. *B. subtilis* was immobilized through limestone powder (LSP) before adding into cement matrix. Self-healing proficiency of *B. subtilis* was deliberated in terms of mechanical strength regain after cracking at 3, 7, 14, and 28 days. To examine the microstructure and characterization of healing precipitate, micrographical (field emission scanning electron microscopy), chemical (energy dispersive X-ray), and thermal (thermogravimetric analysis) analyses were performed after the healing period of 28 days. The results revealed evident signs of calcite precipitation in nano-/microcracks subsequent to microbial activity. Furthermore, immobilized LSP improved the compressive strength of the analyzed formulations.

1. Introduction

Concrete, an extensively used construction material, is a source of anthropogenic greenhouse gas (GHG) emissions, depleting the raw materials, consuming the fossil fuels, and intensifying the environmental concern [1]. Additionally, it is susceptible to crack under tensile stresses, ensuing in the dramatic increase in its porosity, consequences in declined strength, and durability [2]. Numerous advanced cementitious systems have been investigated and practiced for limiting the ingress of deleterious chemicals through pores for enhancing the durability of cementitious systems [3]. But they involved external interventions which are labor dependent and cost extensive [4]. Researchers are probing for sustainable solutions to reduce cost as well as the environmental impacts. One of the potential solutions is self-healing concrete, which can repair its cracks itself [5]. Self-healing mechanisms in concrete are categorized as autogenous and autonomous healing [6]. Autogenous self-healing can be achieved by adding anhydrous cement particles or some pozzolanic material. When cracks appear in humid environments, anhydrous particles endure secondary hydration that seals the internal microcracks and fissures. But it is a limited process with minimal recurring rate having ability of 0.1-0.2 mm trivial crack-healing widths [7, 8]. For accomplishing distinct crack-healing widths by persistent process of repairs, autonomous healing is recommended [9]. Concrete, autonomously, can be healed through addition of engineered cementitious crystalline admixtures, polymers, shape-memory alloys, and microbes [10]. Microbial calcite precipitation attained imperative position among all of them since calcite formation is a way of emulating sustainability in cementitious systems [11]. Microbes secrete calcite under favorable surroundings; the secreting process consumes CO_2 from the environment and acts as sink for CO_2 dumping [12]. But problems associated with such healing systems are survival of bacteria for relatively longer periods and reduction in mechanical properties of concrete [13]. Moreover, the healing rate depends upon the type of microbial strain, its endurance in high alkalinity, food source, and immobilization techniques [14]. Microbes having ability of sporulation can stay relatively long in dormant stage and are therefore preferred in the bio-influenced cementitious systems [15]. Microbes are either directly induced through mixing water or immobilized using different techniques [16]. These immobilization techniques include encapsulation, entrapment, and adsorption of microbes [17]. These immobilization techniques are reported efficient for enhancing the microbes' survival but deficient in attaining intended mechanical properties [18, 19].

Numerous researchers are probing for optimization of immobilization techniques and struggling for appropriate immobilizers. Wiktor and Jonker embedded microbe's *B. alkalinitrilicus* and calcium lactate into porous expanded clay (EC) particles for formulating healable cementitious mortar. Both components are adsorbed on the surface and entrapped into pores of EC. EC particles released microbes on ingress of water and achieved maximum of 0.46 mm crack-healing width [20]. In another study, *B. sphaericus* were adsorbed in diatomite earth (DE) and offered 0.15 to 0.17 mm crack-healing widths. When *B. sphaericus* immobilized through polyurethane sheet or silica gel, they contributed in lowering the permeability of the system [18, 21]. Then, microencapsulation technique was used for immobilization of microbes into melamine capsules using the condensation process. Maximum crack-healing width of 0.97 mm was attained, which was 40% to 80% higher as compared to purely melamine capsules, but it reduced the compressive strength by 15–34% [22]. Hydrogels were also employed for microbial immobilization, and microbes were attached to hydrogels through the cross-linking process. Hydrogels repaired cracks up to 0.5 mm in mortar [19]. Natural diatomite has been investigated for bacterial adsorption and entrapment into the pores with final insertion made in the form of pellets. Addition of pellets enhanced compressive strength but lowered the permeability of the resultant matrix [23]. Ceramsite (sand) was also used as a carrier, but microbes and nutrients were immobilized separately in that case, eventually providing an accomplished repair rate of 87.5% [24]. Expanded perlite (EP) was also tried for enhancing microbial survival, and the results were compared to direct induction and EC [25]. Furthermore, zeolite powder, graphite nanotubes (GNPs), and light weight aggregate (LWA) have also been explored for immobilization of microbes [15, 16]. The state of the art highlighted that each bioimmobilizer has some related pros and cons. Researchers are still struggling to explore viable alternatives to successfully immobilize microbes in cementitious systems for relatively longer periods.

This work is basically a contributing effort to explore potential usage of LSP as an immobilizer in self-healing cementitious systems. LSP has been employed in the construction industry since eras, and it is still divulging contemporary applications owing to its versatility. It is a by-product of aggregate formation from rocks and a prime source of calcium oxide (CaO) provision in the cement manufacturing industry [26]. Moreover, it has been investigated as secondary raw material (SRM) in the cementitious systems for dense microstructure and imparting durability as a cement and sand replacement for fabricating a sustainable concrete to shrink CO_2 footprints [26–28]. Furthermore, LSP is an inert media and acts as a filler, but it reacts with aluminates phases to form carboaluminates and influence the hydration rates of cementitious systems [29]. Initially, hemicarboaluminate component was formed, and then, it converted into monocarboaluminate (Al_2O_3-Fe_2O_3-mono) AFm group [30]. The newly formed AFm improved the microstructure of cementitious systems and enhanced its durability. In a recent study, LSP was used as a cement replacement to enhance calcite precipitation rate via CO_2 curing. Supplementary content replacement of LSP helped in more calcite formation owing to pore structure and nucleation site provision by LSP [31]. The promising results of that study motivated the researchers to investigate the feasibility of LSP as a microbial immobilizer in self-healing cementitious systems as calcite production is a key for microbial activity. Moreover, researchers supported the argument that LSP addition influenced the mechanical properties of cementitious systems positively such as melamine microencapsulation that reduced the compressive strength [22]. Furthermore, its chemical nature resembles instigated calcite that makes it compatible to provide sufficient sites for precipitation as GNPs serving as microbial immobilizers killed the microbes [16]. Additionally, LSP retains its shape and position by filling the empty voids that ensure its homogeneous distribution inside the host matrix for uniform healing.

2. Experimental Program

2.1. Materials

2.1.1. Cement and Sand. Ordinary Portland cement CEM-I (grade 53), conforming to ASTM C-150, was used for all mortar formulations. The average particle size of cement grains is 16.4 μm with density of 3.17 g/cm^3. The oxide composition as determined via X-ray fluorescence (XRF) test is presented in Table 1.

Locally available sand from Lawrencepur, Pakistan, was used in the study. The fineness modulus of sand was determined according to the ASTM C-136, and the calculated value was 2.018 with specific gravity of 2.65 having $D_{50} = 0.215$ mm. Absorption capacity of sand was 2.4% as determined according to ASTM C-128.

2.1.2. Microorganisms. Cementitious composite's environment is highly alkaline in nature having pH around 11–13 [32]. So, bacterial strains must be capable of enduring high pH and have ability of sporulation for ensuring survival within the harsh environment of cementitious composites [33]. Moreover, bacterial strains must be proficient in production of copious amount of calcite, which is

Table 1: Chemical composition of OPC and LSP (%).

Parameters	CaO	SiO_2	MgO	Al_2O_3	Fe_2O_3	TiO_2	Na_2O	K_2O	P_2O_5	MnO	LOI
OPC	65.00	19.19	2.23	4.97	3.27	0.29	0.58	0.51	0.08	0.04	3.84
LSP	52.67	3.00	0.67	0.69	0.27	0.04	0.30	0.10	—	0.01	42.24

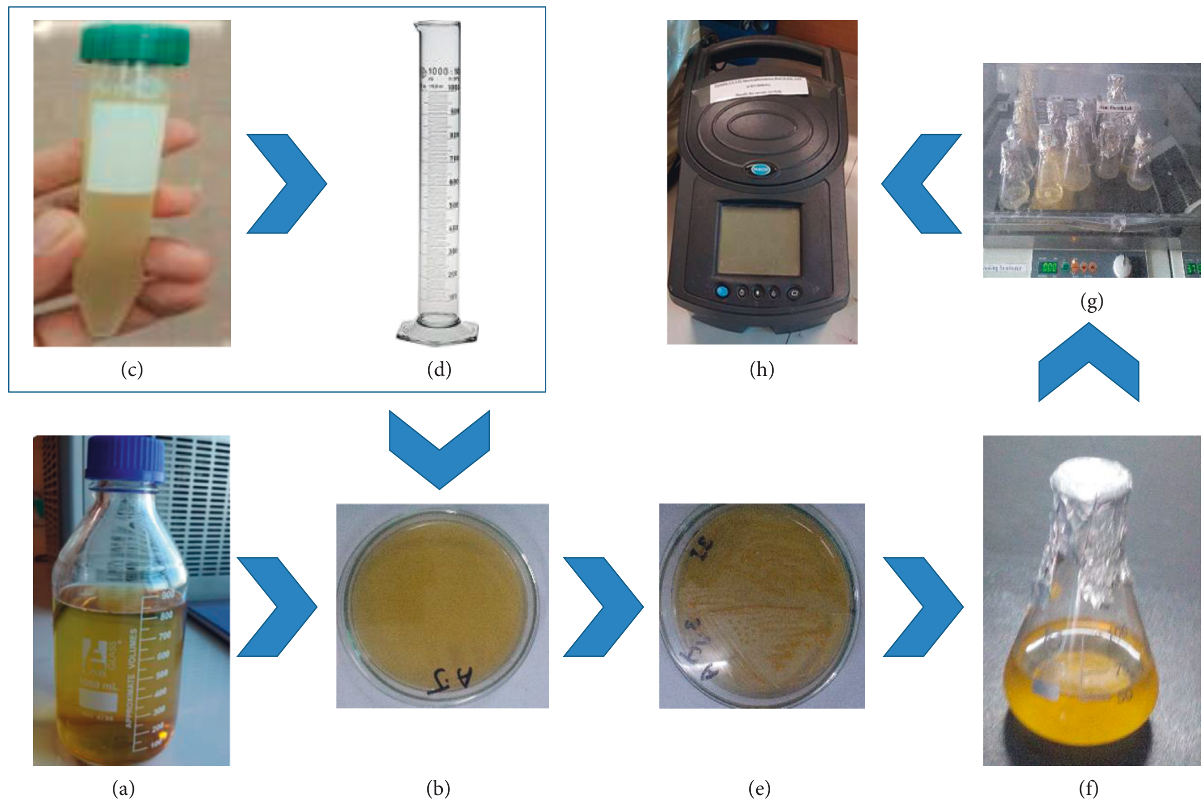


Figure 1: Microbial solution preparation. (a) LB media. (b) Agar plating for growth of microbial colonies. (c) *B. subtilis* spores. (d) Distilled saline. (e) Microbial colonies. (f) Inoculum in LB. (g) Incubation in shaker. (h) OD measurements.

responsible for crack healings [34]. *Bacillus subtilis*, soil-based bacteria, was selected for the purpose. *B. subtilis* is a gram-positive alkaliphilic bacterium having characteristics of sporulation and can survive for prolonged durations [35].

B. subtilis was revived from glycerol stock. 1 ml of frozen glycerol was mixed with 5 ml of 0.9% autoclaved saline solution. Then, this solution was spread on agar media by cotton swab and incubated for 24 hours. Nutrition broth used for *B. subtilis* was standard Luria broth (LB) consisting of 5 g tryptone, 5 g NaCl, and 2 g of LG broth in 1000 ml of distilled water. Incubation was done for 6 to 8 hours at 37°C while shaking at 200 rpm; after that, 15 ml of LB was inoculated on satirized colony of *B. subtilis*.

For sporulation, Difco sporulation medium (DSM) was used. DSM consisted of 2.5 g of peptone, 0.1 g of KCl, 1.5 g of meat extract, 0.5 ml of $MgSO_4$ (1 M), and 0.25 g of $MnSO_4$ in 500 ml of water and sterilized by autoclave. 0.25 ml of $CaCl_2$, 0.5 ml of $FeSO_4$, and 2.5 ml of LB was added into DSM shown in Figure 1(a). The entire solution was incubated for 4 days with shaking at 200 rpm at 37°C. Bacterial cells were pelletized at 9000 rpm for 20 minutes then washed 8 to 10 times. Spores were identified by light microscope. Bacterial solution is shown in Figure 1(c). Calcium lactate was used as food source for microbes.

The concentration of bacteria in the solution was calculated by HACH DR 2400 portable spectrophotometer shown in Figure 1(h). Spectrophotometer was calibrated by using 0.5 ml blank solution at 600 nm wavelength. Bacteria concentration in the solution was measured using the expression $Y = 8.59 \times 10^7 X^{1.3627}$ [36], where Y is bacterial concentration and X is wavelength value at OD_{600}. The cell concentration during the mixing of the mortar samples was kept constant at 6×10^6 cells/cm^3.

2.1.3. Limestone Powder. LSP used in the study was taken from local source and produced by milling of limestone rock. Average particle size of LSP was 22.3 μm with a specific surface area of 3048 m^2/kg and absorption capacity of 26% having density of 2.72 g/cm^2. Particle size distribution of LSP is given in Figure 2 and XRF analysis results are presented in Table 1.

The field emission scanning electron microscope (FESEM) image of LSP is shown in Figure 3. It is evident from SEM that the LSP particles are rhombohedral in nature with sharp indents having rough superficial textures and apparently seem porous. These characteristics contribute in

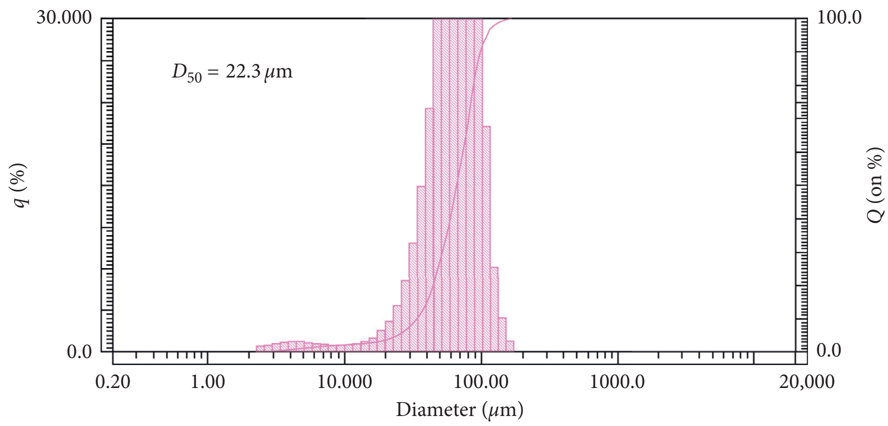


Figure 2: Particle size distribution of LSP.

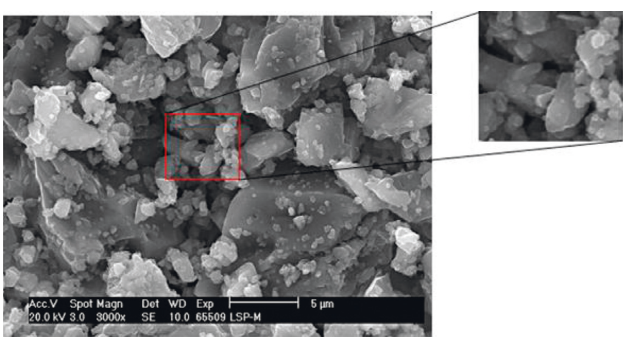


Figure 3: FESEM micrograph of LSP.

developing good bond among grains of cementitious matrix [37, 38].

2.2. Mixing and Testing Regimes. Two types of formulations were investigated with their mix proportions mentioned in Table 2. Standard consistency was ensured in accordance with ASTM standard C191-11. Initial and final setting times of mortar formulations were monitored as per the standard set forth in ASTM C187-11. The analyzed formulations were designated as CM and LSP-B. CM corresponds to the controlled formulation without any addition of LSP, whereas LSP-B contains *B. subtilis* immobilized via 10% LSP in replacement to cement. Mix proportion of cement to sand was set as (1 : 1.4) with constant water to cement ratio (w/c) of 0.4. Quantities of cement and sand were 930 kg/m^3 and 1400 kg/m^3 with water 372 l/m^3. Calcium lactate of 18.7 kg/m^3 was used, which was 2% of the cement in both formulations, whereas bacterial solution of 7.6 Liter/m^3 was only added in LSP-B formulation. Limestone powder particles were soaked in bacterial solution for 24 hours to ensure maximum adsorption and entrapment.

All ingredients were mixed in Hobart mixer of 5 L capacity in accordance with ASTM C-305. Mortar cube specimens of $50 \times 50 \times 50\ mm^3$ dimensions were prepared for both the formulations. Specimens were demolded after 24 hours and moist cured till the age of testing in controlled conditions (25°C temperature, 100% relative humidity). Compressive resistance of specimens was gauged at the age of 3, 7, 14, and 28 days in accordance with ASTM C-109.

For healing inspection, specimens were precracked at 3, 7, 14, and 28 up to 80% of their maximum compressive strength ($0.8 \times fc$) of respective days to induce internal microcracks. The precracked specimens were immersion cured for the healing period of 28 days. After that, the specimens were again subjected to compressive strength analysis, and healing was estimated in terms of percentage regain in compressive strength using the following relation:

$$\mathrm{RCS\%} = \left[1 - \frac{\mathrm{Cu}_{28} - \mathrm{Cr}}{\mathrm{Cu}_{28}} \times 100\right] \quad (1)$$

where Cu_{28} = ultimate compressive strength at 28 days and Cr = regained compressive strength after 28 days of curing.

In total, 48 samples were casted, and reported values are the average of three specimens. Field emission scanning electron microscope (FESEM), energy dispersive X-ray analysis (EDX), and thermogravimetric analysis (TGA) were employed to evidence the precipitation of $CaCO_3$.

3. Results and Discussions

For assessment of self-healing efficiency, two types of testing were conducted, and their results are discussed here. One was based on compressive strength analysis while the other was related to the affirmation of calcite precipitates in the induced cracks, through FESEM, EDX, and TGA.

3.1. Compressive Strength Analysis. Compressive strength of mortar specimens was measured using MCC8 compression-testing machine at a controlled loading rate of 0.2 MPa/sec conforming to standard ASTM C-109. The attained compressive resistance of the investigated formulations is given in Figure 4 at the age of 3, 7, 14, and 28 days. It can be seen that the addition of *B. subtilis* immobilized by LSP improved the strength of mortar specimens in compression during the entire hydration tenure.

The calcite precipitated by microbes within the cementitious matrix actually plugs the pores, and hence, contributes in improvement of compressive strength.

Table 2: Mix proportions of mortar formulations having mixing ratio of (1 : 1.4) with w/c of 0.4.

Specimens Units	Cement kg/m^3	Fine aggregates kg/m^3	Water cement ratio	Calcium lactate kg/m^3	Bacterial solution Liter/m^3	Immobilization media
CM	930	1400	0.4	18.7	—	None
LSP-B	840	1400	0.4	18.7	7.6	LSP (10% of C)

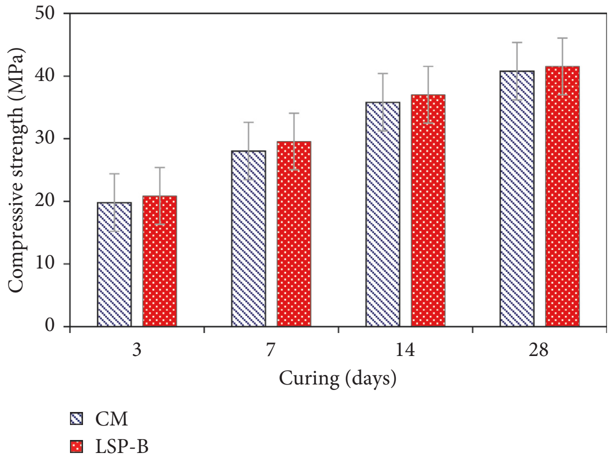


Figure 4: Compressive strength of mortar formulations with and without immobilized microbes at different curing ages.

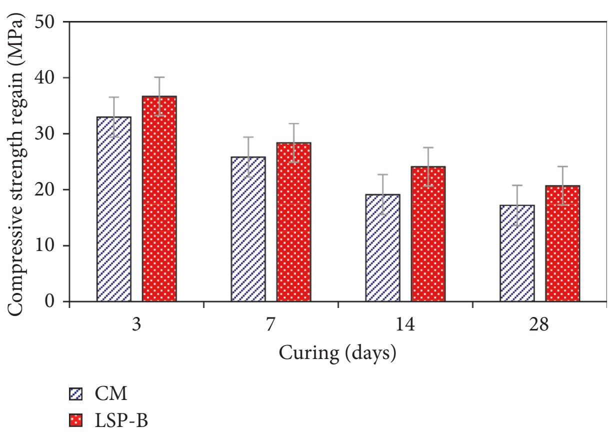


Figure 5: Compressive strength regain in mortar formulations with and without immobilized microbes at different precracking periods.

The reaction kinetics in the presence of microbes also endorses the formation of additional CSH gel as a result of CO_2 consumption as revealed by Li et al., which further adds in compressive strength [39]. The involved chemical reaction is as follows:

$$C_3S/C_2S + CO_2 + H_2O \longrightarrow \text{C-S-H} + CaCO_3 \quad (2)$$

Generally, the addition of microorganisms through an immobilizer reduces the compressive strength of the cementitious matrix [19, 21, 22]. But on the contrary, immobilization via LSP increased the compressive strength of mortar formulations due to its filling ability that plugs the inner pores and homogenized distribution [40, 41]. At the age of 3 days, CM and LSP-B indicated 19.81 MPa and

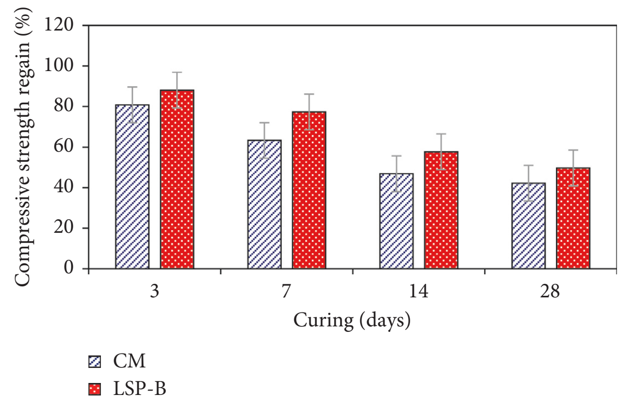


Figure 6: Percentage regain in compressive strength of mortar formulations with and without immobilized microbes at different precracked ages.

20.87 MPa values of compressive strength. LSP-B showed 5% more strength than CM formulation. Similar trend was observed at 7-day strength of both the formulations. At 14 days, CM and LSP-B showed 35.81 MPa and 37 MPa values of compressive strength, respectively, and LSP-B possessed 3.5% more strength than CM. At 28 days, both formulations attained maximum compressive strength showing values of 40.77 MPa and 41.98 MPa. The reported increase further enhanced to a maximum of 11.5% (instead of 5%) while focusing the isolated behavior of microbes with reference to 10% LSP diluted cement formulation. This reveals LSP as quite promising media that adds to the compressive strength of mortar specimens.

3.2. Compressive Strength Regain. Self-healing efficiency was evaluated via regain in compressive strength after precracking in mechanical mode. The compressive strength regain values are plotted in Figure 5 while Figure 6 shows the percentage regain in compressive strength using (1).

Both formulations showed regain in compressive strength. In CM formulation, compressive strength regain is attributed to autogenous healing due to secondary hydration of unhydrated cement grains and carbonation of calcium hydroxide into calcite crystals [42, 43]. While in LSP-B, microbial activity is responsible for regain in compressive strength of specimens. LSP-B exhibited more compressive strength regain than CM at each stage of curing as a result of calcite precipitation induced by microbial actions. The analyzed formulations showed maximum strength regain at 3 days which was around 81% for CM and 88% for LSP-B specimens. At 7 days of curing, compressive strength regain was reduced to 63% and 77% for CM and LSP-B, respectively. Then at 14 and 28 days of curing, similar trend was observed in regained strength with a noticeable

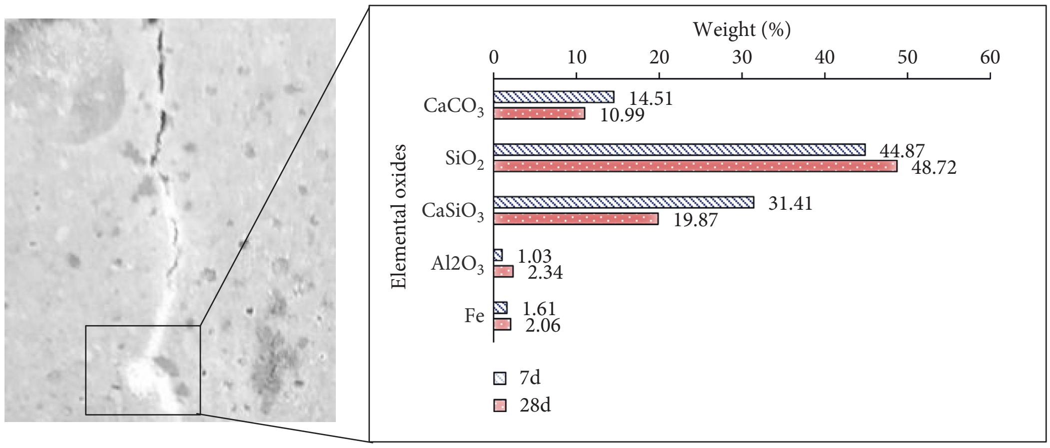


Figure 7: EDX spectrum of extracted powder out of healed crack in LSP-B formulation.

reduction to 42% and 50% in CM and LSP-B formulations, respectively.

The decrease in compressive strength regain with increase in the testing age of specimens may be attributed with reduction in microbial activity at later ages. Microbes' survival depends on food and carrier media. The involved phenomena that can justify the reduction in compressive strength regain are as follows: first, the production of microbial calcite ceased due to lack of food, and second, LSP did not sustain pressure at later stages subsequent to densified microstructure of mortar matrix in later ages ensuing in microbial crushing. However, the comparison of results of microbial formulation to referenced CM gives an isolated effect of bio-influenced self-healing process in the regain of mechanical strength. LSP showed higher preservation efficiency in the earlier phase of hydration that reduces in the later part.

3.3. Energy Dispersive X-Ray Analysis. Energy dispersive X-ray (EDX) spectroscopy is a chemical mode of microstructural analysis. Precracked LSP-B specimens at 7 and 28 days were subjected to EDX spectroscopy after 28 days of healing period to inspect the instigation of possible calcite. The EDX spectrograph of LSP-B samples are given in Figure 7.

The results made the manifestion of calcite precipitation in both the formulations at the age of 7 and 28 days. But, at 7 days, calcite content was more as compared with 28 days. It is further concluded that microbial activity slowed down with the passage of time. However, it remained active till the age of 28 days that renders the investigated media efficient enough to effectively shield *B. subtilis* for relatively longer durations.

3.4. Field Emission Scanning Electron Microscopy. To further explore the instigated calcite, field emission scanning electron microscopy (FESEM) was used. The FESEM micrograph was carefully sketched along the healed crack to observe any possible signs of calcite precipitation. FESEM micrograph of LSP-B formulations is shown in Figure 8 at the precracking age of 7 and 28 days.

In the presence of *B. subtilis*, calcium lactate converted into calcite, by consuming oxygen, and produced rhombohedra crystals. The reaction activity is shown in (3) [44].

$$CaC_6H_{10}O_6 + 6O_2 \longrightarrow CaCO_3 + 5CO_2 + 5H_2O \quad (3)$$

Fabrication of calcite crystals can be clearly seen in both the micrographs of cryofractured cementitious formulations at 7 and 28 days of precracking consequent to microbial healing that ensures the persistence of microbes till the age of 28 days.

3.5. Thermogravimetric Analysis. The thermogravimetric analysis (TGA) is a mode of thermal analysis which is performed in an instrument named as the thermogravimetric analyzer. Mass, time, and temperature are considered basic measurements of TGA. The analyzer continuously measures the mass of the substance while temperature of the sample changes continuously at a constant rate [45]. This change in mass of the substance assists in recognition of different chemical compounds categorized by their decomposition temperature.

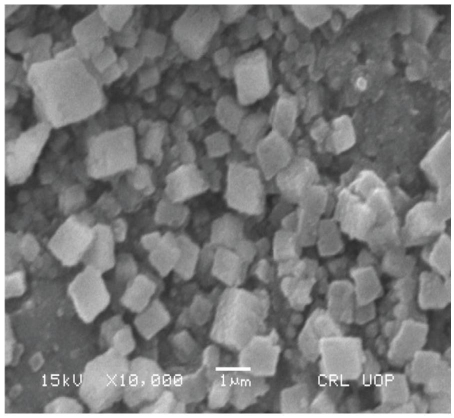
TGA was conducted on the white powder scratched from the surface of healed specimens for confirmation of precipitated calcite after 28 days of healing. The decomposition temperature of $CaCO_3$ crystals ranges from 600 to 850°C [46]. The decomposition reaction of $CaCO_3$ is as given in (4), and the TGA result of healed powder is plotted in Figure 9.

$$CaCO_3\,(s) \longrightarrow CaO\,(s) + CO_2\,(g) \quad (4)$$

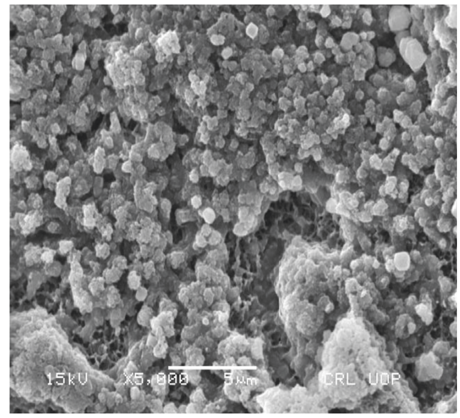
It is clearly evident from the curve that major mass loss of 6.2% occurred in the predefined range of 600–850°C that endorses the presence of calcite ensuing in strong microbial action. Hence, it can be inferred that LSP effectively preserved the microbes "*B. subtilis*" up till the formation of cracks in the mortar formulations.

4. Concluding Remarks

LSP is locally available, cost-efficient alternative to immobilize *B. subtilis* in highly alkaline cementitious environment. The addition of microbes immobilized on LSP



(a)



(b)

Figure 8: (a) FESEM micrograph of LSP-B specimen. (b) FESEM micrograph of LSP-B specimen precracked at 7 and 28 days.

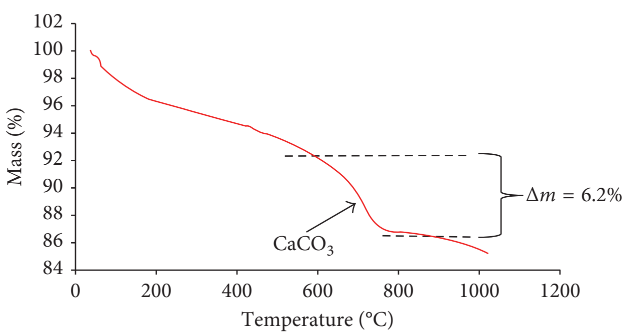


Figure 9: TGA curve of extracted powder out of healed crack in LSP-B formulation.

contributed in the enhancement of compressive strength at 3, 7, 14, and 28 days of curing with maximum increment of 5.75% at 14 days comparative to CM formulation. The regained compressive strength gauging the healing efficiency in mechanical mode endorsed the maximum regain at 3 days of precracking age, which declined later on with increased age of specimens. LSP-B formulation exhibited more regained compressive strength at each testing age. Moreover, micrographical analysis through FESEM, chemical analysis by EDX, and thermal analysis via TGA on the powdered specimens from the near vicinity of healed cracks further evidenced visible signs of calcite precipitation consequent to microbial activity. Hence, LSP can be claimed as a promising carrier media for *B. subtilis* ensuring its preservation for relatively longer durations in highly alkaline cementitious environment.

Abbreviations

FESEM: Field emission scanning electron microscopy
EDX: Energy dispersive X-ray
TGA: Thermogravimetry analysis
LSP: Limestone powder
XRF: X-ray florescence
CSH: Calcium-silicate-hydrate.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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