

Biofilm and Microbial Applications in Biomineralized Concrete

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1. Introduction

Biomineralization is defined as a biologically induced process in which an organism creates a local micro-environment with conditions that allow optimal extracellular chemical precipitation of mineral phases (Hamilton, 2003). The synthesis of these minerals by prokaryotes is broadly classified into two classes: Biologically controlled mineralization (BCM) and Biologically induced mineralization (BIM) (Lowenstam, 1981; Lowenstam & Weiner, 1989). In the case of biologically controlled mineralization, minerals are directly synthesized at a specific location within or on the cell and only under certain conditions. In most cases, BCM happens intracellularly, where lipids, proteins, polysaccharides, etc. make a stable matrix for cations to condense and minerals to grow in a constrained space. Minerals that form by biologically induced mineralization processes generally nucleate and grow extracellularly as a result of metabolic activity of the organism and subsequent chemical reactions involving metabolic byproducts. Bacterial surfaces such as cell walls or polymeric materials (exopolymers) exuded by bacteria includes slimes, sheaths, or biofilms, or even dormant spores, can act as important sites for the adsorption of ions and mineral nucleation and growth (Beveridge, 1989; Konhauser, 1998; Banfield & Zhang, 2001; Bäuerlein, 2003).

Bacterially induced and mediated mineralization is a research subject widely studied in the past decades (Banfield & Hamers, 1997; Douglas & Beveridge, 1998; Ehrlich, 2002). Due to its numerous consequences, bacterially induced precipitation of calcium carbonate, so-called carbonatogenesis (Rodriguez-Navarro et al., 2003), has attracted much attention from both basic and applied points of view. It has implications for: (1) atmospheric CO₂ fixation through carbonate sediment formation and lithification (Krumbein, 1979; Monger et al., 1991; Chafetz & Buczynski, 1992; Folk, 1993) and dolomite precipitation (Vasconcelos et al., 1995) (2) solid-phase capture of inorganic contaminants (Warren et al., 2001) (3) pathological formation of mineral concretions, such as gallstones and kidney stones in humans (Keefe, 1976) (4) the possibility of understanding extraterrestrial biological processes such as Martian carbonate production by bacteria (McKay et al., 1996; Thomas-Keprta et al., 1998).

Bacterially induced mineralization has recently emerged as a method for protecting and consolidating decayed construction materials. In nature, building and remediation of

structures with local materials occurs without any requirement of extreme energy usage. Calcification and polymerization occur at ambient conditions as can be seen from the sustainability of ant hills and coral reefs. This occurs through the application of microorganisms which deposit carbonates (as part of their basic metabolic activities), one of the most well known minerals. These deposits (commonly called as calcium carbonate crystals/ calcite crystals/ microbial concrete) act as binders between loose substrate particles and reduce the pores inside the substrate particles. The use of bacteria for remediating building materials seems like a new idea, but this conservation method mimics what nature has been doing for eons, since many carbonate rocks have been cemented by calcium carbonate precipitation from microbes. The technology of using microbes for calcium carbonate deposition or microbial concrete, called as Microbially induced calcium carbonate precipitation (MICCP) can be used for solving various durability issues of construction materials. Microorganisms are abundant in nature, which paves the way for massive production of bacterial calcium carbonate crystals/ calcite/ concrete. As the microorganisms can penetrate and reproduce themselves in soil or any such environments, there is no need to disturb the ground or environment unlike that of cement. This technology also offers the benefit of being novel and eco- friendly.

Undoubtedly, broad a range of products are available in the market for protection of concrete surfaces (Basheer et al., 1997; Ibrahim et al., 1997; Basheer et al., 2006). Several of these products are organic coatings consisting of volatile organic compounds. The air polluting effect of these compounds during manufacturing and coating has led to the development of new formulations such as inorganic coating materials. Traditional inorganic coatings consist of calcium-silicate compounds, which exhibit a composition similar to cement (Moon et al., 2007). Surface treatments with water repellants like epoxy injections, with pore blockers and various synthetic agents like silanes or siloxanes are also available today, but with a number of disadvantages like degradation with time, need for constant maintenance and environment pollution (De Mynck et al., 2006).

In the case of carbonate stones like limestone, dolostone, and marble, progressive dissolution of the mineral matrix as a consequence of weathering leads to an increase of the porosity, and as a result, a decrease of the mechanical features (Tiano et al., 1999). In order to decrease the susceptibility to decay, many conservation treatments have been applied with the aim of modifying some of the stone characteristics. Water repellents have been applied to protect stone from the ingress of water and other weathering agents. The use of stone consolidants aim at re-establishing the cohesion between separated grains of deteriorated stone. Due to problems related to incompatibility with the stone, both water repellents and consolidants have often been reported to accelerate stone decay (Clifton & Frohnsdorff, 1982; Delgado Rodriguez, 2001; Moropoulou et al., 2003).

Another issue related with conventional building materials is the high production of green house gases and high energy consumed during production of these materials. The emission of green house gases during manufacturing processes of building materials is contributing a detrimental amount to global warming. Along with this, high construction cost of building materials is another issue that needs to be dealt with.

The above mentioned drawbacks of conventional treatments have invited the usage of novel, eco- friendly, self healing and energy efficient technology where microbes are used

for remediation of building materials and enhancement in the durability characteristics. This technology may bring new approaches in the construction industry.

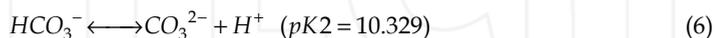
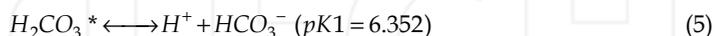
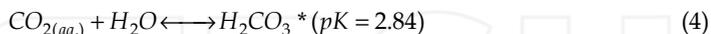
2. Production of microbial concrete/calcite

Bacteria from various natural habitats have frequently been reported to be able to precipitate calcium carbonate both in natural and in laboratory conditions (Krumbein, 1979; Rodriguez et al., 2003). Different types of bacteria, as well as abiotic factors (salinity and composition of the medium) seem to contribute in a variety of ways to calcium carbonate precipitation in a wide range of different environments (Knorre & Krumbein, 2000; Rivadeneyra et al., 2004). Calcium carbonate precipitation is a straight forward chemical process governed mainly by four key factors: (1) the calcium concentration, (2) the concentration of dissolved inorganic carbon (DIC), (3) the pH and (4) the availability of nucleation sites (Hammes & Verstraete, 2002). CaCO_3 precipitation requires sufficient calcium and 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 with the K_{so} the saturation state (Ω) of the system can be defined; if $\Omega > 1$ the system is oversaturated and precipitation is likely (Morse, 1983):



$$\Omega = a(\text{Ca}^{2+})a(\text{CO}_3^{2-}) / K_{so} \text{ with } K_{so \text{ calcite}, 25^\circ} = 4.8 \times 10^{-9} \quad (2)$$

The concentration of carbonate ions is related to the concentration of DIC and the pH of a given aquatic system. In addition, the concentration of DIC depends on several environmental parameters such as temperature and the partial pressure of carbon dioxide (for systems exposed to the atmosphere). The equilibrium reactions and constants governing the dissolution of CO_2 in aqueous media (25°C and 1 atm) are given in Eqs. (3)–(6) (Stumm & Morgan, 1981):



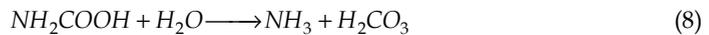
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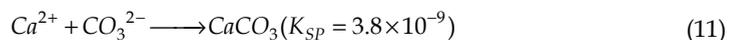
Microorganisms can influence precipitation by altering almost any of the precipitation parameters described above, either separately or in various combinations with one another (Hammes & Verstraete, 2002).

Different pathways appear to be involved in calcium carbonate precipitation. The first pathway involves the sulphur cycle, in particular sulphate reduction, which is carried out by

sulphate reducing bacteria under anoxic conditions. A second pathway involves the nitrogen cycle, and more specifically, (1) the oxidative deamination of amino acids in aerobiosis, (2) the reduction of nitrate in anaerobiosis or microaerophily and (3) the degradation of urea or uric acid in aerobiosis (by ureolytic bacteria). Another microbial process that leads to an increase of both pH and the concentration of dissolved inorganic carbon is the utilization of organic acids (Braissant et al., 2002), a process which has been commonly used in microbial carbonate precipitation experiments. The precipitation pathways described in the aforementioned are generally found in nature which accounts for the common occurrence of microbial carbonate precipitation (MCP) and validates the statement by Boquet et al (1973) that under suitable conditions, most bacteria are capable of inducing carbonate precipitation. Due to the simplicity, the most commonly studied system of applied MICCP is urea hydrolysis via the enzyme urease in a calcium rich environment. Urease catalyzes the hydrolysis of urea to CO_2 and ammonia, resulting in an increase of pH and carbonate concentration in the bacterial environment. During microbial urease activity, 1 mol of urea is hydrolyzed intracellularly to 1 mol of ammonia and 1 mol of carbonate (Eq.7), which spontaneously hydrolyzes to form additional 1 mol of ammonia and carbonic acid (Eq.8) as follows:



These products equilibrate in water to form bicarbonate, 1 mol of ammonium and hydroxide ions which give rise to pH increase



K_{sp} is the solubility product in Eq.11.

Hammes & Verstraete (2002) investigated the series of events occurring during ureolytic calcification emphasizing the importance of pH and calcium metabolism during the process (Fig.1). The primary role of bacteria has been ascribed to their ability to create an alkaline environment through various physiological activities.

Bacterial surfaces play an important role in calcium precipitation (Fortin et al., 1997). Due to the presence of several negatively charged groups, at a neutral pH, positively charged metal ions could be bound on bacterial surfaces, favouring heterogenous nucleation (Douglas, 1998; Bauerlein, 2003). Commonly, carbonate precipitates develop on the external surface of bacterial cells by successive stratification (Pentecost & Bauld, 1988; Castanier et al., 1999) and bacteria can be embedded in growing carbonate crystals (Rivadeneira et al., 1998; Castanier et al., 1999).

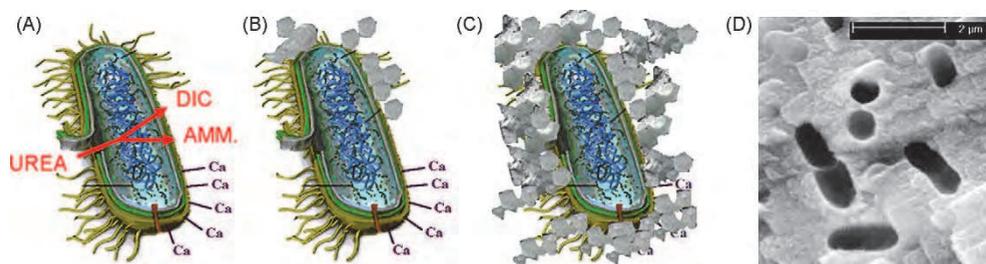
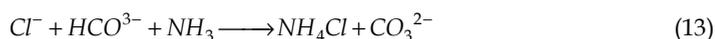
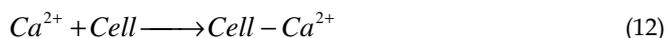


Fig. 1. Simplified representation of the events occurring during the microbially induced carbonate precipitation. Calcium ions in the solution are attracted to the bacterial cell wall due to the negative charge of the latter. Upon addition of urea to the bacteria, dissolved inorganic carbon (DIC) and ammonium (AMM) are released in the microenvironment of the bacteria (A). In the presence of calcium ions, this can result in a local supersaturation and hence heterogeneous precipitation of calcium carbonate on the bacterial cell wall (B). After a while, the whole cell becomes encapsulated (C), limiting nutrient transfer, resulting in cell death. Image (D) shows the imprints of bacterial cells involved in carbonate precipitation (Source: Hammes & Verstraete., 2002).

Possible biochemical reactions in urea- CaCl_2 medium to precipitate CaCO_3 at the cell surface can be summarized as follows:



The actual role of the bacterial precipitation remains, however, a matter of debate. Some authors believe this precipitation to be an unwanted and accidental by-product of the metabolism (Knorre & Krumbein, 2000) while others think that it is a specific process with ecological benefits for the precipitating organisms (Ehrlich, 1996; Mc Connaughey & Whelan, 1997).

However, a number of applications involving MICCP have been attempted as in the removal of heavy metals (Warren et al., 2001) and radionucleotides (Fujita et al., 2004), removal of calcium from wastewater (Hammes et al., 2003) and biodegradation of pollutants (Simon et al., 2004; Chaturvedi et al., 2006). Another series of applications aims at modifying the properties of soil, i.e. for the enhancement of oil recovery from oil reservoirs (Nemati & Voordouw, 2003; Nemati et al., 2005), plugging (Ferris & Stehmeier, 1992) and strengthening of sand columns (DeJong et al., 2006; Whiffin et al., 2007). Moreover, microbially induced precipitation has been investigated for its potential to improve the durability of construction materials such as cementitious materials, sand, bricks and limestone.

This technology has been investigated for its potential in consolidation and restoration of various building materials by many research groups on cement mortar cubes (Achal et al.,

2011b), sand consolidation and limestone monument repair (Stocks – Fischer et al., 1999; Bachmeier et al., 2002; Dick et al., 2006; Achal et al., 2009b), reduction of water and chloride ion permeability in concrete (Achal et al., 2011a), filling of pores and cracks in concrete (Bang et al., 2001; Ramakrishnan 2007; De Muynck et al., 2008a,b) and enhanced strength of red bricks (Sarda et al., 2009).

3. Applications of microbial concrete

The use of microbial concrete in Bio Geo Civil Engineering has become increasingly popular. From enhancement in durability of cementitious materials to improvement in sand properties, from repair of limestone monuments, sealing of concrete cracks to highly durable bricks, microbial concrete has been successful in one and all. This new technology can provide ways for low cost and durable roads, high strength buildings with more bearing capacity, long lasting river banks, erosion prevention of loose sands and low cost durable housing. The next section will illustrate detailed analysis of role of microbial concrete in affecting the durability of building structures.

3.1 Microbial concrete in cementitious materials

Concrete is a strong and relatively cheap construction material used world wide. It is estimated that cement production alone contributes 7% to global anthropogenic CO₂ emissions, what is particularly due to the sintering of limestone and clay at a temperature of 1500°C, as during this process calcium carbonate is converted into calcium oxide while releasing CO₂ (Worrell et al., 2001). There is a great concern and emphasis in reducing the greenhouse gases emission into the atmosphere in order to control adverse environmental impacts.

Another aspect of concrete is its liability to cracking, a phenomenon that hampers the material's structural integrity and durability. It is generally accepted that the durability of concrete is related to the characteristics of its pore structure (Khan, 2003). Degradation mechanisms of concrete often depend on the way potentially aggressive substances can penetrate into the concrete, possibly causing damage. The permeability of concrete depends on the porosity and connectivity of the pores. The more open the pore structure of the concrete, the more vulnerable the material is to degradation mechanisms caused by penetrating substances. The deterioration of concrete structures usually involves movement of aggressive gases and/or liquids from the surrounding environment into the concrete, followed by physical and/or chemical reactions within its internal structure, possibly leading to irreversible damage (Claisse et al., 1997). The quality of concrete structures depends majorly on three parameters: compressive strength, permeability & corrosion. Crack problems in concrete are mostly dealt by manual inspections and repair by impregnation of cracks with epoxy based fillers, latex binding agents like acrylic, polyvinyl acetate, butadiene styrene etc. But there are many disadvantageous aspects of traditional repair systems such as different thermal expansion coefficient compared to concrete, weak bonding, environmental and health hazards along with being costly. So many researchers investigated the application of bacterial calcite in enhancing the durability of cementitious buildings and restoration of structures. Overview of different applications of microbial concrete in cementitious materials is given in table 1.

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

Table 1. Overview of different applications where microbial concrete is used as biocement in cementitious materials.

3.1.1 Improvement in compressive strength of concrete

Compressive strength is one of the most important characteristic of concrete durability. It is considered as an index to assess the quality of concrete. More is the compressive strength, more is the durability of concrete specimen. 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.

The applicability of microbial concrete to affect the compressive strength of mortar and concrete was done by several studies (Bang et al., 2001; Ramachandran et al., 2001; Ghosh et al., 2005; De Muynck et al., 2008a,b; Jonkers et al., 2010; Achal et al., 2011b) where different microorganisms have been applied in the concrete mixture. Ramchandran et al (2001) observed the increase in compressive strength of cement mortar cubes at 7 and 28 days by using various concentrations of *Bacillus pasteurii*. They found that increase of strength resulted from the presence of adequate amount of organic substances in the matrix due to microbial biomass. Ghosh et al (2005) studied the positive potential of *Shewanella* on compressive strength of mortar specimens and found that the greatest improvement was at cell concentration of 10^5 cells/ ml for 3, 7, 14 and 28 days interval. They reported an increase of 17% and 25% after 7 and 28 days. But no noticeable increase was recorded in case of specimens treated with *Escherichia coli* (low urease producing). This concluded that choice of microorganism plays the prime role in improvement of strength characteristics. Jonkers & Schlangen (2007) studied the addition of bacterial spores ($10^8/\text{cm}^3$) of *Bacillus pseudofirmus* and *Bacillus cohnii* to concrete specimens and reported an increase of 10% in the compressive strength. Achal et al (2009a) treated mortar cubes with *Sporosarcina pasteurii* and observed 17% improvement in compressive strength. Another research group by Park et al (2010)

observed 22% increase in the strength of mortar cubes treated by *Arthrobacter crystallopoietes* which was the higher compared to *Sporosarcina soli*, *Bacillus massiliensis* and *Lysinibacillus fusiformis* used along with. Upon addition of *Bacillus* sp. CT-5 to cement mortar specimens, 36% increase in compressive strength was reported (Achal et al., 2011b). Significant increase in compressive strength was observed at the age of 28 days as compared to earlier days. Microbial calcite might have precipitated on the surface of cells and eventually within the pores leading to their plugging which further lead to stoppage of flow of oxygen and nutrients to the cells. The cells either die or turn into endospores and act as organic fiber which enhances the compressive strength of mortar cubes (Ramachandran et al., 2001).

Further studies to confirm the role of microbial concrete in cement mortar specimens and concrete specimens were done by scanning electron microscope (Fig. 2), energy dispersive X-Ray spectrum (EDX) and X-ray diffraction (XRD) analysis of calcite crystals precipitated on concrete surfaces. The results confirmed the presence of calcite in the newly formed layer on the surface of concrete specimens (Ramachandran et al., 2001; Ghosh et al., 2005; De Muynck et al., 2008a; Achal et al., 2009a, 2011). Rod shaped bacteria were found embedded in calcite crystals which proved that bacteria act as the source of nucleation.

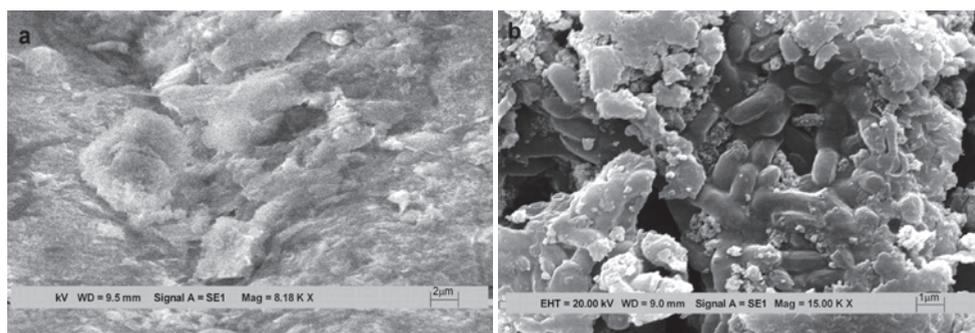


Fig. 2. Scanning electron micrographs of cement mortar specimens: (a) matrix of cement mortar prepared without bacteria (b) showing dense calcite precipitation as calcite crystals with rod-shaped impressions housed by *Bacillus* sp. CT-5 (Achal et al., 2011b).

3.1.2 Reduction in permeability

Permeability of concrete is another important characteristic of concrete that affects its durability. Concrete with low permeability has been reported to last longer (Nolan et al., 1995). Permeation is required for controlling the ingress of moisture, ionic and gaseous species into the concrete. Once they get into concrete structure, the structure no longer maintains its structural integrity; the lifespan is reduced, and the general safety of the public is severely in danger. Many conventional techniques like application of chemical admixtures (plasticizers, water reducing agents etc.) are known which improve the workability of concrete by reducing intergranular friction finally affecting porosity. However, they come along with various disadvantages: a) incompatibility of protective layer and underlying layer due to differences in thermal expansion coefficient b) disintegration of protective layer over time c) need for constant maintenance along with contributing to pollution (Camaiti et al., 1988; Rodriguez- Navarro et al., 2003).

Due to these shortcomings, 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.

The biodeposition by microbial concrete should be regarded as a coating system. This is because of the fact that precipitation is mainly on the surface due to limited penetration of bacteria in the porous matrix. Ramakrishnan et al (1998) reported an increase in resistance of concrete towards alkali, freeze thaw attack, drying shrinkage and reduction in permeability upon application of bacterial cells.

De Muynck et al (2008b) studied the effect of biodeposition of calcite on permeability characteristics of mortar by *B. sphaericus*. The presence of biomass contributed to a large extent in the overall decrease of the gas permeability. Significant differences in carbonation depth between treated and untreated specimens were noticeable after 2 weeks of accelerated carbonation in treated mortar specimens. Bacterial treated specimens were found to have better resistance towards chloride penetration as compared to untreated mortar specimens. The increased resistance towards the migration of chlorides of cubes treated with biodeposition was similar to that of the acrylic coating and the water repellent silanes and silicones and larger than in the case of the silanes/siloxanes mixture, which were all reported to be effective in decreasing the rate of reinforcement corrosion (Basheer et al., 1997; Ibrahim et al., 1997).

Achal et al (2011a) reported the decreased water permeability of bioremediated cement mortar cubes treated by *Sporosarcina pasteurii*. The lower permeability of the bioremediated cubes compared with that of the control cubes was probably due to a denser interfacial zone formed because of calcite precipitation between the aggregate and the concrete matrix. The penetration of water at the sides was found to be higher than that at the top. This is due to better compaction and closing of pores at the top. This demonstrated the profound effect of microbial calcite on the permeability of concrete. The same group studied the effect of *Bacillus pasteurii* on water impermeability in concrete cubes and found the reduction in penetration of water which was more significant on the top side as compared to sides because of better compaction and closing of pores at the top surface (Achal et al., 2010b). Six times reduction in absorption of water was reported upon treatment of mortar cubes with *Bacillus* sp. CT-5 as compared to untreated specimens (Achal et al., 2011b).

3.1.3 Reduction in corrosion of reinforced concrete

Corrosion of steel and rebar structures in concrete is one of the main reasons for failure of structures. Corrosion initiates due to ingress of moisture, chloride ions and carbon dioxide through the concrete to the steel surface. Corrosion and permeation are somehow correlated. The permeability of water and pollutants are amongst the major threats to reinforced concrete. Such penetrations lead to ingress of moisture and chlorides which is responsible for early leakages and corrosion of steel. Corrosion products (iron oxides and hydroxides) lead to stresses that crack and spall the concrete cover which in turn exposes reinforcement to direct environmental attack that results in accelerated deterioration of the structure (Neville, 1995).

Application of microbial calcite may help in sealing the paths of ingress and improve the life of reinforced concrete structures (Jonkers et al., 2007; Fig. 3). Mukherjee et al (2010) reported four fold reduction in corrosion of reinforced concrete specimens upon application of *Bacillus sp. CT-5*. The same group observed reduction in water and chloride ion permeability upon use of calcite by *Sporosarcina pasteurii*. Qian et al (2010) used *B. pasteurii* to check its effect on permeability resistance and acid attack and reported that bacterial calcite improves surface permeability resistance and resist the attack of acid ($\text{pH} > 1.5$).

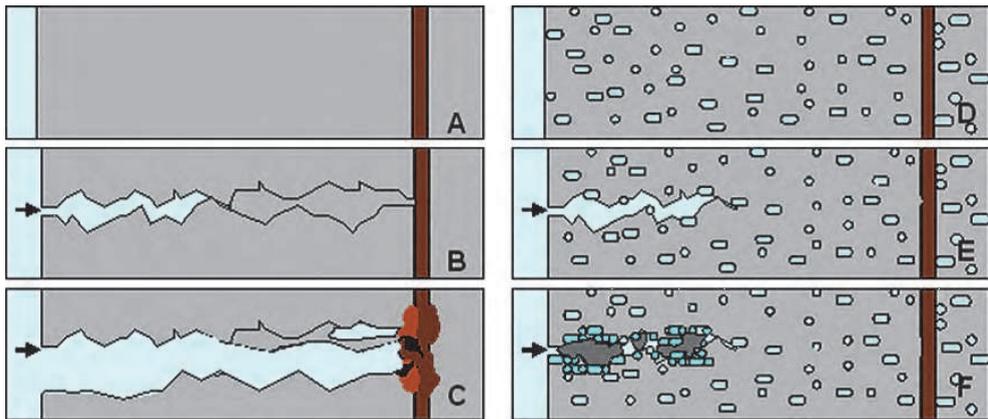


Fig. 3. Schematic drawing of conventional concrete (A-C) versus bacteria-based self-healing concrete (D-F). Crack ingress chemicals degrade the material matrix and accelerate corrosion of the reinforcement (A-C). Incorporated bacteria-based healing agent activated by ingress water seals and prevents further cracking (D-F) (Jonkers et al., 2007).

3.2 Microbial concrete in crack remediation

Use of microbial concrete has exhibited high potential for remediation of cracks in various structural formations such as concrete and granite (Gollapudi et al., 1995; Stocks-Fisher et al., 1999). Microbiologically enhanced crack remediation has been reported by Bang and Ramakrishnan (2001) where *Bacillus pasteurii* was used to induce calcium carbonate precipitation. Ramachandran et al (2001) proposed microbiologically enhanced crack remediation (MECR) in concrete. Specimens were filled with bacteria, nutrients and sand. Significant increase in compressive strength and stiffness values as compared to those without cells was demonstrated. The presence of calcite was limited to the surface areas of crack because bacterial cells grow more actively in the presence of oxygen. Extremely high pH of concrete germinated the need for providing protection to microbes from adverse environmental conditions. Polyurethanes were used as vehicle for immobilization of calcifying enzymes and whole cells because of its mechanically strong and biochemically inert nature (Klein & Kluge 1981; Wang & Ruchenstein, 1993). Bang et al (2001) investigated the encapsulation of bacterial cells in polyurethanes and reported positive potential of microbiologically enhanced crack remediation by polyurethane immobilized bacterial cells. They also studied the effect of immobilized bacterial cells on strength of concrete cubes by varying the concentration of immobilized cells per crack. Highest compressive strength was obtained with cubes remediated with 5×10^9 immobilized cells crack⁻¹ for 7 days while after

that, increase in strength was found to be marginal. SEM pictures also depicted the clear involvement of immobilized bacterial cells in sealing of cracks (Fig. 4).

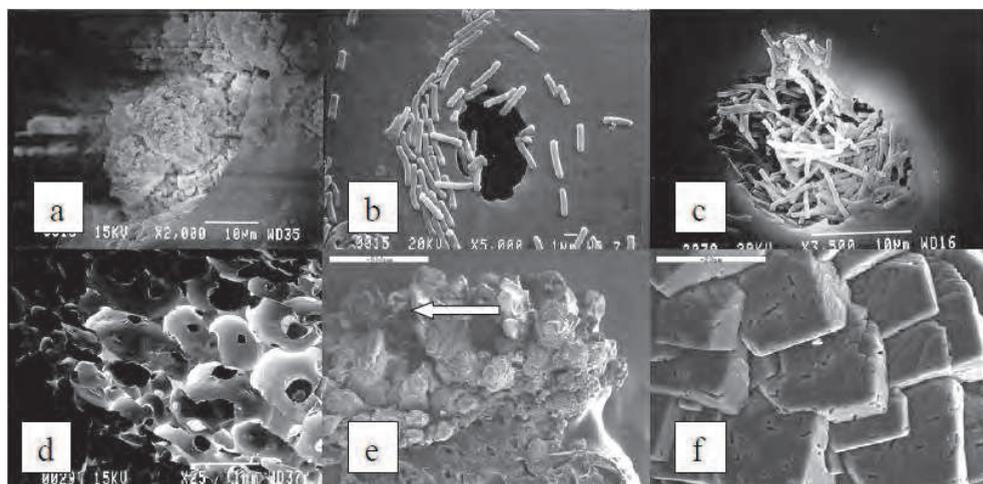


Fig. 4. Scanning electron micrographs of calcite precipitation induced by *B. pasteurii* immobilized in Polyurethanes (PU) (a) Porous PU matrix without microbial cells showing open-cell structures. Bar, 1 mm (b) Distribution of microorganisms on the PU surface. Bar, 1 μm (c) Microorganisms densely packed in a pore of the PU matrix. Bar, 10 μm . (d) Calcite crystals grown in the pore (shown in c) of the PU matrix. Bar, 10 μm . (e) Calcite crystals grown extensively over the PU polymer. Bar, 500 μm . (f) Magnified section pointed with an arrow in e shows crystals embedded with microorganisms. Bar, 20 μm . (Bang et al., 2001).

De Belie & De Muynck (2008) reported positive potential of microbiologically induced carbonate precipitation for the repair of cracks in concrete by *B. sphaericus* while Qian et al (2010) also reported that compressive strength of treated specimens could be restored to 84% upon treatment of bacterial calcite.

3.3 Microbial concrete in restoration of stone buildings

The pyramids of Egypt have been built with durable carbonate stones. With time, the calcareous matrix of the stone shows progressive increase in its porosity and a significant decrease of its mechanical characteristics due to the calcite leaching process (Amoroso, 1983). This leads to breakage of the materials into smaller particles and finally back into constituent minerals. Several conservative treatments are available with inorganic and organic products (Lazzarini & Laurenzi Tabasso, 1986) which can slow down the deterioration process of monuments. However, they offer several drawbacks due to their chemical composition and thermal expansion coefficient as they differ a lot from that of the stone. There is long-term incompatibility of the substrate and the new cement used for consolidation (Clifton, 1980) and the plugging of pores in the treated material induced by the new cement or protective layers (Lazzarini & Tabasso, 1980). These products are also formulated and applied in solvents at very low concentration that lead to huge waste of organic solvents in the environment. More over, their efficiency is inconsistent and in

certain cases, they can have a detrimental effect for the conservation of the stone material itself. Shortcomings of conventional techniques have drawn the attention to bacterially induced carbonate precipitation for reducing the permeation properties and thereby, enhancing the durability properties of ornamental stone. Various researchers have applied this technology for remediation of stone (Le Me'tayer et al., 1999; Rodriguez-Navarro et al., 2003; Dick et al., 2006; Tiano et al., 1995, 1999, 2006; Jimenez- Lopez et al., 2007). Overview of different applications of microbial concrete on stone is given in table 2 (De Muynck et al., 2010).

Mediator	Organism/ molecule	Reference
Microorganisms	<i>Bacillus cereus</i>	Le Metayer- Levrel et al (1999)
	<i>Micrococcus sp. Bacillus subtilis</i>	Tiano et al (1999)
	<i>Myxococcus xanthus</i>	Rodriguez- Navarro et al (2003)
	<i>Bacillus sphaericus</i>	Dick et al (2006)
	<i>Pseudomonas putida</i>	May (2005)
Organic matrix molecules	<i>Mytilus californianus shell extracts</i>	Tiano (1995) and Tiano et al (2006)
	Aspartic acid	
Activator medium	Bacillus cell fragments	
	Microbiota inhabiting the stone	Jimenez- Lopez et al (2007)

Table 2. Different applications of microbial concrete on stone are given in table 2 (De Muynck et al., 2010).

3.3.1 Reduction in permeation of stone by microbial concrete

The main target for consolidation of stone aims at reducing the permeation and water absorption by providing surface treatment as this layer plays the most important protective role. Adolphe et al (1990) were among the first to consider the use of microbially induced carbonate precipitation (MICP) for the protection of ornamental stone. In addition, the role of calcite layer in providing resistance against erosion was reported. Bacterially induced calcium carbonate was compatible with the substrate and significantly reduced the water sorption of the treated stone (Le Me' tayer-Levrel et al., 1999). However, the layer of the new cement induced by *B. cereus* was very thin - only a few microns-thick. Along with this, the formation of endospores and uncontrolled biofilm by *Bacillus* provides a potential drawback in stone conservation.

Rodriguez-Navarro et al (2003) proposed the use of *Myxococcus xanthus*, an abundant Gram-negative, non-pathogenic aerobic soil bacterium which belongs to a peculiar microbial group whose complex life cycle involves a remarkable process of morphogenesis and differentiation. This bacterium has been known to induce the precipitation of carbonates, phosphates and sulfates in a wide range of solid and liquid media (González-Muñoz et al., 1993, 1996; Ben Omar et al., 1995, 1998; Ben Chekroun et al., 2004 & Rodriguez-Navarro et al., 2007). Upon application of this bacterial suspension on stone specimens, no fruiting

bodies were observed and there was no uncontrolled bacterial growth. Calcium carbonate cementation was observed up to a depth of several hundred micrometers ($> 500\mu\text{m}$) without any plugging or blocking of the pores. Plugging occurs mainly due to film formation by extracellular polymeric substance (EPS) (Tiano et al., 1999). In this case, only limited EPS production occurred in stones.

Tiano et al. (1999) commented on the use of viable cells for the formation of new minerals inside monumental stones. They studied the effect of microbial calcite crystals on Pietra di Lecce bioclastic limestone by using of *Micrococcus* sp., and *Bacillus subtilis*. Significant decrease in water absorption was observed due to the physical obstruction of pores. Furthermore, the authors commented on some possible negative consequences, such as (1) the presence of products of new formation, due to the chemical reactions between the stone minerals and some by-products originating from the metabolism of viable heterotrophic bacteria and (2) the formation of stained patches, due to the growth of airborne micro-fungi related to the presence of organic nutrients necessary for bacterial development. In order to avoid these short comings, the authors proposed the use of natural and synthetic polypeptides to control the growth of calcite crystals in the pores. The use of organic matrix macromolecules (OMM) extracted from *Mytilus californianus* shells was proposed (Tiano et al., 1992; 1995) to induce the precipitation of calcium carbonate within the pores of the stone. In this case, there was slight decrease in porosity and water absorption by capillarity (Tiano, 1995). Due to the complexity of extraction procedure as well as low yields of usable product, this method was not beneficial (Tiano et al., 1999). In place of these bio inducing macromolecules (BIM) rich in aspartic acid groups, Tiano et al (2006) put forward the proposal of using acid functionalized proteins such as polyaspartic acid. For calcite crystal growth, calcium and carbonate ions were supplied by addition of ammonium carbonate and calcium chloride solution or a saturated solution of bicarbonate, supplemented in some cases by calcite nanoparticles so as to maintain saturated carbonate solution in the pores over a prolonged period. Spraying was used to introduce proteins, calcium ions and nanoparticles. This was found to be suitable for marble statues and objects of high aesthetic value which require minimum change in the chemistry of the object. The consolidating effect in this case was very low compared to ethylsilicates (Tiano et al., 2006).

Dick et al. (2006) reported 50% reduction in water absorption by treating limestone cubes with two strains of *B. sphaericus*. Various researchers in Biobrush consortium worked on improving the methodologies for delivering bacterial cells to stone surfaces and controlling side effects of bacteria to the stone. Various carrier materials were looked upon. Ranalli et al (1997) used sepiolite for delivering *Desulfovibrio vulgaris* and *D. desulfuricans* as it provided anaerobic conditions, humidity and shortened treatment time. Capitelli et al (2006) reported the superiority of Carbogel as a delivery system for bacteria because of higher retention of viable bacteria and less time needed for entrapment of cells by this. Zamarreno et al (2009) investigated the application of calcite crystals precipitated by fresh water bacteria on limestone and found significant reduction in pore sizes of the bacterial treated substrate stone specimens as compared to the untreated ones (Fig. 5). Bacterial calcite crystals had deposited around and inside open pore spaces. The application of calcite crystals resulted in filling 43-49% of the open pore spaces which was 20% more than the application of the medium alone.

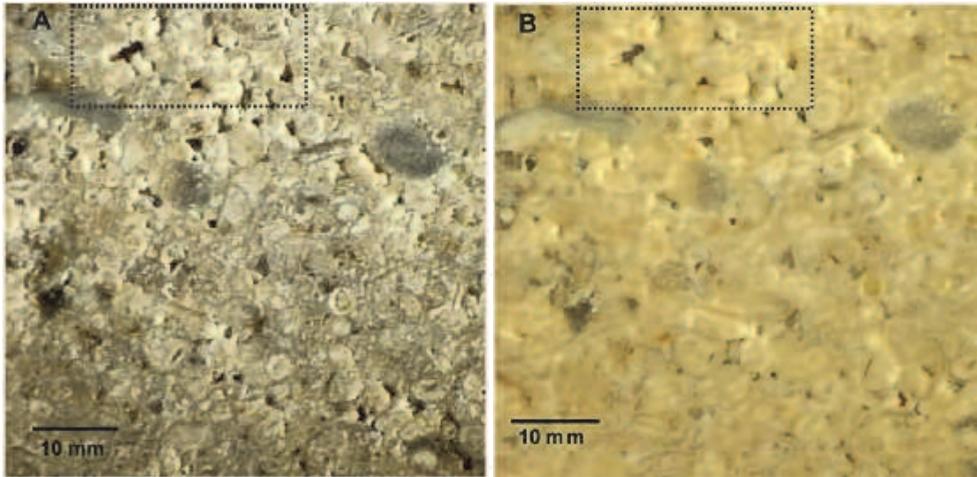


Fig. 5. Bacterial isolate on limestone before treatment (A) and (B) after Bacterial biocalcifying treatment for 21 days of incubation at 30°C (Zamarreno et al., 2009).

3.4 Bio-mediated ground improvement

The liquefaction of loose sands in hydraulic fills and manmade or natural (underwater) slopes is a major problem in geo-engineering. Piping and liquefaction are associated with sudden and catastrophic failures and often lead to loss of life and massive financial consequences. Mitigation strategies such as various methods of compaction or ground improvement methods such as jet-grouting or soil mixing are not always suitable or require high amounts of energy, high costs and materials with significant impact on the environment (van Paassen et al. 2010). Microbial-induced carbonate precipitation by urea hydrolysis has shown promising role for ground improvement. Recent research initiatives (Whiffin et al. 2005; Mitchell & Santamarina, 2005; DeJong et al. 2006; Whiffin et al. 2007; Ivanov & Chu 2008) have shown that the calcite crystals form cohesive “bridges” between existing sand grains, increasing strength and stiffness of sand with limited decrease in permeability. Proper understanding of key parameters, which control the *in situ* distribution of CaCO_3 and related engineering properties in both naturally and induced cemented sands, still prove to be insufficient and therefore represents the greatest challenge for further development of the bio-mediated ground stabilization technology.

In order to induce MICP in the soil subsurface, reagents and catalysts need to be injected and transported to the location where strengthening is required. Mixing bacteria and reagents prior to injection results in immediate flocculation of bacteria and crystal growth. While this method can be applied for treatment of surfaces, very coarse grained materials and mixed in place applications (Le Metayer- Levrel et al., 1999), this would cause rapid clogging of the injection well and surrounding pore space for many (fine) sands. In order to prevent crystal accumulation around the injection point and encourage a more homogeneous distribution of CaCO_3 over large distance, a two-phase injection for bacterial retention has been suggested by Whiffin et al. (2007). Microbial transport in saturated porous media is well studied (Murphy & Ginn, 2000). Many physical, chemical and

biological factors which influence the transport of bacteria have been investigated, including: fluid properties like chemistry and flow regime (Torkzaban et al., 2008), cell wall characteristics like hydrophobicity, charge and appendages (van Loosdrecht et al., 1987; Gilbert et al., 1991) and solid properties, like grain size distribution, surface texture and mineralogy (Scholl et al., 1990; Foppen and Schijven, 2005).

The fixation methodology, suggested by Whiffin et al. (2007), is based on the effect of ionic strength on microbial transport. To achieve homogeneous strength, Harkes et al. (2010) used BioGrout, a microbially induced calcium carbonate to improve the strength of ground. They developed a procedure to enhance fixation and distribution of bacterial cells and their enzyme activity in sand in order to improve the potential of microbially induced carbonate precipitation as ground reinforcement technique in fine-grained sand. The procedure comprises a multi-step injection in which first a bacterial suspension is introduced, potentially followed by a fixation fluid (i.e. a solution with high salt content) before the cementation fluid is introduced.

The new ground reinforcement techniques developed based on microbially induced carbonate precipitation (Whiffin et al. 2007; Harkes et al. 2010) use microbially catalyzed hydrolysis of urea to produce carbonate. In the presence of dissolved calcium this process leads to precipitation of calcium carbonate crystals, which form bridges between the sand grains and hence increase strength and stiffness. In addition to urea hydrolysis, there are many other microbial processes which can lead to the precipitation of calcium carbonate. van Paassen et al. (2010) evaluated various factors such as substrate solubility, CaCO_3 yield, reaction rate and type and amount of side-product. They found that the most suitable candidate as alternative MICP method for sand consolidation turned out to be microbial denitrification. In this process organic compounds, like acetate, can be oxidized to produce carbonate ions and alkalinity, which are required for the precipitation of calcite, while nitrate is reduced to nitrogen gas. Using calcium salts of both the electron donor and acceptor results in a high CaCO_3 yield. The rate of calcium carbonate formation by denitrification is far lower than the urease process, it requires further optimization for practical applications.

From the aforementioned applications, microbial concrete brings a new aspect to the construction industry. Promising results on the use of microorganisms for improvement of the durability of building materials have drawn the attention of research groups from all over the world. However, there are several challenges which must be addressed before wide acceptance of these strategies for construction materials.

4. Challenges to the study

4.1 Reduction of cost

One of the major factors hindering the use of MICCP technology is the high cost required for its production. The cost required is attributed to price of microbial product and the number of applications required. For better precipitation of carbonates, more time is required during which the building material has to be wet. With increasing times of precipitation, increasing amounts of EPS production, biofilm formation and hence, plugging can be expected. In order to ensure the presence of a sufficient amount of water, multiple applications of

nutrients over several days (Le Metayer-Levrel et al., 1999) or the application of a carrier material (May, 2005) have been proposed. Both these measures add to the total cost of the treatment. De Muynck et al (2010) analyzed the cost of biodeposition treatment based on the price of the microorganisms and the price of the nutrients. The calculated price of 1 kg lyophilized bacteria was about US \$1,500 (1,100 €) and 2-3 g m⁻² is applied which costs about US \$4 (3 €) m⁻². The cost of nutrients is estimated to be about US \$250 (180 €) per kg. The dosage for biodeposition on concrete surface generally ranges between 0.04 and 0.08 kg m⁻², bringing the cost of nutrients to US \$7-15 (5-10 €) m⁻². In case of carrier materials, the costs are even higher. Successful adoption and commercialization of this technique requires economical alternatives of the bacteria and the nutrients.

Economical alternatives to the medium ingredients, which can cost high as 60% of the total operating costs, need to be developed (Kristiansen, 2001). The nutritional profile of bacterial cultures indicate a high preference for protein based media as for *S. pasteurii* (Morsdorf & Kaltwasser, 1989). So for economizing this technology, researchers have looked for available cheap nutrient sources. There are many industrial effluents that are rich in proteins. If released in the altered form, they are hazardous for the atmosphere. So, the dual benefits of cost reduction and environment protection is feasible. Two such wastes are lactose mother liquor (LML) and corn steep liquor (CSL). Lactose mother liquor is an industrial effluent of the dairy industry. Its composition is given in table 3. Achal et al (2009a) investigated the effect of LML as sole source of growing bacterium *S. pasteurii* and compared the calcification effect of its usage. LML served as a better nutrient source for the growth of bacteria and also for calcite precipitation as compared to nutrient broth and yeast extract media which are quite expensive. Another by-product of the corn wet milling industry used by Achal et al (2010b) for economization of microbial calcite technology was corn steep liquor. Its constituents are listed in table 3. Corn steep liquor can typically be available locally with a price of nearly US \$2 (1.5 €) per liter, which is very economical compared with standard nutrient medium. The biodeposition cost by this comes to US \$0.5-1.0 (0.3-0.7 €) m⁻². 1.5 % CSL media along with NaCl, urea and CaCl₂ was used to investigate its effect on water and chloride ion permeability along with compressive strength improvement in cement mortar cubes and compared with Nutrient broth and yeast extract. The performance of CSL was significantly better than standard laboratory nutrients in terms of microbial concrete production. CSL offered an economic advantage over the standard nutrient medium and the overall process cost reduced dramatically. The usage of such byproducts not only reduces the cost, but also serve to prevent environmental pollution.

4.2 Usage of industrial by products

The traditional construction materials such as concrete, bricks, hollow blocks, solid blocks, pavement blocks and tiles are being produced from the existing natural resources. This is damaging the environment due to continuous exploration and depletion of natural resources. Many authorities and investigators are now working to have the privilege of reusing the wastes in environmentally and economically sustainable ways (Aubert et al., 2006). Different types of wastes along with their recycling and utilization potentials are listed in table 4.

LML		CSL	
Component	Measure	Component	Measure
pH	6.2	pH	3.86
Solids (%)	5.5	Solids (%)	46-50
Lactose (%)	15.4	Carbohydrates (%)	5.8
Proteins (%)	8	Proteins (%)	24
Fats (%)	2	Fats (%)	1
Ash (%)	0.53	Minerals (%)	8.8
Calcium (mg/l)	353	Arginine (%)	0.4
Phosphorus (mg/l)	35	Cystine (%)	0.5
Potassium (mg/l)	186	Glycine (%)	1.1
Sodium (mg/l)	44	Isoleucine (%)	0.9
Chloride (mg/l)	90	Inositol (mg/ 100g)	602
Sulphur (mg/l)	15	Choline (mg/ 100g)	351

Table 3. Physico chemical characteristics of lactose mother liquor (LML) and corn steep liquor (CSL) (Achal et al., 2009a, 2010b).

Fly ash (FA) generated during the combustion of coal for energy production is one of the industrial byproduct that is recognized as an environmental pollutant. Addition of fly ash to concrete has become a common practice in recent years. Reports have been published concerning the effect of fly ash on concrete porosity and resistivity, pore solution chemistry, oxygen and chloride ion diffusivity, carbonation rates and passivation (Mangat & Gurusamy, 1987; Thomas & Matthews, 1992; Montemor et al., 2000). Rice husk ash (RHA) obtained from burning of rice husk is another major agricultural byproduct and can be used successfully in construction materials such as bricks and blocks without any degradation in the quality of products (Nasly & Yassin., 2009). The utilization of above mentioned by-products as partial replacement of clay in bricks can serve important economical, environmental and technical benefits such as the reduced amount of waste materials, cleaner environment, reduced energy requirement, durable service performance during service life and cost effectiveness. However, problems associated with ash bricks are low strength, higher water adsorption, low resistance to abrasion, low fire resistance and high porosity (Kumar & Palit, 1994). An attempt has been made to study the role of microbial calcite to enhance the durability of ash bricks (FA and RHA) and it is found to be very effective in reducing permeability and decreasing water absorption leading to enhanced durability of ash bricks (Dhami et al. 2011).

Similar efforts need to be accomplished for other industrial wastes as well as with more detailed studies of energy inputs for alternative materials, so that efficiency can be improved in comparison to traditional materials.

Type of wastes	Source details	Recycling and utilization potentials
Industrial waste (inorganic)	Coal combustion residues, fly ash, steel slag, construction debris	Bricks, blocks, tiles, cement, paint, fine and coarse aggregates, concrete, wood substitute products, ceramic products
Agro waste (organic)	Baggage, rice and wheat straw and husk, saw mill waste, ground nut shell, jute, sisal, cotton stalk	Cement boards, particle boards, insulation boards, wall panels, roof sheets, binder, fibrous building panels, bricks, acid proof cement, coir fiber, reinforced composites, polymer composites
Mining/ mineral wastes	Coal washeries waste, mining waste tailing from iron, copper, zinc, gold industries	Bricks, fine and coarse lightweight aggregates, tiles
Non hazardous waste	Waste gypsum, lime sludge, lime stone waste, broken glass and ceramics	Blocks, bricks, cement clinker, hydraulic binder, fibrous gypsum boards, gypsum plaster, super sulfated cement
Hazardous waste	Contaminated blasting materials, galvanizing waste, metallurgical residues, sludge from waste water and waste water treatment plants	Boards, bricks, cement, ceramics, tiles

Table 4. Different types and sources of solid wastes and their recycling and utilization potentials for construction materials (adapted from Pappu et al., 2007).

4.3 Concentration of ammonia and salts

The production of ammonia during hydrolysis of urea might raise some issues of environmental concern because of the fact that atmospheric ammonia is being recognized as a pollutant. Atmospheric ammonia is known to contribute to several environmental problems, including direct toxic effects on vegetation, atmospheric nitrogen deposition, leading to the eutrophication and acidification of sensitive ecosystems, and to the formation of secondary particulate matter in the atmosphere, with effects on human health, atmospheric visibility and global radiative balance (Sutton et al., 2008).

However, when the concentration of ammonia generating compounds does not exceed the concentration of the calcium salt, it is possible to decrease the emission of ammonia to a great extent. The presence of ammonium might also present some risks to the stone. First of all, the presence of an ammonium salt might present some risks related to salt damage. Secondly, ammonium can be converted to nitric acid by the activity of nitrifying bacteria, resulting in damage to the stone. So, if higher concentrations of ammonium are to be

produced, as might be the case for the hydrolysis of urea, the use of a paste might offer an attractive solution. The latter is one of the most commonly applied methods for the removal of salts from building materials (Woolfitt & Abrey, 2008; Carretero et al., 2006).

After ammonia, calcium salt concentration has a major impact on the performance of treatment. High dosage of calcium salt could also lead to an accumulation of salts in the stone, which could give rise to efflorescence or damage related to crystallization. So, detailed studies need to be done in this regard for prevention of such harmful effects.

4.4 Survival of bacteria

The size of bacterial inoculum and survival of bacteria potentially influences bacterial calcification. Zamarreno et al (2009) studied the survival of bacteria inside carbonate crystals for up to 330 days. Significant reduction in the viable cells was noticed after 13 days interval and after 330 days, no cells are viable.

Survival of bacteria inside cracks and other building materials also needs to be studied in detail, such that the efficacy of this treatment can be evaluated.

4.5 Microbial concrete in low carbon buildings

The art and science of building constructions started with the usage of natural materials like soils, stones, leaves, unprocessed timber etc. Hardly any energy is spent in manufacturing and usage of such materials, but they are not much in use because of durability issues unlike materials like burnt clay bricks, lime, cast iron products, aluminium, steel, Portland cement, etc. These modern materials require huge energy reservoirs, are non-recyclable, and as well as are harmful to the environment. The construction sector is responsible for major input of energy resulting in large share of CO₂ emissions (22% in India) into the atmosphere (Reddy & Jagadish.,2001). The emission of these green house gases during manufacturing processes of building materials is contributing a lot to global warming. Its time to put emphasis on reducing the emission of these gases into the atmosphere and save energy by minimizing usage of conventional building materials, methods, techniques and working on some other substitutes. For reduction of indirect energy use in building materials, either alternative for bricks, steel and cement have to be found, or vigorous energy conservation measures in these segments of industry have to be initiated. Energy requirements for production and processing of different building materials, CO₂ emissions and the implications on environment have been studied by many researchers (Suzki *et al.*, 1995; Oka *et al.*, 1995; Debnath *et al.*, 1995; table 5).

Type of material	Thermal energy (MJ/kg)
Cement	5.85
Lime	5.63
Lime Pozollana	2.33
Steel	42.00
Aluminium	236.80
Glass	25.80

Table 5. Energy in basic building materials (Reddy & Jagadish, 2001).

Reddy & Jagadish (2001) reported soil cement blocks with 6-8% cement content to be most energy efficient building material. These materials have low cost, are easily recyclable and environmental friendly as the soils are mixed with additives like cement, limestone etc. As there is no burning involved, this type of stabilized mud block helps in conserving huge amounts in energy. Attempts are being made in our lab to apply Microbial calcite technology to these eco friendly low carbon building materials so that it can pave the way for more sustainable, cheap and durable building materials.

4.6 Enhancing the efficiency of calcifying bacteria

It is imperative to use local bacterial strains that are well conditioned for the environment for production of microbial calcite. There are evidences that indicate the implementation of mutagenesis through UV for strain improvement (Wu et al., 2006). Attempts to enhance the efficiency of calcifying bacterial culture of *S. pasteurii* were done by Achal et al (2009a). They got more promising results with UV induced mutants of as compared to wild type strains for consolidation of sand columns. Further steps are required to produce better calcifying cultures through other methods so as to get better consolidation of various construction materials.

4.7 Other factors

Rodriguez-Navarro et al (2003) reported that fast precipitation of bacterial carbonates could result in a lower efficiency of the calcite deposition. Along with this, the presence of well developed rhombohedral calcite crystals result in a more pronounced consolidating effect compared to the presence of tiny acicular vaterite crystals. So, detailed studies need to focus on different types of nutrients and metabolic products used for growing calcifying microorganisms, as they influence survival, growth, biofilm and crystal formation. More work should be done on the retention of nutrients and metabolic products in the building material. Detailed microbial ecology studies are also needed in order to ascertain the effects of the introduction of new bacteria into the natural microbial communities, the development of the communities at short, mid and long-term, and the eventual secondary colonization of heterotrophic microorganisms using bacterial organic matter and dead cells, such as actinomycetes, fungi, etc. Until now, the practical applications of microbial calcite technology have been mainly limited to France where it has been applied on several historic monuments including a part of the Notre Dame de Paris (De Muyneck et al. 2010).

5. Conclusion

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. Work of various researchers has improved our understanding on the possibilities and limitations 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. Cementation by this method is very easy and convenient for usage. This will soon provide the 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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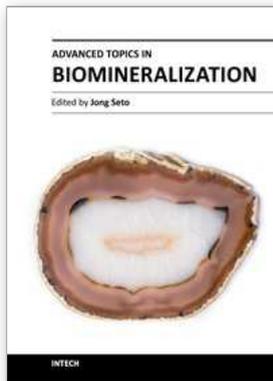
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