



A Mini Review on Prospects and Challenges of Harnessing Fungi for Concrete-Crack Healing

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Abstract

There has been a continual upsurge on research pertaining to bio-based/microbial healing of cracks in concrete (a pre-requisite component when it comes to construction design). Albeit, the application of bacteria in this realm has been documented widely over the years, howbeit, delving into fungus based self-healing under the deleterious ambience of concrete with oxygen and nutrient limitation, moisture deficit and high alkalinity has captured recent research impetus. In this context, we have tried to mine the current contextual information to gauge whether research on fungal-based self-healing concrete could be worthwhile. Recent systematic screening encompassing the application of genetically engineered strains, attests the profound untapped potential of specific fungal species in assisting sustainable self-healing to ensure resilient infrastructure. Known for their adaptability under a plethora of environmental stress-conditions and architecturally endowed with large surface-active biomass, fungi can display both biomineralization and organomineralization, leading to rapid and profuse precipitation of CaCO_3 (a befitting concrete-filler) for prospective sealing of cracks, even of large width, plausibly without any negative trade-off with respect to concrete's strength. This article is thus compiled to mirror the various prospects, practical hitches and future direction of research in using fungi for concrete crack healing.



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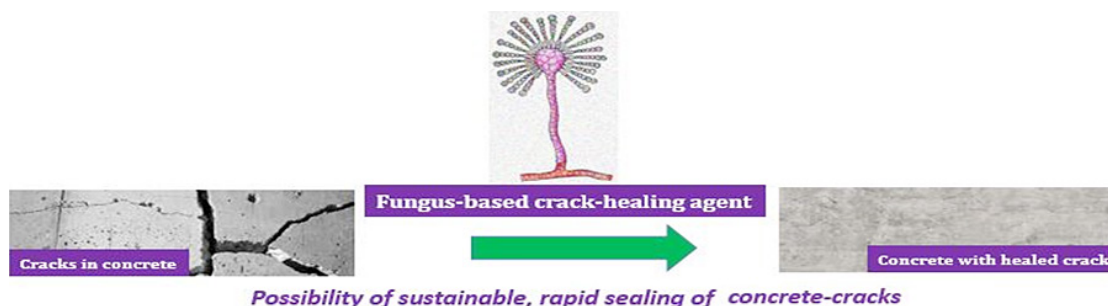


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GRAPHICAL ABSTRACT

**Introduction**

In the domain of civil engineering, the structural failure of concrete, an indispensable constituent when it comes to construction design, remains a pertinent issue.¹ Often, the strength and integrity of the internal structure of concrete is diminished by cracks of various nature that tend to propagate and augment with time. Contaminant ingress like rapid chloride penetration and infiltration of atmospheric water affect the durability of concrete and leads to subsequent corrosion of steel in reinforced concrete.¹ The hunt for sustainable and durable concrete, less prone to cracking has seeded the concept and catalysed the research in 'self-healing'.^{2,3} Amongst others, precipitation of calcium carbonate, admixture-mediated continual hydration, use of shape-memory materials and application of encapsulated/immobilized biobased healing agents have been reported in this context.⁴

On a note of pertinence, microbial healing of concrete-cracks has captured commendable research thrust over the years.^{3, 5-7} The bio-based healing strategy, relying on appropriately selected mineral-precipitating microbes may be projected as a safe, pollution-free, sustainable way to address the afore-stated engineering complicity. Microbe-mediated biomineralization leads to production of calcium carbonate (CaCO_3), the most apposite concrete-filler. A commendable micro-crack sealing potency, durable physicochemical interactions between crack and filler, desirable compatibility with the constituents of the concrete as well as intended thermal expansion have conferred a special niche to the microbial healing strategy in comparison to other approaches.⁵⁻⁸ However, it is pertinent to note that albeit, christened as 'healing based on

micro-organisms' (an umbrella-term), it is the bacterial systems that have captured the lion's share as far as the research reports and patents are concerned till date.⁹⁻²⁵ Bacterial-self healing relies on availability of nutrients and metabolic conversion to precipitate calcium carbonate, creating an optimal extracellular mineral phase-abundant micro-ambience to seal cracks. Electronegative charge in bacterial cell walls and large active surface area facilitate the attraction of calcium cations (Ca^{2+}) from the cementitious matrix and consequently results in calcium carbonate precipitation, which could be equal to or even greater than the cellular weight. It is critical to note that the ambient parameters dictate the efficacy of the bacterial precipitation. Culturing and handling of bacteria in a laboratory setting is not quite complex. Availability of various selective media has facilitated the collection and isolation of bacteria, suitable for *self-healing* protocol. A range of bacteria, with special thrust on the various species of the genus *Bacillus*, has been assessed for the purpose. The various routes/approaches (along with the major challenges) pertaining to bacterial assisted *self-healing* are highlighted in Table 1. On a pertinent note, Jin *et al.*, (2018)²⁶ had assessed the various practical snags in resorting to bacterial *self-healing* approach to seal concrete-cracks. Amongst others, a) diminished bacterial survival vis-à-vis deleterious concrete-ambience, b) negative trade-off with respect to the compressive strength of concrete as a consequence of incorporation of healing agents- bacterial spores and nutrients, besides c) little success in using bacterial cells to mediate accelerated CaCO_3 precipitation for sealing wide cracks demand special mention (although, scientific delving has been underway to address these issues). In this backdrop, of late,

researchers have started to assess another class of micro-organisms, namely fungi for prospective biohealing applications.^{27,28} However, we would like to emphasize upon the fact that the number of documents, reflecting the actual use of fungi for concrete healing, is extremely low as of

now. Nevertheless, the content in this article is streamlined towards understanding the justification, plausibility and expectations when resorting to fungal based crack-sealing in concretes. This write up is compiled as an attempt to gauge the potential of fungi for prospective bio-healing of concretes.

Table 1: Major highlights of the different routes of bacterial self-healing

Route/approach	Mechanism	Critical issue/ Disadvantage
Urea hydrolysis	CaCO ₃ precipitation is facilitated by ureolytic bacteria (e.g., <i>Bacillus sphaericus</i> and <i>B. pasteurii</i>) via urea hydrolysis (with production of two ammonium ions for each carbonate ion)	Excess nitrogen loading into the ambience
Organic compound pathway	Organic acids are metabolically oxidized, generating CO ₂ , leading to production of CO ₃ ²⁻ in an alkaline environment and subsequent precipitation of CaCO ₃ in presence of a calcium source	Requisite for the presence of calcium in high concentrated levels could eventually pile up excess salts in concrete
Dissimilatory nitrate reduction	Denitrifying bacteria mediate oxidation of organic compounds to assist CaCO ₃ generation.	Lesser efficiency to precipitate CaCO ₃ in comparison to ureolysis

Is Research on Fungi-Based Self-healing Concrete Worthwhile? - Mining the Contextual Information

Fungus represents an economically important class of micro-organisms that find applications across multiple agricultural, industrial and biomedical sectors.²⁹⁻³¹ It would be prudent to mine some background information and delve into the milieu that is expected to propel research on fungi-based self-healing concrete, as highlighted underneath.

Robustness and Adaptability of Fungi to a Gamut of Environmental Extremes/stress

Survival-success stories of fungi across various latitudes and longitudes of this globe (including the freezing polar caps, scorching Sahara, hypersaline Dead Sea as well as ~5000 m deep sea sediments³³ are some of the awe-inspiring biological revelations. Few fungal species (e.g., *Paecilomyces lilacinus* and *Chrysosporium* spp.) can proliferate well at even

pH-values of 7.5–11.034. Irrespective of extreme external pH, maintenance of an internal cellular pH at close to neutrality is facilitated by the presence of an osmotic barrier in the fungal cells. Studies have also attested the existence of fungi (survival, possibly facilitated by adept scavenging of nutrients from air and rain-water) as a pertinent constituent of epilithic and endolithic microbial communities in limestone, sandstone, granite, marble and gypsum and even rocks in Antarctica.³⁵ Sporulation is one of the commonly adopted fungal tactics to withstand extremes of nutrient scarcity, temperature, pH, pressure and irradiation.³³ In this milieu, probing into the prospects of using fungi under the deleterious ambient status of concrete with oxygen and nutrient limitation, moisture deficit and high alkalinity seems to be an interesting proposition. It is envisaged that this survival potency of fungi under environmental stress may aid in long-term self-healing of the concrete cracks.

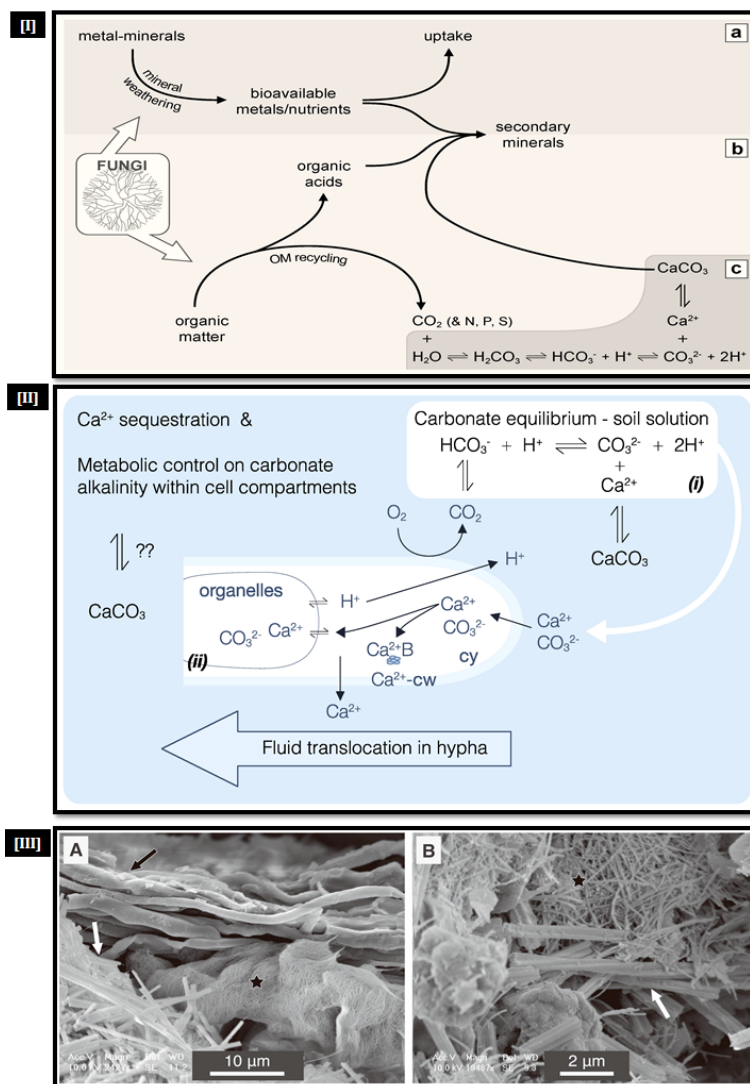


Fig.1: [I] Sketch showing the involvement of fungi in some global biogeochemical cycles; (a) Fungi contribute substantially to mineral weathering, leading to the release of bioavailable metals or nutrients, which eventually may be up taken by living organisms or precipitated as secondary minerals; (b) Fungi as heterotrophs, recycle organic matter (OM). While doing so, they produce metabolites such as organic acids that can also precipitate as secondary minerals (salts). OM recycling eventually releases constitutive elements such as C, N, P and S; (c) CO₂ produced by heterotrophic fungal respiration can dissolve into H₂O and depending on the physicochemical conditions precipitate as CaCO₃ leading to the formation of secondary mineral. [II] Processes potentially leading to fungal CaCO₃ biomineralization at the fungal microenvironment. These processes are mainly linked to both Ca²⁺ sequestration and metabolic control of carbonate alkalinity within cell compartments (cell wall (cw), cytoplasm (cy), and organelles) of fluids, trans located inside the hypha. (i) Carbonate alkalinity levels in the fungal microenvironment influence Ca²⁺ and CO₃²⁻ bioavailability; (ii) Ca²⁺ sequestration rate by fungi in the different cellular compartments influences Ca²⁺ bioavailability. Ca²⁺ can be present within the cell-wall (Ca²⁺-cw) as free cytoplasmic Ca²⁺ bounded to proteins (Ca²⁺-B) or stored in organelles. Finally, fungi may

exert a metabolic control on intra-hyphal alkalinity levels (represented as CO_3^{2-}), through pH regulation for instance (e.g., H^+ excretion). [III] Scanning Electron Microscope (SEM) images of natural samples of secondary CaCO_3 deposit composed of needle fibre calcite (NFC; white arrows) and nanofibres (black stars) from a *calic Cambisol humic calcaric skeletal soil* (WRB 2006) in Villiers, Switzerland. Both images (A) and (B) are from a fungal rhizomorph (fungal hyphae are shown in A by a black arrow) associated to a limestone fragment from deep mineral layers of a soil covered with secondary CaCO_3 deposits. (Reproduced from Bindschedler *et al.*, (2016),³⁶ under Creative Commons Attribution 4.0 International License, Copyright © 2016, The Authors, licensee MDPI, Basel, Switzerland.)

Fungi-Mediated Calcium Mineralization: Exemplification and Plausibility In Harsh Concrete-Ambience to Seal Wide Cracks Rapidly

Fungal Metabolic Activities Promote Biomineralization of Calcite

Induced biomineralization marks bacterial-assisted mineral precipitation. On the other hand, fungi are capable of adroitly employing both induced biomineralization and organomineralization strategies to precipitate calcium carbonate. Figure 1 [I] depicts the participatory role of fungi in some global biogeochemical networks while Figure 1 [II] is illustrative of various routes, directed towards CaCO_3 biomineralization at the fungal micro-ambience. Active pumping out of calcium ions or complexation with cytoplasmic proteins ensure the maintenance of stringent calcium ion gradient, critical for apical growth in fungi.³⁶ Relevantly, various fungal metabolic activities (e.g., nitrate assimilation, urea degradation, organic acid oxidation etc.)³⁶ augment the alkalinity of water that facilitates calcium carbonate biomineralization. As a point of pertinence, Bindschedler *et al.*, (2016)³⁶ had resorted to scanning electron microscopic imaging (Figure 1 [III]) to probe into the fungi mediated secondary CaCO_3 deposition in natural environment.

Architectural Shape and Make

Availability of a number of nucleation sites and skeletal assistance for calcium carbonate precipitates, conferred by the branched architecture and filamentous morphology of fungi is a clear advantage over the minute bacterial cells with simpler shape. Excellent biosorption attribute of chitinous (chitin: a nitrogen-rich polysaccharide) cell walls of fungi has conferred a special niche to this microbial group in metal-uptake studies, biosynthesis of nanobiomaterials and antimicrobial assessment of nanomaterials.³⁷ It is envisaged that

calcite may nucleate and eventually grow on fungal hyphae due to the fact that chitin has the potency to drop the activation energy barrier, required for nucleus formation.³⁸ Consequently, calcium cations on interacting with soluble CO_3^{2-} is expected to yield CaCO_3 deposits on fungal hyphae.

Potency of Organomineralization for Calcium Carbonate Precipitation

Dead and inactive fungal hyphae may also act as substrate since biosorption operates in a manner, free from the dictates of the metabolic status of the fungi.^{36,38} Breakdown of fungal filaments is expected to release the crystals of CaCO_3 , thereby, endowing additional calcite precipitation sites.

Evidence of Calcium Mineral Precipitation in low-Nutrient Environment

Burford *et al.*, (2006)³⁹ had resorted to X-ray diffraction study of crystalline precipitates on the hyphae of *Serpula himantoides* and a *Cephalotrichum* sp. (a limestone fungal isolate). The precipitates of calcite and little whewellite on *S. himantoides* hyphae and calcite or of a mixture of calcite and whewellite on the limestone isolate hyphae attested the deposition of calcite and secondary calcium minerals on fungal substratum even in a nutrient-deficit environment.

Precipitation of Oxalates and Possible Defensive Strategy to Ameliorate Metal-Stress

Release of oxalic acid by the filaments of few fungal species (*Aspergillus niger*,⁴⁰ *S. himantoides*⁴¹) and subsequent precipitation of calcium oxalate in CaCO_3 abundant surrounding is an interesting observation. This is pertinent in the sense that subsequent oxalate-oxidation and fungal metabolic activity-mediated release of CO_2 adds to the carbonate build up in the vicinity and is siphoned towards CaCO_3 precipitation. Precipitation of metallic

minerals onto fungal hyphae has been proposed as a pivotal mechanism to explain their survival and multiplication in apparently metal-polluted areas.^{36,42} In other words, calcium carbonate precipitation could be viewed as a self-protective strategy of fungi to avoid metal-stress. Having said that, concrete, representing a calcium-rich material could create stress conditions in the context of possible calcium cytotoxicity and altered osmotic pressure, thereby, raising the possibility of the desired precipitation in concrete crack-healing.

***In Vitro* Demonstration of Rapid and Copious Precipitation**

Accelerated and copious calcium carbonate precipitation of fungi has been documented previously. The following two exemplary reports vouch for the following:

(a) Appearance of immature calcite crystals post 12 h of exposure of aqueous calcium ions to two different fungal species, followed by maturation to smooth structures (plate-shaped crystals for *Trichothecium* sp. and ring-like superstructures for *Fusarium oxysporum*) after 3 days.⁴³

(b) Profuse CaCO₃ crystallization post 21 days of incubation of *Serpula himantoides* in a micro-niche of carboniferous limestone at 2°C.³⁹ Pertinently, both living and dead fungal biomass were effective in the crystallization.

Widening the Possibility with Genetically Engineered Strains

Recent endeavours have also projected the prospects of genetically engineered fungi for self-healing of concrete. The transcription factor, PacC mediates fungal response to the pH levels of the surrounding.⁴⁴ Trimming the C-terminal region of the pacC regulatory gene leads to generation of gain-of-function pacC^c mutations resulting in alkalinity mimicry via circumventing the requisite of external pH cues and sustained activation of alkaline-responsive genes with super-repression of acid-responsive genes. Such molecular level engineering elicits an ambient-pH independent gene expression-pattern in the mutants analogous to the one exhibited in wild type strains when cultured under alkaline pH, thereby, raising the prospects of the former in *self-healing* applications.

Considering the afore-stated, it is expected that appropriately selected species of fungi would be able to thwart the challenges of the harsh environment of concrete and adroitly be capable of mediating copious CaCO₃ precipitation at a rapid pace to seal even larger cracks.

Can Fungal *Self-Healing* Concrete Meet the Requisites of Compressive Strength?

Self-healing concrete structures would find their real sustainable applications when the compressive strength of the concrete is not compromised with, amongst other factors. It may be argued that comparatively larger size of fungal spores (than the bacterial counterparts) would be a serious practical snag. Use of such large spore-containing immobilization matrix or capsules is expected to drop the compressive strength tremendously. Incorporation of excess healing agents would imply appearance of considerable voids post ripping apart of the capsules. Porous-carrier mediated substitution of definite quota of sand and gravel, the possible dispersal of un-encapsulated nutrients in the vicinity as well as inadequate interaction among the concrete matrix and healing agents are expected to contribute to the probable decline in compressive strength. Pertinently, ~24% augmentation of compressive strength of mortar specimen post *self-healing* of cracks was reported on employing immobilized *S. pasteurii* on porous glass beads.⁴⁵ The point here is the absolute requisite of an optimization of the size-shape-surface chemistry-amount-distribution accord of the fungal based healing agents to ensure sealing of cracks without negatively affecting the compressive strength.

Anticipated Hallmarks of an Ideal Fungal Species for *Self-Healing* Applications

Based on the afore-stated, a few stringent parameters should be appropriately addressed while selecting fungal species for the proposed application of concrete-self healing.

Non-Pathogenicity is a Prime Requisite

Accidental release of spores may prove detrimental to the nearby crops/plants if the latter serve as hosts for germination and proliferation of the former. Although fungal diseases are more apparent in plants, it, however, does not imply that the animal world (particularly the mammals including humans)

is completely free from fungal assaults. Appropriate carrier materials must be assessed to prevent unintended consequences.

Amenability for Inclusion and Preservation of Long-Term Viability Within the Harsh Ambience of the Concrete Infrastructure

The fungal species must be able to withstand mechanical stress while mixing as well as exhibit sustained viability under high-pH conditions of the concrete. During the occurrence of cracks and subsequent entry of water and oxygen, it is expected that germination of the dormant fungal spores, growth and consequent calcium mineral precipitation would lead to in situ healing of cracks at a rapid pace. Post filling of cracks and recreation of stress-ambience, the fungal species is expected to undergo sporulation again. In this regard, alkaliphilic filamentous spore-forming fungi seem to be good option.

As mentioned in the preceding section, genetically manipulated strains have provided a better platform to understand the prospects of using fungi for *self-healing* applications. Availability of the genomic-information of the selected fungus at the public domain is expected to facilitate genetic alterations to amplify its potency for rapid and sustainable crack repair. Well comprehended genetics and availability of wide range of mutants (as in the case of *Trichoderma reesei* and *Aspergillus nidulans*,^{46 47}) with engineered metabolic pathways could be instrumental in this realm,

Recent Exemplary Endeavours

Recently, Jin and colleagues²⁶ had advocated the exploitation of fungi in the domain of crack-sealing in concrete although, Žáková *et al.*, (2019)'s report evinced that *Bacillus pseudofirmus* (a bacterial species) had better edge than *T. reesei* (a fungus) when explored for filling pores and microcracks on cylindrical concrete specimens (diameter: 55 mm and height: 5 mm).⁴⁸ However, as noted in the preceding sections, a number of fungal species may be bracketed together with the potency to promote calcium mineralization and other desirable

facets for prospective *self-healing* applications. Amongst others, a recent report on the exploitation of a urease-positive fungal strain, *Penicillium chrysogenum* CS1 for biocementation of sand in column and documentation of compressive strength as high as 1800 kPa for the biostone, generated as a consequence of fungal mycelia mediated cementing of the sand granules, merit special mention.⁴⁹ Similarly, Martuscelli *et al.*, (2020)'s investigation had testified the CaCO₃ biomineralization potential of a number of urease-positive fungal strains (*Cladosporium herbarum*, *Cladosporium angustitherbarum* and *Penicillium brevicompactum*), isolated from concrete constructions at different sites in Guimarães, Portugal for application in biocementation strategies.⁵⁰

At this juncture, it is crucial to mention that only recently, research has been directed towards systematic endeavouring to screen various fungal species *vis-a-vis* concrete-crack sealing potency.²⁷ Amongst others, researchers²⁷ had assessed the prospects of *T. reesei* (ATCC13631) and *A. nidulans* (ATCC38163), both procured from American Type Culture Collection (ATCC) apart from four other fungal strains, *Cadophora interclivum* (BAG4), *Umbeliopsis dimorpha* (PP16-P60), *Acidomelania panicicola* (8D), and *Pseudophialophora magnispora* (CM14-RG38), isolated from the roots of plants, harboured in nutrient deficit soils. For screening, post overlaying of fungal growth medium onto cured concrete plate, the researchers had aseptically placed mycelial discs at the plate center. Dissolution of Ca(OH)₂ from concrete resulted in shift of pH values towards highly alkaline side (from 6.5 to 13.0). Interestingly, in spite the dramatic pH modulation, germination of the *T. reesei* (ATCC13631) spores into hyphal mycelium and consequent growth were documented, irrespective of the presence or absence of concrete. The other species failed to show similar response. Calcite-precipitation on the fungal hyphae was corroborated by X-ray diffractometric (XRD) analysis and scanning electron microscopic (SEM) imaging. The researchers forwarded that *T. reesei* could be a suitable candidate for bio-based *self-healing* in concrete.

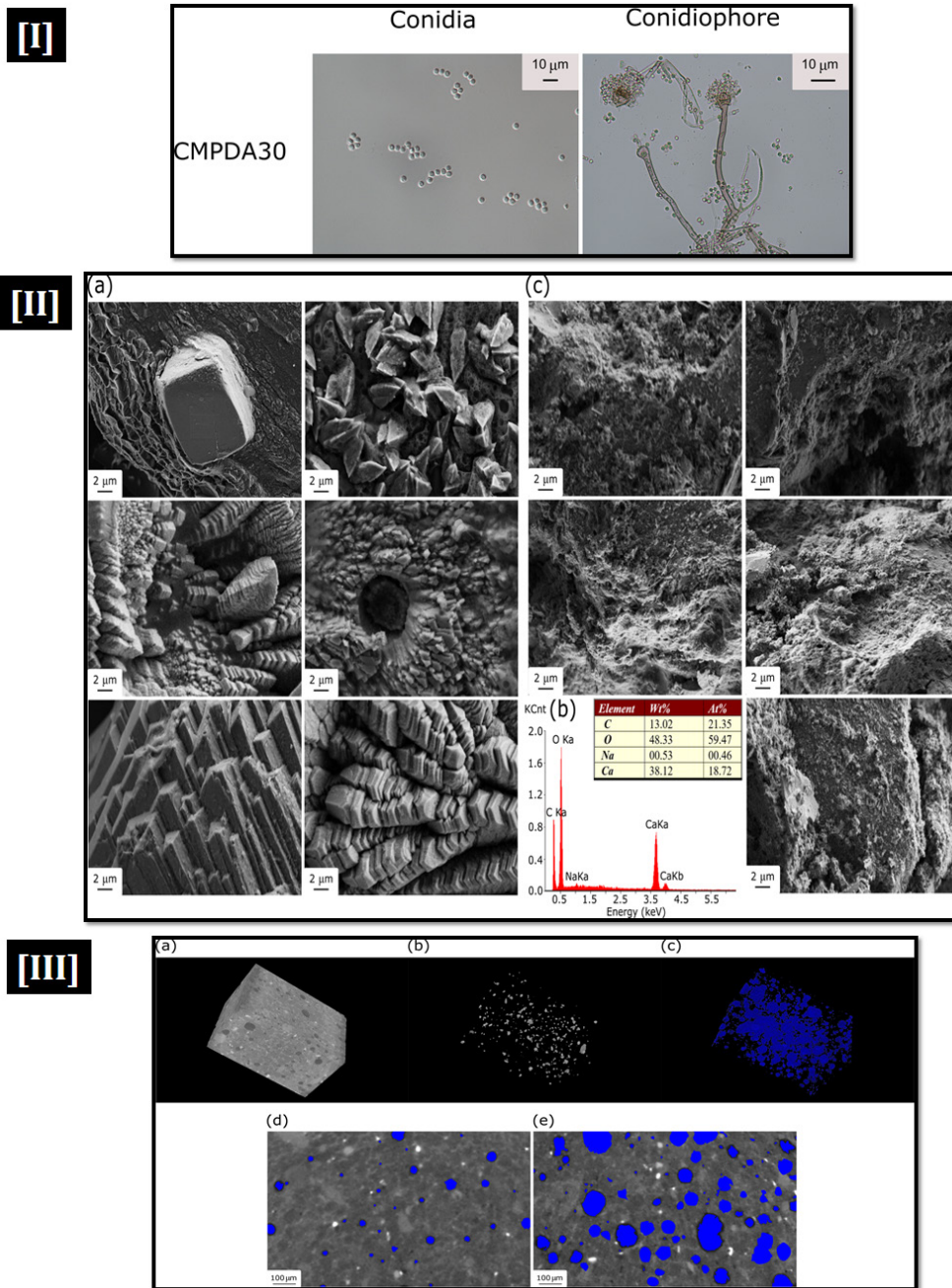


Fig. 2: [I] For *A. nidulans* (MAD1445), plentiful conidia were found in the case of CMPDA 30. The diameter of *A. nidulans* spores is typically larger than 3 μm . [II] SEM and EDS results of the collected solids: (a) *A. nidulans* (MAD1445)-inoculated media; (b) EDS spectra; and (c) fungus-free medium. [III] A 3D rendering of a grayscale volume is shown in (a), the corresponding high-density particles and pores are shown in (b,c), respectively. (d) Pore size distribution inside cement paste specimens after 28 days of curing measured by μCT . (e) Effect of air-entraining agent on pore size distribution measured by μCT . (Reproduced from Mennon *et al.*, (2019),²⁸ under Creative Commons Attribution 4.0 International License, Copyright © The Authors, 2019)

On the other hand, it is pertinent to note that *A. nidulans* represents one among the filamentous fungi, amenable for easy gene manipulation. In this backdrop, three alkalinity-mimicking mutants of *A. nidulans*, i.e., MAD1445, MAD0305, and MAD0306, apart from five wild-type strains (placed in the Biosafety Level 1)- *Rhizopus oryzae* (ATCC22961), *Phanerochaete Chrysosporium* (ATCC24725), *A. nidulans* (ATCC38163), *A. terreus* (ATCC1012), and *A. oryzae* (ATCC1011); as well as *Saccharomyces cerevisiae* (a non-filamentous, single-celled fungus) were assessed for their potential in *self-healing* applications.²⁸ Gene replacement protocol was adroitly employed to obtain the alkalinity-mimicking mutations (alleles *pacC14* and *pacC202*). Similar to previous observation, leaching of $\text{Ca}(\text{OH})_2$ into infrastructure. In this context, myco-informatics and genetic engineering could play an important role in elucidation of genetic make-up and plausible genetic manipulation to augment the fungal adaptability under stress conditions and potential for rapid mineralization. Optimization in terms of loading percent of the healing agents (fungal spores and nutrients) and selection of appropriate carrier materials is prerequisite to avoid negative infliction on concrete strength. Furthermore, deeper insight at the microscopic and molecular levels would open up new portals to comprehend the mechanisms, underpinning the fungus-mediated crystal nucleation and eventual growth that would permit the shape-size tuning of the precipitated minerals. Microscopic assessment of the interface at the concrete crack and the fungal precipitates as well as information related to the bond coherence would be critical to prevent future cracks. On the other hand, numerical simulation studies on the dictates of design-parameters over pace and quality of crack-sealing could reduce expenses in terms of resources/materials and time. In the backdrop of comparative ease in acquiring, handling and culturing fungi in large quantities, and simpler nutritional needs (many species, being oligotrophic) in contrast to bacterial-systems, the cost of fungal based *self-healing* concrete is expected to be low. However, commenting on the life-cycle cost of this strategy would be an imbalanced projection at this moment as current endeavours (highlighted in subsequent sections) attest only the 'proof of concept' status. Understanding the fungi-mediated CaCO_3 based precipitation could open up research in the domain

The researchers in both these studies^{27,28} did not delve into the modulations in the physicochemical attributes of concrete including strength, carbonation resistance etc. in the presence of fungal healing agents. Furthermore, it is crucial to note that the amount of the mineral crystals observed in these studies was meagre, as reported by the authors. Thus, there lies an absolute necessity of optimization-study pertaining to various factors including temperature, fungal spore concentration, fungal-growth medium composition and other additives. Nevertheless, these studies have surely set the platform for further research.

Future Direction and Conclusion

of heavy metal mitigation, carbon sequestration as well as augmented oil recovery. The field is still in its incipient stage and thus calls for greater digging for the unmasking of an economically feasible *self-healing* strategy based on fungi.

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Conflict of interest

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