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An Overview on Fungi as Self Healing Agent in Biomineralization of Calcite

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Abstract: The main objective of the present paper is to discuss the applications of fungi to improve the quality of cement concrete. Though, much work has already been carried on with the use of ureolytic bacteria as the calcite precipitation in bioconcretes, fungi are also known to be involved in doing the same job very efficiently. Several studies have demonstrated the ability of fungi to produce calcite during the last one decade. However, the exact mechanism leading to calcite precipitation by fungi is still unknown requiring further research exploring the facts to complete the various steps involved. The present review described the many fungi inducing the biomineralization of calcite to produce bioconcretes. The review also discusses the overall methodology of culture of fungi, biotechnology of bioconcrete production and the physicochemical and bioengineering properties of bioconcretes in association with fungi.

Key words: Fungi, bioconcrete, self healing, biomineralization, calcite precipitation.

1. INTRODUCTION

Several researches have so far been documented the role of bacteria to verify the fact that they have got natural ability for calcite precipitation via the hydrolysis of urea. It may heal the cracks effectively if mixed in cement concrete (Jonkers *et al.* 2010, Pradeep *et al*. 2015, Fatma *et al.* 2016, Alshalif *et al.* 2017, Vijay *et al.* 2017 and Anitha *et al.* 2018)[2,3,11,20,29,38]. The bacteria have gained a great success and popularity as beginners in achieving the ultimate goal of healing the cracks formed in fresh concretes. But, it appears that still they are bound with some restrictions. Therefore, the present paper deals with the study of a new self healing agent named fungi have been explored to heal the cracks in concrete infrastructures. In this study, a novel self-healing approach is discussed, in which fungi like *Trichoderma reesei* and many others are used to heal concrete cracks by promoting $CaCO_3$ precipitation. This is also called as microbiologically induced calcite precipitation (MICP) and perhaps, one of the most important multidisciplinary works done so far in the fields of microbiology with civil engineering. This is a kind of interdisciplinary research comprising the study of bioconcrete technology incorporating the living fungi with some abiotic materials to improve the quality of concrete and to fill the cracks successfully in future.

2. MATERIAL AND METHODS

The present paper is prepared on the basis of researches done so far in the field of bioconcrete technology. Several research papers were consulted in order to explore the facts found therein and have been discussing in the light of recent researches. We have tried this section to be written in such a way that those interested to work in the same field might make opportunity to repeat the experiments easily.

A. Experimental Design

1. Choice of experimental organisms and culture of fungi

Very few ureolytic fungi have so far been exploited for testing the bioengineering properties of bioconcretes (Table 1). Recently, Jing Luo *et al.* 2018[19] has found an ureolytic fungus named *Trichoderma reesei* showing good results in bioconcrete technology. This was purchased from American Type Culture Collection (ATCC).

Potato dextrose agar (PDA) medium has been found as the best growth medium for *Trichoderma reesei.* The cultures were grown in the same medium at 25 ± 2 ^{0}C for 7 days. The pure and stock cultures were also maintained in the same medium.

Finally, the cultures were refrigerated at 4 ^{0}C for further use. Subculturing was also done in periodic intervals of 3 months. Some of the very common culture media used to culture different fungi for stock cultures, pure cultures and the cultures refrigerated for further use are given as under:

(a) Potato Dextrose Agar (PDA) medium

This is a relatively rich medium for growing a wide variety fungi supplemented with antibiotics to inhibit the growth of bacteria. PDA can be used for growing clinically significant yeasts and molds encouraging luxuriant growth of fungi. An antibiotic used to inhibit the growth of fungi is either streptomycin or chloramphenicol.

Composition of PDA medium

Ingredients		**g/L**
Potato infusion	-	200g
Dextrose	-	20g
Agar-Agar	-	20g

(ph adjusted to 5.6 at 25 ± 2 ^{0}C)

(b) Sabouraud's Dextrose Agar medium

This is also a general medium used to culture a wide variety of fungi, naturally inhibiting the growth of bacteria, though, if required antibacterial agents may be added to augment the antibacterial effect. Antibiotics like chloramphenicol, gentimicin or tetracycline can be used selectively to inhibit the growth of bacteria as and when required.

Composition of SDA medium

Ingredients		**g/L**
Peptone	-	10.00g
Dextrose	-	40.00g
Agar-Agar	-	15.00g

(pH adjusted to 5.6 at 25 ± 2 ^{0}C)

(c) Czapek Dox Liquid medium

This medium is used for the cultivation and maintenance of numerous fungi which are able to utilize sodium nitrate and sucrose as the sole sources of nitrogen and carbon respectively. To solidify the medium 15g agar-agar is also added in the same. The medium is originally developed by Czapek and Dox to grow *Aspergillus niger* and *Penicillium camemberti.* This is used for many saprophytic fungi and soil bacteria.

Composition of CDL medium

Ingredients		**g/L**
Sucrose	-	30.00g
Sodium nitrate	-	02.00g
Magnesium glycerophosphate	-	00.50g
Potassium chloride	-	00.50g
Dipotassium sulphate	-	00.35g

Ferrous sulphate - 00.01g

(pH adjusted to 6.8 ± 0.2 at 25 ± 2 ^{0}C)

(d) Asthana and Hawker's Liquid medium

This is also a good culture medium for fungi investigated by Asthana and Hawker in 1936[6]. To solidify the medium 20g agar-agar is also added in the same.

Composition of AHL medium

Ingredients	-	**g/L**
Glucose	-	5.00g
Potassium nitrate	-	3.50g
Potassium Hydrogen phosphate	-	1.75g
Magnesium sulphate	-	0.75g

(pH adjusted to 5.5 at 25 ± 2 ^{0}C)

The culture medium thus obtained is to be sterilized by autoclaving them at 121 ^{0}C for 20 min. It is cooled to room temperature then either fungal cells or spores were added to the same medium using a device known as laminar air flow chamber. This is incubated at 25 ± 2 ^{0}C on a shaker incubator at 130 rpm. The fungi and their spores are harvested by the separation techniques from 7 days old cultures.

2. Preparation of concrete matrix

With the help of suitable ingredients like cement, aggregates and water, the fresh concrete or mortar of M20 grade (1:1.5:3) is prepared. This is made with or without fungi (control). The following materials are usually required for the formation of bioconcretes of different compositions:

(a) Portland cement (53 grade)

(b) Aggregates

Fine aggregates (Natural river sand)

Specific gravity	-	02.69
Maximum size	-	04.75 mm

Coarse aggregates

Specific gravity	-	02.70
Maximum	-	20.00 mm

(c) Locally available clean water

(d) Fungal sample

Either fungal culture or spores is added in concrete. The fungal spore powder may be added in concrete in association with clay particles. This is done to enhance the viability of bacteria for a longer period of time.

3. Casting of cubes

With the help of cement, aggregates and water, the fresh concrete or mortar of M20 grade (1:1.5:3) is prepared with or without (control) fungi. This is poured into moulds (100mm × 100mm × 100mm) and then left to harden for 24 hours.

After casting of these cubes are demoulded and immediately dipped in clean and fresh water of the curing tank for further period of 24 hours. After curing period is completed these specimens were taken out from water and kept in a shade to dry off. The curing time is fixed depending on the types of experiments to be conducted as 7, 14 and 28 days for the suitability of their bioengineering properties (Kunamineni and Meena 2018)[22].

4. Formation of cracks and self healing in cubes

The 100 mm sized cubes is to be pre-cracked at the age of 28 days by using compression testing mechine and kept in water for curing of 2 weeks.

B. Determination of Bioengineering Properties of Bioconcretes

Very few ureolytic fungi have so far been exploited for testing the various bioengineering properties of bioconcretes (Table 1).The various bioengineering properties of bioconcretes are examined and compared to normal concrete for a maturity period of different intervals. Some of these properties are briefly described as under:

(a) Scanning electron microscopic analysis (SEM)

A Hitachi S-3400N variable pressure scanning electron microscope (VPSEM) equipped with a Oxford Inca Energy 250 energy- dispersive spectrometer (EDS) has been using to visualize and determine the various bioengineering properties of bioconcretes (Jean *et al.* 2017)[18].

(b) Energy dispersive X rays spectroscopy (EDX)

The amount of Ca measured for the specimens with or without fungi have been visualized and estimated with the help of SEM and EDX (Asad and Roshni 2017)[5].

(c) Compressive strength testing of bioconcretes

It is done with the help of automatic compression testing mechine COMPTEST 3000 (Gavimath *et al.* 2012)[14] accordingly as per IS516 : 1964 to record the ultimate loads for failure. The load is applied at a constant rate of 140 Kg/cm^2/min. The compressive strength is calculated using the formula as :

$$\text{Compressive strength} = \frac{P}{N}$$

Where,

P = Load in (N)

A= Area in (mm^2)

(d) Split tensile test (Monishaa and Nishanthi 2017)[28]

This is also tested with the help of compression testing mechine accordingly as per IS 516 : 1964. The Specimen is kept horizontally in the mechine and the pressure applied until failure of the cylinder. The failure load noted and strength is calculated using the formula as :

$$\text{Split Tensile Strength} = \frac{P}{N}$$

Where,

P = Ultimate load (N)

L = Length of cylinder (mm)

D = Diameter of cylinder (mm)

(e) Flexural strength test (Monishaa and Nishanthi 2017)[28]

The flexural strength is the ability of a beam or slab to resist failure in bending and is measured by universal testing mechine accordingly as per IS 516 : 1964. This is also expressed as the "Modulus of rupture" in N/mm^2. This is about 12 to 20 % of compressive strength. The specimen is kept horizontally between the loading surfaces in the mechine and the load applied until failure of the cylinder. The failure load and shorter length from crack to support strength is measured and flexural strength calculated using the formula as :

When $a \geq 133$ mm

$$R = \frac{PL}{bd^2}$$

When $110 < a \leq 133$ mm

$$R = \frac{3Pa}{bd^2}$$

Where,

R = Modulus of rupture in N/mm^2,

P = Maximum load in N,

L = Span in m,

a = Shorter length from crack to support in mm,

b = Average width in mm and

d = Average depth in mm

(f) Evaluation of pore size distribution in aging bioconcrete specimens

It has been determined by mercury intrusion porosimetry (MIP) with the help of a Micromeritics Autopore IV Mercury Porosimeter as followed by Jonkers *et al.* 2010[20]. It is usually carried out with or without incorporated ingredients.

(g) Acid durability test (Gavimath *et al.* 2012)[14]

The specimens to be examined are immersed in 5% solution of sulphuric acid and evaluated in terms of compressive strengths. Similarly, for determining the concrete resistance to the aggressive environment the durability factor as proposed by the philosophy of ASTM C666-1997 is followed.

Acid Durability Factor (ADF) is determined as

ADF = Sr.(N/M)

Where,

M = The number of days when exposure was terminated

N = The number of days at which durability factor is required

Sr. = Relative strength at days (%)

(h) Electrical resistivity (Kunamineni and Meena 2018)[22]

The electrical resistance of concrete is measured using a Leader RCON™ Concrete Electrical Resistivity Meter at one pre-determined location on each test specimens. The electrical resistivity with an average value of the electrical resistance is calculated by the following expression:

$P = RA/l$,

Where,

p is electrical resistivity (unit: Ω· m),

R is electrical resistance (unit : Ω),

A is cross sectional area (unit: m^2) and

l is electrical path length (unit : m)

Table 1. A list of some fungi studied in biomineralization of calcite in bioconcretes

S.N.	Name of fungi	Sources
1.	*Aspergillus niger*	Gharieb *et al*. 1998[15]
2.	*Aspergillus nidulans*	Jing Luo *et al*. 2018[19]
3.	*Neurospora crassa*	Li *et al.* 2014[23]
4.	*Cephalotrichum sp.*	Burford *et al*. 2006[10]
5.	*Morchella sp.*	Masaphy *et al.* 2009[27]
6.	*Trichoderma reesei*	Jing Luo *et al*. 2018[19]
7.	*Cadophora interclivum*	Jing Luo *et al*. 2018[19]
8.	*Serpula himantioides*	Gharieb *et al.* 1998[15]
9.	*Piloderma fallax*	Tuason and Arocena 2009[34]
10.	*Acidomelania panicicola*	Jing Luo *et al*. 2018[19]
11.	*Umbeliopsis dimorpha*	Jing Luo *et al.* 2018[19]
12.	*Beauveria caledonica*	Fomina *et al.* 2005[12]
13.	*Pseudophialophora magnispora*	Jing Luo *et al.* 2018[19]
14.	*Myrothecium gramineum*	Li *et al.* 2015[24]
15.	*Colletotrichum acutatum*	Li *et al.* 2018[25]

3. RESULTS AND DISCUSSION

While bacteria has already been researched as an excellent microorganisms for calcite precipitations in cement concrete recently, the fungi are also being exploited as bioconcrete material. But, it appears that very few fungi have so far been examined despite the fact that they are ubiquitous in their distribution both in aquatic and terrestrial environments. In fact, this is quite a new group of microorganisms introduced in the same field. The basic difference between bacteria and fungi are there in structural as well as genetical organizations as they are prokaryotes and eukaryotes whose cell walls are made of mucopeptide and chitin respectively.

The fungi are a group of eukaryotic organisms usually heterotrophic in nature made of mycelia and hyphae reproducing well with their fruiting bodies and spores. They are able to survive in unfavorable conditions by asexually reproducing spores. These spores lie dormant for a longer period of time and regerminated when conditions become favourable. These are mostly found on soil surface where oxygen is present. However, some of them are able to live in aquatic ecosystem and even in the absence of oxygen. They are also able to promote mineral precipitation via both induced biomineralization and organomineralization (Manoli *et al.* 1997, sayer *et al.* 1997, and Gharieb *et al.* 1998)[15, 26, 31].

The $CaCO_3$ has ubiquitously been found to be associated with fungi but their biomineralization studies have only begun within the last decade. The urease producing fungi like *Neurospora crassa* and *Alternaria* sp. induced the biomineralization of calcium carbonate (Ahmad *et al.* 2004 Rautaray *et al.* 2004, Hou *et al.* 2011, Li *et al.* 2014 & 2015)[15,23,24,26,31]. Some other fungi studied in the biomineralization of $CaCO_3$ are *Serpula himantioides, Cephalotrichum*, *Morchella sp., Aspergillus niger, Piloderma fallax, Beauveria caledonica, Pseudophialophora magnispora Myrothecium gramineum* and *Colletotrichum*

acutatum (Gharieb *et al.* 1998, Fomina *et al.* 2005, Burford *et al.* 2006, Masaphy *et al.* 2009, Tuason and Arocena 2009, Li *et al.* 2015 & 2018 and Jing Luo *et al.* 2018)[10,12,15,19,24, 25, 27, 34].

Similarly, Jing Luo *et al.* 2018[19] have chosen six fungi for their study as *Trichoderma reesei, Aspergillus nidulans, Cadophora interclivum, Umbeliopsis dimorpha, Acidomelania panicicola and Pseudophialophora magnispora.* They have reported that only *Trichoderma reesei* has been found to be able to grow well on the concrete plates promoting calcium carbonate precipitation. This is an outcome of the dissolution of CO_2 reacting with Ca $(OH)_2$ in concrete. Further, this is well supported by the facts that some fungi are able to repair the cracks more efficiently via the mineralization of calcium (Manoli *et al.* 1997, Sayer *et al.* 1997, Gharieb *et al.* 1998, Verrecchia 2000, Takey *et al.* 2013 and Bindschedler *et al.* 2016)[7,15,26,31,33,36].

As fungi are found to be involved in calcite precipitations, their metabolism can also directly or indirectly influence the alkalinity and the calcium concentrations. Further, it has been observed that the apical growth of fungal hyphae is under the strict control of the concentrations of Ca^{2+} ions. The fungal cell wall material, chitin has also been found to be a well known polymer to bind the calcium ions. The calcification of fungal filaments is a very complex phenomenon which is still to be studied in much detail to elucidate the various steps involved. Since, fungi form a beautiful three-dimensional network of mycelium, it appears this is quite advantageous that it provides some extra sites for the deposition of calcite. (Manoli *et al.* 1997 Sayer *et al.* 1997 Gharieb *et al.* 1998 Sterfingler 2000, Boswell *et al.* 2007, Boven *et al.* 2007 Burford *et al.* 2006, Kolo *et al.* 2007 and Hou *et al.* 2013, and Jing Luo *et al*. 2018)[8,9,10,15,16,19,21,26,31,32].

Further, as the fungal cell walls are usually having a very high mechanical resistance, it has been postulated that they might be actively engaged in drilling mineral substrates (Van Scholl *et al.* 2008)[35]. The fungi grew in cracks easily due to mechanical pressures and tighten the cleaves as a result of differential bioweathering. Both active and passive mechanisms are associated with metals and minerals immobilizations leading to formation of biominerals especially the metal oxalates (Sterfingler 2000, Burford *et al.* 2006, Gadd 2007 and Tuason and Arocena 2009)[10,13,32,34]. The excretion of organic acids especially the oxalic acid helps in the reprecipitation of calcium minerals. These secondary cementations in concrete by oxalate salts in fungi could also be degraded to carbonates (Verrechia *et al.* 1993, Arnott 1995, Burford *et al.* 2006 and Tuason and Arocena 2009)[4,10,34,37].

In addition, the fungi to be chosen as experimental material for biomineralization of calcite in bioconcrete technologies should follow the following norms given as under:

- must not be allergic and pathogenic to human health
- should survive in the harsh environment of cement concrete
- should be easily available and cultured in laboratory environments
- must be ureolytic in nature if producing calcite in cement concrete

Lastly, though fungi have also played an alternative role in bioengineering of bioconcrete technologies nonetheless, they are in a juvenile stage and the exact mechanism of calcite precipitation in bioconcrete seems to be more thoroughly assessed in future (Bindschedler *et al*. 2016)[7].

4. CONCLUSION

The fungi have got natural ability for calcite precipitation via the hydrolysis of urea. It may heal the cracks efficiently if mixed with cement concrete. The present paper has documented the role of fungi in producing calcium carbonate in bioconcretes and biogrouts. But, the exact mechanism involving fungi to lead the precipitation of $CaCO_3$ is still unknown requiring further research. Therefore, the present review is an attempt to explore the various facts involved. As this is a kind of multidisciplinary research work involving civil engineering with life sciences, it could be a novel strategy to restore the concrete quality to obtain more sustainable ecofriendly microbial technology providing us high quality, cheap and more durable building materials in future.

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6. CONFLICT OF INTEREST

The authors have declared no conflict of interest. They have approved the final version of the manuscript contributing equally.

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