

Influence of bio-immobilized lime stone powder on self-healing behaviour of cementitious composites

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Abstract. For preserving cementitious systems and inhibiting the ingress of chemicals via cracks and fissure, persistent repairs are inevitable. Thus, self-healing of harmful cracks and fissures, without external intervention and onerous labours emerged as a viable approach for enhancing concrete durability. Among all, bio-inspired self-healing secured imperative position in autonomous healing of cementitious system, latterly. Microbes are potentially efficient in intrinsic healing of cementitious systems due to their ability of calcite precipitation. Instigated calcite filled the void and restrained the ingress of deleterious chemicals. *Bacillus subtilis* was selected as self-healing microbe due to its relatively longer reported survival in high pH environment. Microbes are inducted directly through water and immobilized via immobilizer. In this study, viability of lime stone powder (LSP), as bacterial immobilizer, is explored. Self-healing ability of *Bacillus subtilis* was gauged in terms of mechanical strength after cracking at 3, 7 and 28 days. For inspecting the microstructure and characterization of healing field-emission scanning electron microscopy (FESEM) and energy dispersive x-ray spectroscopy (EDX) were performed at 28 days. Hence, immobilized LSP enhanced the compressive strength of cementitious system and endorsed as a potential carrier for sustaining microbes for relatively extended periods. Furthermore, LSP is an economical locally available raw material, it eventually helped in formulating sustainable, cost-effective and proficient in crack repairing cementitious system.

1. Introduction

Concrete's durability and serviceability are of prime concern in advanced concrete systems. Cracking is inevitable in cementitious systems and make it susceptible for ingress of aggressive agents. Moreover, crack and fissures stimulates permeability that degrades the durability, shortens the service life of cementitious systems and necessitates the routine maintenance [1, 2]. To restrain the adverse effects of cracking in concrete systems, undue repairing is involved which adds on cost [3]. For limiting the external intervention in the cementitious systems to refrain adverse effects consequent to repairing, different techniques are employed to device the inherent system of crack repairing [4]. These systems can be categorized as autogenous and autonomous healings systems [5]. Autogenous healing is subsequent of secondary hydration of anhydrous particles, pozzolanic activity and re-expansion of pivotal constituent gels [6-8]. While autonomous healing can be attained through microbes, polymers, crystalline and mineral admixtures [9-11]. Autonomous healing by microbial actions secured imperative position owing to its obvious advantages [12].

Bio-influenced healing system is comprising of microbes as healing agent. Microbes secrete calcium carbonate consequent to microbial activity by consuming appropriate compounds and CO₂ from surrounding environment [13]. Inadequacy of mentioned healing system is the endurance of microbes in highly alkaline localities of cementitious matrix for longer durations [14]. Alkaliphilic microbial species have being used due to their tolerance to alkaline pH. But, their survival is limited owing to chemical and physical changing within cementitious matrix [15]. For enhancing microbial survival immobilization techniques are employed [6]. In these techniques, microbes are imprinted, adsorbed,



entrapped, encapsulated and bonded with suitable media. These media's prevent the microbes from crushing during mixing and in hardened state in addition to provision of dormancy [16]. Dormant microbes triggered on ingress of water upon formation of cracks and fissures on requisite stage [17]. Several materials have been investigated as healing media for the boosted microbial survival. Initially, *Bacillus* genus microbes were immobilized via expanded clay (EC) particles and calcium lactate was used as substrate. This system amplified the microbial survival rate and attained 0.46 mm healing crack width [6]. Then polyurethane sheet and silica gel were used for immobilization of *Bacillus sphaericus* microbes and polyurethane presented better results by attaining 60% strength regain and lower permeability [18]. Furthermore, feasibility of diatomite earth (DE) was investigated for immobilization of *Bacillus subtilis* and 0.17 mm crack healing width was achieved by 60% W/V of bacterial solution [8]. Micro-encapsulation technique was used for protecting microbes and melamine capsules having a size of 5 μm was formulated. Maximum crack healing width of 0.97mm was achieved with 80% higher crack healing rate and strength degradation was observed up to 34% [19]. *Bacillus subtilis* and nutrients immobilized by natural diatomite as control releasing agent, increased the compressive and flexural strength of concrete by reducing the permeability of self-healed concrete samples. Authors concluded that use of immobilized diatomite *Bacillus subtilis* pellets as 0.5% replacement of coarse aggregates are more effective as compared to the solution with 0.5% replacement of mix water [20]. Bacteria spores embedding on hydrogels was established as a viable solution for longer bacterial life without compromising compressive strength and gave 0.5mm crack healing width [10]. The ceramsite carrier was used in for immobilization of microbes and nutrients separately, which exhibited successful enhancement of area repair rate up to 87.5% complimented by an increase in mechanical properties [21]. Khaliq and Ehsan [22] investigated the effect of microbial intrusion method using *Bacillus subtilis* in self-healing concrete using. These are direct intrusion, immobilization with carrier media namely light weight aggregate (LWA) and graphite nano platelets (GNPs). GNPs were found effective at an early age while LWA immobilization exhibited greater crack healing at later ages [22]. Immobilized bacteria using Zeolite carrier was used in RCC and fiber reinforced mortar to enhancing micro structural and crack healing capacities. Chlorides ion penetration was reduced along compressive strength amendments [23]. In an other study perlite was used for immobilization in which effects of direct induction and immobilization through expanded perlite (EP) were compared. Experimentation results proved that (EP) immobilized bacteria exhibit efficient crack healing (up to 0.79 mm) and strength which was better than expanded clay (EC) immobilized self-healing concrete which results in crack healing up to 0.45 mm [16].

LSP is a sedimentary rock, composed of calcite (CaCO_3) used as a construction material [24]. The application of LSP in construction industry started a century ago and still acquainting renowned interest due to its versatility [25-27]. Firstly, it was used as binding agent in all types of masonries, then used for production of hydraulic cement. It has been used as secondary raw material in cementitious systems for microstructural improvements and replacing cementitious content for fabricating a sustainable concrete [28]. In numerous studies, LSP used as replacement of cement to reduce the cost and enhancing the durability of the cementitious systems [28, 29]. LSP is also used as accelerator for achieving early strength in case of rapid hardening cementitious systems [30]. Moreover, LPS does not hold pozzolanic properties, but it indicates chemical reactivity with the aluminate phases of cement to form an alumina+ferric oxide+mono-sulfate (Afm) phase [31].

Aforementioned studies accentuate the potential feasibilities of various materials as immobilization media for enhancing the microbial survival rate without influencing mechanical properties of cementitious matrix. It is evident from literature, researchers are still struggling for viable media for mitigating the problem of microbial survival. Moreover, some bio-influenced healing systems influenced the strength negatively owing to compatibility glitches associated with poor bonding [19]. In current study, LSP was subjected for examining the potential as healing agent immobilizer to overcome compatibility problems. By morphological characterization, it is polymorph of calcite [29]. Microbial cementitious systems secrete calcite as well, as a result of microbial activity to seal the cracks [32]. In investigated system, both healing agent and its carriers are polymorph of calcite and used for eliminating possible inter transitional zones films which causes hindrance in formation of optimised bond.

2. Materials

2.1. *Bacillus subtilis* and calcium lactate

The microbes “*Bacillus subtilis*” were selected as a healing agent owing to their tolerance towards high pH. The microbes used in the study was imported from Germany and retrieved from glycerol stock. LB agar (5g of trypton, 10g yeast extract, 5 g NaCl and 12 g Ager)/l was used for microbial growth. For enhancing their survival, sporulation was performed using difco sporulation medium (DSM). Nutrients and vegetative cells were removed while washing. The microbial spore concentration used in the study was 6×10^6 cells/cm³ as shown in figure 1 (a).

Calcium lactate (C₆H₁₀CaO₆) was used as food source for microbes for enhancing the calcite production. Calcium lactate (shown in figure 1(b)) was selected consequent to its good influence on calcite content and mechanical properties of cementitious systems [33]. The content of calcium lactate added into formulated mix was 2% of cement content. In the presence of microbes, calcium lactate was converted into calcite by consuming oxygen as shown in Equation 1 [34].

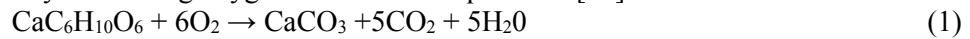


Figure 1: (a) Microbial Solution (b) Calcium lactate solution

Moreover, by product of Equation 1 CO₂ and water adds on in calcite production further. These by-products make it more efficient organic calcium source. Additionally, organic calcium food source produced 2 mol of ammonia resulting in production of nitrogen which contributes in global warming [35].

2.2. Cement and sand

A locally available Type-I ordinary Portland cement (OPC) conforming to ASTM C150 was used in the study. The density of cement was 3.16 g/cm³ having an average particle size of 16.4 μm. X-ray fluorescent analysis of cement is given in Table 1. Locally available siliceous sand was obtained from a local source having fines modulus value of 2.11 with specific gravity of 2.67.

2.3. LSP

LSP used in the study was acquired from local source in form of lumps (figure 2 (a)). It was converted into powder form by milling as shown in figure 2(b). The average diameter D₅₀ of LSP powders was 22.27 μm having surface area of 3043 m²/Kg. the estimated absorption capacity of LSP was 26%. Chemical composition of LSP determined by XRF is presented in Table 1. FESEM micrograph of LSP was shown in figure 2(c)

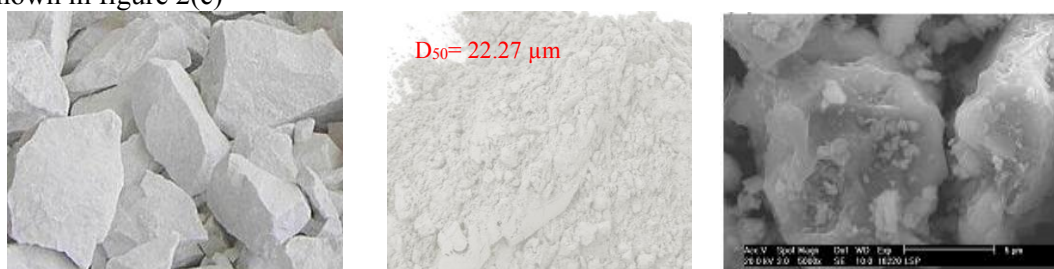


Figure 2: (a) Raw LSP (b) Powdered LSP (c) FESEM of LSP

Table 1 XRF analysis of OPC and LSP (%)

Parameters	CaO	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	MgO	TiO ₂	Na ₂ O	K ₂ O	P ₂ O ₅	MnO	LOI
OPC	65.00	19.18	4.97	3.25	2.21	0.25	0.58	0.55	0.08	0.04	3.84
LSP	52.63	3.12	0.69	0.26	0.66	0.04	0.30	0.18	-	0.01	42.24

3. Experimental program

3.1. Mixing regimes

For evaluating the effects of immobilized *Bacillus subtilis* on healing of cracks and mechanical properties of mortar, two formulations were subjected to investigation. The mix proportion of investigated formulations is given in Table 2. Standard consistency of mortar mix was measured according to ASTM standard C191-11 and found within range. Initial and final settings were checked against ASTM C187-11. The investigated formulations were designated as CM and B-LSP. CM is control mix which was formulated for comparison. CM didn't include LSP and *Bacillus subtilis*. Formulation B-LSP contained immobilized *Bacillus subtilis* through LSP. Mix design was set as (1:1.4) having water to cement ratio (w/c) of 0.4. Total quantities of cement and fine aggregates 930 kg/m³ and 1400 kg/m³ using water of 372 l/m³. Calcium lactate used was 18.7 kg/m³ with 2% replacement of cement was used while microbial solution of 7.6 liter/m³.

For mixing particles of LSP were soaked in microbial solution for the period of 24 hours for maximum adsorption and entrapment of microbes. All components were mixed in Hobart mixer designed conforming to ASTM C-305. Specimens of mortar were casted in cubes having size of 50x50x50 mm³. **Tamping** was done during casting and specimens were demolded after the period of 24 hours. Then moist curing was done till the age of testing in controlled environment (25°C temperature; 100% relative humidity).

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

Specimens	Cement	Fine Aggregate	Water Cement ratio	Calcium Lactate	Bacterial solution	Immobilization media
Units	Kg/m ³	Kg/m ³		Kg/m ³	Liter/m ³	Kg/m ³
CM	930	1400	0.4	18.7	--	None
B-LSP	837	1400	0.4	18.7	7.6	LSP(93)

3.2. Testing regime

Two types of testing were performed on analysed formulation. First, mechanical testing was gauged in terms of compressive resistance of specimens at the age of 3, 14 and 28 days conforming to ASTM C-109. Healing efficiency was measured in terms of compressive strength gained. Second type of testing was done for confirmation of calcite in the pores of specimens as a result of microbial action. Morphological characteristics were examined through field emission scanning electron microscope (FESEM) and energy dispersive analysis (EDX) after the age of 28 days of specimens.

A total of 36 specimens of mortar were casted for above mentioned testing. Reported values are average of three values of tested specimens.

4. Results and discussions

4.1. Compressive strength analysis

Most common performance measure of cementitious systems is its compressive strength. Which is quantifiable measure of concerned properties of hardened cementitious systems [36]. Compressive

strength of formulated mixes of mortar was determined according to standard ASTM C-109. The compressive strength values of investigated formulations were given in Figure 1 and computed at the age of 3, 7 and 28 days.

It is evident from figure 3, immobilized microbes via LSP slightly increased compressive strength of mortar specimens at all ages of testing. At 3 days of testing, specimens of CM attained 19.8 MPa while specimens of formulation B-LSP showed 20.9 MPa compressive strength. Compressive strength values of both formulations kept on increasing owing to on-going hydration reaction. After 7 days of curing, CM's specimens endured 28 MPa compressive strength while B-LSP achieved 29.6 MPa compressive strength value. Similar trend of compressive strength increase was observed in both formulations after 28 days of curing testing. CM exhibited 40.8 MPa compressive strength value while B-LSP attained 41.6 MPa compressive strength value. It was established from the results that microbes didn't influence the strength negatively. Results are contrasted to the general studies in which the addition of microbes via immobilizer reduced the compressive strength of cementitious matrix [10, 18, 19]. Moreover, gradual strength gain in entire tenure of 28 days of B-LSP formulation depicted that LSP protected the microbes effectively. Effectively preserved microbes plugged the pores by precipitation of calcite subsequent to microbial activity.

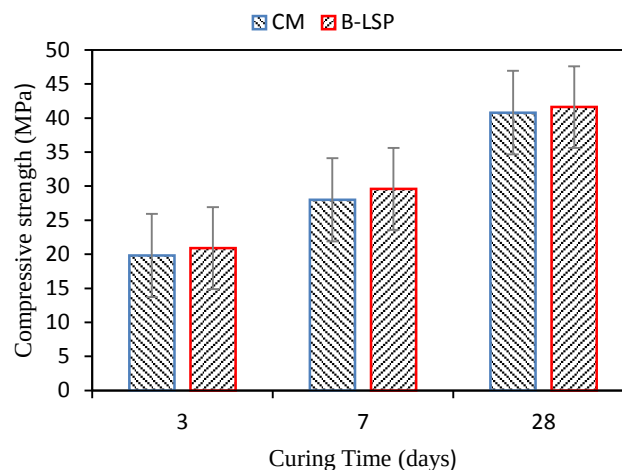


Figure 3: Compressive strength of mortar formulations with and without immobilized microbes at different curing ages

4.2. Field emission scanning electron microscopy

FESEM was employed for confirmation of microbial survival in bio-immobilized LSP formulation. For this purpose specimens were inspected by FESEM after 28 days of curing for inspecting traces of calcite. As, calcite is responsible for crack repairing, its presence is indication of self-healing cementitious system. The FESEM micrograph of B-LSP formulation was shown in figure 4.

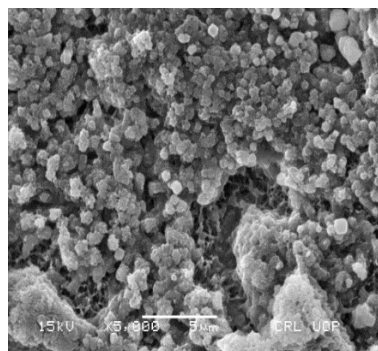


Figure 4: FESEM micrograph of LSP-B specimen pre cracked at 28 days

Precipitation of calcite crystals is clearly evident from FESEM micrograph. It was concluded that LSP preserved the microbes and microbes converted calcium lactate into calcite successfully.

4.3. Energy dispersive x-ray analysis

For further confirmation of healing activity EDX analysis was performed. EDX is used for chemical elemental investigation. EDX spectrum of B-LSP formulation is shown in figure 5. Specimen of B-LSP formulation was cracked after 28 days of curing and healing precipitate was scratched carefully and subjected to EDX analysis.

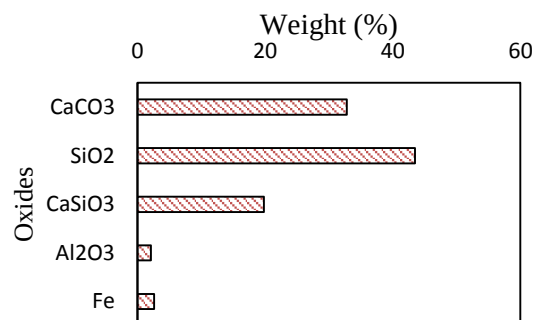


Figure 5: EDX spectrum of extracted powder out of healed crack in LSP-B formulation

The results evident the microbial survival as calcite content was 33%. It can be concluded that LSP protected the microbes and they remained active even later stages. Hence, immobilized media is proficient in securing *Bacillus subtilis* for relatively longer durations.

5. Conclusions

In the study LSP was used as an innovative media for enhancing *Bacillus subtilis* survival in self-healing mortar systems. Based on mechanical and micro-structural properties following conclusions are drawn.

1. Addition of *Bacillus subtilis* immobilized via LSP improved the compressive strength of mortar specimens. Moreover, LSP facilitated the uniform dispersion of the microbes in to the cementitious matrix.
2. FESEM micrograph confirmed the calcite production ensuing to microbial activity and endorsed LSP as proficient media for investigated microbial survival.
3. EDX spectrum of healing powder supported the calcite evidence consequent to microbial action and indication of relatively longer survival of microbes.

Hence, LSP ascertained as a proficient media for *Bacillus subtilis* intrusion into cementitious environment for ensuring its preservation for relatively longer durations. Furthermore, LSP is raw material, assisted in formulation of sustainable, cost-effectively and durable self-healing mortar system.

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