Transactions on Science and Technology Vol. **5**, No. 4, 245 - 256, 2018

Microbially Induced Carbonate Precipitation Via Ureolysis Process: A Mini-Review

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ABSTRACT Microbially induced carbonate precipitation is a relatively new technology that uses biocementation treatment method for the improvement of soils. This process which relies on microbial and chemical reactions to produce biominerals, has drawn the interest of scientists, engineers and entrepreneurs. MICP can be employed for numerous biotechnological and engineering applications. Biocementation is often used as an alternative to conventional chemical treatment techniques (i.e. lime, asphalt, sodium silicate, and cement) for soil enhancement or embarkment. This eco-friendly and energy saving method binds soil particles together at ambient temperature through biominerals (such a calcium carbonate), thereby leading to enhanced strength and stiffness soils. In this review, the fundamentals of MICP, its metabolic processes and its applications are discussed. The challenges facing this technology and recently reported attempts to solve the problems are also discussed.

KEYWORDS: Biocementation; *Sporosarcina pasteurii*; Calcium carbonate; Soil treatment; Urease

I Received 1 May 2018 **II Revised** 4 December 2018 **II Accepted** 7 December 2018 **II Online** 26 December 2018 **I**

INTRODUCTION

Enzyme technology is a well-established branch of biotechnology undergoing a developmental phase and their functional significance suggests novel applications for numerous purposes (Binod *et al.*, 2013). Microbial enzymes are industrially relevant, relatively more stable with more diverse properties than enzymes derived from plants and animals (Alves *et al.*, 2014). In recent decades, urease enzyme has become vehemently useful in geotechnical engineering discipline via a technology termed 'microbially induced carbonate precipitation (MICP)' to produce a cementing mineral used for soil stabilization. This cross-disciplinary technology involving scientist and engineers have evolved a new discipline called 'Construction Biotechnology' which is rapidly experiencing an exponential development (Stabnikov *et al.*, 2015). The prospect of using non-pathogenic microbes for bio-geotechnical engineering applications was first reported to show a novel permeability reduction process with the use of urease enzyme from *Sporosarcina pasteurii*, which hydrolyzes urea to produce calcium carbonate ($CaCO_3$) precipitates as a mineral plugging and cementing agent (Ferris *et al.*, 1997). This idea inspired numerous studies on the utilisation of MICP technology for soil improvement that does not harm the environment (Kim & Youn, 2016).

BIOMINERALISATION

Biomineralisation is the reformation of chemicals in a microenvironment caused by the activity of microorganisms which result in the precipitation of minerals (Stocks-Fischer *et al.*, 1999). In nature, biomineralisation results in the formation of sixty (or more) various biological minerals which exist as extracellular or intracellular inorganic crystals. Biominerals are distinguished based on their properties such as size, shape, crystalline nature and elemental composition (Sarayu *et al.*, 2014). Some of these minerals are formed through biologically induced mineralisation via passive surface-mediation which includes iron, manganese, carbonates, phosphonates and silicates.

MICROBIAL INDUCED CARBONATE PRECIPITATION

MICP has been exploited in recent decades as an alternative building material to Portland cement through either direct substitution or complementary usage (DeJong *et al.*, 2013, Kavazanjian & Hamdan, 2015). MICP application requires lesser energy for production, low production cost and less contribution to the greenhouse gas emission (Achal, 2015). MICP process refers to carbonate precipitation from a supersaturated solution in a microenvironment that occurs due to microbial and biochemical activities (Anbu *et al.*, 2016). During MICP process, these microbes produce metabolic products (CO_3^{2-}) that react with ions (Ca^{2+}) in the microenvironment which results in the precipitation of $CaCO_3$ mineral. Biocementation is an alternative ground improvement technique which makes use of MICP process to improve the geotechnical properties of soil in a way similar to ordinary cement (Chu *et al.*, 2009). Studies have also shown that MICP process is able to significantly improve soil's shear strength and reduce permeability by filling the pores of the soil with minerals precipitated (Soon, 2013). Calcite, aragonite and vaterite are the three polymorphs of $CaCO_3$while calcite is the most stable and preferred form of $CaCO_3$ for soil biocalcification.

Cement is a major construction material of choice for building structure and ground improvement applications in order to meet the increasing demand of rapid industrialisation and urbanisation (Siddique *et al.*, 2016). However, the use of cement is associated with certain challenges such as energy, resource conservation, the cost of production and greenhouse gas emission (Kavazanjian & Hamdan, 2015). It is estimated that production of cement clinker solely contributes about 7% global CO_2 emission, making it an unsustainable construction material (Jonkers *et al.*, 2010). The CO_2 emission generated due to cement production is expected to reach up to 260% by 2050 (Cuzman *et al.*, 2015). Green building construction has been developed as a strategy to reduce energy consumption and the overall impact of the built environment on our natural environment (Ohueri *et al.*, 2018). Hence, MICP can serve as a suitable technology to replace the use of conventional cement in construction industry. Exploratory research involving MICP has gained an increased interest in the last 20 years, with the primary focus of research in biotechnology, applied microbiology, geotechnical and civil engineering, due to the numerous applications of MICP (Dhami *et al.*, 2014). Urease activity and the amount of $CaCO_3$ precipitated during MICP process are based on various environmental factors, including pH, temperature, bacterial size and cell concentration (Soon, 2013). Other known conditions which affect the efficiency of MICP process includes the concentration of reactants, the presence of dissolved inorganic carbon, essential nutrients and the availability of nucleation site.

Metabolic pathways of MICP

$CaCO_3$ precipitation is very slow under normal conditions which require a long geological time, however, MICP can produce a large amount of carbonate mineral in shorter duration (Dhami *et al.*, 2013). This precipitation is a rather straightforward chemical process often governed by four main key factors calcium concentration, the concentration of dissolved inorganic carbon, pH and availability of nucleation sites (Hammes & Verstraete, 2002). Various microbial species are capable of inducing calcite precipitates in alkaline environments rich in Ca^{2+} ions and other mechanisms in natural habitats (Ehrlich, 1996, Rivadeneyra *et al.*, 2004). There are six metabolic pathways microorganisms make use for precipitation of induce $CaCO_3$ (Figure 1), namely: (i) photosynthesis (Rodríguez-Martínez *et al.*, 2012), (ii) ammonification (González-Muñoz *et al.*, 2010), (iii) denitrification (Erşan *et al.*, 2015), (iv) sulphate reduction (Plee *et al.*, 2010), (v) methane oxidation (Reeburgh, 2007) and (vi) ureolysis (Wei *et al.*, 2015). $CaCO_3$ precipitation by bacteria through ureolysis is the most straightforward and easily controlled mechanism of MICP with the ability to induce a high amount of $CaCO_3$ in a short duration of time (Sarayu *et al.*, 2014). The use of ureolysis pathway has made MICP technology highly useful due to the its easy understanding of the pathway and cultivation of microbes necessary to aid in producing urease for sufficient $CaCO_3$ precipitates.

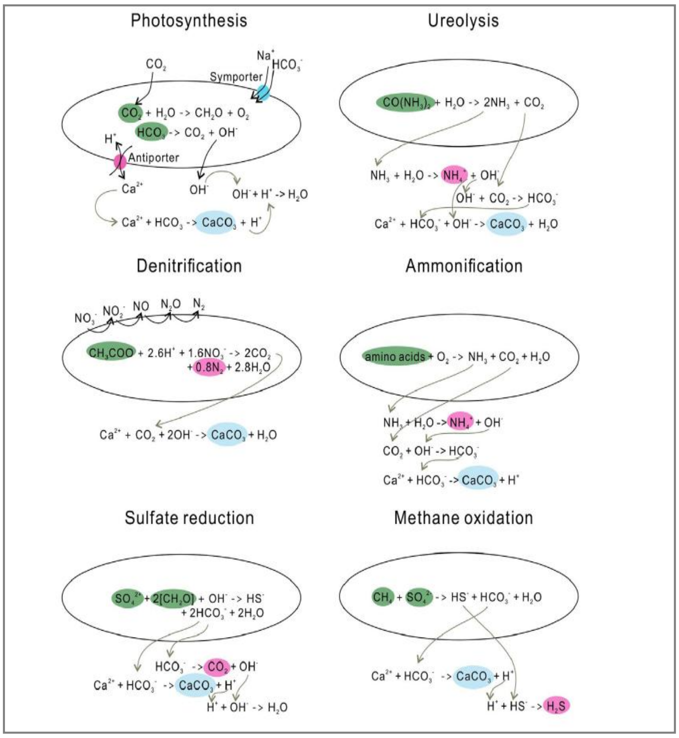

Figure 1. Various metabolic pathways of MICP which leads to precipitation of $CaCO_3$ (Zhu & Dittrich, 2016). These pathways utilize microbes for biomineralization process to occur.

Urease pathway and sources

Microbial urease catalyses the hydrolysis of urea to produce ammonium and carbonate ions, which react with calcium ions to form $CaCO_3$ mineral (Hammes & Verstraete, 2002). In a study on precipitation of $CaCO_3$ via ureolysis, De Muynck *et al.*, (2010) suggested that the bacteria breakdowns the urea releases ammonium and dissolved inorganic acids. The attraction of calcium ions towards the bacterial cells leads to a localised occurrence of super-saturation, resulting in the precipitation of $CaCO_3$ on the bacterial cell wall. Urease activity which is the urea hydrolysis activity produced by the enzyme urease per minute was determined by Whiffin (2004) for urease assay. Determination of urease activity and specific urease activity using conductivity method were elucidated in reports by Omoregie *et al.*, (2017) and Zhao *et al.*, (2014). Urease is produced by many diverse bacterial species which includes normal flora and non-pathogens (Mobley, 2001). *Sporosarcina pasteurii* has been reported in various studies to have high urease activity, hence it serves as the preferred choice of MICP agent. They are often chosen as a bio-agent of biocement applications because of their non-pathogenic nature, able to produce high amount of urease enzyme, can survive in high alkaline (above pH 8.5) environments and high calcium ions concentrations (Stabnikov & Ivanov, 2016).

Urease enzyme was previously studied from clinical evaluation on patients infected with pathogenic microorganisms (Cheng & Cord-Ruwisch, 2013). However, the usage of urease on biocementation application for improvement of soil strengthening has been the subject of various research from the Microbial biotechnology, geotechnical engineering and civil engineering (Whiffin, 2004). These have led to an increase in screening for non-pathogenic ureolytic bacteria from local environments. Majority of urease producing bacteria which reported in the literature with calcite precipitating potentials were mostly from soils and sludge samples. In a review by Sarayu, et al. (2014), a list of bacteria that have been reported to induce $CaCO_3$ precipitates such as *Bacillus cereus*, *Bacillus sphaericus* and *Bacillus subtilis* was tabularized. However, very limited numbers of these microbes are non-pathogenic. It is important to screen for non-pathogenic microbes from non-exploited regions for possible novelty strains. The diversity of bacteria from speleothems samples in Colombia and their ability to precipitate carbonates were studied using conventional microbiological methods and molecular tools, such as temporal temperature gradient electrophoresis (Garcia *et al.*, 2016). Figure 2 elucidates ureolytic bacterial isolates with different distinctive morphologies were recovered from limestone cave samples in Sarawak, Malaysia. In a recent study by Mkwata (2018), the isolation of lytic bacteriophages from the aforementioned caves and evaluated prospective potential application as biocontrol agents of *Pseudomonas aeruginosa* was reported. Recent studies have started exploring the use of locally isolated fungi strains capable of precipitating $CaCO_3$ for biocementation of soils (Fang *et al.*, 2018, Dhami *et al.*, 2017). More studies should focus on

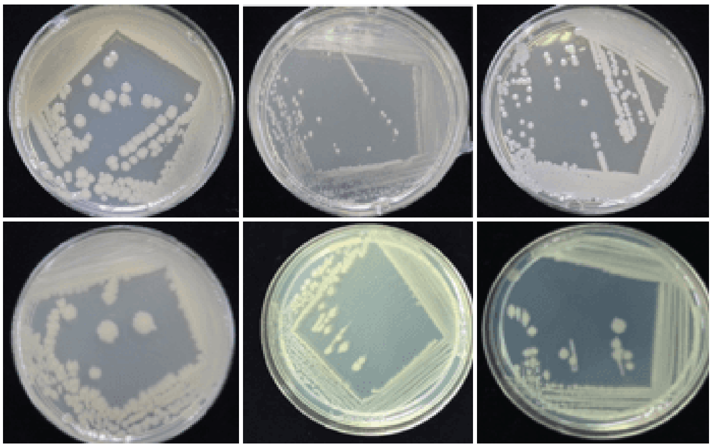

Figure 2. Sub-cultured colonies of various ureolytic bacterial species isolated from limestone caves samples of Sarawak.

CURRENT APPLICATIONS

Biocement production and creation of biological mortars

Biocement or biosandstone was proposed as a novel method for cementing loose sands to produce structural materials, consisting of Alkaliphilic urease producing bacteria, a substrate solution (urea), a calcium source and sand (Achal, 2015). However, a typical set-up for sand consolidation experiment to develop biocementation was simplified by Reddy *et al.*, (2012), where sand is either mixed with bacterial culture or later injected directly into the sand columns. The sand was plugged through a plastic column, and the cementation fluid which consisted of nutrient media, urea, and calcium ions were then injected at a specific rate in the column using gravimetric free flow direction. Another study on calcite deposition in sand columns using *Sporosarcina pasteurii* by Achal *et al.*, (2009) found that 40% of calcite deposited in the sandstone resulted and led to a reduction of porosity and permeability in the sandstone. Biocement treatment involving premix with bacterial culture or no premix leads to better solidified sand samples Omoregie *et al.* (2018a). The use of 1 M urea and calcium chloride alongside overnight grown ureolytic bacterial culture aid evenly distributed $CaCO_3$ precipitates when treatment is slowly added into the sand samples. The knowledge obtained with MICP treatments resulted in the development of biological mortar for remediation of small cavities on limestone surfaces (De Muynck *et al.*, 2010). The purpose of using initiating biological mortars was to avoid some of the problems related to chemical and physical incompatibilities of commonly used mortars with the underlying materials, specifically in the case of brittle materials (Castanier *et al.*, 1999). The resistance of mortar specimens and surface deposition to degradation process can be improved via microbial calcite precipitation (Al-Thawadi, 2008).

Bioremediation of cracks in concrete and biodeposition

In concrete, cracking is common due to relatively low tensile strength (De-Belie & De-Muynck, 2008). Several mechanisms such as shrinkage, freeze-thaw reactions, mechanical compressive and tensile forces lead to the formation of cracks (Alhour, 2013). Cracking on concrete surfaces also results in enhanced deterioration of embedded steel through easy ingress of moisture and ions that react with reinforcements in concrete and expansive stressed which leadings to spalling (Achal *et al.*, 2013, Gavimath *et al.*, 2012). Thus, it is practical to use adhesive for sealing of concrete cracks so that the strength and durability of the concrete will be improved (Wong, 2015). A conventional approach used in repairing cracks involves injecting epoxy resin or cement grout into the concrete. However, they result in various thermal expansions, environmental and health hazards (De-Belie & De-Muynck, 2008). Investigation on the potential of using bacteria to act as self-healing agent in concrete to fix a crack, specifically with the use of alkali-resistant spore-forming bacteria, *Bacillus pseudofirmus* (type strain DSM 8715) and *Bacillus cohnii* (type strain DSM 6307) (Jonkers, 2007; Jonkers & Schlangen, 2007; Jonkers *et al.*, 2010). Their findings showed that bacterial cement stone specimens appeared to produce a solid result of crack-plugging.

A recent *in situ* application on cracked was carried out by Jonkers *et al.*, (2016). Their finding showed that concrete repair using MICP is inexpensive, improved the durability of the material and also lowered the environmental impact of civil engineering activities. The emergence of microbial involvement in carbonate precipitation has led to the exploration of this process in a variety of fields, including environmental, civil and geotechnical engineering (De Muynck, *et al.*, 2010). Among these applications, MICP has been used for biogenic-carbonate-based surface treatments, a process known as biodeposition (Achal *et al.*, 2010). Biodeposition of bacterial calcite is a viable method of surface treatment for cement-based materials that can be explored in a sustainable approach (Wong, 2015). Considering the size of bacterial cells are around 1 μm, both the cells and their media containing the reactants (urea and calcium ions) can permeate deep into the pores and interface between aggregates or paste of the concrete structure (Ramachandran *et al.*, 2001). Hence, this enables microbial cementation to take place within and on the surface of such materials which then provides reinforcement and protection (Wong, 2015).

Biogrout

Nemati & Voordouw (2003) described the use of urease to cement porous medium. Their study showed that reducing the permeability of porous medium by enzymatic $CaCO_3$ precipitation using *Canvalia ensiformis* was successful. They used between 0.1 and 1.0 M (>33 $g.L^{-1}$) of cementation reagents with high urease activity for a successful plugging of the sand core. Unfortunately, the strength build-up was not monitored. Stocks-Fischer *et al.*, (1999) reported that injection of bacteria and reagents together at low flow rates can result in full clogging of the system near the injection point. An investigation on Biogrout ground improvement using MICP was also performed by van Paassen (2009). This study was successful in developing an unprecedented 100 m^3 field scale experiment and 40 m^3 of the sand were treated using MICP process within a duration of 12 days Although in both scale up experiments significant increase of the average strength was obtained, different variable mechanical properties were observed in the sand. It could be affected by induced flow field, bacteria distribution, the supply of reagents and crystallization process (van Paassen, 2009). Another study by Suer *et al.* (2009) investigated the potential of using biogrouting as an alternative approach to jet grouting to seal the contact between sheet pilling and bedrock. Their finding showed that biogrouting process was cheaper than jet grouting and had much lower environmental impact. Biogrouting also consumed less water and produced less landfilled waste.

Other essential applications

MICP is also used for the removal of calcium ions (Ca^{2+}), removal of polychlorinated biphenyls (PBs) and Industrial by-products. Calcium-rich wastewater is a problem some industries face due to calcification during downstream processing (Hammes *et al.*, 2003). High concentration of calcium ions ranging from 500-1500 $mg.L^{-1}$ in the wastewater can cause substantial scaling in pipelines and reactors as a result of calcium formation as carbonate, phosphate, and gypsum (Al-Thawadi, 2008). A novel application for the process of MICP as an alternative mechanism for the potential removal of Ca^{2+} from industrial wastewater instead of chemical precipitation approach has been developed (Hammes *et al.*, 2001). Calcium removal more than 90% was achieved throughout the experimental period while the effluent pH remained at a reasonable level (Hammes *et al.*, 2003).

Polychlorinated biphenyls (PCBs) is a recalcitrant contaminant which surfaces on concrete when PCBs containing oils leaks from the equipment. (Phillips *et al.*, 2013). The last two decades have seen an increase in the use of bioremediation for the removal of contaminants, which includes PCBs (Dhami *et al.*, 2013). Microbial process using MICP process has been initiated as an alternative measure to remove PCBs. The use of *Sporosarcina pasteurii* for the treatment of PCB-coated cement cylinders leading to surficial encapsulation of PCB-containing oils have been promising (Okwadha & Li, 2011).

Construction materials such as concrete, brick and pavement blocks are all produced from natural existing resources Their production has affected our environment due to continuous exploration limitation of natural resources. It has led researchers to explore other means of building materials which are environmentally friendly, affordable and sustainable (Aubert *et al.*, 2006). There are different types of waste such as slag, fly ash, wheat straw, saw milk waste, cotton stalk, mining waste tailing and waste gypsum which are currently being recycled for potential utilisation (Pappu *et al.*, 2007). The production of fly ashes during combustion of coal for energy is one of the industrial by-products recognised as an environmental pollutant (Dhami *et al.*, 2013).

MICP LIMITATIONS AND RECENT IMPROVEMENTS

Major issues affecting MICP is bioclogging which occurs during treatment and prevents uneven distribution of $CaCO_3$ within the treated samples. Majority of biocement studies in laboratory-scale trials noted the intrinsic obstacles of an uneven uniformity or distribution of carbonate precipitation in tested sand columns and also bioclogging formation at regions around the injection points (Cheng *et al.*, 2013, Dhami *et al.*, 2016, Rowshanbakht *et al.*, 2016). This affect shear strength and stiffness of the treated samples. Uneven distribution of MICP treatment remains an issue due to the transportation and retention of bacterial culture and the cementation reagents which ends up closing the treatment injection points, thereby limiting the amount of solution that passes through into the soil samples (El Mountassir *et al.*, 2018).

Previosouly, van Paassen *et al.*, (2010) and Michael *et al.*, (2016) performed biocement treatment test using injection method in large-scale experiment of soil volume of 100 m^3 and 1.7 m^3, respectively. Their findings also reported the uneven distribution of $CaCO_3$ precipitation along the bio-treated sand volume. A different treatment method called surface percolation was used by

Cheng & Cord-Ruwisch (2014) and Mahawish *et al.*, (2016) to improve even distribution of $CaCO_3$ content. Their findings also showed that formations of bioclogs on the external surfaces of the treatment soil columns. The formation of these bioclogs at regions around the injection points prevents the movement of the cementation solution or bacterial culture during the treatment process. In another study by Cheng & Shahin (2016) in an attempt to resolve the bioclogging and homogeneity problem in treated soil samples, they reported the use of a new urease active material called "bioslurry". Their finding showed an improved uniformity of $CaCO_3$ content distributed along the pipes (330 mm), but highlighted that at large scale (metres), uniformity of $CaCO_3$ contents is still challenging. Recently, a report on effect of temperature on MICP by Sun *et al.*, (2018) showed how the addition of urea concentration (20 g/L) during inoculation of *Bacillus megaterium* and *Sporosarcina pasteurii* can aid in precipitating more $CaCO_3$. This biocement treatment can be useful in resolving the issue of bioclogging and also low $CaCO_3$ contents at low temperature. Cheng *et al.*, (2018) also recently reported the use of a one-phase low-pH injection method of biocementation to replace the existing multiple injection for soil solidification. It was shown that the use of a one-phase low-pH injection method prevent the bioclogging problem and also reduces the ammonia gas by 90% which has often been an issue during MICP treatment, hence making this technology environmentally friendly.

Another obstacle inhibiting the possibility of large-scale or field-scale work is mass production of bacterial culture. The ingredients which are necessary for bacterial growth medium often ranges between 10 to 60% of the total production cost for biocement treatment process (Whiffin, 2004). This has affected or delayed the progress of commercialising MICP technology because the cost of MICP process is very expensive due to bacterial growth media. However, recent studies from Yoosathaporn *et al.* (2016) and Cuzman *et al.* (2015) reported the potential use of diary and brewery waste to cultivate ureolytic bacteria with the prospect for biocement application. Their findings suggested that the use of chicken manure and lactose mother liquor were a good source of nutrients for a low-cost cultivation. There is need for more studies on alternative growth media for ureolytic bacteria in comparison with standard analytical-grade media to reduce bacterial production cost especially at a scale-up volume. Omoregie *et al.*, (2018b), recently reported the use of the use of inexpensive food-grade yeast extract media (15 g/L) to cultivate *Sporosarcina pasteurii* strain for biocalcification, which is typically used cooking and bakery purpose. Gowthaman *et al.*, (2018) also reported the use of Beer yeast media to grow *Psychrobacillus soli*, which is primarily used in food industry to break down sugars. Roslan *et al.*, (2018) showed that vegetable waste could serve as substrate for growth of microorganisms capable of precipitating calcite, thus be used for biocementation of sandy soils. These recent studies which explore the cultivation of ureolytic bacteria from cost reduction perspective, have shown that these media could potential be used for large-scale cultivation of ureolytic bacteria suitable for field-scale MICP application.

CONCLUSION

MICP is a process that has emerged as an attractive alternative ground improvement technique in Geotechnical engineering using ureolytic bacteria for soil strengthening and stabilisation. Various microbes are capable of precipitating $CaCO_3$, essential for binding soil particles together, however, *Sporosarcina pasteurii* is often preferred as a bioagent to produce urease. The idea of utilizing microbes for geotechnical engineering applications has revolutionized the function of urease enzyme previously relevant from clinical studies to now the improvement of soils. Despite the advantages and prospects of MICP, more future studies need must be performed to nullify the problems affecting the homogeneity of $CaCO_3$ contents after biocement treatments and minimise drawback affecting large-scale or commercial applications.

ACKNOWLEDGEMENT

This research was supported by by Bachy Soletanche (France) and the School of Research Office through Swinburne Sarawak Research Grants (SSRG 2-5162 and SSRG 2-5535).

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