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USE OF BACTERIA TO REPAIR CRACKS IN CONCRETE

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ABSTRACT

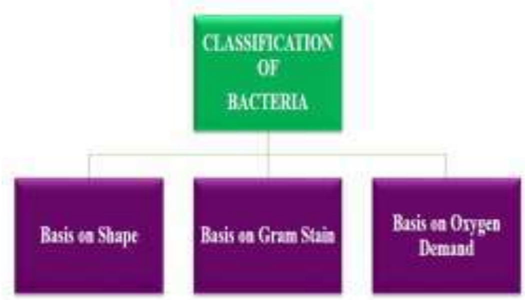
Concrete which forms major component in the construction Industry as it is cheap, easily available and convenient to cast. But drawback of these materials is it is weak in tension so, it cracks under sustained loading and due to aggressive environmental agents which ultimately reduce the life of the structure which are built using these materials. This process of damage occurs in the early life of the building structure and also during its life time. Synthetic materials like epoxies are used for remediation. But, they are not compatible, costly, reduce aesthetic appearance and need constant maintenance. Therefore bacterial inducedCalcium Carbonate (Calcite) precipitation has been proposed as an alternative and environment friendly crack remediation and hence improvementof strength of building materials..

INTRODUCTION

A novel technique is adopted in re-mediating cracks and fissures in concrete by utilizing Microbiologically Induced Calcite or Calcium Carbonate ($CaCO3$) Precipitation (MICP) is a technique that comes under a broader category of science called bio-mineralization. MICP is highly desirable because the Calcite precipitation induced as a result of microbial activities is pollution free and natural. and stiffness of cracked concrete specimens.

The technique can be used to improve the compressive strength and stiffness of cracked concrete specimens. Research leading to microbial Calcium Carbonate precipitation and its ability to heal cracks of construction materials has led to many applications like crack remediation of concrete, sand consolidation, restoration ofhistorical monuments and other suchapplications. So it can be define as "The process can occur inside or outside the microbial cell or even some distance away within the concrete. Often bacterial activities simply trigger a change in solution chemistry that leads to over saturation and mineral precipitation. Use of these Bio mineralogy concepts in concrete leads to potential invention of new material called —Bacterial Concrete"

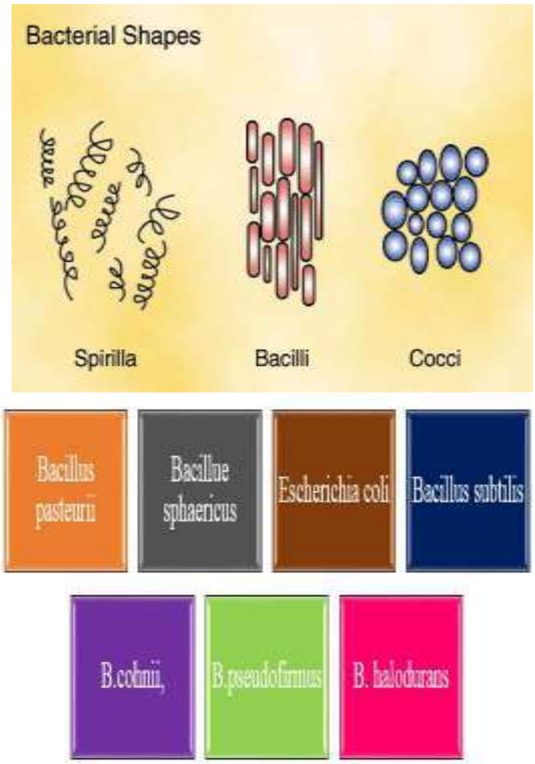
CLASSIFICATION OF BACTERIA



Bacteria is generally classified in three category Basis on Shape, Basis on Gram Stain and Basis on Oxygen Demand which shown in Fig. 1and sub types of each category also can be shown in Fig. 2,Fig. 3**.**

VARIOUS TYPES OF BACTERIA USED IN CONCRETE

There are various types of bacteria were used in construction area, from literature review it is as shown in **Fig. 5**and other application of bacteria are shown in **Table.1** and **Table 2**.



Type of Microorganism	System	Crystal type	Ref.
Photosynthetic organism : Synechococcus GL24	Meromictic lake	Calcite ($CaCO_3$)	Tai C.Y. and Chen F.B., 1998
Photosynthetic organism :Chlorella	Lurcene Lake	Calcite ($CaCO_3$)	Sanchez-Moral S., Canaveras J.C 2003
Sulphate Reducing Bacteria: Isolate SRB LVform6	Anoxic hypersaline lagoon	Dolomite (Ca(Mg) CO_3)	González-Muñoz M.T. 2000
Nitrogen cycle Bacillus subtilis	Urea degradation in synthetic medium	Calcite ($CaCO_3$)	Castanier S. 2000
Nitrogen cycle Bacillus cereus	Ammonification and nitrate reduction	Calcite ($CaCO_3$)	Castanier S. 1999
Nitrogen cycle Bacillus subtilis JC3	Ammonification (Ammo acid degradation)	Calcite ($CaCO_3$)	Seshagiri Rao M.V 2012

BIO-CONCRETEWORK

HOW DOES BIO-CONCRETE WORK?

Self-healing concrete is a product that will biologically produce limestone to heal cracks that appear on the surface of concrete structures. Specially selected types of the bacteria genus Bacillus, along with a calcium-based nutrient known as calcium lactate, and nitrogen and phosphorus, are added to the ingredients of the concrete when it is being mixed. These self healingagents can lie dormant within the concrete for up to 200 years. However, when a concrete structure is damagedand water starts to seep through the cracks thatappear in the concrete, the spores of the bacteriagerminate on contact with the water andnutrients. Having been activated, the

Application	Micro organism	Metabolism	Nutrients	REF.
Biological mortar	Bacillus cereus	oxidative deamination of amino acids	Growth media (peptone, extract yeast, KNO_3, NaCl) + $CaCl_2.2H_2O$, Actical, Natamycine	De Muynck W 2008
Crack in concrete remediation	Bacillus subtilis	Hydrolysis of urea	Nutrient broth, urea, $CaCl_2.2H_2O$, NH4Cl, $NaHCO_3$	Ramachandran S.K., 2001
Crack in concrete remediation	Bacillus sphaericus	Hydrolysis of urea	Extract yeast, urea, $CaCl_2.2H_2O$	De Muynck W., 2010
Bacterial concrete	Bacillus subtilis	Hydrolysis of urea	Nutrient broth, urea, $CaCl_2.2H_2O$, NH_4Cl, $NaHCO_3$	De Muynck W 2008
Bacterial concrete	Bacillus subtilis	oxidative deamination of amino acid	Peptone: 5 g/lit., NaCl: 5 g/lit., Yeast extract: 3 g/lit.	Seshagiri Rao M.V., 2012

bacteriastart to feed on the calcium lactate.

As thebacteria feeds oxygen is consumed and thesoluble calcium lactate is converted to insolublelimestone. The limestone solidifies on thecracked surface, thereby sealing it up. It mimicsthe process by which bone fractures in thehuman body are naturally healed by osteoblastcells that mineralise to re-form the bone.The consumption of oxygen during the bacterialconversion of calcium lactate to limestone hasan additional advantage. Oxygen is an essentialelement in the process of corrosion of steel andwhen the bacterial activity has consumed it all itincreases the durability of steel reinforcedconcrete constructions.

The two self-healing agent parts (the bacterialspores and the calcium lactate-based nutrients)are introduced to the concrete within separateexpanded clay pellets 2-4 mm wide, whichensure that the agents will not be activatedduring the

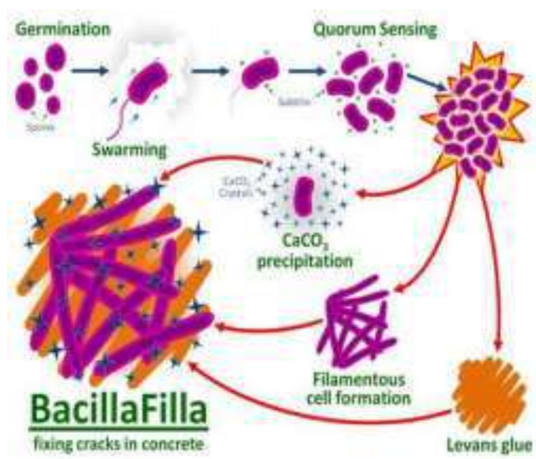
In the crack fixing process the anaerobic typebacteria which can be using along with concretecan be fix that crack by step by step.At first germination of germs by spores and
swarming themselves and quorum sensing andgrowing from proper medium in large amount inparticular time and from the metabolism process -levans glue is produce and making such type offilamentous cell formation and precipitationCaCO3. This both material combine with each otherand making cementations material.

MATERIALS AND METHODS:

cement-mixing process. Only whencracks open up the pellets and incoming waterbrings the calcium lactate into contact with thebacteria do these become activated.Testing has shown that when water seeps intothe concrete, the bacteria germinate and multiplyquickly. They convert the nutrients intolimestone within seven days in the laboratory.Outside, in lower temperatures, the process takesseveral weeks.

PROCESS OF FIXING CRACKS IN CONCRETE BY BACTERIA

Process of fixing cracks in concrete by bacteriain such a process can be shown in **Fig.6**



Bacterial strains and spore formation:

Starter cultures of alkaliphilic (i.e. alkali-resistant) spore-forming bacteria were obtained from the German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany. These cultures were initially cultivated according to the suppliers' recommendations in yeast extract based medium. Subsequently, growth and spore-forming potential (sporulation) was further tested in mineral medium amended with different organic carbon sources (6 g

Na-citric acid or 5 g peptone plus 3 g yeast extract per liter).

Mineral medium contained per liter of Milli-Q ultra-pure water: 0.2g NH_4Cl, 0.02g KH_2PO_4, 0.225g $CaCl_2$, 0.2g KCl, 0.2g $MgCl_2.6H_2O$, 1 ml per liter trace elements solution SL12B, 0.1g yeast extract and 8.4g sodium bicarbonate. The pH of this medium was 9.2. Aerobic batch cultures were incubated in 2-l Erlenmeyer flasks on a shaker table at 150 rpm. Growth was monitored by microscopy and cell numbers and percentage of sporulating cells were quantified by microscopy using a Burger-Turk counting chamber.

Concrete compatibility of bacteria and organic substrates:

A series of tests were performed in order to investigate whether the incorporation of bacteria or organic substrates (needed for bacterial calcium carbonate formation) in the concrete matrix do not negatively affect strength characteristics. Therefore concrete bars with and without (control) bacteria or organic substrates were prepared for flexural tensile- and compressive strength determination. For the preparation of bacterial concrete, a dense culture of S.pasteurii, grown in medium, was obtained and total cell number was quantified by microscopy using a Burger-Turk counting chamber.

Cells were harvested after a double washing step by centrifugation (20 min x 10000g) and suspension of the cell pellet in tap water. The washed cells were finally suspended in a 20-ml aliquot of tap water which was used as part of the needed make up water for concrete bar preparation. The quantity of the tested organic compounds was 0.5% of cement weight. The individual organic compounds (Na-aspartate, Na-glutamate, Na-polyacrylate, Na-gluconate, Na-citric acid and Na-ascorbic acid) were firstly dissolved in the needed make up water, prior to concrete bar preparation.

Bacterial-, organic compound- and control sets of concrete bars for flexural tensile- and compressive strength testing were prepared. Each set consisted of three replicate bars (dimensions 16 x 4 x 4 cm) made from ordinary portland cement (ENCI CEMI 32.5R), make up water and aggregates. Aggregate (gravel and sand) composition and quantities of used components are listed in Table 1. The concrete bars were uncased after an initial curing period of 24 hours and were subsequently further cured in tap water-filled separate plastic containers at room temperature. The sets of three replicate bars were tested for flexural tensile- and compressive strength after 28 days curing, following the procedure according to EN 196-1 Standard Norm.

Table 1: Cement, water and aggregate composition needed for the production of 3 concrete bars of dimensions 16 x 4 x 4 cm used for flexural tensile- and compressive strength characterization of bacterial-, organic compound-, and control (no bacteria added) concrete

CHEMICAL PROCESS TO REMEDIATECRACKS BY BACTERIA

Bacteria from various natural habitats havefrequently been reported to be able to precipitatecalcium carbonate both in natural and in laboratoryconditions (Krumbein, 1979; Rodriguez et al.,2003).Different types of bacteria, as well as abioticfactors (salinity and composition of the medium)seem to contribute in a variety of ways to calciumcarbonate precipitation in a wide range of differentenvironments (Knorre & Krumbein, 2000;Rivadeneyra et al., 2004).Calcium carbonate precipitation is a straightforward chemical process governed mainly by fourkey factors:

(1) The calcium concentration,
(2) The concentration of dissolvedinorganic carbon (DIC),
(3) The pH and
(4) The availability of nucleation sites(Hammes & Verstraete, 2002).

$CaCO_3$ precipitation requires sufficient calciumand carbonate ions so that the ion activity product(IAP) exceeds the solubility constant (*K*so) (Eqs.(1) and (2)). From the comparison of the IAP withthe *K*so the saturation state (Ω) of the system canbe defined; if Ω > 1 the system is oversaturated andprecipitation is likely (Morse, 1983):

$Ca^{+2} + CO_3^{2-} \leftrightarrow CaCO_3$……… (1)

$\Omega = a(Ca^{+2})\, a(CO_3^{2-}) / k_{SO}$…… (2)

With kSOcalcite, 25˚C =4.8×10-9)The concentration of carbonate ions is related to theconcentration of DIC and the pH of a given aquaticsystem. In addition, the concentration of DICdepends on several environmental parameters suchas temperature and the partial pressure of carbondioxide (for systems exposed to the atmosphere).

Compound:	Weight (g):
Cement (ENCI CEMI 32.5)	390
Water	195
Organics (see text)	19.5
Aggregate (gravel and sand):	
4 - 8 mm	562
2 - 4 mm	378
1 - 2 mm	283
0.5 - 1 mm	283
0.25 - 0.5 mm	243
0.125 - 0.25 mm	132

The equilibrium reactions and constants governingthe dissolution of CO2 in aqueous media (25°C and1 atm) are given in Eqs. (3)–(6) (Stumm&Morgan, 1981)

$CO_2(g) \leftrightarrow CO_2(aq.)\ (pK_H=1.468)$………(3)

$CO_2(aq.)+H_2O \leftrightarrow H_2CO_3^*\ (pK=2.84)$…(4)

$H_2CO_3^* \leftrightarrow H^+ + HCO_3^-\ (pK_1=6$……..(5)

$HCO_3^- \leftrightarrow CO_3^{2-} H^+\ (pK_2=10.329)$…...(6)

With $H_2CO_3^* = CO_2(aq.) + H_2CO_3$

Microorganisms can influence precipitation byaltering almost any of the precipitation parametersdescribed above, either separately or in variouscombinations with one another,-(Hammes &Verstraete, 2002).Different pathways appear to be involved incalcium carbonate precipitation.**The first pathway** involves the sulphur cycle, inparticular sulphate reduction, which is carried outby Advanced Topics in Bio mineralization sulphatereducing bacteria under anoxic conditions.

A second pathway involves the nitrogen cycle,and more specifically,

(1) The oxidative deamination of amino acids inaerobiosis,
(2) The reduction of nitrate in anaerobiosis ormicroaerophily and
(3) The degradation of urea or uric acid inaerobiosis (by ureolytic bacteria).

Another microbial process that leads to an increaseof both pH and the concentration of dissolvedinorganic carbon is the utilization of organic acids(Braissant et al., 2002), a process which has beencommonly used in microbial carbonateprecipitation experiments. The precipitationpathways described in the aforementioned aregenerally found in nature which accounts for thecommon occurrence of microbial carbonateprecipitation (MCP) and validates the statement byBoquet et al (1973) that under suitable conditions,most bacteria are capable of inducing carbonateprecipitation.

Due to the simplicity, the mostcommonly studied system of applied MICCP isurea hydrolysis via the enzyme urease in a calciumrich environment.Urease catalyses the hydrolysis of urea to CO_2 andammonia, resulting in an increase of pH andcarbonate concentration in the bacterialenvironment. During microbial urease activity, 1mol. of urea is hydrolysed intracellularly to 1 molof ammonia and 1 mol of carbonate (Eq.1), whichspontaneously hydrolyzes to form additional 1 molof ammonia and carbonic acid (Eq.2) as follows:(with bacteria)

$CO(NH_2)_2+H_2O \rightarrow NH_2COOH+NH$……(7)
$NH_2COOH+H_2O \rightarrow NH_3+H_2CO_3$……(8)
These products equilibrate in water to formbicarbonate, 1 mol. of ammonium and hydroxide
ions which give rise to pH increase
H_2CO_3 □ $2H^{+}+2CO_3^{2-}$……………..(9)
NH_3+H_2O □ $NH_4^{-}+OH^{-}$……………. (10)
$Ca^{2}+CO_3^{2-}$ □ $CaCO_3$ (KSP = 3.8)…(11)
(KSP is the Solubility of product in Eq.11)Hammes & Verstraete (2002) investigated the Series of events occurring during ureolyticcalcification emphasizing the importance of pH andcalcium metabolism during the process.

The primary role of bacteria has been ascribed totheir ability to create an alkaline environmentthrough various physiological activities.Bacterial surfaces play an important role in calciumprecipitation (Fortin et al., 1997). Due to thepresence of several negatively charged groups, at aneutral pH, positively charged metal ions could bebound on bacterial surfaces, favouringheterogeneous nucleation (Douglas, 1998;Bauerlein, 2003). Commonly, carbonateprecipitates develop on the external surface ofbacterial cells by successive stratificationand bacteria can be embedded in growingcarbonate crystals.

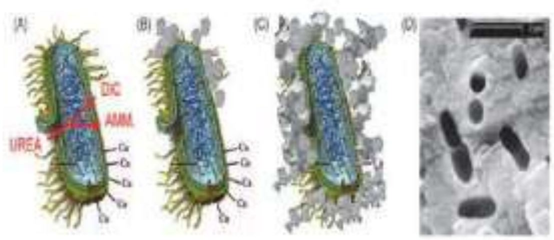


Fig.8 shows Simplified representation of the eventsoccurring during the microbially induced carbonateprecipitation. Calcium ions in the solution areattracted to the bacterial cell wall due to thenegative charge of the latter. Upon addition of ureato the bacteria, dissolved inorganic carbon (DIC)and ammonium (AMM) are released in themicroenvironment of the bacteria (A). In the presence of calcium ions, this can result in a localsupersaturation and hence heterogeneousprecipitation of calcium carbonate on the bacterialcell wall (B). After a while, the whole cell becomesencapsulated (C). Limiting nutrient transfer,resulting in cell death.

Image (D) shows theimprints of bacterial cells involved in carbonatePrecipitationPossible biochemical reactions in urea-CaCl2medium to precipitate CaCO3 at the cell surfacecan be summarized as follows

Ca^{+2} + cell $\rightarrow$ cell- Ca^{+2}………..… (6)

Cell- Ca^{+2} + CO_3^{-2} $\rightarrow$ cell-$CaCO_3$... (7)

Simplified representation of the events occurringduring the microbially induced carbonate

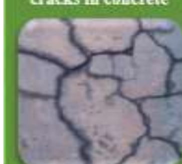
precipitation. Calcium ions in the solution areattracted to the bacterial cell wall due to the

negative charge of the latter. Upon addition of ureato the bacteria, dissolved inorganic carbon (DIC)and ammonium (AMM) are released in themicroenvironment of the bacteriaA. In the presence of calcium ions, this can resultin a local super saturation and henceheterogeneous precipitation of calciumcarbonate on the bacterial cell wallB.

After a while, the whole cell becomesencapsulatedC. Limiting nutrient transfer, resulting in celldeath.D. Shows the imprints of bacterial cells involvedin carbonate precipitationthe actual role of the bacterial precipitationremains, however, a matter of debate. Someauthors believe this precipitation to be anunwanted and accidental by-product of themetabolism (Knorre & Krumbein, 2000) whileothers think that it is a specific process withecological benefits for the precipitatingorganism.

APPLICATION OF BACTERIA INCONSTRUCTION AREA

The use of microbial concrete in Bio Geo CivilEngineering has become increasingly popular.From enhancement in durability of cementitiusmaterials to improvement in sand properties, fromrepair of limestone monuments, sealing of concretecracks to highly durable bricks, microbial concretehas been successful in one and all. Application ofvarious bacteria in construction area by variousauthor shown in **Table 3** and other application ofbacteria in construction area shown in **Fig.8**

APPLICATION	ORGANISM	REFERENCE
Cement mortar and Concrete 	Bacillus cereus Bacillus sp. CT-5 Bacillus pasteurii Shewanella Sporosarcina pasteurii	Le Metayer- Leverel et al (1999) Achal et al., 2011b Ramachandran et al (2001) Ghosh et al (2005) Achal et al (2011a)
Remediation of cracks in concrete 	Sporosarcina pasteurii Bacillus pasteurii Bacillus pasteurii Bacillus sphaericus Bacillus sphaericus	Bang et al (2001) Ramachandran et al (2001) Ramakrishnan (2007) De Belie et al (2008) De Muynck et al (2008a, b)
Self-Healing 	Bacillus pseudifirmus Bacillus cohnii	Jonkers et al (2007)

This new technology can provide ways for low costand durable roads, high strength buildings withmore bearing capacity, long lasting river banks,erosion prevention of loose sands and low costdurable housing. The next section will illustratedetailed analysis of role of microbial concrete inaffecting the durability of building structures.

Another issue related with conventional buildingmaterials is the high production of green housegases and high energy consumed during productionof these materials. The emission of greenhousegases during manufacturing processes of buildingmaterials is contributing a detrimental amount toglobal warming. Along with this, high constructioncost of building materials is another issue thatneeds to be dealt with.

The above mentioned drawbacks of conventionaltreatments have invited the usage of novel, eco-friendly,self-healing and energy efficienttechnology where microbes are used forremediation of building materials and enhancementin the durability characteristics. This technologymay bring new approaches in the constructionindustry.Thus, bacterial material as a smart material than itcan be utilise in various construction area toimprove the performance if structure in new era

ADVANTAGES OF BACTERIAL CONCRETE:

1. Microbial concrete in Crack Remediation:

- Specimens were filled with bacteria, nutrients and sand. Significant increase in compressive strength and stiffness values as compared to those without cells was demonstrated.

2. Improvement in Compressive Strength of Concrete:

- Compressive strength test results are used to determine that the concrete mixture as delivered meets the requirements of the job specification. So, the effect of microbial concrete on compressive strength of concrete and mortar was studied and it was observed that significant enhancement in the strength of concrete and mortar can be seen upon application of bacteria.

3. Better Resistance towards Freeze-Thaw Attack Reduction:

- Application of microbial calcite may help in resistance towards Freeze-thaw Reduction due to bacterial chemical process and also it can reduce the permeability than freezing process decreased.

4. Reduction in Permeability of Concrete:

- Effect of microbial concrete on permeation properties was studied by different researchers. Permeability can be investigated by carbonation tests as it is increasingly apparent that decrease in gas permeability due to surface treatments results in an increased resistance towards carbonation and chloride ingress. Carbonation is related to the nature and connectivity of the pores, with larger pores giving rise to higher carbonation depths..

RESULT

The main objective of this study was to investigate whether bacteria can potentially act as a self-healing agent in concrete. The bacteria tested are known to be alkali-resistant, i.e. they grow in natural environments characterized by a relatively high pH (10-11). In addition, these strains can produce spores which are resting cells with sturdy cell walls that protect them against extreme environmental mechanical- and chemical stresses (Schlegel 1993). Therefore these specific bacteria may have the potential to resist the high internal concrete pH values (12-13 for Portland cement-based concrete), and remain viable for a long time as well, as spore viability for up to 200 years.

Concrete-immobilized spores of such bacteria may be able to seal cracks by bio mineral formation after being revived by water and growth nutrients entering freshly formed cracks. The experimental data presented support hypothesis, as cement stone samples with immobilized bacteria but not control samples precipitated minerals on surfaces exposed to growth medium. Although the exact nature of the produced minerals still needs to be clarified, they appear morphologically related to calcite precipitates.

The mechanism of bacterially-mediated calcite production likely proceeds via organic carbon respiration with oxygen what results in carbonate ion production under alkaline conditions. The produced carbonate ions which can locally reach high concentrations at bacterially active 'hot spots' precipitate with excess calcium ions leaking out of the concrete matrix. This microbial calcium carbonate precipitation mechanism is well studied and occurs worldwide in natural systems such as oceans, biofilms, microbial mats and stromatolites. For an autonomous self-healing mechanism all needed reaction components, or self-healing agents, must be present in the material matrix to ensure minimal externally needed triggers. In the bio mineral precipitation experiment, however, the organic substrate needed for bacterial carbonate ion production was still supplied as part of the incubation medium.

As it should ideally also be part of the concrete matrix we tested the compatibility of bacteria as well as different organic components, which can act as bacterial growth substrates. The obtained flexural tensile- and compressive strength data indicate that the incorporation of high numbers of bacteria (10^9 cells cm^{-3}) and the amino acids aspartate and glutamate (0.5% of cement weight) in the concrete matrix, do not result in a significant loss of strength after a 28 days curing period. However, the same experiment also revealed that apparently only specific organic components are suitable for incorporation, as some others resulted in dramatic strength loss.

Another aspect that was not considered in this study but what is of major importance for the long term self-healing potential of bacterial concrete is the long term viability of concrete-immobilized bacterial spores. As most concrete structures are build to last for 50 years or more the viability of immobilized spores should keep up with that. One advantage of application of bacteria as self-healing agent is that a healing event not only revives bacterial cells but also potentially results in the production of fresh spores what resets the viability status.

The application of bacteria as a self-healing agent in concrete appears promising. We have demonstrated that concrete-immobilized bacterial spores revive and produce copious minerals after stimulation by suitable medium, i.e. water containing an organic growth substrate. To further improve the autonomous bacterial self-healing mechanism current research focuses on long term viability and selection of best adapted bacterial species to the concrete environment as well as the incorporation of compatible bio mineral-producing organic substrates to the concrete matrix.

CONCLUSION

1. Microbial concrete technology has proved to be better than many conventional technologies because of its eco- friendly nature, self-healing abilities and increase in durability of various building materials.

2.Work of various researchers has improved our understanding on the possibilities andlimitations of biotechnological applications on building materials.

Enhancement of compressive strength, reduction in permeability, water absorption,reinforced corrosion have been seen in various cementitious and stone materials.

3.Cementation by this method is very easy and convenient for usage. This will soon providethe basis for high quality structures that will be cost effective and environmentally safe but, more work is required to improve the feasibility of this technology from both an economical and practical viewpoints.

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