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Research Article

ASSESSMENT OF UREOLYTIC BACTERIA FOR SELF-HEALING CONCRETE

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ABSTRACT

Concrete is a very resilient and versatile construction material. Although Concrete can be spoiled by many channels, such as the spreading of corrosion from reinforcement bars, freeze-thaw conditions of trapped water, sun rays, fire, sea water effect, leaching, erosion by fast-gushing water, physical damage and chemical damage (from chlorides, sulfates etc.). Construction impediments and mix design intricacies using natural waste materials is the reasonable evidence for a need for another type of concrete. Scientists enthused by nature have formulated self-healing concrete which is able to repair itself as a consequence of the metabolic activity of bacteria. One noteworthy area of curiosity is bacterial concrete. In the past decade, a lot of researchers have put their effort into this field. It has transpired as a promising advancement to extend the service life of infrastructure in the construction field. In this review, the basic concept of bio-concrete along with research works performed is mentioned. Evaluation of self-healing performance and application of bio-concrete in fieldwork is illustrated.

INTRODUCTION

Concrete is an imperative building material used for construction purposes at large magnitude. Even after using so many safety measures in design mix and using better materials, it ends up with cracks. Concrete cracks with time; it is an inherent property [1]. This causes durability, appearance and strength damage to concrete. The passage of water through these cracks and exposure to acidic conditions (such as acid rain, sewer water) is detrimental to concrete. Thereby it can be said that primary cause of structural failure is crack formation.

An environmental price has to be paid in the form of carbon dioxide emissions due to cement production, raw material consumption, landscape marring due to mining. For this reason, a true innovation is required. To decrease the probability of crack formations, a structure desires preservation on a customary basis which will add to the maintenance cost. Hence, we need a structure that can restore to health itself naturally. Epoxy treatments are currently used but they are harmful to environment and health as lethal fumes emanated may cause skin and breathing issues. These treatments usually encompass chemical constituents such as epoxy resins, chlorine infused rubbers, acrylics, and siloxane. While passive treatments are appropriate for numerous standing concrete constructions, they have countless restrictions which deter their practice. Shortcomings in the use of chemical treatments are having inferior weather endurance, moisture vulnerability, low heat confrontation, un-sustainability, poor adherence with concrete, exposure to decomposition and de-lamination with time and diverse thermal expansion coefficients between concrete and chemical treatments [2]. Consequently, a more natural way to instigate self-healing mechanism is to use bio-materials, such as micro-organisms which work on the basis of biomineralization. Since the most compatible substance with concrete compositions is calcium carbonate; microbial metabolic pathways can replace techniques of conventional treatment of cracks [3-6].

Muynck and Ramakrishnan have performed noteworthy studies. Generally, cement after coming in contact with water during the primary reaction creates calcium silicate hydrated (C-S-H) gel and calcium hydroxide in aqueous form [7, 8]. There are materials which do not possess cementitious properties initially but when they react with $Ca(OH)_2$ in the presence of H_2O they tend to behave like one. The existence of pozzolanic material inside the concrete triggers secondary hydration and fabricates additional C-S-H gel that accelerates the strength of final concrete is thereby increased. Though, the pozzolanic materials such as silica fume are exclusive and

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limited. Besides, the cement manufacturing releases a large number of greenhouse gases [9].Therefore, living ingredients such as microorganisms can be a substitute to fabricate a precipitation that encompasses properties similar to the pozzolanic material. This living ingredient generates a precipitate which springs from the micro-organism activity.
In this review, the basic concept of bio-concrete along with research works performed for different micro-organisms is discussed. The evaluation and application of bio-concrete in fieldwork is illustrated.

Accomplished Researches

The pH of concrete is suitable for micro-organisms to thrive and perform biomineralization pathways to generate calcium carbonate crystals in an alkaline surrounding. In the alkaline environment, the heterotrophic precipitation of calcium carbonate takes place and it has comparatively superior efficiency in comparison to other pathways, and thus they are the most apt mechanisms in bio self-healing concrete [10].

A number of studies on microbes within concrete are proven to be advantageous. H.M. Jonkers scrutinized the impact of a healing agent on the concrete and the ability to fill the cracks [4]. The outcome of the precipitation of calcium carbonate due to activation of the microbial compound was an increase in compressive strength of the concrete sample. Achal *et al.* introduced a microbial healing agent consisting of *Bacillus sphaericus* in mortar [11].

It was established that to fill up the cracks and porosities the precipitation of calcium carbonate through ureolytic pathway was required. Their analysis demonstrated that the bio-treated mortar soaked up six times less water than crude mortar. The principle of the analysis executed by Wang *et al.* found out the effect of the microbial agent on the crack healing capacity and water permeability [12]. It was established that the crack healing capability amplified from 20-48 % to 50-80 % in the company of the microbial agent. A tenfold reduction in water permeability was observed by the precipitation of calcium carbonate through the ureolytic pathway. To establish the competence of the bio self-healing methodology distribution and the amount of calcium carbonate precipitate across the concrete formation are the core prerequisite. They scrutinized the distribution of the bio-precipitates and conclude that crack filling takes place throughout concrete sample. For the crack width ranges, 0.05-0.3 mm the healing ratio of 70-100 % was discovered. Regardless of a moderately well allocation of precipitates all through the specimen, the healing was mostly limited to the crack width less than 0.3 mm.

With the biogenic treatment given to concrete mix, many researchers admitted that use of bacteria helped in reducing the water permeability [3, 7, 11].The ureolytic activity biologically produces calcite layer which congests the pores subsequently diminishing porosity [13].

The application of bacterial culture has enriched durability of concrete. One of the concrete durability aspects is the chemical attack [14]. Cracking; strength loss and disintegration strike concrete when endangered to acid attack. The acidic strike is subjective to the dissolution processes of the cement paste components. The acid attack menace can be diminished by filling the corridors present inside the concrete formation. Calcium Carbonate, from the microorganism as grout material can shrink the pores and mend the concrete durability. Andalib *et al*. conducted an acid attack test on 50 MPa grade concrete. The concluded that the durability studies of bacterial concrete based on acid attack showed less depletion of concrete, the strength loss was less and weighed more than controlled 50 MPa concrete [15].

Microbiologically Induced Calcite Precipitation (MICP)

Concept of MICP

The major perception following the precipitation of $CaCO_3$ by means of biogenic methods is MICP (Microbiologically Induced Calcite Precipitation). Biomineralization can be interpreted as the chemical modification of atmosphere owing to the microbial behaviour that consequently leads minerals to precipitate [16]. MICP implythe development of calcium carbonate originating from a super-saturated suspension attributable to the existence of their microbial cells in addition to biochemical actions [17].

The propensity of urease to stimulate carbonate precipitation in micro-organisms has formerly been discussed [18-20]. Urease has an effect on the chemical activity linked with the generation of bio-minerals in accordance with dissimilar constraintsfor example pH, calcium concentrations, dissolved inorganic carbon concentrations along with the accessibility of nucleation sites [3, 18]. In saturation state, Calcium concentration, pH, and dissolved ionic concentration control the concentration of carbonate ions (CO_3^{2-}), whereas the last constraint is crucial for steady and incessant calcium carbonate development [21]. In the biomineralization process, nucleation sites are served by means of bacteria.

Importance of Urea

Addition of urea is extremely recommended. Bacteria are acknowledged to hydrolyze urea by urease to:(1) increase the general pH [22];(2) exploit it as a nitrogen source [23]; and (3) consume it as a reserve of energy [24]. B. pasteurii is identified to yield a huge quantity of urease in soil atmospheres [25]. The pH of aqueous medium's ionic potency decides the solubility of calcite, supplemented urea and calcium chloride in the media that encourages microbial sporulation [26-27]. Attributable to hydrolysis of urea, the pH of the medium was expressively amplified, and the isolates were capable to endure in this setting.

Mechanism

1mol of carbamate and 1 mol of ammonia is the result of hydrolysis of 1mol urea (Eq. 1).

$$CO\,(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3 \quad (1)$$

This later hydrolyzes into carbonic acid and 1mol ammonia (eq. 2).

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \quad (2)$$

Consequently, these products react with water to produces 2mol of hydroxide ions, bicarbonate and 2 mol of ammonium (Eq. 3 & 4).

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+ \quad (3)$$

$$2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^- \quad (4)$$

Hydroxide ions are responsible for increasing pH. Carbonate ions develop due to the changed equilibrium of bicarbonate (Eq.5).

$$HCO_3^- + H^+ + 2NH_4^+ + 2OH^- \leftrightarrow CO_3^{2-} + 2NH_4^+ + 2H_2O \quad (5)$$

$CaCO_3$ precipitation takes place when Carbonate ions come across soluble calcium ions (Eq.6).

$$CO_3^{2-} + Ca^{2+} \rightarrow CaCO_3 \text{ (precipitation)} \quad (6)$$

The system is indicated in Eq. 7-

$$CO(NH_2)_2 + 2H_2O + Ca^{2+} \rightarrow 2NH_4^+ + CaCO_3 \downarrow \quad (7)$$

Calcium carbonate precipitation in aqueous environment represents the following equation [27]-

$$CO_3^{2-} + Ca^{2+} \rightarrow CaCO_3 \downarrow \quad (8)$$

$CaCO_3$ thus formed is influenced by pH and ionic capability in the aqueous medium. When a medium a provided with the medium that favors microbial growth e.g. Urea-$CaCl_2$ medium, the ions NH_4^+, Cl^-, Na^+, OH^- and H^+ controls precipitation at varied pHs [28].

Reactions taking place at cell surface are illustrated below [3]:-

$$Ca^{2+} + \text{Cell} \rightarrow \text{Cell} - Ca^{2+} \quad (9)$$

$$\text{Cell} - Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 - \text{Cell} \downarrow \quad (10)$$

Factors Influencing MICP

There are many environmental factors that affect the efficiency of MICP.

Microbe Selection

The first is the type of microbe used. It decides the urease production capability. Urease production occurs in a plentiful stretch of micro-organisms, but some strains fabricate predominantly immense amount of urease as shown in Table I.

Table I Urease production of various organisms [29]

S.No.	Bacteria	Ureaseactivity	Calcite-precipitation
1	*B.sphaericus*sp. CR2	431 U/ml	2.32 mg/cell mass (mg)
2	*L. sphaericus* CH5	--	980 mg/100 ml
3	*S.pasteurii*	550 U/ml	--
4	*B. pasteurii* NCIM 2477	18 U/ml	--
5	*K. flava*CR1	472 U/ml	--
6	*B. megaterium*SS3	690 U/ml	187 mg/100 ml
7	*B. thuringiensis*	620 U/ml	167 mg/100 ml
8	*Halomonassp. SR4*	374.5 U/ml	--

Microbial Cell concentration

Augmentation in the urease concentration intended for hydrolysis of urea accounts for high concentrations of microbe cells (from 10^6 to 10^8cells/ml) thereby escalating the quantity of calcite precipitation by MICP [30]. As a result, urea hydrolysis has an unwavering association with bacterial cell concentrations [31]. Table II shows compressive strength test conducted by Shaikh *et al*. They concluded that optimum level of concentration as 10^5cells/ml. If the concentration is increased further; the strength of concrete tends to decrease [32].

Table II Compressive Strength test with Different Concentration of bacteria [32]

Concrete	3 days	7 days	28 days	56 days	90 days
Conventional Concrete	14.4	21.8	36.5	41.9	42.3
Bacteria 10^3 cells/ml	16.4	25.8	44.6	51.7	53.3
Bacteria 10^5cells/ml	17.4	27.8	46.4	54.3	57.0
Bacteria 10^7 cells/ml	14.6	24.5	40.6	46.9	47.8

The pH effect

The third important factor is pH which influences calcite precipitation. Urea hydrolysis will happen, once urease enzyme will attain specific pH values which would result in Calcite precipitation, which is subjective to pH. Many researchers have proclaimed that the optimum pH in favor of urease is 8.0, beyond which the activity of enzyme dwindles [16, 33]. An increased pH is essential for ammonia via urea hydrolysis. Cell breathing allows aerobic bacteria to release CO_2, which is complemented by a boost in pH owing to ammonia creation [31]. The carbonate is liable to liquefy than to precipitate if the pH levels reduce [34].

Temperature

Temperature plays an important role in catalysis process of urea by means of urease. The most favorable temperature stretches from 20 to 37 °C [30]. Urease is totally stable &steady at 35 °C [35]; however, beyond 55°C there is 47% decline in enzyme activity.

Ca+ Concentrations

The periphery of microbes' cells is negatively charged. They work as foragers of cations, especially calcium ion. The nucleation sites served by microbes combine themselves to their cell [16, 28]. This manifests the need for an ideal calcium supplement with specific concentration for $CaCO_3$ precipitation. If the concentration of urea and $CaCl_2$ surpasses 0.5M, the calcite precipitation gets slower [30]. For calcite precipitation, ideal concentration of urea is 0.5 M and $CaCl_2$is0.25 M [36].

The concentration of Ca^{2+}concludes the amount of $CaCO_3$ precipitation than urea concentrations [30].

Evaluation of Self-Healing Concrete

With the unending progress of self-healing expertise for concrete, there is an increasing necessity to cultivate means that are competent to precisely estimate the efficiency of this technique [37-40]. X-ray induced micro-tomography (X-ray µCT) has demonstrated to be beneficial in giving a three-dimensional (3D) multilayered vision inside the crack to computably create the quantity and dispersal of internal precipitates [41].

Table III History of Self Healing Evaluation techniques

Parameter	Technique Used	Work done by
Geometric modification(sealing of surface cracks)	Optical microscopy or computed tomography	[42-44]
The recuperation of mechanical properties	Compression	[40,45]
	Pure tension	[46,47]
	Bending tests	[48]
The difference in the durability properties	Water permeability	[3,46]
	Air permeability	[49]

Comparative transformation in the material properties	Ion diffusivity tests	[50]
	Ultrasound characteristics	[43,44,47]
	Impedance	[50]
	Resonance frequency	[50]
Ultrasonic Nondestructive test methods	Ultrasonic pulse velocity (UPV)	[43,44]
	Surface-wave transmission	[37]
	Diffusion in ultrasound	[38]
	CW interferometer	[39,40]
	Acoustic emission	[51]

From Research to Field Work

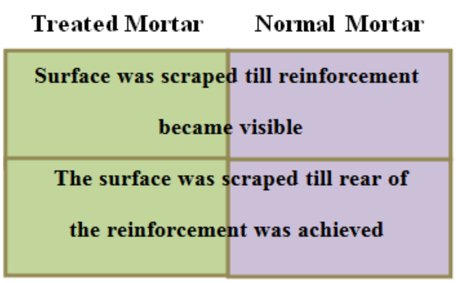
To safeguard the existing structures biogenic repairs systems have also been established which more environment-friendly than the prevailing repair provisions.

Laboratory research of bio-concrete is based on data obtained from small cubes or cylinders. Lately, Felipe Silva developed an upscale system in which beams of dimension 150X250X3000 mm^3 was casted and subjected to 4-point bending. Less healing was observed in the large-scale beam than smaller specimens of beam. The average crack healing ratio of large scale beam was about 24% and for smaller specimens it was 40% using the same culture [52].

In Ecuador on July 2014, first field application of bio-concrete was established in Andean highlands where constant water supply was required as the local economy is based on agriculture. The yield of canals built 100years ago was very low because of infiltration through the soil. Therefore, new canals with concrete lining were built but they soon developed cracking. Bio-concrete was suggested as the possible solution to improve the performance of irrigation system. The design mix was prepared from locally available ingredients. Laboratory results showed increased compressive strength in bio-concrete specimens. Later this mix was applied to the section that had no lining. Till now, no cracking has been observed [53].

Healing agent flakes were added to locally available repair mortar and a 16m^2 square wall was built divided into four parts. The treated mortar and normal mortar was applied on half portion each vertically. The upper horizontal surface was scraped till reinforcements became visible and on the lower horizontal portion the surface was scraped till rear of the reinforcement was achieved. It is represented in figure 1. This was done to check exposure in outside environment. It was reported that after 2 days treated mortar portion showed clean surface finish and reinforcements were completely intact with no further damage [53].

Figure 1 Application of treated mortar



Benefits of Bio-Concrete

1. The bio-mineralization course will not hinder with the setting time of the concrete [54].
2. Density and uniformity of bio-concrete are better than normal concrete against hostile conditions [15].
3. A significant increase in Stiffness and strength.
4. It can be used as a crack healer, surface treatment, and water purifier [55].
5. Lesser permeability reduces ingression of foreign compounds.
6. Increased service life.

CONCLUSION

Bio-concrete has emerged as a distinguished building material. Nature and science are working parallel to create a biogenic technology. It not only increases the service life but also enhance durability of concrete structures. The bio-material treatment can be applied to existing as well new structures.

Bio-concrete works on the principle of MICP which is a complex procedure and depends upon various factors such as the amount of Urea and Calcium ions, type of bacteria used and pH effect.

Various techniques to judge the healing capacity have also emerged. From using Strength and durability tests to non - destructive methods to impedance test have come out as precise evaluation test methods for bio-concrete.

Laboratory work and research on bio-concrete are done extensively but field applications are very less. Hence, constructions incorporating bio-concrete should be practiced by engineers.

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