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An overview on the role of photosynthetic microbes in biomineralization of calcite in bioconcretes

Mohammad Salim[1*], Waseem Khan[2], Arvind Kumar Tripathi[3], Mohammad Shahid Masroor[4], Mohammad Rizwan Khan Bhina[5]

[1] Department of Botany, S.G.S. Govt. Autonomous P. G. College, Sidhi, Madhya Pradesh, India
[2] Department of Civil Engineering, UIT, RGPV, Bhopal, Madhya Pradesh, India
[3] Department of Physics, S.G.S. Govt. Autonomous P. G. College, Sidhi, Madhya Pradesh, India
[4] P.C.D.S. and R.C., People's University, Bhopal, Madhya Pradesh, India
[5] Department of Civil Engineering, HIT, Harmaya University, Harar, Ethiopia

Abstract
The main objective of the present paper is to discuss the applications of microalgae to improve the quality of cement concrete. It deals with the study of urease producing algae like *Chlorella vulgaris, Spirulina platensis, Synechocystis, Synechococcus, Scytonema, Picocyanobacteria* and many others to produce bioconcretes in future. Microalgae are the photosynthetic microorganisms capable of producing calcite in bioconcretes. This is also called as microbiologically induced calcite precipitation (MICP) and could be a novel strategy to restore the concrete structures with the help of biomineralization of $CaCO_3$ using microalgae in future. The review discusses the overall methodology of the culture of microalgae, biotechnology of bioconcrete production and the physicochemical and bioengineering properties of bioconcrete in association with microalgae.

Keywords: photosynthetic microalgae, biomineralization, calcite, bioconcrete

1. Introduction
In recent past, it has already been proved that the infrastructures made of bioconcretes enhanced the durability of building materials (Pradeep *et al*. 2015, Alshalif *et al*. 2017 and Kunamineni and Meena 2018) [1, 25, 35]. As the qualities of bioconcretes mainly depend upon the types of microbes they contain, these microbes perpetuate accordingly to fill the cracks in future. It autonomously decreases the permeability and increases the durability of concrete matrix. (Jonkers *et al*. 2010) [23]. The added microbes into concrete produces copious amounts of materials required to plug the freshly formed cracks (Ferris and Stechmeir, 1992) [13]. The specific chemical reactions of bioconcretes strengthen the whole material in such a way that it fills and tighten the gap successfully (Bang *et al*. 2001, Ramchandran *et al*. 2001, Ramkrishnan 2007, Muynck *et al*. 2008 and Jonkers *et al*. 2010) [6, 23, 32, 36, 37].

Though, microbial bioconcrete technology has begun successfully mainly with the use of bacteria (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) [1, 2, 12, 23, 35, 42], still several other microorganisms like fungi (Hou *et al*. 2013, Bind and schedler *et al*. 2016, Li *et al.* 2014, 2015 & 2018, Jing Luo *et al.* 2018) [9, 20, 22, 27, 28, 29] and algae (Arianti *et al.* 2011 & 2012 and Tingting *et al.* 2018) [3, 4, 41] and have also been tried recently to improve the cementation of concrete technology for the same job.

This is an interdisciplinary approach reviewing the researches done so far in the field of bio concrete technology using a new self-healing agent named microalgae. In recent past, several studies have been put forward to demonstrate the ability of algae to produce calcite in bio concrete. But, the exact mechanism to produce calcite in bio concrete is still in dark requiring further research exploring the facts to complete the missing links involved. In the present course of study algae has been explored severely to heal the cracks in concretes partially. This is a multidisciplinary review discussing the types of microalgae involved cultural techniques and the scope of calcite mineralization in bioconcretes with designing the bioengineering properties of bioconcretes for the evaluation of concrete quality in future.

2. Methodology
The present review 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. The basics of these bioconcrete technologies are briefly described as under:

2.1 Experimental Design
i) Choice of experimental organisms and the culture of microalgae
Though, very few ureolytic microalgae (Table 1) have so far been exploited for bioconcrete technologies, the isolation of a required species can be done by adapting the following methods (Perumal *et al.* 2012) [34].

1. Washing method or centrifugation
2. By exploiting the phototactic movement
3. By agar-plate method
4. Nutrient medium

Some of the media found suitable for the growth of microalgae are given as under:

(a) Schreiber's medium

Potassium nitrate	-	0.10 g
Sodium orthophosphate	-	0.02 g
Soil extract	-	50 ml
Sea water	-	1 L

The soil extract is prepared by dissolving 1 kg of garden soil in 1L of clean water. This is then boiled, cooled and decanted and stored in a bottle for further use. 50 ml of soil extract is added in 1L of filtered and sterilized sea water.

(b) TMRL medium

Potassium nitrate	-	10.0 g
Sodium orthophosphate	-	01.0 g
Ferric chloride	-	00.3 g
Sodium silicate	-	00.1 g

Each chemical is dissolved in 100 ml of clean water separately. And then 1 ml of each solution is added in 1 L of distilled water.

(c) F/2 Medium

This is prepared as given under

$NaNo_3$ (75.0g/L dH_2O	-	1.0 ml
$NaH_2PO_4.H_2O$ (5.0g/L dH_2O	-	1.0 ml
$Na_2SiO_3.9H_2O$ (30.0g/L dH_2O	-	1.0 ml
f/2 Trace metal solution	-	1.0 m
f/2 vitamin solution	-	0.5 ml
filtered seawater	-	1.0 L

(Mixed the solutions and autoclaved)

Preparation of f/2 Trace metal solution

$FeCl_3.6H_2O$	-	3.15 g
$Na_2EDTA.2H_2O$	-	4.36 g
$CuSO_4.5H_2O$(9.8 g/L dH_2O	-	1.00 ml
$Na_2MoO_4.2H_2O$(6.3g/L dH_2O	-	1.00 ml
$ZnSO_4.7H_2O$ (22.0g/L dH_2O)	-	1.00 ml
$CoCl_2.6H_2O$ (10.0g/L dH_2O	-	1.00 ml
Distilled water	-	1.00 L

Preparation of f/2 vitamin solution

Vitamin B_{12} (Cyanocobalamine, 1.0g/L dH_2O)	-	01.00 ml
Vitamin B_7 (Biotin, 0.1g/L dH_2O)	-	10.00 ml
Vitamin B_1 (Thiamine HCL)	-	200.00 mg
Distilled water	-	1.00 L

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

(d) Conway's or Walne's medium

This is prepared as described 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	-	1L

Nutrient solution B

$ZnCl_2$	-	2.1 g
$CoCl_2.6H_2O$	-	2.0 g
$(NH_4)6Mo7024.4H_20$	-	0.9 g
$CuSO_4.5H_2O$	-	2.0 g
Distilled water	-	100 ml

Vitamin solution C

Vitamin B_{12} (Cyanocobalmine)	-	10.0 mg
Vitamin B_7 (Biotin)	-	10.0 mg
Vitamin B_1 (Thiamine	-	200.0 µg
Distilled water		100 ml

Mixed the solutions as under

Nutrient solution A	-	1.0 ml
Nutrient solution B	-	.5 ml
Vitamin solution C	-	0.1 ml
Distilled water	-	1 L

After screening the desired microalgae it was transferred into a series of Petri-plates containing the medium and was exposed to sunlight or an artificial light. The optimal temperature for the growth of microalgae is 20 to 24 ºC. Similarly, the pH ranges for most of the algae species is 7 to 9. The filtered air and CO_2 passed through a flow meter will keep the pH of the culture medium between 7 to 9. Further, to solidify the medium, 1.5% agar is added to 1L of the required medium and sterilized by autoclaving the same at 120 ºC under 150 lbs pressure for 15 minutes. The pure cultures of the same microalgae were also maintained.

ii) 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 algae (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 size	-	20.00 mm

(c) Locally available clean water

(d) Algal sample

iii) 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) algae. This is poured into moulds (100 mm × 100 mm × 100 mm) and then left to harden for 24 hours. After casting these cubes are demoulded and immediately dipped in clean, 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) n [25].

iv) Formation of cracks and self-healing in cubes

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

2.2 Determination of bioengineering properties of bioconcretes

Very few ureolytic microalgae have so far been exploited for testing various bioengineering properties of bioconcretes (Table 1). These 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:

Table 1: A list of some photosynthetic microorganisms studied in biomineralization of calcite in bioconcretes

Sr. No.	Name of photosynthetic microorganisms	Class of microorganisms	Sources
1.	*Muriellopsis* sp.	Chlorophyceae	Giordano *et al.* 2005, Harun *et al.* 2010 and Ariyanti *et al.* 2012
2.	*Mychonastes* sp.	Chlorophyceae	Castanier *et al.* 1999 and Muynck and Belie 2010
3.	*Chlorella vulgaris*	Chlorophyceae	Ramnan *et al.* 2010, Perez-Garcia *et al.* 2011 and Ariyanti *et al.* 2012
4.	*Dunaliella salina*	Chlorophyceae	Giordano *et al.* 2005, Harun *et al.* 2010 and Arianti *et al.* 2012
5.	*Haematococcus pluvialis*	Chlorophyceae	Giordano *et al.* 2005, Harun *et al.* 2010 and Arianti *et al.* 2012
6.	*Porphyridium cruentum*	Rhodophyceae	Giordano *et al.* 2005, Harun *et al.* 2010 and Arianti *et al.* 2012
7.	*Spirulina platensis*	Cyanophyceae	Ramanan *et al.* 2010, Kumar *et al.* 2011 and Arianti *et al.* 2012
8.	*Arthrospira platensis*	Cyanophyceae	Giordino *et al.* 2005, Harun *et al.* 2010 and Arianti *et al.* 2012
9.	*Synechocystis* sp.	Cyanophyceae	Zhu *et al.* 2018
10.	*Synechococcus* sp.	Cyanophyceae	Sweety and Marjadi 2017 and Zhu *et al.* 2018
11.	*Scytonema* sp.	Cyanophyceae	Sweety and Marjadi 2017
12.	*Anabaena* sp.	Cyanophyceae	Hasan 2000
13.	*Anacystis nidulans*	Cyanophyceae	Hasan 2000
14.	*Picocyanobacteria*	Cyanophyceae	Dittrich *et al.* 2004
15.	*Coccochloris peniocystis*	Cyanophyceae	Miller and Colman 1980
16.	*Brevibacterium ammoniagenes*	Cyanophyceae	Hasan 2000
17.	*Nostoc calcicola*	Cyanophyceae	Hasan 2000
18.	*Nannochloris atomus*	Eustigmatophyceae	Sweety and Marjadi 2017

(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) [21].

(b) Energy dispersive x rays spectroscopy (EDX)

The amount of Ca measured for the specimens with or without algae have been visualized and estimated with the help of SEM and EDX (Asad and Roshni 2017) [6].

(c) Compressive strength testing of bioconcretes

It is done with the help of automatic compression testing mechine COMPTEST 3000 (Gavimath *et al.* 2012) [15] accordingly as per IS 516:1964 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:

Compression strength = P/N

Where, P = Load in (N)

A = Area in (mm^2)

(d) Split tensile test (Monishaa and Nishanthi 2017) [30]

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:

Split Tensile Strength = 2P/πLD

Where,

P = Ultimate load (N)

L = Length of cylinder (mm)

D = Diameter of cylinder (mm)

(e) Flexural strength test (Monishaa and Nishanthi 2017) [30]

The flexural strength test 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", 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 ≥ 133mm

$R = PL/bd^2$

When

110 < a ≤ 133mm

$R = 3Pa/bd^2$

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 Mercury Porosimeter as followed by Jonkers *et al.* (2012) [23]. It was usually carried out with or without incorporated ingredients.

(g) Acid durability test (Gavimath *et. al.* 2012) [15]

The specimens to be examined were immersed in 5% solution of sulphuric acid and are evaluated in terms of compressive strengths. Similarly, for determining the concrete résistance to the aggressive environment the durability factor as proposed by the philosophy of ASTMC666-1997 was 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) [25]

The electrical resistance of concrete is measured using a Leader RCON^{TM} 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).

3. Results and Discussion

Biomeralization is the process of minerals formation by living microorganisms like bacteria, fungi, algae and so on. The present paper deals with the study of microalgae material to improve the bioengineering properties of concrete technology. Biomeralization of calcite with the help of urea is an important manifestation of urease in nature. It provides the carbonate ions with an increase of pH generated by the reaction offering enormous potential in innovative engineering applications as an ecofriendly technique. It includes the strengthening of cement concrete as consolidation of sand particles as well as the restoration of stone and plugging of concrete cracks (Barbara 2018) [7].

Photosynthetic microbiologically induced carbonate precipitation (MICP) consisting of series of complex biochemical reactions are summarized as under (Ariyanti *et al*. 2011 & 2012) [3, 4].

(i) $CO_2 + H_2O \rightarrow (CH_2O) + O_2$

(ii) $2HCO_3 \rightarrow CO_2 + CO_3^{2-} + H_2O$

(iii) $CO_3^{2-} + H_2O \rightarrow HCO_3^- + OH^-$

(iv) $Ca^{2-} + HCO_3^- + OH \rightarrow CaCO_3 + 2H_2O$

Cracks thus healed by biologically producing limestone precipitating microorganisms would make the concrete more durable as well as more sustainable in future. Further, microalgae also use urea to hydrolyse them into ammonia and bicarbonate with the help of urease enzyme (Ariyanti *et al*. 2011 & 2012) [3, 4]. Recently, it has been observed that the algae are being exploited as bioconcrete material though, bacteria and fungi have already been researched as an excellent microorganisms for calcite precipitation in cement concrete (Gadd 2010) [14]. But, while considering the microbial biomineralizations, it appears that very few algae have so far been examined for the same, despite the fact that they are ubiquitous in their distribution both in aquatic and terrestrial environments.

In fact, algae are quite a new group of microorganisms to be introduced in the field of concrete technology. The basic differences among bacteria, fungi and algae are there in structural and genetical organizations as they are prokaryote and eukaryotes whose cell walls are made of mucopeptide, chitin and cellulose respectively. Similarly, in bacteria, fungi and algae their reserve food materials are either glycogen or starch.

The algae are a group of eukaryotic organisms usually autotrophic in nature made of unicellular to multicellular filamentous structures reproducing well with their fruiting bodies and spores. These are mostly found in water where oxygen, carbon dioxide and lights are present. They are also found in soil and sometimes, in desert in association with other microorganisms. Further, they are also able to promote mineral precipitations both by biologically controlled and biologically induced mineralizations (Barbara 2018) [7].

As microalgae have proved their potential in improving the quality of bioconcrete we hope photosynthetic microorganisms will also be exploited maximally in future to get the multipurpose benefit in concrete technology (Ariyanti *et al*. 2011 & 2012, Tingting *et al*. 2018 and Zhu *et al*. 2018) [3, 4, 41, 44]. Theses microorganisms especially the cyanobacteria have been found to be involved in massive carbonate precipitations. They have got natural ability to induce the precipitation of calcium carbonate (Ariyanti *et al*. 2012) [4]. Whiting events caused by the *Picocyanobacteria* is an outcome of high potential of calcification of the same bacteria (Table 1) (Thompson *et al*. 1997 and Zhu and Dittrich 2016) [40, 43].

Photosynthesis leads to calcite precipitation by conducting a $^{HCO-}{}_3/OH^-$ exchange process via the cell membrane increasing the pH value in and around the cell (Miller and Colman 1980 and Zhu and Dittrich 2016) [29, 43]. Similar pathway of calcite precipitations in cyanobacteria like *Anabena cycadae, A. cylindrica, A.doliolum, A. variabilis, Anacystis nidulans, Spirulina maxima, Nostoc calcicola, N.muscorum, Synechococcus sp., Arthrospira platensis, Syctonema sp.* and *Brevibacterium ammoniagenes* has been found to be the hydrolysis of urea with the help of urease enzyme as urea is a nitrogen source for a variety of microorganisms (Bekheet and Syrett 1977, Hasan 2000, Dittrich *et al*. 2004, Giordano *et al*. 2005, Harun *et al*. 2010, Ariyanti *et. al*. 2012, Sweety and Marjadi 2017 and Tingting *et al*. 2018) [4, 8, 11, 17, 18, 19, 39, 41] (Table 1).

Another algae like *Chlorella vulgaris, Muriellopsis* sp., *Myconastes* sp., *Dunaliella salina, Hematococcus pluvialis* and *Porphyridium cruentum* also utilized urea as a nitrogen source with the help of urease enzyme to produce ammonia and bicarbonate (Giordano *et al.* 2005, Harun *et al.* 2010, Muynck *et al*. 2010, Perez-Garcia *et al.* 2011 and Ariyanti *et al.* 2012) [4, 17, 18, 31, 33] (Table 1).

Several bioengineering properties of bioconcretes have been determined as to study the effects of added bacteria in the same. Some of the parameters studied and discussed are as compressive strengths, acid durability test, permeation properties and porosity, scanning electron microscopy (SEM), energy dispersive X rays spectrum (EDX) and X rays diffraction studies (XRD) of calcite crystals precipitated on concrete surfaces (Gavimath *et al.* 2012, Asad and Roshni 2017, Jean *et al.* 2017 and Kunamineni and Meena 2018)[5,15,21,25].

One of the most important characteristic properties of concrete is their durability determined via compressive strength. The compressive strength is directly related to the durability of a concrete and could be considered as an index of the quality of concrete. The permeability is another important factor that affects the durability of a concrete. It depends upon the various permeation properties of concrete like pore structure, size of the pore and other permeation properties of the concrete. These permeation properties can also be checked by the visualization of scanning electron microscopic studies (SEM) and EDX analysis of the concrete. It has also been observed that the test cubes usually have the high electrical resistivity and this is comparatively decreased only when the cracks are formed. Similarly, the microbial concretes are more durable in acid attack test with 5 % H_2SO_4. Ramchandran *et al.* 2001, Ghosh *et al.* 2005, Muynck *et al.* 2010, Gavimath *et al.* 2012, Pradeep *et al.* 2015, Asad and Roshni 2017, Jean *et al.* 2017 and Kunamineni and Meena 2018) [5, 15, 16, 21, 25, 31, 35, 36].

Further, microalgae have got some advantages as media due to their photosynthetic activity. They are easy to grow reducing the CO_2 emission which produced in conventional

cement production. Lastly, though, microalgae have got ability to produce calcium carbonate yet the exact mechanism still lack for calcite precipitation. In future, more researches are required to fill the gaps.

4. Conclusion

The involvement of microorganisms in carbonate precipitation in bioconcretes technology is quite a new field of civil engineering. It refers to the deposition of $CaCO_3$ by microbial activity with the help of urease enzyme by ureolytic microbes like bacteria, fungi and microalgae. The present paper has documented the role of microalgae in inducing the carbonate precipitation and their future prospects to be used as bio concrete. Microalgae being photosynthetic in nature have been very prospective to be used in bio concrete technology. They are photosynthetic as nature's gift of good renewable resources to be used in bio concrete technology. Also, they can easily be cultured and maintained properly. But, as the researches on microalgae to be used as bio cement is still in a juvenile stage, more researches are needed to produce the good quality of bio concrete in association with the same microbe.

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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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