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# BACTERIA BASED SELF-HEALING CONCRETE

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## ABSTRACT

*Concrete is one of the most widely used construction material worldwide. Concrete is a mixture of cement, fine aggregates, coarse aggregates, water and admixtures in definite proportion. Cement is mainly composed of calcium oxide (CaO) which is produced by sintering of limestone ($CaCO_3$) and clay at a temperature of 1500˚C.*

## INTRODUCTION

Concrete is strong in compression but weak in tension which is a measure cause of cracking in concrete structures. One of the most common causes of structural damage/deterioration is cracking, which is considered by designers, contractors, and clients. Cracking is inevitable in concrete and is affected by many natural factors such as:

1. Corrosion of steel
2. Freeze and thaw action
3. Chemical attack
4. Abrasion/ erosion
5. Fire
6. Plastic and drying shrinkage
7. Overloading

1. **Corrosion of steel** can cause concrete cracking due to the expansion of rust, which exerts pressure on the surrounding concrete.
2. **Freeze and thaw** action can lead to concrete cracking as water inside the concrete expands when freezing and contracts when thawing, causing stress.
3. **Chemical attack** may result in concrete cracking when aggressive substances chemically react with and degrade the concrete, weakening its structure.
4. **Abrasion/erosion** can cause concrete cracking by wearing down the surface and reducing its strength over time.
5. **Fire** can lead to concrete cracking as high temperatures cause thermal stress, potentially weakening the material.
6. **Plastic and drying shrinkage** can result in concrete cracking during the curing process as the material shrinks and develops internal stresses.
7. **Overloading** can cause concrete cracking when excessive loads exceed the material's capacity, leading to structural failure.

Corrosion of reinforcement is one of the measure reasons behind concrete deterioration When crack occurs in concrete structures, steel gets exposed to the water, air and chlorides. Penetration of such chemicals reacts with the reinforcement and starts corroding it which further causes widening of cracks. When external load is applied, it causes a chain of cracks. Cracking in concrete reduces its strength and durability and decreases the expected lifespan.

- Cracking is the prime cause which trigger Deterioration process.
- Steel loss of 0.05 to 1 mm causes spelling of concrete.
- This process can initiate in 1 to 5 years from the appearance of first crack.
- Deterioration of concrete can decrease its life span between 5-25 years & demand Repairing

However, fine cracks (200 to 300 micron) exposed to moist condition can sometimes close completely. This property of concrete is known as "Autogenous self-healing" in which Un-hydrated cement particles react with water if concrete begins to crack and allow it to heal naturally. If the crack exceeds 300 micron size, the concrete structure will be subjected to deterioration and after particular time it will require repair or rehabilitation.

Hence, regular RCC structures requires frequent maintenance and repair to prevent the reinforcement from corroding, avoid cracking and preserve the structural integrity.

There are different methods of repair/rehabilitation of concrete structure such as:

- Epoxy injection

- Routing and sealing
- Crack repair by stitching method
- Additional reinforcement for repair
- Drilling and plugging method
- Shotcrete method
- Retrofitting with FRP

1. **Epoxy injection** involves filling cracks with epoxy resin to restore structural integrity and prevent further deterioration
2. **Routing and sealing** entails widening cracks, then sealing them with a flexible material to prevent moisture infiltration and further cracking.
3. **Crack repair by stitching method** involves installing steel staples or stitching bars across cracks to strengthen and stabilize the concrete.
4. **Additional reinforcement for repair** includes adding steel bars or fiber-reinforced polymers (FRP) to enhance the structural capacity of the damaged concrete.
5. **Drilling and plugging method** entail drilling holes at crack ends and filling them with grout to stop crack propagation.
6. **Shotcrete method** involves spraying or applying concrete mix to damaged surfaces to restore strength and durability.
7. **Retrofitting with FRP** involves bonding fiber-reinforced polymers to existing concrete structures to enhance their load-carrying capacity and longevity.

**Market rate for different methods of concrete repair in India: (Source: IndiaMART)**

- Epoxy injection method cost around □ 20 per Square feet which include Crack Filling, Damaged Concrete Repair, Potholes, Undulations Repair, Levelling Floors etc.
- Crack repair by stitching method cost around □ 70 per Square feet and service charges Depends on Quantity and location.
- Concrete Repair & Retrofitting cost around □ 300 per Square feet.
- Shotcrete method cost around □ 65 per Square feet.

These methods of concrete repair require high investment and labour cost which makes the structure uneconomical for longer run. Therefore, we need an economical solution to repair cracks inside the concrete and slow down the deterioration process which is caused by chemical attacks by external agents such as water, chlorides, air etc. and stop the penetration of external agents in concrete structures.

One way to achieve these is to improve self-healing ability of concrete to repair its cracks when they form. However, autogenous self-healing is restricted to microcracks smaller than 300 µm. Therefore, researchers are considering other environmentally friendly alternatives to enable and enhance autonomous self-healing of cracks in concrete structures, such as admixtures (including microcapsules containing mineral healing agents or bacterial spores, shape memory polymer tendons and vascular networks.)

Our research mainly focuses on self-healing of concrete based on microbially induced calcium carbonate precipitation Or **Bacteria based self-healing concrete**.

**Aim Of Research:**

The aim is to study the crack healing ability of concrete when mixed with the bacteria which are capable of producing calcium carbonate in presence of calcium source, understanding the effect of bacterial application on the different properties of concrete such as its Compressive Strength, Setting Time, Permeability etc.

**Objective:**

- To study the Crack-healing ability of concrete by microbially induced calcium carbonate precipitation.
- To study the change in compressive strength of the normal concrete and concrete casted with bacterial application.

**Limitations of Bacterial Concrete:**

- **Precise mixing and handling**: Bacterial concrete requires careful mixing and handling to ensure that the bacteria remain viable and evenly distributed throughout the concrete.
- **Limited crack size healing**: Bacterial concrete is most effective at healing relatively small cracks, typically those up to a few milli meters in width. It may not be as effective at repairing larger or more extensive structural damage.
- **Activation time**: Bacterial concrete's self-healing process relies on moisture and oxygen penetration to activate the bacteria. This means that it may not be suitable for applications where the concrete remains consistently dry.
- **Compatibility issues**: Bacterial concrete may not be fully compatible with certain concrete additives, admixtures, or construction practices.
- **Long-term monitoring**: To ensure the effectiveness of self-healing in bacterial concrete, long-term monitoring and maintenance are required.

- **Limited widespread adoption**: Bacterial concrete is still a relatively new technology, and widespread adoption may be hindered by a lack of familiarity among contractors and engineers.

**LITERATURE REVIEW**

1. **Hesam Doostkami et al. "Self-healing capability of conventional, high-performance, and Ultra High-Performance Concrete with commercial bacteria characterized by means of water and chloride penetration" Vol. 401, 2023**

The viability of bacteria directly embedded in the concrete matrix was reported to be restricted to about two months, bacteria cells could be damaged during the mixing stage, setting, and hardening of the cementitious composite. To ensure the bacteria's viability, immobilization or encapsulation of the bacteria in a protective carrier is recommended.

Concrete mixtures containing encapsulated bacteria have shown a higher healing capability of 500 µm cracks under wet-dry cycles conditions & maximum healed crack width of 970 µm under water immersion conditions.

In this research, commercially available bacteria were used to reduce cost of self-healing techniques. Serenade® Max from BAYER is Bacillus subtilis encapsulated in Diatomaceous Earth, formulated as a wettable powder of natural origin containing 15.67% p/p (g/g) bacteria and bacterial content of $5.13\times10^{10}$ CFU/g. bacteria was added in the dosage of 7.5 kg/m3 and 15 kg/m3.

Crack heal test, water permeability test and chloride penetration test was performed on the different samples. The crack's width was measured the same day after pre-cracking and after 28 days of healing under various conditions One week of water immersion significantly improved the crack closure capability compared to specimens healed in a humidity chamber. Chloride penetration reduction of specimens containing bacteria was much greater than that of specimens containing no bacteria. Water tightness of specimens healed in water immersion conditions is higher than in other conditions.

2. **Md Montaseer Meraz, Nusrat Jahan Mim et al. "Self-healing concrete: Fabrication, advancement, and effectiveness for long-term integrity of concrete infrastructures" vol. 73, 2023**

This literature discusses key parameters such as the compatibility of healing materials with cementitious materials, self-healing effectiveness, and reliability. Concrete production costs ($60–80 per cubic meter), an estimated $147 per cubic meter is needed for maintenance and repair.

In Autogenous self-healing 20 to 30 percent of the cement remains un-hydrated. Un-hydrated cement particles react with ingressing water if concrete begins to crack. This reaction starts the hydration process again and makes hydration products fill the cracks. This process only applies to very young concrete. Autogenous healing has been observed to have a maximal crack width of between 200 and 300 micron.

$H_2O + CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO^-_3 \rightleftharpoons 2H^+ + CO_3^{-2}$

$Ca^{2+} + CO_3^{-2} \rightleftharpoons CaCO_3$

$Ca^{2+} + HCO_3^{-2} \rightleftharpoons CaCO_3 + H^+$

In Autonomous Self-Healing chemicals or biological agents are introduced, tubular networks and capsules can be used to deliver external agent. Microencapsulated bacteria-based concrete, has been shown to mend cracks of up to 970 micron.

Bacterial precipitation: The main advantage of using this technique is that it is environmentally friendly and compatible with cement matrix. Among the alkaliphile bacteria strains, the use of Bacillus Sphaericus, Bacillus Pasteurii and Bacillus Subtilis in the production of bacterial concrete is the most suitable for self-healing applications. The true survival rate of bacteria after introduction into concrete is still unknown

3. **Manpreet Bagga, Charlotte Hamley-Bennett et al. “Advancements in bacteria based self-healing concrete and the promise of modelling”, Vol. 358 (2022)**

In this literature various bacteria and their experimental pathways of producing calcium carbonate are discussed. There are different pathways through which microorganisms precipitate calcium carbonate, and these pathways can be broadly classified into two groups: autotrophic (e.g., photosynthesis or methane oxidation) and heterotrophic (e.g., sulphate reduction, organic acid oxidation or nitrogen cycle).

First large-scale applications to test BBSHC were done in the Netherlands for a wastewater purification tank and a water reservoir. The results are not yet conclusive as more time is needed for concrete to age and develop cracks. A more recent large-scale application installed in Antwerp, Belgium, consists in a roof slab made of BBSHC including a mixed ureolytic culture and anaerobic granular bacteria. The monitoring of the slab is ongoing as in one year no cracks have been observed.

Limitations of BBSHC and scope for modelling: Despite the relatively extensive set of reported experimental results, there are still various aspects of MICP in concrete that are not fully understood, due to the complexity of the underlying mechanisms. Extensive research is required to understand the genetic factors associated with MICP for different pathways, as this will guide the selection of the most suitable species for a particular application. the biological self-healing of materials has brought a lot of optimism, but limited engineering progress was made in this area. If we want to understand and predict the true limits of this process, we will need effective, reliable, and detailed models to complement experimentation and practice at larger scales.

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4. **Kunamineni Vijay, Meena Murmu, Shirish V. Deo. "Bacteria based self-healing concrete – A review" Volume 152, 2017.**

This literature elaborates the decomposition of urea by bacteria with aid of bacterial urease enzyme. As a component of metabolism, bacteria species gives urease, that catalyzes urea to carbonate and ammonium that results in an increase of pH and carbonate concentration in the bacterial surroundings. As per literature the healing agent can be applied in concrete by two methods: Direct application and Encapsulation.

In encapsulation method polymer based coating is used for the mixing of bacteria with light weight aggregates. impregnation of lightweight aggregates by bacteria solution and then their encapsulation in a polymer- based coating layer.

As soon as the crack ruptures the embedded microcapsules, the healing agent is released into the crack faces. In direct application healing agent is directly incorporated in LWA along with their nutrients. The nutrients to bacteria are supplied in the form of calcium lactate, calcium nitrate, and calcium formate.

The compressive strength of concrete with Sparcina pasteurii accompanied with Bacillus subtilis bacteria (2 X $10^9$ cells/ml) is found 20% more than concrete without bacteria as observed for 28 days.

The Rapid chloride permeability test is performed by monitoring the amount of electrical current that passes through a sample. The resistance of concrete towards chloride permeation can be enhanced by including bacteria in concrete.

5. **E. Tziviloglou, V. Wiktor, H.M. Jonkers, E. Schlangen. "Bacteria-based self-healing concrete to increase liquid tightness of cracks", Volume 122, 30 September 2016**.

A test on bacterial concrete was done in this literature. The healing agent was encapsulated, in order to immobilize and protect it from crushing during mixing and from the high alkalinity of the cement matrix. Agent is embedded into LWA, upon crack formation the weak lightweight capsules break; the healing agent activates and fills the open crack by precipitating CaCO3. The healing agent is incorporated in LWA via Impregnation under vacuum with calcium lactate, yeast extract and bacteria spores. Active bacteria cells convert the calcium lactate ($CaC_6H_{10}O_6$), present in the healing agent to $CaCO_3$ by using oxygen.

$$CaC_6H_{10}O_6 + 6O_2 \rightarrow CaCO_3 + 5CO_2 + 5H_2O$$

Damage was introduced in specimens by applying load until the large crack (0.35mm). specimens were placed in water for crack healing for 28 days. The results of this study revealed that replacement of sand with LWA in the mortar specimens led to considerable reduction of the density and the compressive strength of the cementitious material, as expected. The lightweight mortar with incorporated bacteria-based healing agent shows improved crack sealing, particularly when subjected to a more realistic healing regime, i.e. wet-dry cycles.

6. **Amirreza Talaiekhozan, Ali Keyvanfar, Arezo Shafaghat et al. "A Review of Self-healing Concrete Research Development", Volume 2, 2014.**

This literature covers the classification of self-healing concrete namely 1) Natural self-healing processes 2) Chemical self-healing processes 3) Biological self-healing processes.

**Natural self-healing process** include (a) Calcium carbonate or calcium hydroxide formation, (b) Blocking cracks by impurities in the water, (c) Further hydration of the unreacted cement or cementitious materials etc.

**Chemical self-healing processes** include Vascular networks with external link to supply glue, capsules, Shallow pipettes to supply glue.

**Biological self-healing process** includes Precipitation of calcium carbonates using spore forming bacteria. Most of the bacteria cannot survive pH, temperature, and serious limitation of water. Hence spore forming bacteria which is capable of producing urease enzyme is used in bio concrete. And the reaction includes hydrolysis of urea to carbon dioxide and ammonia can be **catalyzed by urease enzyme**.

This literature reviews intensively about the great potential of biological method, using the bacteria capable of precipitating calcite. The precipitation of calcite will form calcium carbonate that would help in healing concrete cracks.

7. **Henk M. Jonkers, Arjan Thijssen, Gerard Muyzer et al. "Application of bacteria as self-healing agent for the development of sustainable concrete", Volume 36 (2010).**

In most of these studies ureolytic bacteria of the genus Bacillus were used as agent for the biological production of calcium carbonate-based minerals. The mechanism of calcium carbonate formation by these bacteria is based on the enzymatic hydrolysis of urea to ammonia and carbon dioxide. A potential drawback of this reaction mechanism is that for each carbonate ion two ammonium ions are simultaneously produced which may result in excessive environmental nitrogen loading.

According to this literature calcium lactate ($CaC_6H_{10}O_6$) in presence of oxygen produces calcium carbonate ($CaCO_3$)

Bacillus pseudofirmus and B. cohnii were cultured in liquid media supplemented with manganese to enhance sporulation. some organic compounds (yeast extract, peptone, calcium acetate and calcium lactate) were added to the cement paste mixture in a quantity of 1% of cement weight. Samples were prepared and various test were performed to measure permeability, crack healing and compressive strength of samples.

The process of bacterial mineral formation from calcium lactate represents an alternative mechanism to the urease-based system, metabolic conversion of calcium lactate does not result in production of massive amounts of ammonia what drastically increases the risk of reinforcement corrosion.

**METHODOLOGY**

**Self-Healing Concrete:**

A Self-Healing Concrete can be defined as the ability of concrete to repair its small cracks autonomously. A perfect self-healing system should sense the damage or cracks and release the healing agent to repair them. Self-healing techniques are good approaches for rehabilitation of micro-cracks in concrete.

Types of Self-Healing Concrete:


1. **Natural Self-Healing Process:**

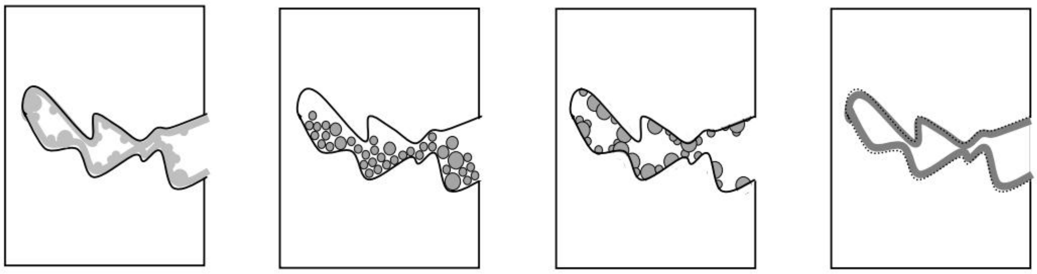
In natural processes, four following processes can block crack

(1) The formation of calcium carbonate or calcium hydroxide is another process to block crack.

(2) Crack is blocked by impurities in the presence of water.

(3) Crack is further blocked by hydration of the unreacted cement or cementitious material.

(4) Crack is blocked by the expansion of hydrated cementitious matrix in the crack flanks.



(A) Formation of calcium carbonate (B) Blocking by impurities (C) Hydration of unreacted cement (D) Expansion of the hydrated cement

In conventional concrete, 20 to 30 percent of the cement remains un-hydrated. Un-hydrated cement particles react with ingressing water if concrete begins to crack. This reaction starts the hydration process again and makes hydration products fill the cracks. Among the proposed self-healing mechanisms in the natural process, formation of calcium carbonate ($CaCO_3$) and calcium hydroxide ($Ca(OH)_2$) are the most effective methods to heal concrete naturally.

The fundamental mechanisms for the formation of calcium carbonate is as follows:

$H_2O + CO_2 \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \Leftrightarrow 2H^+ + CO_3^{-2}$

$Ca^{2+} + CO_3^{-2} \Leftrightarrow CaCO_3$

$Ca^{2+} + HCO_3^{-2} \Leftrightarrow CaCO_3 + H^+$

However, this process only applies to very young concrete and the formation of calcium carbonate most likely causes self-healing at later ages. Natural self-healing can be useful for cracks with widths up to 0.1–0.2 mm.

## 2. Chemical Self-Healing Process:

Chemical healing process mainly refers to the artificial healing by injecting chemical compounds into the crack for healing. Self-healing concrete is designed by mixing chemical liquid agents such as glue with fresh concrete in small containers.

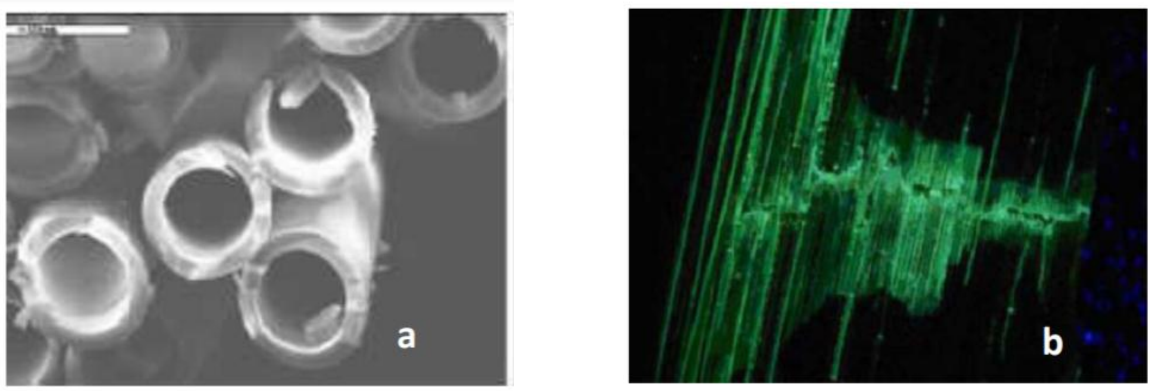
The chemical self-healing mode for concrete can be divided into two categories: a) active mode b) passive mode. Active mode uses vessel network linked with external supply of glue for the distribution of glue whereas the passive mode uses hollow pipettes, capsules or vessel network to distribute glue that is not linked to an external glue source.

Two common chemical methods that make use of glue addition to the concrete for healing purposes:

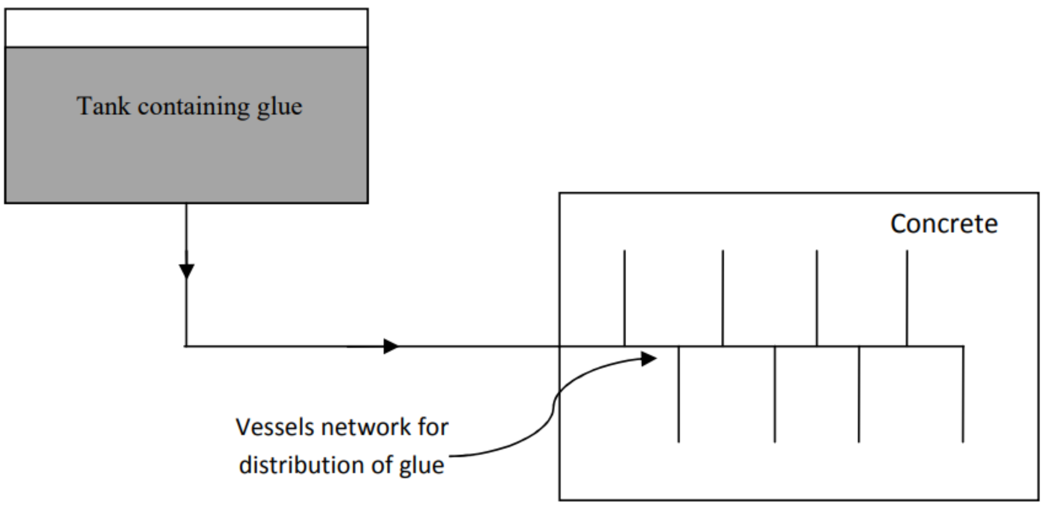
(1) hollow pipettes and vessel networks containing glue

(2) encapsulated glue

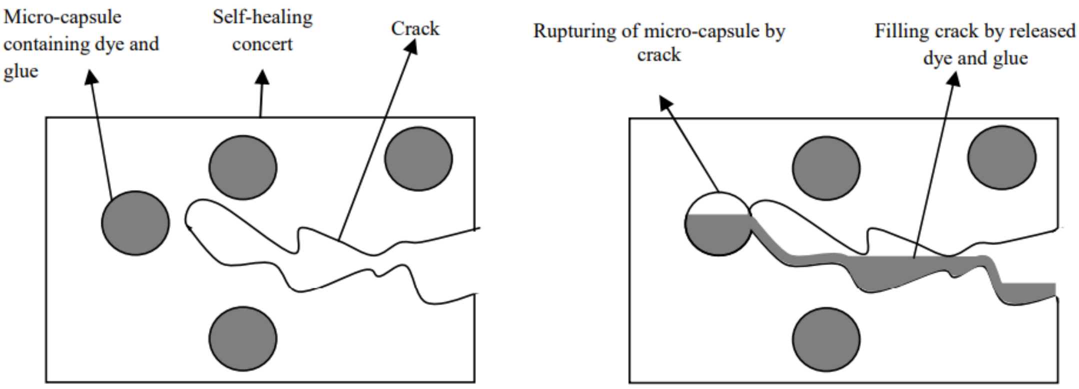
Hollow pipettes contain glue that can be mixed with fresh concrete and will be ruptured during crack propagation. The mixture of glue and fluorescent dye is released into the cracks and finally it heals the crack. Self-healing concrete containing hollow pipettes is inspired from blood vessels in creatures.




In Vessel network, network is placed inside a concrete specimen with one end linked to the supply of glue and the other end sealed.




Encapsulated glue: The size for capsules containing glue used for self-healing concrete varies from microcapsules to nanocapsules. Normally cracks would rupture the embedded microcapsules hence the glue is released into the crack faces through capillary action and the crack gets filled.




**(A)** Crack is being propagated into concrete

**(B)** The crack ruptures the microcapsule and glue released into the crack and is filled

3. **Biological self-healing process:**

Microorganisms can grow almost everywhere such as soil and water. Microorganisms are mostly divided into three important categories: bacteria, fungi, and viruses. Among these microorganisms, special strains of bacteria capable of precipitating certain chemicals are used to design the biological self-healing concrete. Microorganisms can be added to the biological self-healing concrete through different approaches. The bacteria produce calcium carbonate in presence of calcium source and other nutrients by biological process.

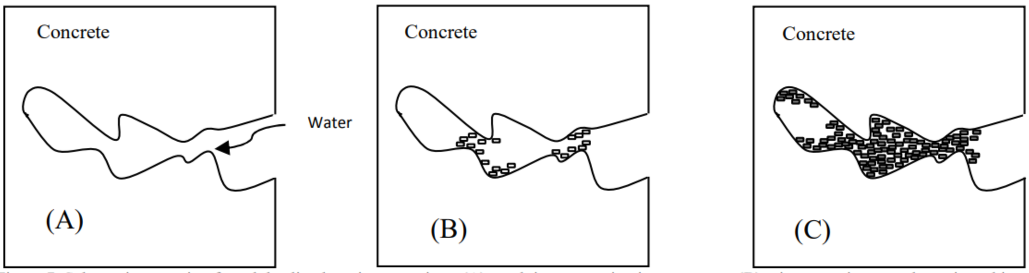



Figure 7: Schematic scenario of crack healing by microorganisms (A) crack is propagating into concrete; (B) microorganisms can be activated into crack; (C) microorganisms grow and precipitate calcium carbonate around their wall cells and can fill the crack

Further explanation of Bacterial Concrete is discussed below;

**Bacterial Concrete:**

The use of microorganisms to design self-healing concrete has been categorized as biological strategy by several researchers. Special strains of bacteria capable of precipitating certain chemicals are used to design the biological self-healing concrete. The involvement of microorganism in calcite precipitation can increase the durability of concrete. The bacteria produce calcium carbonate ($CaCO_3$) in presence of calcium source and other nutrients by biological process.

Special type of bacteria's known as Bacillus are used along with calcium nutrient known as Calcium Lactate. While preparation of concrete, this products are added in the wet concrete when the mixing is done. This bacteria's can be in dormant stage for around 200 years. When the cracks appear in the concrete, the water seeps in the cracks. The spores of the bacteria germinate and starts feeding on the calcium lactate consuming oxygen. The soluble calcium lactate is converted to insoluble limestone. The insoluble limestone starts to harden. Thus filling the crack, automatically without any external aide. Other advantage of this process is, as the oxygen is consumed by the bacteria to convert calcium into limestone, it helps in the prevention of corrosion of steel due to cracks. This improves the durability of steel reinforced concrete construction.

In self-healing concrete, formation of any cracks, leads to activation of bacteria from its stage of hibernation. When a concrete structure damages and water starts to penetrate in the cracks present in it the bacteria start to feed on the calcium lactate consuming oxygen and converts the soluble calcium lactate into insoluble limestone. The limestone formed thus seals the cracks present. By the metabolic activities of bacteria, during the process of self-healing, calcium carbonate precipitates into the cracks healing them. Once the cracks are completely filled with calcium carbonate, bacteria return to the stage of hibernation. In future, if any cracks form the bacteria gets activated and fills the cracks. Bacteria act as a long lasting healing agent.

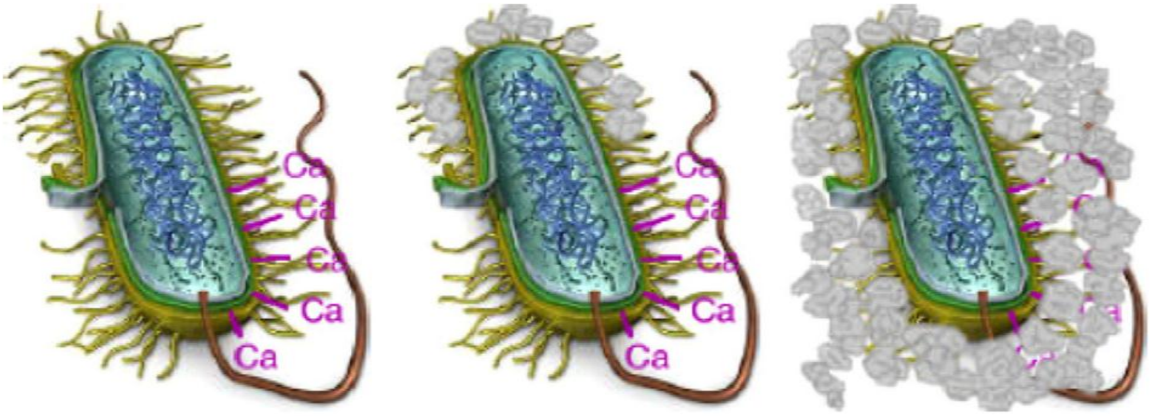



**Fig. 1.** Calcium carbonates formation on bacterial cell wall.

The pH of fresh concrete is usually between 10 to 13. The temperature of fresh concrete can go up to 70◦C. After the drying of concrete, there is not enough water. Therefore, the selected bacteria need to exhibit high resistance against high pH, temperature, and serious limitation of water. Bacillus Sphaericus can precipitate CaCO3 in the high alkaline environment.

Microbial calcium carbonate can be precipitated as a by-product during photosynthesis, urea hydrolysis, and sulfate reduction.

1. **Photosynthesis**: During photosynthesis by cyanobacteria, dissolved carbon dioxide ($CO_2$ reacts with water to form carbonic acid. In addition, exchanges occur between bicarbonate ($HCO^{3\ -}$) and hydroxyl ($OH^{-}$) ions, and bicarbonate ions dissociate into carbon dioxide ($CO_2$) and hydroxyl ($OH^{-}$) ions, increasing the pH of the environment. In the presence of $Ca^{2+}$ ions in the nutrient medium, calcium carbonate ($CaCO_3$) is formed by bacteria with the effect of high pH and precipitated as crystals.
2. **Ureolysis**: Ureolysis is recognized as one of the main pathways of microbial biomineralization of carbonates resulting from photosynthesis and sulfate reduction, as well as from the increase of both dissolved inorganic carbon and alkaline produced by bacterial degradation of urea. the precipitation of carbonates induced by ureolytic bacteria is strongly associated with urease activity, the enzyme responsible for catalyzing urea hydrolysis. A series of complex reactions in urea hydrolysis are driven by the enzymes urease and carbonic anhydrase. Microorganisms can easily adsorb Ca2+ ions on their surfaces due to their negative charge Under adverse conditions, the cell survives by allowing the entry and accumulation of calcium ions. This causes protons to be ejected. When a suitable environment is created, the cell compensates for the loss of protons by expelling Ca2+ ions. Although supersaturation of carbonate causes the precipitation of calcium carbonate on the cell surface.

3. **Sulfate reduction**: In anaerobic environments rich in organic matter, sulfate ($SO_4^{2-}$), sulfide ($SO_3^{2-}$ ) is reduced as a result of sulfate-reducing bacteria activity. On the other hand, sulfate-reducing bacteria can increase the local $Ca^{2+}$ concentration by degrading the extracellular polymeric substances of cyanobacteria or by secreting $Ca^{2+}$ from the cells. During this reaction, bicarbonate ($HCO3^{-}$) ion with organic carbon transforms into carbon dioxide (CO2) and hydroxyl ion ($OH^{-}$), increasing the pH of the environment. With the effect of increasing pH, $CO_2$ and $OH^{-}$ ions react with $Ca^{2+}$ ions in the environment and indirectly cause the precipitation of calcium carbonate minerals ($CaCO_3$).

This paper mainly focuses on urea hydrolysis process in self-healing concrete.

Several factors affecting the precipitation rate of biological calcite are (1) concentration of the dissolved inorganic carbon content, (2) pH, (3) concentration of calcium ions (4) presence

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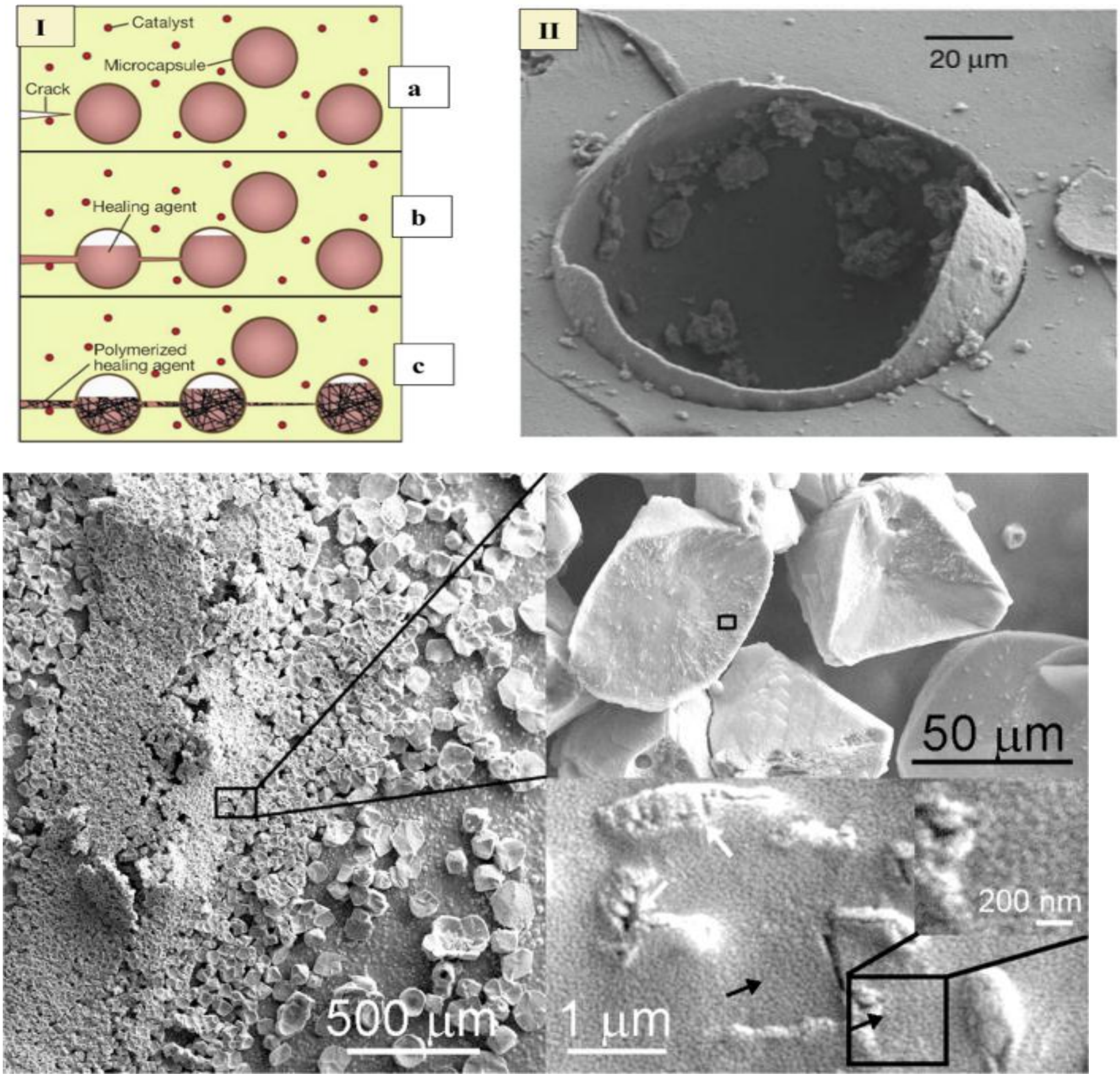
of nucleation sites. Microbial metabolisms can provide first three of these factors and the cell wall of the bacteria acts as nucleation site.

The primary role of bacteria to precipitate calcium carbonate is attributed to their ability to increase the pH of the environment through different bacterial metabolisms. Apparently, biological calcium carbonate precipitation using ureolytic bacteria is one of the most popular ways to design self-healing concrete. These bacteria are able to produce extracellular urease enzyme. The hydrolysis of urea to carbon dioxide and ammonia can be catalyzed by urease enzyme. The hydrolyzed ammonia and carbon dioxide increases the pH and carbonate concentration in the bacterial environment, respectively.



**Chemical Reaction:**

As a component of metabolism, bacteria species gives urease, that catalyzes urea to carbonate and ammonium that results in an increase of pH and carbonate concentration in the bacterial surroundings. These components further hydrolyze to ammonia ($NH^{4+}$) and carbonic acid ($CO_3^{2-}$) that leads to the formation of calcium carbonate. The process of making urease for the hydrolysis of urea CO $(NH_2)_2$ into carbonate ($CO_3^{2-}$) and ammonium ($NH^{4+}$) is be as follows:

1. Urea is hydrolyzed to Carbamic acid and ammonia in the presence of urease

$$CO\,(NH_2)_2 + H_2O \longrightarrow NH_2COOH + NH_3$$

2. Carbamate is spontaneously hydrolyzed to form ammonia and carbonic acid

$$NH_2COOH + H_2O \longrightarrow NH_3 + H_2CO_3$$

3. Carbonic acid is hydrolyzed to form carbonate ion and hydrogen ion

$$H_2CO_3 \longleftrightarrow HCO_3^{-} + H^{+}$$

4. Ammonia spontaneously hydrolyses to form ammonium and hydroxide ion

$$2NH_3 + 2H_2O \longleftrightarrow 2NH_4^{+} + 2OH^{-}$$

5. The reaction in Eqn. 4 continuously produces hydroxide ion, this gives rise to a pH increase, which shifts the overall equilibrium of bicarbonate ion ($HCO_3^{-}$) towards the formation of carbonate ions.

$$HCO_3^{-} + H^{+} + 2NH_4^{+} + 2OH^{-} \longleftrightarrow CO_3^{2-} + 2NH_4^{+} + 2H_2O$$

6. Bacterial cell wall has negative charge and for this reason, cell wall is able to draw positively charged calcium ions ($Ca2^{+}$) to deposit on their cell wall surface. The $Ca2^{+}$ ions then react with the $CO_3^{2-}$ ions leading to the precipitation of calcium carbonate ($CaCO_3$ ).

$$Ca^{2+} + Cell \longrightarrow Cell - Ca^{2+}$$

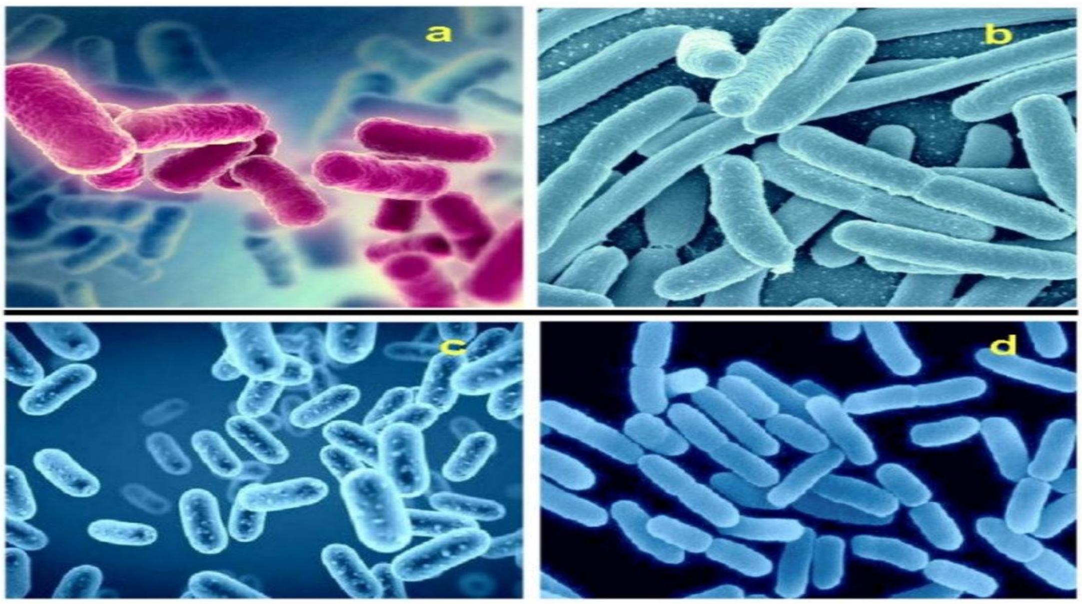
$$Cell- Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3 \downarrow – Cell$$

In this way, bacteria produce calcium carbonate precipitate. Based on the microbial pathway for the precipitation of calcium carbonate, urea has to be present in the self-healing concrete to initiate the necessary biochemical reactions.

A large domain of bacteria has been reported to produce self-healing concrete. Unfortunately, the pH of the fresh concrete is between 10 and 13, which is rather harsh for the survival of majority of bacteria. In addition, the temperature of the fresh concrete (70˚C) is also too high for cell growth. Upon drying of the concrete, there is not enough water for cell growth to occur. Therefore, the suitable bacteria have to exhibit high resistance against high pH, temperature, and serious limitation of water.Unlike the live bacteria, bacterial spore is very resistant against the harsh conditions as mentioned and some bacterial spores can live for more than 60 years.

The range of bacteria strains useful to design biological self-healing concrete using ureolytic process are mentioned below:

- Sporosarcina Pasteurii (or Bacillus Pasteurii)
- Bacillus megaterium
- Halomonas euryhaline
- Myxococcusxanthus
- Deleya halophila
- Bacillus sphaericus
- Bacillus lentus
- Acinobacter species
- Escherichia coli
- Pseudomonas aeruginosa
- Shewanella species
- Bacillus Cohnii
- Bacillus pseudofirmus
- Bacillus amyloliquefaciens
- Bacillus alkalinitrilicus




**Bacteria type:**

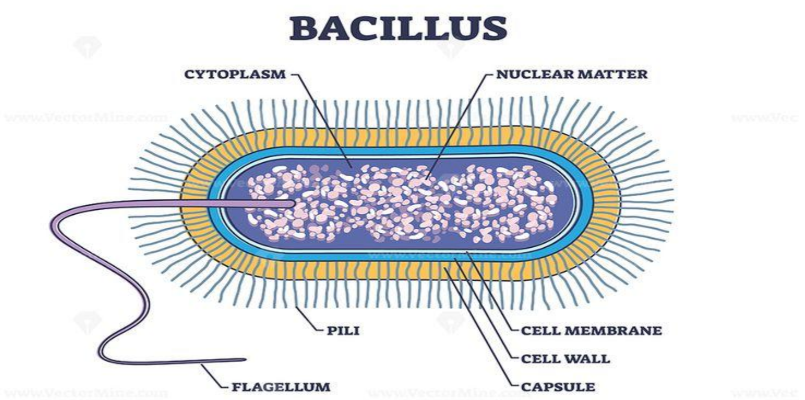
It is understood that the bacteria used in concrete that precipitate CaCO3 can be aerobic or anaerobic. Anaerobic bacteria are bacteria that have the ability to live and grow without oxygen. Examples of anaerobic bacteria are Pseudomonas, Thiobacillus, Denitrobacillus, Alcaligenes, Micrococcus, and Diaphorobacter.

Aerobic bacteria are bacteria that need oxygen to grow and multiply. Bacillus sporasarcina pasteurii, Bacillus sphaericus, Escherichia coli, Bacillus subtilis, Bacillus cohnii, Bacillus pseudofirmus, Bacillus halodurans, and Bacillus massiliensis are examples of aerobic bacteria used in self-healing concrete mixes and obtained favorable results. Some bacteria, such as Bacillus, can form more robust resistant spores in cells in harsh environments such as drought and freezing. According to the literature, some spores can sleep for hundreds or even thousands of years.

**Bacillus megaterium** is an aerobic, spore-producing, gram-positive bacterium. Cells are usually formed in pairs and chains by bonding with polysaccharides in the cell wall. It was reported that Bacillus megaterium has good temperature resistance and can grow in the temperature range of 3–45 ˚C.

**Bacillus cohnii** is a spore-forming, mesophilic gram-positive, nonureolytic bacterium isolated from soil. Bacillus cohnii and Bacillus pseudofirmus are gram-positive spore-forming bacteria that can remain dormant for many years. It can survive in highly alkaline conditions (pH > 10). The optimum growth temperature is 37 ˚C for Bacillus cohnii and 30 ˚C for Bacillus pseudofirmus.

**Bacillus sporosacina pasteurii** is a facultative anaerobic bacterium. It can grow aerobically or anaerobically but prefers the presence of oxygen and becomes metabolically active through aerobic respiration. It can withstand highly alkaline environments and is a gram-positive bacterium.Bacillus subtilis is a rod-shaped, gram-positive bacterium with a hard protective outer surface. It can survive in extreme climatic conditions and can be found naturally in soil or any vegetation. It can withstand temperatures between 25 and 37 ˚C.




**Nutrient medium:**

Ureolytic bacteria use free $Ca^{2+}$ ions in the environment while generating CaCO3 by urea hydrolysis. The calcium ion present in the environment increases the formation of calcium hydroxide, thus increasing the rate of precipitation.

Different nutrient media, such as calcium acetate, calcium formate, calcium lactate and calcium nitrate, are used as calcium sources. Yeast extract is nutrient source for the survival of bacteria. Urea is a source essential in case of ureolytic process.

**Healing Capacity:**

- The maximum filling depth of 35mm crack was reported when bacteria were microencapsulated.
- In direct application method, maximum filling depth of 27 mm crack was reported.
- Healing of maximum crack width of 0.970 mm was reported as per the literatures.

**Methods of applying bacteria in concrete:**

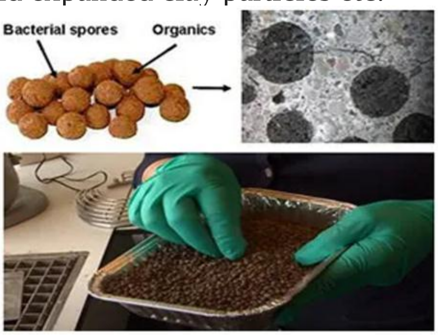
There are mainly two ways to apply the bacteria in concrete as per the literature.

1. **Microencapsulation**:

In microencapsulation method spores are encapsulated in a carrier to retain their viability for a longer time inside the concrete matrix. As soon as the crack ruptures the embedded microcapsules, the healing agent is released into the crack faces by using capillary movement.

By encapsulation method the bacteria and its food i.e. calcium lactate, are placed inside treated clay pellets and concrete is prepared. About 6% of the clay pellets are added for making bacterial concrete. When concrete structures are made with bacterial concrete, when the crack occurs in the structure and clay pellets are broken and the bacteria germinate and eat down the calcium lactate and produce limestone, which hardens and thus sealing the crack.

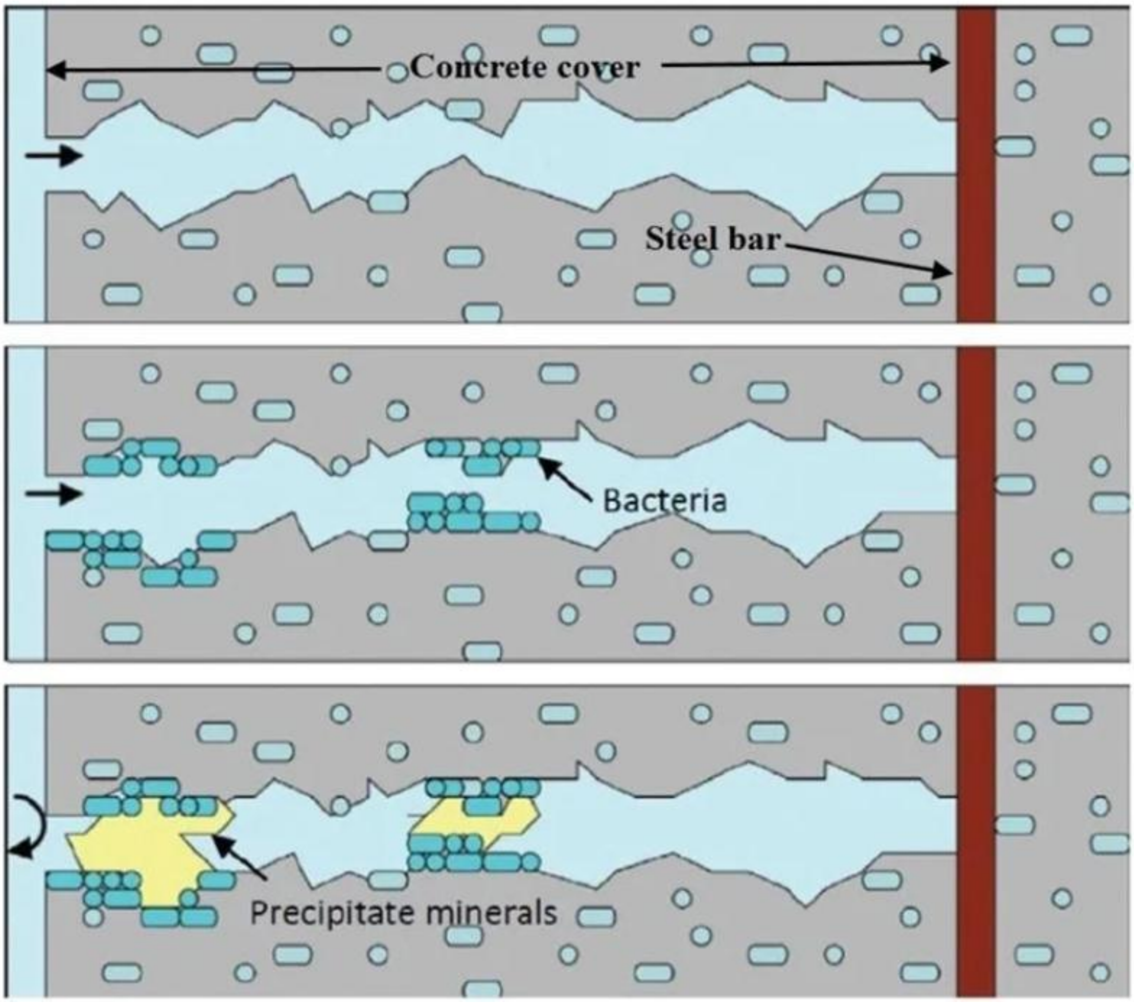
The tested encapsulation materials include aerated concrete granules, ceramsite silica gels, polyurethane, hydrogels and expanded clay particles etc.




2. **Direct application:**

In the direct application method, bacterial spores and calcium lactate is added into concrete directly when mixing of concrete is done. The use of this bacteria and calcium lactate doesn't change the normal properties of concrete. When cracks are occurred in the structure due to obvious reasons. The bacteria are exposed to climatic changes. When water comes in contact with this bacteria, they germinate and feed on calcium lactate and produces limestone. The bacteria precipitate calcium carbonate, thus sealing the cracks.

Following image shows the embedded bacteria into the concrete matrix. When crack occurs due to any reason, exposed bacteria gets activated and they starts feeding the calcium food source (calcium lactate) mixed in concrete and produces calcium carbonate which then accumulate and seals the crack. Once crack is sealed, bacteria goes back to hibernation stage.




**Ingredients typically used:**

i. **Cement:** Ordinary Portland Cement or any suitable type of cement.

ii. **Aggregates:** Sand, gravel, or crushed stone.

iii. **Water:** Clean water for mixing.

iv. **Bacteria:** Ureolytic or non-ureolytic bacteria, such as species of Bacillus or Sporosarcina, commonly used for self-healing properties.

v. **Nutrients:** calcium lactate, Yeast extract, Urea etc.

**Recipe of Bacterial concrete:**

**Prepare the Concrete mix:** Mix the cement, aggregate, and water in appropriate proportions based on the desired concrete strength. Follow standard concrete mixing practices to achieve a uniform concrete mixture.

**Bacteria incorporation:** Incorporate the bacteria into the concrete mix. This can be achieved in different ways:

Direct Mixing: Bacteria can be introduced into the concrete mix by spraying a bacterial solution onto the aggregates or mixing the bacteria directly into the water used for making the concrete.

Micro encapsule: Bacteria can also be encapsulated within microcapsules designed to release them when cracks occur, ensuring they remain dormant until activated by moisture.

**Nutrient sources:** Bacteria require specific nutrients to survive and thrive. The nutrients to bacteria are supplied in the form of “calcium lactate, calcium nitrate, and calcium formate etc.” (3% of cement) which are calcium sources, “Yeast extract” (0.85% of cement) which is nutrient source for the survival of bacteria and “Urea” (2.5% of cement) which is a source essential in case of ureolytic process. Mix all these nutrient sources in required proportion to the cement content.

**Homogeneous Distribution:** Ensure that the bacteria are uniformly distributed throughout the concrete mixture to facilitate their activation and functionality when cracks form.

**Curing:** After mixing the ingredients, proceed with the curing process as per standard concrete curing practices. This allows the concrete to gain strength and provides an environment conducive to bacterial activation and growth.

**Activation:** Once the concrete is placed, the bacteria remain dormant until they come into contact with water from cracks or external sources. When activated by moisture penetrating the concrete, the bacteria start their metabolic activities, initiating processes such as ureolysis or non-ureolytic mineral precipitation to aid in self-healing.

**Case study 1:**

**Refernece:** Hesam Doostkami et al. *"Self-healing capability of conventional, high-performance, and Ultra High-Performance Concrete with commercial bacteria characterized by means of water and chloride penetration" Vol. 401, 2023*

The primary aim of this literature was to study the effect of bacterial application on water permeability and chloride penetration of the concrete. Commercially available bacteria were used to reduce cost of self-healing techniques. Serenade® Max from BAYER is Bacillus subtilis encapsulated in Diatomaceous Earth, formulated as a wettable powder of natural origin containing 15.67% p/p (g/g) bacteria and bacterial content of $5.13 \times 10^{10}$ CFU/g. bacteria was added in the dosage of 7.5 kg/m3 and 15 kg/m3.

This work studies three different types of concrete: a reference mix of conventional concrete (C30/37) labeled CC, a High-Performance Concrete (C70/85) labeled HPC, and an Ultra High-Performance (160).

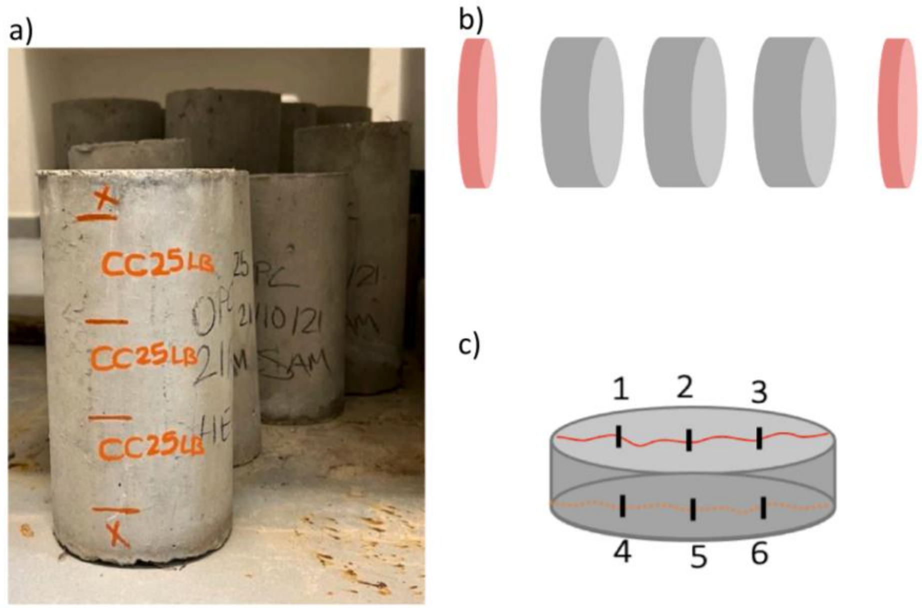



**Fig. 1.** A) cylinder marks before cutting the disks, b) cut of cylinders to obtain disks, c) crack measurement points after pre-cracking.

The liquid bacteria, SerBiotec solution consisting of a combination of 66.3% Bacillus bacteria, 26.2% denitrifying bacteria, and 7.5% photosynthetic bacteria, containing in total $6.81 \times 10^5$ CFU/l bacteria. This product was incorporated in substitution of the water content of the reference mix.

**For crack heal test**, 8 cylinders of ϕ100 X 200 mm per mix were prepared. Samples are pre-cracked, targeting cracks near 500 µm during loading. The 21-day-old disks were pre-cracked using a splitting test. The crack's width was measured the same day after pre-cracking and after 28 days of healing under various conditions.

{28 days immersion in water (WI), 28 days in a humidity chamber (HC) and 7 days in water immersion followed by 21 days in the humidity chamber (WH)}

**For permeability test**, a day after the disks were sealed, the bottom surface was wrapped with PVC tape to prevent water leakage before the test began. After preparing the water permeability setup, the tubes were filled with a 200 mm water column.

**For chloride penetration test**, samples were placed on the setup, and the tubes were filled with a 200 mm water column containing 33 g NaCl/litre. Two areas appear: the dark area presents the regions without penetration of the chloride, and the lighter area indicates the region containing chloride.

**Result:**

- One week of water immersion significantly improved the crack closure capability compared to specimens healed in a humidity chamber.
- Chloride penetration reduction of specimens containing bacteria was much greater than that of specimens containing no bacteria.
- Water tightness of specimens healed in water immersion conditions is higher than in other conditions. **Case study 2:**

**Refernece:** M. Pourfallahi et al. ***"Effect of direct addition of two different bacteria in concrete as self-healing agent"*** Vol. 28, 2020.

In this paper, an attempt has been made to analyze and study the feasibility of self-repairing bacterial concrete in a special way. This method is based on two principles: 1) Extraction of bacteria compatible with the concrete from concrete components 2) Adding bacteria in the solution directly to the concrete mixing plan.

The nutrient broth culture medium was considered to propagate the bacteria. According to the instructions 13 g of Nutrient broth powder was poured into 1 L of distilled water, and the solution was thoroughly mixed and clarified by heating. The medium was then sterilized in an autoclave at 121 ◦C for 15 min.  concrete components such as washed sand and 1.5 cm crushed sand were then prepared In the designs containing bacterial, the water-substituting bacteria were utilized for 5% by the weight of components. After the complete mixing of the materials, fresh concrete was poured into 150 mm molds.

To generate different levels of damage on the concrete samples with/ without bacteria, on the 28th day, 50 tons of force was applied to create micro-cracks in the sample. To prove the self-healing after cracking, some photos were taken from all samples, and then the cracked samples were wetted and dried. the bacterial concrete was placed in ordinary underwater for 24 h in a laboratory environment. It was then dried in the open air for 24 h. After 6 weeks of wet and dry cycle, all cracked samples were photographed.

The compressive strength tests of samples on 7 and 28 days of construction were conducted. The average compressive strength of three samples was considered for each group.

To perform the water absorption tests t first the bacterial and control concrete samples were dried in the incubator for 24 h at 105 ◦C, and they were immersed in water for half an hour after cooling. The weighing of the samples was carried out

water absorption (%) = $(W_{\text{saturated}} - W_{\text{oven dried}}) / W_{\text{oven dried}} \times 100$

**Result:**

- The 7-Day compressive strength of bacterial concrete with PPC was 1.94% higher than its control sample. Moreover, bacterial concrete with cement PC2 was 5.65% lower than its Table control sample.
- The water absorption percent of the bacterial concrete samples with PPC and PC2 cements was 0.07 and 0.19% higher than that of their control samples

**Application areas of bacterial concrete**

- **Infrastructure projects:** Bacterial concrete can be used in bridges, roads, tunnels, and other critical infrastructure to increase their lifespan and reduce maintenance costs.
- **Buildings:** It can be used in the construction of residential and commercial buildings, reducing the need for frequent repairs and associated expenses.
- **Runways and pavements:** in airports, bacterial concrete can be used in runways and pavements to minimize damage from heavy aircraft and vehicles.
- **Highways and roads:** Bacterial concrete can be applied to highway and road construction to extend the life of these structures and reduce road maintenance.
- **Harbors and ports:** Bacterial concrete can protect marine structures such as harbor walls and breakwaters from the corrosive effects of seawater and wave action.
- **Underwater structures:** It can be used in underwater structures like piers and docks, helping to maintain their integrity in harsh marine environments.
- **Mines**: In the mining industry, self-healing concrete can be used for infrastructure within mines to withstand the challenges of constant vibration, heavy loads, and harsh working conditions.
- **Offshore platforms:** Bacterial concrete can be applied to offshore drilling platforms to enhance structural integrity and prevent corrosion due to exposure to seawater and harsh environmental conditions.
- **Water treatment plants:** Bacterial concrete can be used in the construction of tanks and structures in wastewater treatment plants to resist chemical corrosion and extend service life.
- **Sewage system:** It can help improve the durability of sewage pipes and reduce leakage, preventing environmental contamination.

**Advantages of Bacterial Concrete in Construction field**.

- **Self-healing capability**: Bacterial concrete has the unique ability to heal small cracks that develop over time. When cracks occur, moisture penetrates the concrete, activating the dormant bacteria, which then produce calcite to fill in the gaps. This self-healing property extends the lifespan of concrete structures and reduces the need for costly repairs.
- **Durability**: The self-healing feature enhances the durability and longevity of structures made with bacterial concrete. By preventing the ingress of water and harmful substances, it reduces the risk of corrosion and deterioration.

- **Reduced maintenance costs**: The self-healing nature of bacterial concrete reduces the need for frequent maintenance and repair, ultimately saving on maintenance costs and minimizing downtime for infrastructure projects.
- **Environmental benefits**: Bacterial concrete is considered more environmentally friendly than traditional concrete because it can extend the service life of structures, reducing the need for replacement or significant repairs. This helps to conserve resources and reduce the carbon footprint associated with the construction industry.
- **Improved sustainability**: Bacterial concrete aligns with sustainable construction practices by promoting long-lasting structures, reducing waste, and decreasing the need for resource-intensive maintenance activities.
- **Enhanced waterproofing**: Bacterial concrete can enhance the waterproofing properties of concrete structures, reducing the risk of water penetration and damage. This is particularly beneficial for infrastructure exposed to harsh environmental conditions.
- **Potential for use in various applications**: Bacterial concrete can be used in a wide range of applications, including bridges, roads, buildings, and marine structures. It offers versatility and adaptability in construction projects.

**Disadvantages of Bacterial Concrete:**

- **Initial cost**: Bacterial concrete can be more expensive than traditional concrete due to the incorporation of bacteria and special nutrients.
- **Precise mixing and handling**: Bacterial concrete requires careful mixing and handling to ensure that the bacteria remain viable and evenly distributed throughout the concrete.
- **Limited crack size healing**: Bacterial concrete is most effective at healing relatively small cracks, typically those up to a few milli meters in width. It may not be as effective at repairing larger or more extensive structural damage.
- **Activation time**: Bacterial concrete's self-healing process relies on moisture and oxygen penetration to activate the bacteria. This means that it may not be suitable for applications where the concrete remains consistently dry, as there would be no trigger for healing.
- **Compatibility issues**: Bacterial concrete may not be fully compatible with certain concrete additives, admixtures, or construction practices. The addition of chemicals or treatments that inhibit bacterial activity can reduce or negate its self-healing properties.

- **Long-term monitoring**: To ensure the effectiveness of self-healing in bacterial concrete, long-term monitoring and maintenance are required.
- **Environmental concerns**: Some environmental concerns have been raised regarding the use of bacteria in concrete, including the potential for these organisms to spread in the environment, particularly in aquatic ecosystems.
- **Limited widespread adoption**: Bacterial concrete is still a relatively new technology, and widespread adoption may be hindered by a lack of familiarity among contractors and engineers.
- **Research and development**: Ongoing research and development are needed to refine the use of bacteria in concrete, improve its effectiveness, and address the limitations and challenges associated with this technology.

Despite these disadvantages, ongoing research and innovation in the field of bacterial concrete are likely to lead to improvements and a broader range of applications in the future.

## CONCLUSION

In the course of this report, we have explored the intricacies of bacterial concrete, from the underlying science of self-healing mechanisms to the practical applications and real-world case studies. The key insights from this research can be summarized as follows:

**Ureolitic Process:** The ureolitic process, which involves the conversion of urea and calcium sources into calcium carbonate, is the core mechanism that enables the healing of cracks in concrete. This process, mediated by bacteria, plays a pivotal role in repairing structural damage.

**Bacterial Diversity:** Various types of bacteria have demonstrated the capacity to facilitate the ureolitic process and contribute to self-healing. Bacteria such as Bacillus Pasteurii, Bacillus Cohnii etc. are among those studied and employed in different applications.

**Nutrients and Food Source:** Understanding the nutritional requirements of the bacteria, as well as the provision of suitable food sources, is crucial for the successful implementation of self-healing concrete. calcium lactate, Yeast extract, Urea etc.

**Application Method:** This report has elucidated the various methods used for introducing bacteria into concrete, including encapsulation and in situ activation / Direct mixing, each with its own advantages and challenges

**Case Studies:** Real-world case studies and examples have demonstrated the practicality and effectiveness of bacterial concrete across a range of applications, from infrastructure projects to historical monument preservation.

In conclusion, bacterial concrete is not just a novel concept; it is a sustainable, eco-friendly, and cost-effective solution to enhance the longevity and durability of concrete structures. As the construction industry continues to seek innovative ways to address infrastructure challenges and promote sustainability, the adoption of bacterial concrete is poised to grow. Nevertheless, it is important to recognize that, like any technology, bacterial concrete has its limitations, and further research and development are essential to fully unlock its potential.

## REFERENCES


1) Fadi Althoey, Osama Zaid, et al. “Physical, strength, durability and microstructural analysis of self-healing concrete: A systematic review”. Vol 18, 2023
2) Md Montaseer Meraz, Nusrat Jahan Mim, et al. “Self-healing concrete: Fabrication, advancement, and effectiveness for long-term integrity of concrete Infrastructures”. Vol 73, 2023:
3) Manas Sarkar, Moumita Maiti, et al. “Bio-concrete: Unveiling self-healing properties beyond crack-sealing”. Vol 74, 2023:
4) Hassan Amjad, Muhammad Shah Zeb, et al. “Impacts of biomimetic self-healing of Lysinibacillus boronitolerans immobilized through recycled fine and coarse brick aggregates in concrete”. Vol 76, 2023:
5) Vanessa Giaretton Cappellesso , Tim Van Mullem, et al. “Bacteria-based self-healing concrete exposed to frost salt scaling”. Vol 139, 2023:
6) Xi Xiao, Cise Unluer, et al. “Single bacteria spore encapsulation through layer-by-layer self-assembly of poly (dimethyldiallyl ammonium chloride) and silica nanoparticles for self-healing concrete”. Vol 140, 2023:
7) Mahmoud Sadeghpour, Mohammadreza Baradaran. “Effect of bacteria on the self-healing ability of fly ash concrete”. Vol 364, 2023:
8) Hassan Amjad, Rao Arsalan Khushnood, Shazim Ali Memon. “Biomimetic robust self-healing of Bacillus Subtilis immobilized through sisal fiber for next-generation concrete infrastructure”. Vol 368, 2023:
9) Harry Hermawan, Virginie Wiktor, Elke Gruyaert, Pedro Serna. “Experimental investigation on the bond behaviour of steel reinforcement in self-healing concrete”. Vol 383, 2023:
10) Xiaoqi D, Zheng Si, et al. “Optimization of spore production and activation conditions of concrete crack healing bacteria and research on crack repair effect”. Vol 394, 2023:
11) Bowen Guan, Qian Tian, et al. “Selecting bacteria for in-depth self-healing of concrete at both room and low temperature”. Vol 394, 2023:

12) Hesam Doostkami, Javier de Jesús Estacio Cumberbatch,et al. “Self-healing capability of conventional, high-performance, and Ultra High-Performance Concrete with commercial bacteria characterized by means of water and chloride penetration”. Vol 401, 2023:

13) Aihua Deng, Tiantian Wang. “Adaptive mechanisms of Bacillus to near space extreme environments”. Vol 886,2023:

14) Rama Mohan Rao Pannem, Bhaskar Bashaveni, et al. “The effect of fly ash aggregates on the self-healing capacity of bacterial Concrete”. Mar 2023:

15) Burcu Aytekin, Ali Mardani, S¸ emsi Yazıcı. “State-of-art review of bacteria-based self-healing concrete: Biomineralization process, crack healing, and mechanical properties”. 2023:

16) Diego J. De Souza, Leandro F.M. Sanchez, et al. “Understanding the efficiency of autogenous and autonomous self-healing of conventional concrete mixtures through mechanical and microscopical analysis”. 2023:

17) Mehrab Nodehi, Togay Ozbakkaloglu, Aliakbar Gholampour. “A systematic review of bacteria-based self-healing concrete:Biomineralization, mechanical, and durability properties”. Vol 49, 2022:

18) Xianzhi Wang, Jing Xu, Zhongping Wang, Wu Yao. “Use of recycled concrete aggregates as carriers for self-healing of concrete cracks by bacteria with high urease activity”. Vol 337, 2022:

19) Manpreet Bagga, Charlotte Hamley-Bennett, et al. “Advancements in bacteria based self-healing concrete and the promise of modelling”. Vol 358, 2022:

20) Manpreet Bagga, Charlotte Hamley-Bennett, et al. “Advancements in bacteria based self-healing concrete and the promise of modelling”. Vol 358, 2022:

21) Chereddy Sonali Sri Durgaa, et al. “Comprehensive microbiological studies on screening bacteria for self-healing concrete”. Vol 15, 2021:

22) Yihong Tang , Jing Xu. “Application of microbial precipitation in self-healing concrete: A review on the protection strategies for bacteria”. Vol 306, 2021:

23) Hassan Amer Algaifi, Suhaimi Abu Bakar, et al. “Bio-inspired self-healing of concrete cracks using new B. pseudomycoides species”. March 2021:

24) M. Pourfallahi, A. Nohegoo-Shahvari, M. Salimizadeh. “Effect of direct addition of two different bacteria in concrete as self-healing agent”. Vol 28, 2020:

25) Mr. Sk. Alisha, Mr. P. Rohit, et al. “Self-Healing Concrete Using Bacteria”. Nov 2020:

26) How-Ji Chen, et al. “Self-Healing Concrete by Biological Substrate”. Dec 2019:

27) Hana Schreiberov, Petr Bílý, et al. "Impact of the self-healing agent composition on material characteristics of bio-based self-healing concrete". May 2019:

28) Muhd Afiq Hizami Abdullah1, et al. "Development and Performance of Bacterial Self-healing Concrete - A Review". Vol 431, 2018:

29) B.R.Gautam1. "Bacteria Based Self-Healing Concrete – A Bacterial Approach". 2018:

30) Mohamed alazhari, et al. "Application of expanded perlite encapsulated bacteria and growth media for self-healing concrete". Nov 2017:

31) Kunamineni Vijay, Meena Murmu, Shirish V. Deo. "Bacteria based self healing concrete – A review". July 2017:

32) E. Tziviloglou, V. Wiktor, "Bacteria-based self-healing concrete to increase liquid tightness of cracks". Vol 122, June 2016:

33) Mian Luo, Chun-xiang Qian, Rui-yang Li. "Factors affecting crack repairing capacity of bacteria-based self-healing Concrete". April 2015:

34) Salmabanu Luhar, Suthar Gourav. "A Review Paper on Self Healing Concrete". Jan 2015:

35) Amirreza Talaiekhozan, Ali Keyvanfar et al. "A Review of Self-healing Concrete Research Development". Vol 02, Feb 2014:

36) J. Y. Wang • N. De Belie • W. Verstraete. "Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete". Sep 2011:

37) Rafat Siddique, Navneet Kaur Chahal. "Effect of ureolytic bacteria on concrete properties". May 2011:

38) Henk M. Jonkers, Arjan Thijssena. "Application of bacteria as self-healing agent for the development of sustainable concrete". Dec 2008:

39) Henk M. Jonkers & Erik Schlangen. "Development of a bacteria-based self healing concrete". May 2008:

40) H. M. Jonkers1. "Bacteria-based self-healing concrete".