 doi:10.20944/preprints202104.0019.v1

Review

An Overview on Bioconcrete and the Utilization of Microbes in Civil Engineering

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Abstract: The advancement of bioconcrete over cementitious composites has brought us to the application of microbes in the field of construction materials. Certain microbes like bacteria, algae, and fungi have been discussed in the review. The purpose of applying these microbes in the matrix is mainly to enhance the concrete's strength and other properties such as durability, resistance, and self-healing ability. As these microbes are able to induce calcite biomineralizations, the process is also known as Microbiologically Induced Calcite Precipitation (MICP). Some known microorganisms with their mentioned ability are *Bacillus subtilis* and *Bacillus cohnii* (bacteria), *Chlorella vulgaris* and *Spirulina platensis* (algae), and *Trichoderma reesei*, *Aspergillus niger*, and *Neurospora crassa* (fungi). The paper provides a "state-of-the-art" review of research into the effects of bioconcrete and discusses the overall methodologies of every medium with their physiological, physicochemical and bioengineering properties in the light of recent researches done so far in the same field.

Keywords: bioconcrete; microbes; biomineralization; self-healing; calcite precipitation

1. Introduction

Cement concrete is arguably the most widely used construction material because it is strong, cheap, easy to shape, and deteriorated slowly. The researches are continuously being done to improve the quality, cost, and sustainability of the same concrete. However, recent innovations have proved that the durability of the same concrete could further be enhanced by using some bioengineering technologies. It is partly done by using a process known as calcium carbonate ($CaCO_3$) biomineralisation [1-3] or Microbiologically Enhanced Crack Remediation (MECR) and Microbiologically Induced Calcite Precipitation (MICP) [4-7].

As concretes are highly susceptible to cracks, various researches have been carried on to reduce them. These cracks are either microcracks or macrocracks. Microcracks almost happen to occur in every reinforced concrete structure and are usually required to activate the steel reinforcement by allowing it to elongate. As these microcracks are further propagated into macrocracks, they cause steel activation in such a way to weaken and harm the structure by percolating the water and deteriorating them slowly. While microcracks usually occur due to certain abiotic phenomena such as drying, shrinkage, and external forces, the macrocracks arise from the concrete inability to resist a considerable amount of tensile forces [8-10]. Therefore, an approach to prevent or rejuvenate these cracks would be highly beneficial.

Early research collaborations between bioengineering and civil and material engineering used bacteria to heal concrete cracks to improve general concrete quality. Thus,

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the specimens are named as bioconcrete and biogrouts, and their characteristics and qualities are found to be dependent on the type of bacteria used [1,11-13]. These bacteria proliferate accordingly to fill the gaps and cracks based on their particular chemical reactions, recovering its cracks having its strength, durability, and resistance increased [11,14-18]. Furthermore, the bacteria also produce a handful of minerals necessary to fill the newly formed cracks [19]. It also decreases the permeability, thus increasing the concrete matrix's durability, resulting in huge benefits in inspection and repair costs [19].

The benefits of bacteria employed in bioconcrete production have triggered the use of several other microorganisms such as algae [20-22] and fungi [23-38]. Though similar in action, fundamental differences exist among them, such as the medium, process, and, more importantly, the strengths and weaknesses. While algae are exceptionally known for adjoining photosynthesis in the calcite precipitation process, fungi are known for their ability to perform organomineralisation using spores to reproduce and lie dormant for an extended period of time [20-21, 29-31]. Since the microorganisms are different in their mode of living, the results obtained to compare the strength and bioconcretes are still in paradox. The present paper gives a comprehensive review of the applications of certain microorganisms in the field of bioconcrete technology.

2. Materials and Methods

This section mainly comprises the cultural techniques of microorganisms with the objective of minimising the complexities of methods in such a way that one can repeat the experiments easily in the future. Several research papers were consulted to explore the methods. The basics of cultural techniques and mechanics of the same research are being described as under:

2.1. Experimental Design

2.1.1. Choice and Culture of Microorganisms

There are very few microbes studied and have been used in the process of bioconcrete. Every microbe requires a specific medium to culture in the laboratory.

2.1.1.1. Selection and Culture of Bacteria

Recent researchers use reasonably diverse ureolytic bacteria for making bioconcrete and biogrouts [15, 18, 32-35, 37]. Most of them use *Bacillus subtilis* as an experimental organism due to their availability and compatibility of the bacterium [1,3,38]. The pure and stock cultures were maintained on nutrient agar medium as striped and agar slants. The culture is kept at a temperature of 35±2°C for three days and then refrigerated at 4°C for subsequent use. Subculturing can be done every 3 months at periodic intervals.

Most research works show that *Bacillus subtilis* does not require a specific culture medium. They use different culture media based on their growth and sporulation demand. The nutrient media is prepared under a unit volume of 1 liter with a pH of 10. Some of the media discussed are given as under:

1. Liquid medium [3]

Peptone	05.00 g
Meat extract	03.00 g
Yeast extract	05.00 g

(1.5g agar is added if solid media is required)

2. Alkaline medium [18]

NH_4Cl	00.20 g
KH_2PO_4	00.02 g
$CaCl_2$	00.225 g
KCl	00.20 g
$MgCl_2.6H_2O$	00.20 g
$MnSO_4.2H_2O$	00.01 g
$NaHCO_3$	04.20 g

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Na_2CO_3	05.30 g
Yeast extract	00.10 g
Citric acid trisodium salt	05.16 g
Trace element solution (SL12B)	1.00 ml

3. Nutrient broth medium [18]

Peptone	05.00 g
$NaHCO_3$	00.42 g
Na_2CO_3	00.53 g
Meat extract	03.00 g

The preparation of culture media requires sterilisation using autoclaving at 121°C for 20 minutes and then cooled under room temperature. The bacterial cells or spores are added using a laminar airflow chamber. Subsequently, the bacterial quantity in the culture was maintained using an optical density test with the help of a spectrophotometer. It is kept as 1x 10^5 cells/ml and incubated on a shaker incubator at 130 rpm at 35±2°C temperature. After 72 hours, the bacterial cells and spores are ready to be harvested using various separation techniques.

Some researchers are using urea [$(CO(NH_2)_2$] in their culture media to provide carbonate ions (CO_3^{2-}) for the bacterial cells [38-39]. Similarly, they have also added manganese for better sporulation in bacteria which is finally visualised by using ESEM [18].

The application of bacterial culture or spore to the concrete matrix follows the method as presented [3]. The bacterial culture or spore is added to 0.5% cement content with 2 million cfu/g concentration before the concrete is mixed. The bacteria can also be added using clay particles, silica gel, diatomaceous earth, or insulated form. This is simply done to extend the bacterial viability and longevity in the concrete [9, 18, 40-45]. Further, a most-probable-number (MPN) viability test is also performed for the bioconcrete or biogrout specimens [46]. The test is done to determine the number of viable bacterial cells or spores present in the concrete matrix. This is carried on in ageing concrete, as the viability of bacteria cells or spores is decreased during the course of time [39].

2.1.1.2. Selection and culture of Fungi

Several ureolytic fungi have also been used to generate bioconcrete. *Trichoderma reesei* obtained from American Type Culture Collection (ATCC) has shown promising results [48]. It has been found that using potato dextrose agar (PDA) with a temperature of 25 ± 2 °C for seven days is the best growth medium for *Trichoderma reesei*. Further, both pure and stock cultures are conserved in the same medium and were kept refrigerated at 4 °C for subsequent use. Subculturing of *Trichoderma reesei* can be done every three months time interval. Some of the common media which are commonly being used up for the culture of fungi for the same purposes are described as under:

1. Potato dextrose agar (PDA) medium

PDA is a rich medium for the cultivation of fungi sometimes supplemented with antibiotics differentially. These antibiotics act as bacterial inhibitors, and the commonly used antibiotics are streptomycin and chloramphenicol. PDA is a commonly used medium for the growth of yeasts and moulds. The ingredients used per litre of the medium are commonly prescribed as under:

Potato infusion	200.00 g
Dextrose	20.00 g
Agar-agar	20.00 g
(pH adjusted to 5.6 at 25 ± 2°C)	

2. Sabouraud's dextrose agar medium

Sabouraud's dextrose agar is also a general medium to grow a wide variety of fungi. It can naturally inhibit bacterial growth, but antibacterial agents may be added if required. Common selective antibiotic used for Sabouraud's dextrose agar medium is chloramphenicol, gentamicin, and tetracycline. The medium uses the ingredients as:

Peptone	10.00 g

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Dextrose	40.00 g
Agar-Agar	15.00 g

(pH adjusted to 5.6 at 25 ± 2°C)

3. Czapek Dox liquid medium

Czapek Dox liquid medium used for several fungi uses sodium nitrate ($NaNO_3$) and sucrose ($C_{12}H_{22}O_{11}$) as the sole sources of nitrogen and carbon. This medium uses 15g of agar-agar for the solidification of the medium. The medium is initially developed by Czapek and Dox to grow *Aspergillus niger* and *Penicillium camemberti*, and has now been used for many saprophytic fungi and soil bacteria.

Sucrose	30.00 g
Sodium nitrate	02.00 g
Magnesium glycerophosphate	00.50 g
Potassium chloride	00.50 g
Dipotassium sulphate	00.35 g
Ferrous sulphate	00.01 g

(pH adjusted to 6.8 ± 0.2 at 25 ± 2°C)

4. Asthana and Hawker's liquid medium

This is also a suitable culture medium for fungi investigated by Asthana and Hawker in 1936 [49]. To solidify the medium, 20g agar-agar is added in the same.

Glucose	05.00 g
Potassium nitrate	03.50 g
Potassium Hydrogen phosphate	01.75 g
Magnesium sulphate	00.75 g

(pH adjusted to 5.5 at 25 ± 2°C)

The culture medium is further sterilised using autoclave at 121°C for 20 min. It is then cooled at room temperature, and subsequently, the fungal cells or spores were added to the medium using a laminar airflow chamber. The inoculated medium with fungi is incubated at 25 ± 2°C on a shaker incubator at 130 rpm. The mould spores were usually cultured on a solid medium in Petri dishes and are harvested with the help of an especially designed spore collector for the same purposes [102]. And, this is usually done from seven days old culture.

2.1.1.3. Selection and culture of Photosynthetic Microorganisms

Recent researchers have also used different microalgae to generate a bioconcrete [21,69-80]. These microalgae can be isolated using the methods given as under [47].

1. washing method or centrifugation
2. by exploiting the phototactic movement
3. by agar-plate method
4. nutrient medium

Similarly, these microalgae, as described in the review, are cultured with the help of some specific media described as under:

1. Schreiber's medium

Potassium nitrate	00.10 g
Sodium orthophosphate	00.02 g
Soil extract	50.00 ml
Seawater	1 Liter

The soil extract is composed of 1 Kg of garden soil in 1-Liter of clean water. The mixture is further boiled, cooled, decanted and stored in a bottle.

2. F/2 medium

$NaNO_3$ (75.0 g/L dH_2O)	01.00 ml
$NaH_2PO_4.H_2O$ (5.0 g/L dH_2O)	01.00 ml
$Na_2 SiO_3.9H_2O$ (30.0 g/L dH_2O)	01.00 ml
f/2 Trace metal solution	01.00 ml
f/2 vitamin solution	00.50 ml
filtered seawater	1.0 L

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(mixed the solutions and autoclaved)

where the f/2 Trace metal solution consists of:

$FeCl_3.6H_2O$	03.15 g
$Na_2EDTA.2H_2O$	04.36 g
$CuSO_4$.$5H_2O$ (9.8 g/L dH_2O)	01.00 ml
$Na_2MoO_4.2H_2O$ (6.3 g/L dH_2O)	01.00 ml
$ZnSO_4.7H_2O$ (22.0 g/L dH_2O)	01.00 ml
$CoC_{12}.6H_2O$ (10.0 g/L dH_2O)	01.00 ml
Distilled water	1.0 L

and the f/2 vitamin solution consists of:

Vitamin B12 (Cyanocobalamine, 1.0 g/L dH_2O)	01.00 ml
Vitamin B7 (Biotin, 0.1 g/L dH_2O)	10.00 ml
Vitamin B1 (Thiamine HCL)	200.00 mg
Distilled water	1.0 L

(Sterilised by filtration, stored in plastic vials and refrigerated for further use)

3. Conway's or Walne's medium

The medium is a composition of three solutions prescribed as under:

- Nutrient solution A

$FeCl_3.6H_2O$	01.30g
$MnCl_2.4H_2O$	00.36g
H_3BO_3	33.60g
EDTA (disodium salt)	45.00g
$NaH_2PO_4.2H_2O$	20.00g
$NaNO_3$	100.00g
Distilled water	1.0 L

- Nutrient solution B

$ZnCl_2$	02.10 g
$CoCl_2.6H_2O$	02.00 g
$(NH_4)_6Mo_7O_{24}.4H_2O$	00.90 g
$CuSO_4.5H_2O$	02.00 g
Distilled water	100.00 ml

- Vitamin solution C

Vitamin B12 (Cyanocobalmine)	10.00 mg
Vitamin B7 (Biotin)	10.00 mg
Vitamin B1 (Thiamine)	200.00 µg
Distilled water 100 ml	

The medium used the three prescribed solutions utilising the composition of:

Nutrient solution A	01.00 ml
Nutrient solution B	00.50 ml
Vitamin solution C	00.10 ml
Distilled water	1.0 L

The microalgae are further evaluated and transferred into a series of Petri dishes containing the medium. Microalgae species are best suited with pH between 7 to 9 with sun or artificial light exposure with optimal temperature between 20 to 24 ^oC. The pH in a suitable range using filtered air and CO_2 were regulated with a flow meter. The medium is further solidified by adding 1.5% agar to a 1-litre medium followed by sterilising using autoclave at 120 ^oC under 150 lbs pressure for 15 minutes.

2.1.2. Preparation of Concrete Matrix

The concrete matrix is prepared in the form of concrete or mortar using fresh materials as cement, aggregates, and clean water. The fresh concrete matrix follows the M20 grade with a mix ratio of 1:1.5:3. It can be made with or without bacteria to examine and compare the enhancement in terms of compressive strength and self-healing ability. Below are the listed materials usually used to construct a bioconcrete or biogrout:

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1. Portland cement (53 grade)
2. Aggregates

- Fine aggregates (natural river sand):
  Specific gravity 02.69
  Maximum size 04.75 mm
- Coarse aggregates:
  Specific gravity 02.70
  Maximum size 20.00 mm

3. Precursors to provide Ca^{2+} ions
   Calcium lactate ($[CH_3CH(OH)COO]_2Ca$) [3]
   Calcium gluconate ($C_{12}H_{22}CaO_{14}$) [39]
   Calcium chloride ($CaCl_2$) [16]
4. Locally available clean water (H_2O)
5. Microbes sample

2.1.3. Casting of Cubes

As usual, both normal concrete and bioconcrete are required to undergo a slump test to examine the consistency, wetness and workability of the fresh concrete. A cube mould with a dimension of 100 x 100 x 100 mm^3 is used for casting. The fresh concrete is left in mould for 24 hours and then placed in clean and fresh water for curing for another 24 hours. The specimens thus produced are dried in a shady place or inside the room. The curing time is modified accordingly to the type of experiments conducted. Finally, the specimens are tested to examine their qualities and associated properties. This is usually done on days 7, 14, and 28 [3].

2.1.4. Formation of Cracks and Self-healing in Cubes

The pre-casted bioconcrete cubes of 100 mm size will be pre-cracked at the age of 28 days and put in water for curing of 2 to 3 weeks to develop self-healing properties.

2.2. Testing of Bioengineering Properties of Bioconcrete

The bioengineering properties of bioconcretes have been tested based on several parameters for the inclusion of various ureolytic microbes are briefly discussed as under:

2.2.1. Scanning electron microscopic analysis (SEM)

Hitachi S-3400-N Variable Pressure Scanning Electron Microscope (VPSEM) together with Oxford Inca Energy 250 Energy-dispersive Spectrometer (EDS) were used to visualise and examine various bioengineering properties of bioconcrete and biogrouts [39].

2.2.2. Energy dispersive x-rays spectroscopy (EDX)

EDX is used to obtain clear visualisation of calcium (Ca) in either normal concrete or bioconcrete effectively with or without SEM [36].

2.2.3. Compressive strength testing of bioconcrete

The compressive strength of normal concrete and bioconcrete is determined by using the compression testing machine based on IS 516:1964. COMPTEST 2000 was used to obtain the compressive test for the concrete specimens [6]. The load is applied at a constant rate of 140 Kg/cm^2/min. The compressive strength is derived as:

$$\text{Compressive Strength} = P / A \quad (1)$$

where,
P = compressive load (N)
A= specimen cross-sectional area (mm^2)

2.2.4. Split tensile test

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The split tensile test is done using the same instrument as the compressive strength test based on IS 516:1964. The split tensile strength is obtained by giving the load to the cylinder that is put horizontally [50]. It is calculated as:

$$\text{Split Tensile Strength} = 2P/\pi LD \quad (2)$$

where,
P = ultimate load (N)
L = length of cylinder (mm)
D = diameter of cylinder (mm)

2.2.5. Flexural strength test [50]

The flexural strength test is also done using the same instrument as the compressive strength test as per IS 516:1964. The flexural strength determines the material's ability to resist flexural load, mostly the tension strain caused by the flexure. It is also expressed as the "Modulus of rupture," N/mm^2 and usually has a value between 12 to 20% of compressive strength. The test is also done with the specimen put in the horizontal position. The failure load and the shorter length from crack to support are measured. The flexural strength is calculated using the formulae:

$$R = PL/bd^2 \ (a \geq 133 \text{ mm}) \quad (3)$$

$$R = 3Pa/bd^2 \ (110 < a \leq 133 \text{ mm}) \quad (4)$$

Where,
R = modulus of rupture (N/mm^2)
P = maximum load in (N)
L = span (m)
a = shorter length from crack to support (mm)
b = average width (mm)
d = average depth (mm)

2.2.6. Self-healing of cracks

The 28 days old cube specimens are cracked using the compressive testing machine and were kept in water for curing for two to three weeks.

2.2.7. Evaluation of pore size distribution in ageing bioconcrete specimens

The permeability of a bioconcrete is examined by mercury Intrusion Porosimetry (MIP) with the help of a Micromeritics Autopore IV Mercury Porosimeter [18]. The test can be done with or without incorporated ingredients.

2.2.8. Acid durability test [6]

A specimen's resistance against an acidic environment is tested by observing the compressive strength reduction after a limited time submersion in a 5% solution of sulphuric acid. Also, the concrete healing ability against an aggressive environment is presented in ASTM C666-1997. The Acid Durability Factor (ADF) is determined as:

$$ADF = Sr.\ (N/M) \quad (5)$$

Where,
M = acid exposure duration (days)
N = required duration of durability factor (days)
Sr. = relative strength corresponding to the duration (%)

2.2.6 Electrical resistivity [3]

An electrical resistivity test is done by using Leader RCONTM Concrete Electrical Resistivity Meter at a certain location on a specimen. The electrical resistivity is determined by the formula as:

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$$p = RA/\iota \quad (6)$$

Where,
p = electrical resistivity (Ωm)
R = electrical resistance (Ω)
A = cross-sectional area (m^2)
ι = electrical path length (m)

3. Discussion

Cement concrete is one of the popular and most used building materials due to its versatility, availability and economy of the ingredients all over the world. It has got the extra ability to be moulded in any shape. But, it also degenerated due to micro and macro cracks, fracture, and decays. The decaying process may further be accelerated as the water percolated inside the concrete and the mineralisation process took place. This in fact is an unwanted process for construction works where minerals are dissolved to form gases.

Currently, it appears that it would be beneficial to stop the microcracks propagation further. Similarly, on the other hand, as Portland cement is responsible for 7 to 8% of anthropogenic emission worldwide, there should be some alternative technologies like bioconcrete, bioengineering has become necessary to be introduced in the same field.

The benefits of bioengineering of bioconcrete technology are increased compressive strength, increased environmental resistance such as lower permeability, lower absorption, resistance against acid, autonomous self-healing ability, and adheres sustainability.

3.1. Bacteria

This paper highlights the role of microbes in enhancing concrete materials. The most prominent bacteria to form a bioconcrete is *Bacillus subtilis*. It is a type of Gram-positive ureolytic bacteria that produces calcites ($CaCO_3$), a similar mineral found in cement. The same bacteria is often called grass or hay bacteria, usually found in humans' digestive-tract. In addition, it has got the ability to withstand extreme environmental conditions by forming endospores [1,3,36]. The other bacteria used in bioconcrete technology are as *Bacillus pseudofirmus* [9,18,56], *Bacillus cohnii* [9,56,57], *Sporosarcina pastuerii* [11,54], *Bacillus pastuerii* [11,14,15], *Bacillus sphaericus* [16,17,41,59], *Schewanella* [32], *Myxococcus xanthus* [37], *Bacillus sp. CT-5* [55], *Bacillus cereus* [58], *Bacillus megatherium* [60], *Bacillus licheniformis* [61], *Proteusmirabilis* [62], *Proteus vulgaris* [62] and *Geobacillus thermoglucosidasius* [63].

The expected outcome of bacterial byproduct produced by *B. subtilis* is calcium carbonate. This is also an important component of cement. And, the same component is chemically synthesised by bacteria where calcium is required externally in the forms of either calcium lactate, calcium gluconate or calcium silicate. These chemical substances, with the help of urease enzyme by the process of hydrolysis, transformed them into CO, CO_2 and NH_3. Finally, the calcite precipitation took place on the surface of bacteria to heal the cracks microbiologically via MICP [3,16,39].

It appears that the slightly reduced pH of the produced bioconcrete has got some additional benefits over normal cement as the bacterial spore can live longer under cover of the bacterial wall. Similarly, as the water percolates naturally inside the microcracks, these bacterial spores germinated to heal the cracks as soon as water touches them [51].

The essential biochemical reactions happen to occur during the microbiologically induced calcite precipitation process (MICP) are briefly enumerated as under. However, the biochemistry of these biochemical reactions is still in paradox [16,38,52].

1. $Ca^{2+} + Cell \rightarrow Cell\text{-}Ca^{2+}$
   $Cl^- + HCO_3 + NH_3 \rightarrow NH_4Cl + CO_3^{2-}$
   $Cell + Ca^{2+} + CO_3^{2-} \rightarrow Cell\text{-}CaCO_3$

They are commonly implanted in the concrete matrix after immobilisation on diatomaceous earth and activated when the crack occurs [1].

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2. $CaC_6H_{10}O_6 + 6O_2 \rightarrow CaCO_3 + 5CO_2 + 5H_2O$
(Cal. lactate) *(Cal. Carbonate)*
3. $Ca(OH)_2 + 5CO_2 \rightarrow 5CaCO_3 + 5H_2O$
(Porlandite) *(Cal. Carbonate)*

Table 1. A list of some per cent increase of compressive strengths of bioconcrete using different bacteria

Sr. No	Name of Bacteria	Compressive Strength (%)	Authors
1	*Bacillus cohnii*	10%	[8]
2	*Bacillus pseudofirmus*	10%	[8]
3	*Sporosarcina pasteurii*	17%	[53]
4	*Arthrobacter crystallopoietes*	22%	[64]
5	*Bacillus subtilis*	25%	[36]
6	*Shewanella*	25%	[32]
7	*Bacillus sp.*	36%	[55]

3.2. Fungi

Next in the series, fungi have also been tried for bioconcrete technology further. The fundamental difference between bacteria and fungi is their prokaryotic and eukaryotic nature consisting of mucopeptide and chitin in their cell walls. Fungi are the kinds of heterotrophic microorganisms made of mycelia and hyphae, usually reproducing asexually with the help of a variety of spores. These spores lay dormant for an extended period of time and grew when the conditions become favourable. While they are mostly found on the soil surface where oxygen is abundant, the rest of them can live in an aquatic habitat or even in a place where oxygen is absent. They can also promote mineral precipitation by both induced biomineralisation and organomineralisation [29-31].

Although calcite precipitation had also been associated with fungi quite a long time ago, it is only recently being studied well by scientists. The urease-producing fungi like *Neurospora crassa* and *Alternaria sp.* induced the $CaCO_3$ biomineralisations. Some other fungi studied in the biomineralisations of $CaCO_3$ are *Serpula himantioides*, *Cephalotrichum*, *Morchella sp. Piloderma fallax*, *Beauveria caledonica*, *Pseudophialophora magnispora*, *Myrothecium gramineum*, and *Colletotrichum* [18,25-27,31,88-89,93,96].

Luo *et al.* have chosen six fungi for the biomineralisation studies: *Trichoderma reesei*, *Aspergillus nidulans*, *Cadophora interclivum*, *Umbeliopsis dimorpha*, *Acidomelania panicicola*, and *Pseudophialophora magnispora* [28]. It has been reported that only *Trichoderma reesei* could grow well on the concrete plates promoting calcium carbonate precipitation. The precipitation is a result of CO_2 solution reacting with $Ca(OH)_2$ in concrete. This is appropriately supported by the facts that some fungi can repair the cracks more efficiently by using calcium mineralisation [24,29-31,82,84].

Fungi metabolism can directly or indirectly influence the alkalinity and calcium concentrations since they perform the calcite precipitation. The apical growth of fungal hyphae has been found to be significantly controlled by the concentrations of Ca^{2+} ions. The chitin found in fungal cell walls has also been a recognised polymer to bind the calcium ions. The calcification of fungal filaments is a complex phenomenon with various steps that still need to be explored in further studies. Furthermore, the three-dimensional mycelium network appears to be advantageous to provide some extra sites for calcite deposition. [23,29-31,85-89,90].

Fungal cell walls usually have a very high mechanical resistance, for so, they have been hypothesised to be engined in mineral substrates drilling [91]. Fungi can grow well in cracks because of the mechanical pressures that tighten the cleaves due to differential bioweathering. Both active and passive mechanisms are associated with metals and minerals immobilisation inducing metal oxalates and other biomineral formations [85,88,92-93]. Furthermore, fungi also secrete several organic acids, particularly the oxalic

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acid that helps in the reprecipitation of calcium minerals. These secondary cementations in concrete using oxalate salts could be degraded to carbonates [83,88,93,94].

Additionally, the fungi chosen in the calcite biomineralisation experiments should meet the following criteria:

1. easily available and cultured in laboratory environments
2. able to survive in the harsh environment of cement concrete
3. ureolytic in nature if producing calcite in cement concrete
4. should not be allergic and pathogenic to human health

Fungi studies related to the bioengineering of bioconcrete technologies are still in a juvenile stage needs further research [24].

3.3. Photosynthetic Microorganisms

Microalgae perform biomineralisation of calcite ($CaCO_3$) with the help of urea, $CO(NH_2)_2$. Urea provides carbonate ions (CO_3^{2-}) with a pH to increase. This reaction is vital for calcite precipitation. Calcite precipitation strengthens the concrete matrix by filling the concrete cracks, consolidate the sand, and restore the aggregates comprehensively [65]. Unlike bacteria or fungi, microalgae execute the Microbiologically Induced Carbonate Precipitation (MICP) with the help of photosynthesis. It consists of complex biochemical reactions sequence summarised as under [20,21].

1. $CO_2 + H_2O \rightarrow (CH_2O) + O_2$
2. $2HCO_3 \rightarrow CO_2 + CO_3^{2-} + H_2O$
3. $CO_32- + H_2O \rightarrow HCO^{3-} + OH^-$
4. $Ca^{2-} + HCO^{3-} + OH \rightarrow CaCO_3 + 2H_2O$

At the end of these microalgae chain reactions, the limestone ($CaCO_3$) is produced. The microbiologically precipitated limestone ultimately heals the concrete cracks to increase its durability. Microalgae also hydrolyse urea into ammonia and bicarbonate with the help of the urease enzyme [20-21].

Algae are a group of eukaryotic organisms that belongs to the kingdom Plantae, ranging from unicellular to multicellular and mostly autotrophic. Exclusion to this is blue-green algae (cyanobacteria) that are often misinterpreted as algae although they belong to Empire prokaryote. Algae are primarily found in water but sometimes also can be found in soil or even dessert. They are best suited in places where oxygen, carbon dioxide, and lights are available. Further, algae have many ways of reproducing, from asexual cell division, spores, to sexual reproduction through meiosis. Finally, Algae can promote mineral precipitations by biologically controlled or biologically induced mineralisations [65]. However, only a few of them have been used for microbial biomineralisations, although they are abundant in nature and can live in both land and water [66].

The successful applications of microalgae including cyanobacteria in bioconcretes have also opened a new path for researches in concrete technology [1,20,67]. Whiting events caused by Picocyanobacteria have shown their huge potential in performing calcifications in bioconcretes [22,68,69].

The algae like *Chlorella vulgaris*, *Muriellopsis sp.*, *Mychonastes sp.*, *Dunaliella salina*, *Hematococcus pluvialis*, and *Porphyridium cruentum* have been found to use urea as a nitrogen source to produce ammonia and bicarbonate with the help of urease enzyme [21, 70-73]. The other algae used in bioconcrete technology are as *Arthrospira Platensis* [21,70,71], *Arthrospira platensis, Spirulina platensis, Picocyanobacteria, Synechocystis sp. Synechococcus sp. Scytonema sp. Nannochloris atomus, Anabaena sp. Anacystis nidulans, Brevibacterium ammoniagenes, Nostoc calcicola and Coccochloris peniocystis* [21,69,70-71,74-79].

Finally, the photosynthetic microorganisms are more eco-friendly as compared to bacteria and fungi. Algae are rather easy to grow to reduce a part of carbon dioxide (CO_2) emission produced by cement concrete. It is capable of producing calcium carbonate in a little bit of a different way than bacteria and fungi; however, the exact mechanism for the calcite precipitation is still in the dark and requires further research.

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3.4. Bioengineering Properties of Bioconcrete

To study the various bioengineering properties, different media were used for growing different microorganisms like bacteria, fungi, and photosynthetic microorganisms. The recent paper uses compressive strength, acid durability test, porosity, permeation test, SEM, EDX, and XRD to determine the bioengineering effects on bioconcrete and biogrouts as parameters [14, 32, 17, 53-55].

As the concrete compressive strength and durability are closely related to each other, they are considered as the determinant for other properties such as quality, life, and resistance. A differential increase in compressive strength is obtained with the use of certain microbes (**Table 1**) [8,32,36,53,55,64]. This is in the range of 10% to 36% except for *E. coli* due to low urease production [32]. It is also found that calcium lactate or clay particles added to concrete gives more increase in compressive strength. The enhancement is due to the availability of calcium ions in concrete increasing the self-healing capacity as well. Therefore, the choice of microbes and the media chosen are significantly more important for the characteristics and the qualities of bioconcretes produced [1,3,11,14,16-18, 32,36,63-64, 55,97].

Concrete durability is influenced by the permeability of concrete, which is affected by the type of materials used, pore size and concrete structure and composition. This is simply determined by the carbonation tests [18,38,98]. Biomineralisation of $CaCO_3$ reduces the permeability by healing the pores naturally. For example, six times reduction in water absorption in bioconcrete treated with *Bacillus spp.* have been noted in the same bioconcrete [18,19,55]. The concrete permeation properties can further be reviewed by using SEM and EDX tests [1,3,17,55,59].

Further, the electrical resistivity is also considerably decreased when cracks are formed in concretes. This is increased by filling the pores and cracks. It has also been reported that calcium sources such as calcium lactate further increases electrical resistivity [3]. In addition, bioconcrete is also tested to be more acid-resistant as compared to normal concrete [6,36,99].

Last but not least, in spite of all the merits mention in bioconcretes, there are some drawbacks found in the same technology. Some of them are the production of ammonia by the microbes. Ammonia is considered a powerful pollutant causing human health problems and toxicity to the plants [100]. As this is further converted into ammonium salts and nitric acid, it extended the risk of further damage [101]. Similarly, the excess of calcium salts added crystallises in the concrete also extended another risk for bioconcrete.

Finally, as the deposition of these organic $CaCO_3$ depositions appears to have been more resistant and less soluble in acid rains, the bioconcretes are found rather better than normal concrete. However, more researches are still required to prove the fact [17].

4. Conclusions

The bioconcrete technology is a multidisciplinary field of applying bioengineering to concrete material. A collaboration between civil and material engineers and microbiologists in this field is imperative. The field has many challenges as open to innovation with an admirable future. However, the comprehensive use of microbes may affect human's life adversely. The use of pathogenic microbes should be avoided as much as possible.

Finally, bioconcrete is undeniably one of the most advanced multidisciplinary works done in civil and material engineering with microbiology. The concept and grasp of increased strength, durability, resistance and self-healing ability using microbes induced $CaCO_3$ precipitation are almost done impeccably. This concept is one realisation of making sustainable, economical, yet high-quality building materials. Therefore, incoming research in multi-aspect of bioconcrete is critical but crucial.

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

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