Application of calcifying bacteria for remediation of stones and cultural heritages

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INTRODUCTION

Taking a look at our history, beautiful monuments and sculptures of limestone are seen. Be it European Cathedrals (Milan Cathedral, Italy), Roman and Greek temples, the Taj Mahal and the Pyramids, limestone is everywhere. Numerous limestones and marble quarries encouraged ancient Greek architects to build the Acropolis and Roman architects to build magnificent Forum (Corvo et al., 2000). Limestone, consisting almost entirely of calcite (the most stable polymorph of calcium carbonate) along with a small content of aragonite has been found to be highly durable building material since ages (Graedel, 2000). Though known for its great strength, these materials are highly porous and hydrophilic in nature making them highly susceptible to water (such as acid rain) and environmental pollutants (Tiano et al., 1999). The water carrying harmful and corrosive ions often penetrates into the pores of these stones leading to their deterioration. Architectural and sculptural stones have undergone deterioration due to number of factors such as physical, chemical, and biological weathering (Rodriguez-Navarro and Sebastian, 1996; Wakefield and Jones, 1998; Rodriguez-Navarro and Doehne, 1999). Several environmental pollutants, particulate matters, fly ash, smog and natural causes lead to degradation of these structures (Fernandes, 2006; Chand and Cameotra, 2011). Microorganisms inhabiting these structures, upon interaction with other detrimental biotic and abiotic factors, also promote weathering and corrosion of these building materials (Fernandes, 2006). All these factors are thereby causing great harm to physical and esthetic appearance of such structures. Because of all these reasons, survival of many cultural and historical assets is in threat. One of such examples is the cave of Lascaux in southwest France where infection of Fusarium sp. and other molds deteriorated the floor and banks of main chamber (Rosenbaum, 2006). Paintings in Altamira cave in Santillana del Mar, Spain, and the earliest known Christian paintings adorning Roman catacomb walls have also undergone similar fate.

The problem of deterioration of these historical monuments has fetched the attention of archeologists, geobiologists and bio-conservators to preventive and remedial technologies to safeguard these cultural heritage monuments, which is a big challenging task. Many attempts have been made to remediate such structures by application of conservative treatments using organic and inorganic products (Lazzarini and Laurenci Tabasso, 1986), but these agents have not been as effective as expected due to complex nature of the textures/compositions of materials encountered. Other drawback is that high amounts of organic solvents are often wasted in the environment and in few cases, these treatments even led to detrimental effects on the stone material in context to its texture, physical strength and esthetic appearance (González-Muñoz, 2008). None of the tested conventional treatment methods have proved to be satisfactory for the preservation and consolidation of these deteriorated monuments (Cappitelli et al., 2007; González-Muñoz, 2008) (Table 1). Hence, the durability related issues are causing high impact on national economies as huge sums of money are required for maintenance and repair of such structures. The short comings of conventional methods have encouraged the search and development of new conservation treatments for remediation and protection of these magnificent materials, based on biological methods (Fernandes, 2006).

Microbial geotechnology, i.e., microbial based technology for civil structures is an emerging discipline of science which has developed immensely in the recent years. Microbially induced carbonate precipitation has successfully emerged as a novel method to protect and remediate decayed building structures and
Table 1 | Methodologies for eradication of degradative agents of stone works.

| Method                                      | Advantages                                                                 | Disadvantages                                                                 | References                        |
|---------------------------------------------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------------|-----------------------------------|
| **PHYSICAL METHODS**                       |                                                                            |                                                                                |                                   |
| UV, Gamma, X irradiation                    | Simple, high penetration of gamma and X, effective on insects, UV effective on microbes | Application in movable or small scale objects, poor penetration of UV          | Warscheid and Braams, 2000; Salvadori, 2003 |
| Mechanical removal of biological material by hand or tool | Traditional and widely used                                              | Short lived results, only superficial mycelium removed, microbes redevelop, damage stone | Dakal and Cameotra, 2012          |
| Low pressure water rinsing/ steam cleansing  | Effective for removal of algae, mosses, lichens, no health hazards        | Water retained in pores likely to favor microbial growth                        | Kumar and Kumar, 1999             |
| **CHEMICAL METHODS**                       |                                                                            |                                                                                |                                   |
| Nongaseous biocides                         | Broad and narrow spectrum                                                  | Health hazards, unwanted side effects, inadequate timing of application        | Kumar and Kumar, 1999; Salvadori, 2003; Cappitelli and Sorlini, 2005 |
| Fumigation                                  | Highly and rapidly effective in fungi and insects, organic materials        | Very toxic gases (often carcinogenic)                                           | Kumar and Kumar, 1999; Warscheid and Braams, 2000; Salvadori, 2003 |
| Anoxic atmosphere                           | Fungi are susceptible to oxygen depletion                                  | Long exposure period, expensive equipment                                       | Gu, 2003; Salvadori, 2003          |

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Deterioration of stone works: causative agents

The deterioration of historic monuments and stone works occurs due to numerous factors leading to stone dissolution, staining or color alteration, surface alterations, biocorrosion and transformations into smaller sized crystals etc. (Chand and Cameotra, 2011). In the last decades, alterations have occurred mainly due to microbial biofilm production, deposition of organic and inorganic compounds, formation of black crusts, nitratation, sulphation and, due to residual hydrocarbons and other organic pollutants in dust (Warscheid and Braams, 2000; Fernandes, 2006; Di Pippo et al., 2009).

**Nitrites**

Nitrites, originating from the reaction of numerous oxides of nitrogen present in atmosphere (N₂O, NO, N₂O₃, NO₂, N₂O₅) due to pollution formed through oxidation upon reaction with water vapor produces nitrous acid and more abundant nitric acid (HNO₃) as final products. These acids result in the formation of acid rain and attack the stone structures causing formation of calcium nitrate salts on the stone buildings (Ranalli et al., 1999).

**Sulfates**

Sulfates, which originate from oxidation of sulfur dioxide lead to the formation of sulfuric acid (H₂SO₄) resulting in acid rain. These acid rains cause transformation of insoluble calcium sulfate posing potential risk not only to buildings but also for humans (Ranalli et al., 1999).

**Black crusts**

Black crusts are normally formed as a result of mixing of gypsum crystals with atmospheric particles (pollen, dusts, spores, particulate matter called smog etc.) (Saiz-Jimenez, 1991; Saiz-Jimenez and García del Cura, 1991). Calcium sulfate salt crusts also accumulate particles of soot originating from fossil fuel consumption and form black crusts (Kumar and Kumar, 1999; Warscheid and Braams, 2000).

**Organic matter**

Organic matter is ascribed to the lysis of microbial cells and presence of hydrocarbons originating from combustion of oil. This type of deterioration becomes more evident in the buildings located in the open as atmospheric pollution also contributes to
add up the degradation process (Ranalli et al., 1999). The formation of all these crusts affect stone's texture, crystal structure, composition, coherence, water uptake and strength.

Microorganisms
Microorganisms (Bacteria, Archaea, Fungi, Algae, Lichens), along with mosses and higher plants, have been reported commonly on stoneworks leading to deterioration of several types of materials as stone works, wood, tapestries, papyrus, canvas, paper etc. (Cappitelli and Sorlini, 2005; McNamara and Mitchell, 2005; Ramirez et al., 2005) (Table 2). These microorganisms are amongst the major players of biodegradation of several stone work buildings. The photolithoautotrophic nature of algae and cyanobacteria facilitate colonization of stone by many other microorganisms (e.g., fungi and bacteria) (Warscheid and Braams, 2000; McNamara and Mitchell, 2005). The nitrifying bacteria (Nitrosomonas spp. and Nitrobacter spp.), capable of excreting nitrous and nitric acid and sulfur-oxidizing bacteria (Thiobacillus spp.) which produce sulfuric acid leads to biocorrosion of the stone material (Gómez-Alarcón et al., 1995; Warscheid and Braams, 2000). The acid formed reacts with stone constituents to produce sulfate-based crusts which upon precipitation in pores of stone cause considerable stress in porewalls. Biocorrosion also occurs by chemoorganotrophic microorganisms, including several bacteria and fungi (Acidithiobacillus ferroxidans, Bacillus spp., Leptospirillum spp., Acreobasidium spp.) as well as lichens as they excrete organic acids. These acids have been reported to chelate the metal cations (e.g., Fe, Mg, Mn, Si, Al, Ca etc.) from minerals to form complexes which are quite stable with time (Kumar and Kumar, 1999; Warscheid and Braams, 2000; McNamara and Mitchell, 2005; Rawlings, 2005). Physical penetration by lichens and fungi also contributes to degradation. The hyphae of fungus penetrate deeply beneath the stone surface, causing not only mechanical deterioration but also transport of water and nutrients through the stone facilitating colonization of stone interior by bacteria leading to biochemical deterioration (Gómez-Alarcón and de La Torre, 1994).

Several attempts have been made to decrease the susceptibility to decay by many conservation treatments which includes application of surface sealing or consolidating agents to the substrate resulting in organic/inorganic precipitation of binding material in the pores of stone (Adeyemi and Gadd, 2005). These stone consolidants reestablish the binding between the grains of degraded stone. To protect the stone from water ingress and weathering agents, water repellents have also been applied. These chemicals have short efficacy due to their chemical composition and thermal expansion coefficient which are quite different from that of the stone (De Muynck et al., 2010). But due to incompatibility problems with the stone, consolidants as well as water repellents have been reported to accelerate decay of the stone material (Clifton and Frohsndorff, 1982; Delgado Rodrigues, 2001; Moropoulou et al., 2003). Efforts have been made to introduce methods based on CaCO₃ precipitation into the pores of limestone by few researchers. Application of saturated solution of calcium hydroxide (Lime-water technique) has been used on degraded stones so as to impart a slight water repellent and consolidating effect (Tiano et al., 1999). But, little success has been achieved till now in consolidation of stone with inorganic materials. This is because of the tendency of these materials to generate shallow and hard crusts due to their poor penetration abilities, growth of precipitated crystals, salts formation and stone particle binding ability (Clifton and Frohsndorff, 1982).

To overcome the limitations of these conventional treatments, researchers proposed microbially induced calcium carbonate precipitation as an eco-friendly method to protect and restore degraded ornamental stones (Le Metayer-Levrel et al., 1999; Stocks-Fischer et al., 1999; Ramachandran et al., 2001; Ramakrishnan et al., 2001; De Muynck et al., 2008a,b). Although microorganisms have often been associated with detrimental effects on the integrity of stone structures, affecting mineral integrity or exacerbating powerful physical processes of deterioration (Papida et al., 2000), there is an increase of evidence that they could be used to reverse the deterioration processes on historical objects of art (Atlas et al., 1988; Lal Gauri et al., 1989b; Orial et al., 1992; Castanier et al., 1999; Perito et al., 1999; Ranalli et al., 1999).

Microbially Induced Calcium Carbonate Precipitation
Microbially induced calcium carbonate precipitation (MICCP) is a process where an organism creates a local microenvironment, with conditions that permits precipitation of carbonates (Hamilton, 2003). Bacteria isolated from different natural habitats have been reported for their ability to precipitate calcium carbonate both in natural and laboratory conditions (Krumbein, 1979; Rodriguez-Navarro et al., 2003). Precipitation of carbonates varies based on the types of bacteria, abiotic factors such as salinity and composition of the nutrients in various environments (Knorre and Krumbein, 2000; Rivadeneyra et al., 2004). Calcium carbonate precipitation is a chemical process and influenced by four main factors such as the calcium concentration, amount of dissolved inorganic carbon (DIC), availability of nucleation sites and pH (Hamnes and Verstraete, 2002). Sufficient calcium and carbonate ions are required for CaCO₃ precipitation so that the ion activity product (IAP) exceeds the solubility constant (K_{sp}) Equations (1) and (2). From the comparison of the IAP with the K_{sp}, the saturation state (Ω) of the system can be defined; if Ω > 1, then the system is oversaturated and precipitation is likely to occur as mentioned below by Morse (1983):

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

$$Ω = a(Ca^{2+}) a(CO_3^{2-}) / K_{sp} \text{ with }$$

$$K_{sp,calcite,25^\circ} = 4.8 \times 10^{-9}$$

As mentioned previously, the amount of carbonate ions is related to the amount of DIC and pH of a given aquatic system. However, the amount of DIC depends on several environmental parameters like temperature and partial pressure of carbon dioxide. The equilibrium reactions and constants governing the dissolution of CO₂ in aqueous media (25°C and 1 atm) are given below in Equations
Table 2 | Microorganisms and environmental factors involved in biodeterioration of architectural buildings and artworks (Source: Dakal and Cameotra, 2012).

| Microbial group          | Microorganisms/ environmental factors        | Deterioration type                      | Mechanism                                                                 |
|--------------------------|---------------------------------------------|-----------------------------------------|---------------------------------------------------------------------------|
| Photoautotrophs          | Cyanobacteria                               | Esthetic and chemical deterioration     | Biofilm, color alteration, patina, crust formation, bioweathering         |
|                          | Lichen                                      | Chemical and mechanical deterioration   | Extraction of nutrients from stone surface, oxalate formation, carbonic acid production, physical intrusions |
|                          | Algae                                       | Esthetic and chemical deterioration     | Biofilm, color alteration, black crusts                                  |
|                          | Mosses and Liverworts                       | Esthetic and chemical deterioration     | Discoloration, green gray crusts, extraction of minerals                  |
| Chemoautotrophs          | Sulfur oxidizing, Nitrifying bacteria        | Chemical deterioration                  | Black custs                                                              |
| Chemoheterotrophs        | Heterotrophic bacteria                       | Esthetic and chemical deterioration     | Crust formation, patina, exfoliation, color alteration                    |
|                          | Actinomycetes                               | Esthetic deterioration                  | Whitish gray powder, patina, white salt efflorescence                     |
|                          | Fungi                                       | Esthetic, chemical, physical and        | Fungal diagenesis, color alteration, oxalate formation, bioweathering, physical intrusions, destabilization of stone texture |
|                          |                                             | mechanical deterioration                |                                                                           |
| Chemoorganotrophs        | Sulfur reducing bacteria                     | Chemical deterioration                  | Conversion of sulfate to sulfite                                          |
| Higher plants            | Higher plants                               | Mechanical deterioration                | Intrusion of roots in cracks, pores leading to collapse and detachment of stone structure |

(3)–(6) as suggested by Stumm and Morgan (1981):

\[
\begin{align*}
\text{CO}_2(g) & \leftrightarrow \text{CO}_2(aq) \quad (pK_H = 1.468) \tag{3} \\
\text{CO}_2(aq) + \text{H}_2\text{O} & \leftrightarrow \text{H}_2\text{CO}_3^- \quad (pK = 2.84) \tag{4} \\
\text{H}_2\text{CO}_3^- & \leftrightarrow \text{H}^+ + \text{HCO}_3^- \quad (pK_1 = 6.352) \tag{5} \\
\text{HCO}_3^- & \leftrightarrow \text{CO}_3^{2-} + \text{H}^+ \quad (pK_2 = 10.329) \tag{6} \\
\text{With } \text{H}_2\text{CO}_3^- & = \text{CO}_2(aq) + \text{H}_2\text{CO}_3 \tag{7}
\end{align*}
\]

Hammes and Verstraete (2002) suggested that microorganisms influence precipitation by altering any of the precipitation parameters described above, either separately or in various combinations with one another. MICCP has gained increasing interest in the last 20 years and found to be the primary focus of research in bio geo civil engineering because of its numerous applications.

There are mainly four groups of microorganisms involved in the process, which are: (i) photosynthetic organisms such as cyanobacteria and algae, (ii) sulfate reducing bacteria responsible for dissimilatory reduction of sulfates, (iii) organisms utilizing organic acids, and (iv) organisms that are involved in nitrogen cycle either by ammonification of amino acids/nitrate reduction or hydrolysis of urea (Stocks-Fischer et al., 1999; Hammes and Verstraete, 2002; Jargeat et al., 2003).

In aquatic environments, MICCP is primarily caused by photosynthetic organisms (McConnaughey and Whelan, 1997). Algae and cyanobacterial metabolic processes utilize dissolved CO₂ Equation (8), which is in equilibrium with HCO₃⁻ and CO₃²⁻. Equation (9). The removal of CO₂ induces a shift in this equilibrium, and results in an increase in pH Equation (10) (Ehrlich, 1998) and in presence of calcium ions, this reaction leads to precipitation of calcium carbonate as mentioned by Hammes and Verstraete (2002) Equation (11).

\[
\begin{align*}
\text{CO}_2 + \text{H}_2\text{O} & \rightarrow (\text{CH}_2\text{O}) + \text{O}_2 \tag{8} \\
2\text{HCO}_3^- & \leftrightarrow \text{CO}_2 + \text{CO}_3^{2-} + \text{H}_2\text{O} \tag{9} \\
\text{CO}_3^{2-} + \text{H}_2\text{O} & \leftrightarrow \text{HCO}_3^- + \text{OH}^- \tag{10} \\
\text{Ca}^{2+} + \text{HCO}_3^- + \text{OH}^- & \rightarrow \text{CaCO}_3 + 2\text{H}_2\text{O} \tag{11}
\end{align*}
\]

Calcium carbonate precipitation via this pathway occurs in sea water, geological formations (Packman et al., 1999; Machel, 2001), in landfill leachates (Maliwa et al., 2000) and even during the biological treatment of acid mine drainage (Kaufman et al., 1996). Chiefly, in several of the described examples for this pathway, instead of calcite, dolomite and aragonite are the predominant minerals to precipitate (Packman et al., 1999; Wright, 1999; Warthmann et al., 2000; Machel, 2001).

Calcium carbonates can also be precipitated by heterotrophic organisms, by the production of carbonate or bicarbonate and modification of the environment to favor precipitation (Castanier et al., 1999). The abiotic dissolution of gypsum (CaSO₄,2H₂O) Equation (12) provides an environment that is rich in both sulfate and calcium ions. In the presence of organic matter and absence of oxygen, sulfate reducing bacteria can reduce sulfate to H₂S
and release HCO$_3^-$ Equation (13) (Ehrlich, 1998; Castanier et al., 1999; Wright, 1999). If H$_2$S then degasses from the environment, this results in an increase in pH and favors the precipitation of calcium carbonate Equation (11) (Castanier et al., 1999).

$$\text{CaSO}_4 + 2\text{H}_2\text{O} \rightarrow \text{Ca}^{2+} + \text{SO}_4^{2-} + 2\text{H}_2\text{O} \quad (12)$$

$$2(\text{CH}_3\text{COO})^+ + \text{SO}_4^{2-} \rightarrow \text{HS}^- + \text{HCO}_3^- + \text{CO}_2 + \text{H}_2\text{O} \quad (13)$$

Third pathway includes bacteria which use organic acids as their only source of carbon and energy wherein some common species of soil bacteria are included. Such acids include oxalate, acetate, citrate, glyoxylate, succinate and malate. The consumption of these acids results in pH increase, which leads to precipitation of carbonates in the presence of calcium ions Equations (14)–(16) (Knorre and Krumbein, 2000; Braissant et al., 2002)

$$\text{CH}_3\text{COO}^- + 2\text{O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O} + \text{OH}^- \quad (14)$$

$$2\text{CO}_2 + \text{OH}^- \rightarrow \text{CO}_2 + \text{HCO}_3^- \quad (15)$$

$$2\text{HCO}_3^- + \text{Ca}^{2+} \rightarrow \text{CaCO}_3 + \text{CO}_2 + \text{H}_2\text{O} \quad (16)$$

Numerous heterogenous bacterial groups are linked to this precipitation mechanism. Braissant et al. (2002) speculated that this pathway might be extremely common in natural environments due to the abundance of such low molecular weight acids in the soils (produced by fungi and plants).

**MICROBIAL CARBONATE: REMEDIATION OF LIMESTONE**

Boquet et al. (1973) firstly demonstrated the precipitation of calcium carbonate by soil bacteria under laboratory conditions. Previous researchers showed precipitation of carbonates by marine bacteria only in liquid media while Drew (1911) and Shinano (1972), investigated the carbonate precipitation by soil bacteria on solid media and obtained best results with B4 medium. Among the organisms tested, several *Bacillus* strains and *Pseudomonas aeruginosa* were observed to form crystals. Castanier et al. (1999) reported the microbial origin of limestone while Adolphe et al. (1989) further demonstrated the bacterial origin of the calcite crusts and found great resistance against erosion by this calcite layer. Adolphe et al. (1990) applied patent for the treatment of artificial surfaces by surface coatings produced by microorganisms and formed a company “Calcite Bioconcept.” The promising results of “Calcite Bioconcept” encouraged many researchers to look for different approaches for bioremediation of stone by microbial carbonates. First approach was based on usage of different microbes and metabolic pathways or delivery systems to overcome limitations of “Calcite Bioconcept” technique while in second approach, no microbes were applied directly but instead introducing macromolecules along with supersaturated solution of calcium carbonate and carbonate precipitation by microbiota inhabiting the stone were investigated.

The carbonate precipitation ability of bacteria has been demonstrated under laboratory but further experiments are required to assess the viability and carbonate precipitation ability...
of these bacteria in situ. The collaboration between the University of Nantes, the Laboratory for the research of historic monuments (LRMH) and the "Calcite Bioconcept" (Le Metayer-Levrel et al., 1999) led to the optimization and industrialization of this concept. Upon investigating different bacteria, Castanier et al. (1999) reported the highest performance by *B. cereus* which was further selected for in situ applications (Orial, 2000). This paved way to optimization of the nutrient media with source of proteins for the oxidative deamination of amino acids by aerobiosis and nitrogen source for the dissimilatory reduction of nitrate in anaerobiosis and microaerophilic conditions along with a fungicide to prevent undesirable growth of fungi on the stone (Le Metayer-Levrel et al., 1999). But Orial (2000) suggested that after every 10 years a new biocalcin treatment was needed to restore its protective effect. This technology was also applied on limestone statuaries in different climatic environments where it was found to be highly successful even after 4 years of application. Addition of natural pigments into the nutritional medium created a surficial patina with the biodeposition treatment. The pigments integrated into the biocalcin resulted a persistent light coloring to the stone. This technique concealed some newly replaced stones on a monument (Le Metayer-Levrel et al., 1999). Tiano et al. (1999) studied the effect of microbial calcite crystals on Pietra di Lecce bioclastic limestone by *Micrococcus* spp. and *Bacillus subtilis* and their results showed a significant reduction in water absorption. The authors also commented some negative consequences, such as (i) the formation of new products due to chemical reactions between stone minerals and some by-products originating from the metabolism of bacteria, and (ii) the formation of stained patches because of the growth of air-borne fungi. To avoid such short comings, the authors used some natural and synthetic polypeptides to control the calcite crystal growth in the pores. Use of organic matrix macromolecules (OMM) isolated from *Mytilus californianus* shells was proposed to induce the precipitation of calcium carbonate within the pores of the stone (Tiano et al., 1992; Tiano, 1995). Slight decrease in porosity and water absorption by capillarity was observed in this case (Tiano, 1995). This method was not much beneficial due to the complexity of isolation procedure as well as less yield of usable product (Tiano et al., 1999). Hence, in place of this bio inducing macromolecules (BIM) rich in aspartic acid groups, Tiano et al. (2006) proposed to use acid functionalized proteins such as polyaspartic acid. Calcium and carbonate ions were supplied for
FIGURE 2 | Colonies of 6 different strains of B. sphaericus and B. lentus on agar plates and their ability to encrust themselves in calcium carbonate (Source: Dick et al., 2006).
calcite crystal growth, by addition of ammonium carbonate and calcium chloride solution or a solution of saturated bicarbonate. The consolidating effect was observed to be very low compared to ethylsilicates (Tiano et al., 2006). Dick et al. (2006) observed 50% reduction in water absorption by treating limestone cubes with two strains of *B. sphaericus* (Figure 2).

To improve the methodologies for delivering bacterial cells to stone surfaces and also to control the 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 provides anaerobic conditions, humidity and shortens the treatment time. Cappitelli et al. (2006) proposed Carbogel as a delivery system for bacteria due to its high retention of viable bacteria and less time to entrap cells (Figure 3). Different methodologies where microbial calcite has been deposited as a layer on surface of stone is presented in Table 3.

Precipitation of calcite crystals by fresh water bacteria on limestone significantly reduced the pore sizes of the stone (Zamarreno et al., 2009). Calcite crystals were deposited around and inside open pore spaces. Application of calcite crystals filled 43–49% of the open pore spaces which was 20% higher than the application of the medium alone. De Muynck et al. (2012) reported B. sphaericus to be very efficient strain for consolidation of limestone specimens at range of temperatures (10, 20, 28, 37°C). This isolate led to 64% lower weight loss upon sonication and 46% decreased sorptivity in treated limestone specimens compared to the control specimens. De Muynck et al. (2011) recently applied bacterial calcite in two types of stones: microporous and macro-porous. They reported that application of bacterial carbonates is more successful in macro-porous stone where it occurs to a larger extent and at greater depths than in microporous stone. It has also been shown on laboratory scale that several bacterial strains (such as Pseudomonas stutzeri, P. aeruginosa, D. vulgaris, and D. desulfuricans) are not only able to denitrify and desulfuricate harmful masonry salts such as nitrate and sulfate (Lal Gauri et al., 1989a; Heselmeyer et al., 1991; Ranalli et al., 1999) but also mineralize organic residues or pollutants like carbohydrates, waxes or hydrocarbons that commonly occur in crusts on stonework (Warscheid et al., 1991; Saiz-Jimenez, 1997; Ranalli et al., 1999).

From the above mentioned applications, microbial concrete seems to bring a new revolution in the civil industry. Use of bacteria to improve the durability of building materials has drawn the attention of research groups all over the world. But several challenges have to be met before acceptance of this technology by conservators.

LIMITATIONS AND CHALLENGES

Though there are many advantages of MICCP technology for bioremediation of several stone structures but there are a few limitations also. In comparison to chemical treatments, biobased treatments are found to be more complex because the microbial activity depends on many environmental factors such as temperature, pH, concentrations of donors and acceptors of electrons, concentrations and diffusion rates of nutrients and metabolites. Design of experiments for biodeposition treatments require a huge data of the biological processes (growth, biosynthesis, specific enzymatic activities), chemical reactions accompanied with formation of insoluble compounds, physic—chemical processes as precipitation, crystallization, and adhesion.

Due to this complexity, its usage at large-scale has not been so encouraging. The inconvenient application procedures also are major gaps for successful commercialization. The precipitation of carbonates mainly depends on time required for carbonate formation. If precipitation time increases, then the amounts of EPS production increases, and hence plugging but multiple applications of nutrients and usage of carrier materials have significant influence on the total cost of treatment (Le Metayer-Levrel et al., 1999; May, 2005). Production of ammonia during hydrolysis of urea poses environmental as well as leads to discoloration of stone (Sutton et al., 2008; Tobler et al., 2011). Ammonium is also converted to nitric acid due to the action of denitrifying bacteria which results in significant damage to the stone. Additional research is necessary to overcome this problem. As the amounts of carbonate precipitates formed are dependent on amount of calcium added, increased concentration of calcium leads to accumulation of salts and paves way to efflorescence and damage to crystallization. The survival of bacteria within the stone material also influences the extent of calcification. As the laboratory grade nutrient media limit the economical usage of this technology for commercial scales, there is great need to look for alternative economical and cheap medium ingredients as corn steep liquor and lactose mother liquor (Achal et al., 2009, 2010). Large scale production of bacterial cultures is also a hindrance in the path of success of this technology over traditional treatments. The above mentioned concerns limit the use of MICCP for practical applications in various fields in comparison to the traditional methods.

CONCLUSION

Microbially induced calcium carbonate precipitation technology has been found to be highly promising with potential to successfully remediate and protect several stone structures. The eco-friendly, self-healing and highly durable nature of these bio-binders encourage their biotechnological applications for several purposes. Carbonate formation by this technology has been found to be very easy and convenient. The potential of these bio-binders has brought a new revolution in field of civil engineering but still there has been much to explore in order to bring this environmentally safe, cost effective and convenient technology from lab to field scales. There is need to assess the long term efficacy of microbial carbonates and compared to chemical binders. As the success of this technology needs experts from varying sectors from Microbiologists to Geologists to Civil Engineers, researchers from all around the globe should work together to make this multi-disciplinary research move toward commercial scale applications at a higher pace.

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