

**Title:** Microbially Induced Calcium Carbonate Precipitation: a Widespread Phenomenon in the Biological World

**Name of authors:**

Mostafa Seifan<sup>a</sup> and Aydin Berenjian<sup>a\*</sup>

*Affiliation of authors:*

<sup>a</sup> *School of Engineering, Faculty of Science and Engineering, The University of Waikato, Hamilton, New Zealand*

**Corresponding author:**

Dr. Aydin Berenjian

School of Engineering, Faculty of Science and Engineering, The University of Waikato, Hamilton, New Zealand

T: +64 7 858 5119

Email: aydin.berenjian@waikato.ac.nz

**Abstract**

Bio-deposition of minerals is a widespread phenomenon in the biological world and is mediated by bacteria, fungi, protists, and plants. Calcium carbonate is one of those minerals that naturally precipitates as a by-product of microbial metabolic activities. Over recent years, microbially induced calcium carbonate precipitation (MICP) has been proposed as a potent solution to address many environmental and engineering issues. However, for being a viable alternative to conventional techniques as well as being financially and industrially competitive, various challenges need to be overcome. In this review, the detailed metabolic pathways, including ammonification of amino acids, dissimilatory reduction of nitrate, and urea degradation (ureolysis), along with the potent bacteria and the favorable conditions for precipitation of calcium carbonate, are explained. Moreover, this review highlights the potential environmental and engineering applications of MICP, including restoration of stones and concrete, improvement of soil properties, sand consolidation, bioremediation of contaminants, and carbon dioxide sequestration. The key research and development questions necessary for near future large-scale applications of this innovative technology are also discussed.

**Keywords:** Calcium carbonate; Bacteria; MICP; Concrete; Bioremediation; Soil

## Introduction

Biom mineralization refers to a process by which living organisms carry out reactions that promote mineral precipitation. The bio-deposition of minerals is a widespread phenomenon in the biological world and is mediated by bacteria, fungi, protists, and plants. Biominerals can be found everywhere, from shells, bone, and teeth to limestone caves, and they offer great solutions for many engineering and environmental issues.

Bioprecipitation of minerals by prokaryotes can be achieved through two fundamentally different pathways, namely biologically controlled mineralization (BCM) and biologically induced mineralization (BIM). The degree of control on the biomineralization process is the main difference between these two processes. In a BCM pathway, the organism greatly controls the biomineralization process and is responsible for nucleation and growth of the mineral particles. This process is a highly regulated mechanism which produces more uniform particles with consistent mineral morphologies (Mann 2001) and the mineral precipitates are deposited on or within the organic matrices or vesicles inside the cell (Bazylinski and Frankel 2003; Bazylinski and Moskowitzi 1997; Berenjian et al. 2013). Well-defined mineral structures, such as bones, teeth, shells, and fish otoliths, are formed through the BCM process.

On the other hand, BIM occurs in an open environment as an uncontrolled consequence of microbial metabolic activity, and its effectiveness highly depends on the concentration of dissolved inorganic carbon, nucleation site, pH, temperature and Hartree energy (Eh) (Barton and Northup 2011; Hammes and Verstraete 2002). Carbonate is one of those minerals that can be induced through BIM, and it is widely precipitated in nature. Microorganisms that induce precipitation of calcium carbonate are able to alter the chemistry of microenvironments. The diffusion of metabolic products, such as bicarbonate generated by sulfate-reducing bacteria (SRB), or ions like  $\text{NH}_4^+$  generated by metabolizing nitrogenated organic substances (Douglas and Beveridge 1998) into the environment, can contribute to the formation of biominerals. In the production of complex molecules such as calcium carbonate via living microorganisms, biominerals are formed through the reaction of metabolites produced by a microorganism ( $\text{CO}_3^{2-}$ ) and their surrounding environment enriched in  $\text{Ca}^{2+}$ . Bacterial surface, such as cell walls and polymeric materials discharged by bacteria, provide favorable sites for adsorption of ions and consequently mineral nucleation and crystal growth (Frankel and Bazylinski 2003). Broad particle size distribution, as well as poorly crystalline or even amorphous calcium carbonate (ACC) formation, are the characteristics of the minerals induced in the BIM process. Unlike the narrow size distribution of generated crystals in BCM, the precipitated minerals in BIM have a wide size distribution (Frankel and Bazylinski 2003). Goodwin et al. (Goodwin et al. 2010) reported that ACC is usually found in a monohydrate state ( $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ ) but can also be synthesized in dihydrate form, and its structure consists of a porous calcium-rich framework with interconnected channels containing water and carbonate ions. ACC is the least thermodynamically stable form of calcium carbonate and relatively soluble compared to crystalline polymorphs. When suspended in an aqueous solution at ambient temperature, these amorphous polymorphs usually transform to another stable form of calcium carbonate such as calcite, vaterite and aragonite (Rodriguez-Blanco et al. 2011). Therefore, this characteristic may limit its functionality for those applications that need a stable form of calcium carbonate.

During the last decades, the phenomenon called microbially induced calcium carbonate precipitation or MICP has received considerable attention. More recently, MICP has been proposed as a potential tool to address many engineering and environmental issues due to its advantages such as being relatively inexpensive and eco-friendly.

This paper aims to elucidate the biological routes for precipitation of calcium carbonate and to critically review the potential applications of MICP technology.

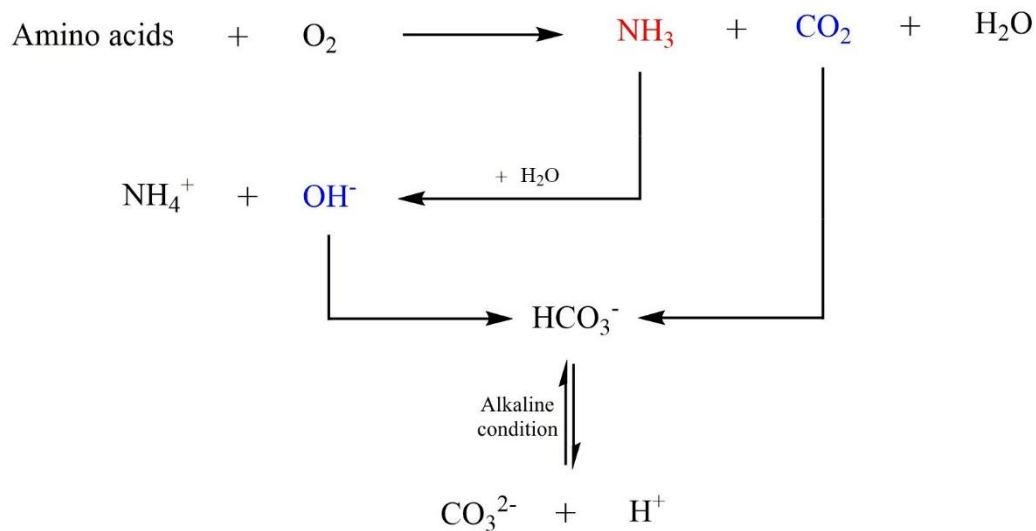
### **MICP pathways, kinetics, and potential microorganisms**

Among all the recognized biominerals, MICP has drawn scientists' attention due to its potential for a variety of applications. In this process, calcium carbonate crystals form through the reaction of metabolites generated by a microorganism ( $\text{CO}_3^{2-}$ ) and their surrounding environment enriched in  $\text{Ca}^{2+}$ . Four key factors, including the concentration of  $\text{Ca}^{2+}$  and dissolved inorganic carbon (DIC), medium pH, and the availability of nucleation sites, have been reported by Hammes and Verstraete (Hammes and Verstraete 2002) as the main influencing parameters on calcium carbonate precipitation.

A wide range of species, including heterotrophic and autotrophic microorganisms, are able to precipitate calcium carbonate crystals in various environments such as soils, oceans, caves and saline/soda lakes (Sarayu et al. 2014). The bioprecipitation of calcium carbonate is classified into two main categories: autotrophic and heterotrophic pathways. Seifan et al. (Seifan et al. 2016a) extensively reported the mechanisms involve in the autotrophic biosynthesis of calcium carbonate through methanogenesis, oxygenic photosynthesis and anoxygenic photosynthesis pathways. Different metabolic pathways have been described for heterotrophic precipitation of calcium carbonate which mainly happen through sulphur and nitrogen cycle. The sulfur cycle is a combination of reactions wherein SRB are responsible for dissimilatory reduction of sulfate as a terminal electron acceptor (Joshi et al. 2017). It has been reported that the largest share of global calcification takes place via biotic processes in the oceans, and the precipitation of calcium carbonate in the absence of microorganisms, particularly cyanobacteria and SRB, is rare due to various kinetic barriers (Olajire 2013). These genera of bacteria can facilitate many biochemical processes, like MICP, in lithifying microbial communities. To accomplish this process, the medium must be rich in organic matter, calcium and sulfate in an anoxic environment. As shown in Eq. 1, this process starts with the abiotic dissolution of gypsum (Hammes and Verstraete 2002). Under this condition, the organic matter is consumed by SRB, and sulfate is removed and subsequently sulfide and metabolic  $\text{CO}_2$  are released into surrounding (Wright 1999). Then calcium carbonate is precipitated as a result of pH increase due to proton consumption. The potent microbial communities, along with metabolic pathways to induce calcium carbonate precipitation, are tabulated in Table 1. The availability of exopolymeric substances (EPS) as a favorable nucleation site has shown to be a significant factor on precipitation of calcium carbonate (Braissant et al. 2007; Zhu and Dittrich 2016). EPS provides a template for adsorption of metal cations to which carbonate ions are attracted to induce local mineral supersaturation (Tourney and Ngwenya 2014). The following reactions represent the overall process of sulfate reduction that mediate calcium carbonate precipitation. Where  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  and  $\text{CH}_2\text{O}$  represent gypsum and an organic carbon source, respectively.



The biosynthesis of calcium carbonate in the nitrogen cycle are achieved through different pathways, namely (i) ammonification of amino acids, (ii) dissimilatory reduction of nitrate (denitrification) and (iii) ureolysis (urea degradation) (Seifan et al. 2016a). Some Gram-negative aerobic microbial strains are capable of using amino acids as their sole source of energy to initiate the biomineralization of calcium carbonate. *Myxococcus* was reported as a potent bacterium for biosynthesis of different minerals including carbonates, phosphates, sulfates, chlorides, oxalates, and silicates (González-Muñoz et al. 2010). Chekroun et al. (Chekroun et al. 2004) showed that *Myxococcus xanthus* is able to induce calcium carbonate precipitation in a medium containing calcium acetate. Authors reported that *Myxococcus xanthus* plays an active role in the biosynthesis of calcium carbonate by modifying the physical chemistry of their microenvironment through active alkalisation. As shown in Fig. 1, ammonia and carbon dioxide are produced by oxidative deamination of amino acids.



**Fig. 1** Schematic representation of the reaction in ammonification of amino acid pathway.

According to the following reaction, the production of ammonia creates an alkaline microenvironment around the cell which is in favor of calcium carbonate precipitation.



Carbon dioxide is another by-product which generates during oxidative deamination of amino acids, and it tends to dissolve and transform into either  $\text{HCO}_3^-$  or  $\text{CO}_3^{2-}$  at elevated pH (Rodriguez-Navarro et al. 2003).

Denitrification pathway is another subclass of the nitrogen cycle, and it mainly occurs where nitrate and organic carbon are available. This metabolic pathway is achieved when nitrate is being used as an electron acceptor by denitrifier bacteria, such as *Bacillus*, *Alcaligenes*, *Denitro bacillus*, *Thiobacillus*, *Spirillum*, *Micrococcus*, *Pseudomonas denitrificans*, *Castellaniella denitrificans* and *Achromobacter*, for oxidizing organic compounds to provide energy and support microbial growth (Karatas 2008; Martin et al. 2013; Van Paassen et al. 2010a; Zhu and Dittrich 2016). As shown in Fig. 2, elevated medium pH is attained by consuming  $\text{H}^+$  to facilitate the biosynthesis of calcium carbonate.  $\text{N}_2$  and  $\text{CO}_2$  are the by-products of denitrification and this process is expected to predominantly happen under  $\text{O}_2$  limited conditions (Erşan et al. 2015a). Although the lack of calcium carbonate precipitation in aerobic conditions is the main drawback of denitrification, it can be widely used to address many



Urea is a source of nitrogen for a variety of microorganisms, and therefore its availability in the medium also contributes to cell growth and further urease production. However, an ideal microbial strain for the MICP process must be able to tolerate high concentrations of urea (Whiffin 2004). It has been reported that a high concentration of urea has an inhibitory effect on the growth of bacteria, and, consequently, the biosynthesis of calcium carbonate will be negatively affected. Xu et al. (Xu et al. 2017) investigated the urea resistance capacity of strain GM-1 isolated from active sludge. It was noted that the bacterial growth was increased from 0.7 to 0.8 (optical density) when the concentration of urea increased from 20 to 40 g/L and the maximum optical density of 0.9 was obtained for a medium supplemented with 60 g/L of urea. However, a further increase in the concentration of urea (80 g/L) substantially decreased the bacterial growth. Bacterial urease response to ammonium is another important factor to be taken into account. According to this, the urease-producing bacteria are divided into two main categories: (i) those whose urease activity is not repressed and (ii) those whose urease activity is repressed (Whiffin 2004). Therefore, selection of those bacteria whose urease activity is not repressed by ammonium can substantially increase the effectiveness of the MICP process. Ureolysis pathway is distinguished by its high calcium carbonate yield compared to the other metabolic pathways. This phenomenon is mainly due to the capability of bacteria to generate a high amount of urease enzyme in a short time as well as the structure of ureases produced by bacteria which consist of two or three polypeptides (Krajewska 2018; Mobley et al. 1995). However, the urease production yield varies from species to species. Among all ureolytic bacteria, *B. sphaericus* and *S. pasteurii* have shown a high urease activity (Parks 2009; Phillips et al. 2013) and suitability for inducing a high amount of calcium carbonate mineral. Both microorganisms can tolerate relatively high pH and they are Gram-positive endospore former and non-pathogenic bacteria. Although *S. pasteurii* has been used as a model organism in numerous studies for MICP, its long-term viability under anaerobic conditions has been questioned (Martin et al. 2012).

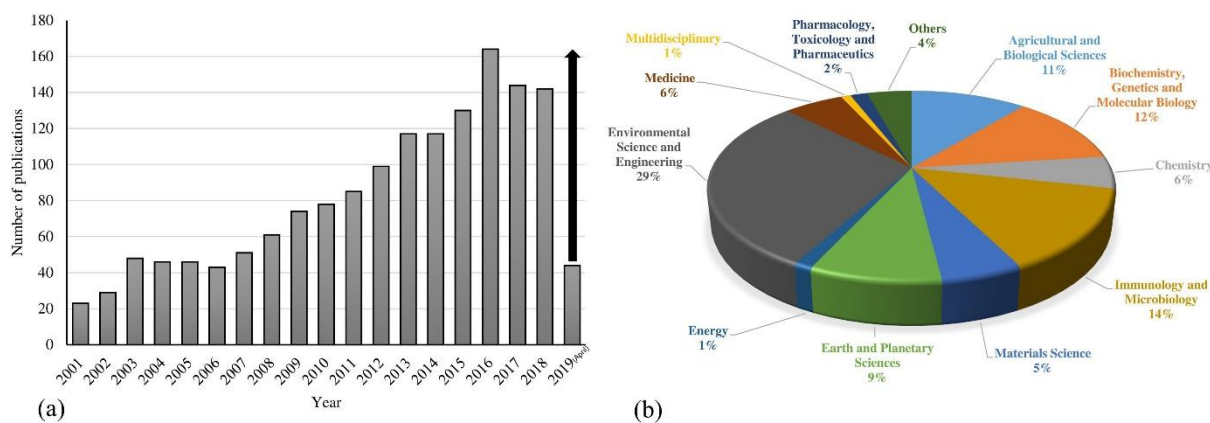
Table 1 Overview of different microorganisms and metabolic pathways to induce calcium carbonate precipitation.

Metabolic pathway	Microorganism	Reference
Sulfate reduction	<i>Desulfovibrio</i> sp.	(Braissant et al. 2007)
Photosynthesis	<i>Spirulina platensis</i>	(Kumar et al. 2011; Ramanan et al. 2010)
	<i>Chlorella vulgaris</i>	(Ramanan et al. 2010; Wang et al. 2010)
	<i>Synechococcus</i>	(Zhu et al. 2015)
Ammonification	<i>Myxococcus xanthus</i>	(Ettenauer et al. 2011; Jroundi et al. 2010; Rodriguez-Navarro et al. 2003)
Denitrification	<i>Pseudomonas denitrificans</i>	(Karatas 2008)
	<i>Castellaniella denitrificans</i>	(Van Paassen et al. 2010a)
	<i>Diaphorobacter nitroreducens</i> .	(Erşan et al. 2015b)
	<i>Pseudomonas aeruginosa</i>	(Erşan et al. 2015a)
	<i>Diaphorobacter nitroreducens</i>	(Erşan et al. 2015a)
	<i>Halomonas halodenitrificans</i>	(Martin et al. 2013)
Ureolysis	<i>Kocuria flava</i>	(Achal et al. 2011d; Achal et al. 2012d)
	<i>Lysinibacillus sphaericus</i>	(Kang et al. 2014b)
	<i>Sporosarcina ginsengisoli</i>	(Achal et al. 2012a)
	<i>Bacillus cereus</i>	(Kumari et al. 2014)
	<i>Halomonas</i> sp.	(Achal et al. 2012c)

	<i>Sporosarcina pasteurii</i>	(Achal et al. 2009; Achal et al. 2011a; Bang et al. 2010; Chahal et al. 2012; DeJong et al. 2006; Gat et al. 2014; Grabiec et al. 2012; Harkes et al. 2010; Kim et al. 2013; Lauchnor et al. 2013; Okwadha and Li 2011; Okwadha and Li 2010; Ramachandran et al. 2001; Warren et al. 2001a; Whiffin et al. 2007)
	<i>Bacillus</i> sp.	(Achal et al. 2011b; Chu et al. 2012)
	<i>Bacillus lentus</i>	(Dick et al. 2006; Wei et al. 2015)
	<i>Proteus vulgaris</i>	(Fujita et al. 2000)
	<i>Bacillus licheniformis</i>	(Helmi et al. 2016)
	<i>Bacillus megaterium</i>	(Achal et al. 2011c; Dhami et al. 2013a; Kaur et al. 2013; Lian et al. 2006)
	<i>Bacillus sphaericus</i>	(Arunachalam et al. 2010; De Muynck et al. 2008a; De Muynck et al. 2008b; De Muynck et al. 2013; Dick et al. 2006; Kim et al. 2013; Seifan et al. 2018a; Seifan et al. 2018b; Seifan et al. 2018c; Seifan et al. 2018d; Seifan et al. 2016b; Seifan et al. 2017a; Seifan et al. 2017b; Seifan et al. 2017c; Van Tittelboom et al. 2010; Wang et al. 2012a; Wang et al. 2012b)
	<i>Bacillus thuringiensis</i>	(Kaur et al. 2013)
	<i>Bacillus aerius</i> U2	(Sensoy et al. 2017)
Conversion of organic acid to calcium carbonate	<i>Bacillus pseudofirmus</i>	(Jonkers et al. 2010)
	<i>Bacillus cohnii</i>	(Jonkers et al. 2010)
	<i>Bacillus pumilus</i>	(Daskalakis et al. 2015)
	<i>Bacillus alkalinitrilicus</i>	(Wiktor and Jonkers 2011)
	<i>Bacillus subtilis</i>	(Khaliq and Ehsan 2016)
	<i>Micrococcus</i> sp.	(Tiano et al. 1999)
	<i>Bacillus subtilis</i>	(Tiano et al. 1999)
	<i>Pseudomonas</i>	(Zamarreño et al. 2009)
	<i>Acinetobacter</i>	(Zamarreño et al. 2009)

### Potential applications of MICP

As shown in Fig. 4a, the statistical reviewing until April 2019 reveals a significant increase in the number of published works related to the MICP process and its applications. The majority of these studies have been published in the field of environmental science and engineering (Fig. 4b). Therefore, in this section, the potential applications of MICP for addressing environmental and engineering issues are discussed.



**Fig. 4** Increasing trend in the number of publications for bacterially induced calcium carbonate precipitation: a) year-sorted and b) subject-sorted ('bacteria' + 'calcium carbonate' were used as keywords searching in the article title, abstract and keywords).

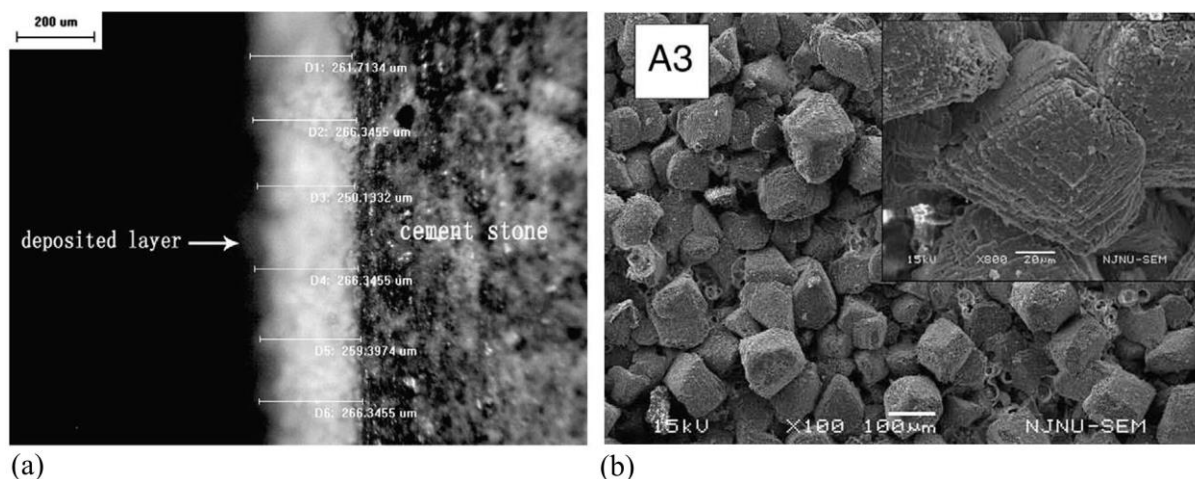
### ***MICP for constructional purposes***

Calcium carbonate is an essential component in the construction industry and its biodeposition can be used to address the shortcomings associated with the constructional materials. The bioremediation of construction materials is achieved through passive and active techniques. Passive treatments are performed manually once the defects are detected, while the active approaches are viable and the remediation process is started intrinsically. Remediation of monumental stones and surface treatment of concrete structures are amongst passive treatment techniques. Monumental stones or, in general, building stones (granites and carbonate rocks), are subjected to the weathering action due to several physicochemical and biological factors (Price and Doehne 2011; Rodriguez-Navarro and Sebastian 1996). As a consequence of this action in calcareous stones, the induction of a progressive mineral matrix dissolution leads to calcite leaching, which contributes to an increase in porosity and decrease in mechanical properties. Application of mineral products offers a sustainable solution to terminate the deterioration of monumental buildings and stones (Castanier et al. 2000). In this technique, the bacteria and nutrients are sprayed or brushed on the surface of stones and calcium carbonate minerals are precipitated as a result of microbial metabolic activity. Oriol et al. (Oriol et al. 1993) examined the formation of sacrificial layers by bacteria and its promising effect on the treatment of historic buildings. Me'tayer-Levrel et al. (Le M'etayer-Levrel et al. 1999) performed an investigation to observe the effectiveness of microbial treatment for surficial protecting coatings of Thouars church tower. The results of permeability tests show that the surficial permeability of treated facades was lower than untreated conditions. Likewise, Tiano et al. (Tiano et al. 1999) evaluated the effect of calcium carbonate bio-deposition by *Micrococcus* sp. and *B. subtilis*. Authors reported that the bioremediation resulted in a decrease in the stone porosity. In a similar study, Rodriguez-Navarro et al. (Rodriguez-Navarro et al. 2003) investigated the potential application of *Myxococcus xanthus* to protect and consolidate porous ornamental stone. They performed sonication tests to determine the attachment efficiency of newly formed calcium carbonate to the matrix. It was found that the new carbonate crystals were strongly attached to the substratum, mostly due to epitaxial growth on pre-existing calcite grains. Further examination revealed that the newly formed crystals were more stress resistant due to their organic-inorganic nature. However, the ineffectiveness for in-depth consolidation and the possibility of formation superficial biofilm are the main disadvantages of this technique (Le M'etayer-



Levrel et al. 1999). The former drawback can be addressed by introducing microbial community and nutrients inside defects and pores. The latter disadvantage is mainly due to the formation of superficial calcium carbonate crystals that has insufficient consolidation or protection effect, however it can be minimized by selecting those bacteria that produce less biofilm while inducing a large amount of minerals over the biosynthesis process.

Another potential passive application of MICP is the remediation of cracks on the surface of concrete and mortar. Concrete is one of the most broadly used construction materials worldwide which is susceptible to cracking. This results in a significant decrease in the concrete's lifespan and leads to allocation of considerable budget for repair and maintenance. In contrast to conventional crack treatment approaches, the bio-deposition of calcium carbonate can act as a barrier against the penetration of aggressive substances. The effectiveness of surface bioremediation relies on both quality and quantity of bio-deposited crystals in terms of density, thickness, cohesion and effective bond with the concrete matrix (Wang et al. 2016). De Muynck et al. (De Muynck et al. 2008a) investigated the effect of pure *B. sphaericus* and ureolytic mixed cultures on the efficiency of concrete surface treatment. The bioremediation was performed in two-steps by immersion of mortar/concrete samples in stock culture for 24 h and then followed by submersion in a nutrient solution. The results showed that the bio-deposition of calcium carbonate on the surface of the specimens resulted in a decrease in capillary water uptake and permeability towards gas. It was also found that the utilization of pure cultures resulted in a more pronounced decrease in the water uptake due to the combined effect of biomass and carbonate precipitation, and the addition of a calcium source to the medium resulted in further reduction of water absorption for the samples treated with pure cultures. To lessen the steps towards remediation, Chunxiang et al. (Chunxiang et al. 2009) used a one-step immersion method by submerging a cement-based sample in a solution of *S. pasteurii*, urea and calcium nitrate. Their results showed that water penetration resistance of the specimen surface could greatly improve when the samples were treated by deposition of calcium carbonate. Fig. 5a clearly shows the thickness of bio-deposited calcium carbonate crystals when the surface of specimen is exposed to the bacteria and nutrients. Interestingly, the thickness of the layers in the samples submerged in a solution containing bacteria at stationary phase were larger than the other counterparts in the solution including bacteria at exponential and decline phases. The layer of precipitated crystals was examined, and the SEM micrograph of samples submerged in 0.1 mol/L of calcium nitrate, urea and bacteria (log phase) is shown in Fig. 5b. The characterization reveals that calcium carbonate crystals exhibited different morphologies at different  $\text{Ca}^{2+}$  concentrations. Biotic and abiotic factors, such as bacterial genotype and concentration, nucleation site, concentration of nutrients (calcium, carbon and nitrogen source), pH and temperature, have been reported to be influencing the biosynthesis of calcium carbonate (Seifan and Berenjian 2018). In another review, Al-Salloum et al. (Al-Salloum et al. 2017) reported the influence of additional factor, nutritional history of bacterial at the time of addition to cementitious materials, on calcium carbonate formation and subsequently the performance of crack healing process.



**Fig. 5** a) The thickness measurement of the deposited layer of sample submerged in 0.3 mol/L of calcium nitrate, urea and bacteria (stationary phase) and b) scanning electron micrograph of calcium carbonate on the surface of cement stone specimen submerged in 0.1 mol/L of calcium nitrate, urea and bacteria (log phase) (Chunxiang et al. 2009).

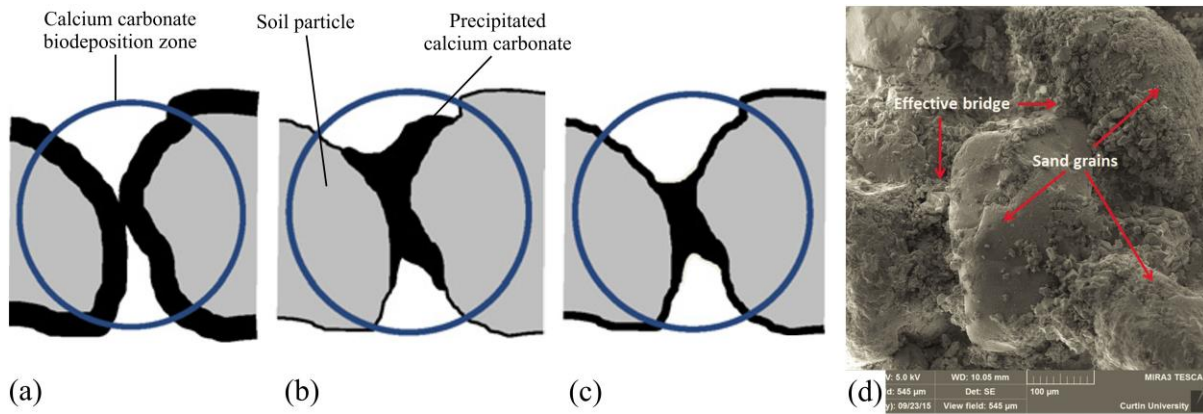
Since the passive treatments are not permanent, they require labor to detect cracks, examine the concrete integrity, and repeat the repair as needed. These challenges result in a high maintenance cost. Most importantly, the passive techniques are limited to the exterior sides and reachable parts of the structures. Currently, the utilization of mineral admixtures is a common practice to produce a self-healing concrete. However, these unprotected admixtures immediately start to react once they come in contact with water over the concrete mixing process (Huang et al. 2016). This phenomenon significantly decreases their effectiveness to heal the cracks in hardened concrete. Recently, attempts have been made to introduce the biological healing agent (including bacteria and nutrients) into the concrete matrix during concrete preparation (Seifan et al. 2018c; Seifan et al. 2018d). However, the protection of bacteria from stresses in alkaline environment of concrete to induce a high affinity of calcium carbonate has remained a challenge (Wang et al. 2012b). Bacteria must endure enough to withstand the stresses, high temperature (during cement hydration), and long periods of inactivated lifestyle before a crack occurs. Immobilization or attachment to carriers that shield the bacteria from such a stresses can be a practical solution to address the bacterial low viability issue. Lee and Park (Lee and Park 2018) noted that an idea carrier for bacteria should be biocompatible, mechanically strong enough to endure the concrete mixing and to minimize the likelihood of rupturing, as well as not being effective on mechanical properties of concrete itself. Therefore, the bacterial cells that mediate the self-healing need protection from the harsh environment and this can be achieved through immobilization. Recently, Seifan et al. (Seifan et al. 2018c) successfully employed a nanotechnological approach to address the current issue associated with the low viability of bacteria in the concrete environment. They fabricated biocompatible magnetic iron oxide nanoparticles that can attach to the cell surface because of the negative charge in the bacterial cell walls (Seifan et al. 2018b). The proposed immobilization approach has superior advantages over other techniques as it facilitates the cement hydration and offers protection at nanoscale which guarantees the integrity of the concrete structure. The additive content in the concrete mixture is a key factor to be considered. To preserve the main characteristics of concrete and being commercially feasible, the

allowable dosage of biological healing agent must be in a range of 2–5% by weight of cement. The latter limitation can be addressed by optimization of MICP in concrete matrix.

### ***Soil strengthening and sand consolidation***

The improper mechanical properties of soil in many regions and industrial sites can cause serious issues. Under this condition, the dikes, dunes, and slopes can become unstable, the roads and railways undergo settlement, and slopes, coasts and rivers are likely to be subject to erosion (Van Paassen et al. 2010a). In another scenario, the seismic loads, such as earthquakes, cause a phenomenon called soil liquefaction which can largely damage infrastructures. Densification of the loose sand in the land reclamation projects is a big concern. Therefore, the improvement in mechanical properties of soil becomes an important research topic. Every year, more than US\$6 billion is spent on projects involving soil improvement around the world (DeJong et al. 2010). To prevent soil erosion, stabilization at the surface can be achieved using constructive, ecological or combined techniques (Jones and Hanna 2004; Normaniza et al. 2008), though these surficial approaches are not sufficient, and therefore in situ strengthening techniques are required. A common practice for the soil improvement is chemical grouting techniques by insertion of synthetic materials, such as micro-fine cement, epoxy, acrylmide, phenoplasts, silicates, and polyurethane (Xanthakos et al. 1994). However, the injection of these materials requires a considerable cost and energy to fabricate a huge number of injection wells for treating a large volume of soil. Moreover, as a consequence of treatment, the permeability of soil is reduced, which may disrupt the groundwater flow. Most importantly, the injection of these chemicals creates environmental concerns, as the majority of them are toxic and/or hazardous (Karol 2003).

Over recent years, researchers have investigated various bio-mediated techniques for soil improvement such as biocementation, bioclogging, bioremediation, and phytoremediation (Shashank et al. 2016). Biocementation or MICP provide a great opportunity to alter the engineering properties of soil. Exploitation of bacteria to induce biominerals in soil contributes to fill the pore space and bind the soil particles together (Fig. 6). The implementation of MICP also has potential for enhancing the stability for retaining walls, embankments, and dams; treating pavement surface; strengthening tailings dams to prevent erosion and slope failure; increasing the bearing capacity of piled or non-piled foundations; reinforcing or stabilizing soil to facilitate the stability of tunnels or underground constructions; reducing the liquefaction potential of soil; and controlling erosion in coastal areas and rivers (Kucharski et al. 2012).



**Fig. 6** Illustration of calcite distribution within the soil pore space (DeJong et al. 2010): a) uniform distribution, b) preferential distribution, c) actual distribution, and d) scanning electron micrograph showing the effective bridge formation caused by MICP (Mujah et al. 2017).

The properties of soil can be evaluated by examination of different geotechnical characteristics such as permeability, stiffness, porosity, microstructure and binding, shear strength, shear wave velocity, and unconfined compressive strength. To improve the soil properties via microbial approaches, the potent microbial strain can be introduced to the soil matrix through three main routes: (i) injection method, (ii) percolation method, and (iii) premixing method. In the first method, the bacteria are injected into the soil resulting in flushing of bacterial solution top to bottom (Mujah et al. 2017). The injection technique is the most commonly preferred approach for introducing the biological healing agent into the soil. In this method, injection parameters, such as pressure and flow rate, can be easily controlled and the biological healing agent can be applied in both vertical and horizontal directions. Bacteria and nutrients can also be introduced into the soil through a simple spraying or trickling, which is called percolation. On an industrial scale, this approach is significantly cheaper than other methods of introducing biological healing agents to the soil matrix. However, its efficiency is limited to the narrow depth of soil as the biological healing agent penetrates due to gravity. For example, Cheng and Cord-Ruwisch (Cheng and Cord-Ruwisch 2014) reported the successful insertion of biological healing agent using this method in a short column of 2 m long. The mechanical mixing of bacteria with soil known as the ‘premix method’ is another way to introduce biological healing agent into the treatment zone. Compared to the trickling method, this technique requires a higher source of energy and cost, though its effectiveness is higher, specifically in deeper levels of soil.

To date, different studies have been performed to investigate the feasibility of microglial grouting for modification of soil properties. For instance, to study the effect of MICP on the properties of soil, Whiffin (Whiffin 2004) injected the bacteria and Ca/urea solution into the core of a sandy soil column. Yasuhara et al. (Yasuhara et al. 2012) evaluated the effect of urease enzyme and calcium chloride solution as the essential elements for initiation of calcium carbonate on the properties of soil. They found that the precipitated crystals could significantly improve the strength of soil. Moreover, the permeability of the treated soil showed one order of magnitude reduction as compared to untreated soil, indicating that the precipitated crystals could effectively occupy the pore space. In another investigation, van Paassen et al. (van Paassen et al. 2010b) utilized the MICP mechanism to increase the strength and stiffness of granular soils at a large scale experiment (100 m<sup>3</sup>). They found that the stiffness of the

soil significantly increased just after a day of treatment as a function of the injected volume of grouting agents and the distance from the injection points. Dhimi et al. (Dhimi et al. 2013a) investigated the effect of *B. megaterium* on the biogenic treatment of soil-cement block. Their experiments showed 40% decrease in water absorption, 31% decrease in porosity and 18% increase in compressive strength in biogenic treated samples as compared to control specimens. As there was no precipitate on the surface of the control samples, the resulted improvement is attributed to the deposition of a whitish layer on the surface of blocks which was attributed to the calcium carbonate formation. In a similar finding, Al Qabany and Soga (Al Qabany and Soga 2013) introduced *S. pasteurii* and various concentrations of urea-CaCl<sub>2</sub> solution into the sand sample. It was noted that all microbial treated samples had higher strength, and the increase in strength was proportional to the concentration of reactants. The same positive effect was also reported for the permeability results where a higher decline in water absorption was obtained in treated samples with concentrated urea-CaCl<sub>2</sub> solution. According to research performed by Ivanov et al. (Ivanov et al. 2015), the introduction of microbial agent into the soft marine clay can contribute to an increase in shear strength clay aggregates and, more surprisingly, it leads to an increase in unconfined compressive strength of aggregates with a size of 5 mm from zero to more than 2 MPa. Despite a large number of investigations performed to evaluate the strength, stiffness and permeability of different soils via the MICP process, there are still challenges that need to be overcome. The mass transfer limitations for transporting nutrients as well as limited metabolic activity of bacteria in deeper subsurface area of treating zones are the main challenges to be addressed for prospective applications (Umar et al. 2016).

#### ***Bioremediation of contaminants from soil and groundwater***

During the last few decades, deterioration and contamination of soil and groundwater have been dramatically increased due to different sources of pollutions mainly from urbanization, industry and intensive agriculture. The contamination sources in soil and groundwater are mainly radionuclides and/or heavy metals such as cadmium, chromium, copper, zinc, arsenic, cobalt, lead, nickel, mercury, silver, selenium, antimony, and thallium. Although the heavy metals are naturally occurring, they become concentrated as a result of anthropogenic activities (Guo et al. 2010; Pérez-Marín et al. 2008). As the majority of contaminants are toxic, non-degradable and persistent to accumulate, the research on toxic waste degradation approaches have been prioritized for immediate conservation of our environment. Conventionally, various types of physicochemical techniques, including chemical precipitation, filtration, oxidation/reduction, ion exchange, electrochemical treatment, membrane technology, reverse osmosis, and evaporation recovery, have been developed for remediation of polluted resources (Wang and Chen 2009; Xiao et al. 2010). However, the majority of these strategies are inefficient, expensive, labor-intensive, and require a considerable amount of chemicals and energy (Chen et al. 2008; Fu and Wang 2011; Guo et al. 2010; Tang et al. 2008). More recently, biological techniques, such as phytoremediation, bioaccumulation, biocoagulation, bioleaching, biosorbents, and bioimmobilization (Arias et al. 2017; Gadd 2000; Gzásó 2001; Lloyd and Lovley 2001; Volesky 2001) have been developed as alternative and/or supplement for chemical approaches. Despite the advances in the removal of heavy metals from contaminated environments through biological approaches, they are ineffective, costly, time-consuming, and, most importantly, lead to the release of immobilized or adsorbed heavy metals back into the environment (Achal et al. 2011d). The confluence of these challenges necessitates the development of a new sustainable alternative which is called bioprecipitation. As a

result of this process, the toxic compounds are changed from soluble heavy metals to insoluble forms. Although the capacity of heavy metals removal by microorganism was reported to be higher than conventional techniques (Leung et al. 2001), the uptake of heavy metals can be selective (Loaïc et al. 1997). Moreover, the bacterial activity can be limited by heavy metals toxicity and the precipitation process highly depends on the pH. In general, the biosorption of heavy metals by bacteria can be achieved through different mechanisms, namely cell surface adsorption, extracellular precipitation, intracellular accumulation through special components, and intracellular accumulation into vacuoles (Mosa et al. 2016). A vast array of microorganisms, such as bacteria, algae, yeasts, and fungi, have been used for heavy metals removal due to their high performance and low cost (Wang and Chen 2009). However, their effectiveness in environmental cleaning differ based on their varied ability of interacting with contaminants. The bioremediation of heavy metals through the MICP process has an advantage over the other biotechnological processes because it can sequester metals as minerals precipitate for a long period (Fujita et al. 2000). Heavy metals can be removed through a direct precipitation process where metal carbonate is precipitated, or by a co-precipitation, in which heavy ions, such as  $Cd^{2+}$ ,  $Cu^{2+}$ ,  $Zn^{2+}$ ,  $Fe^{2+}$ , and  $Pb^{2+}$ , are incorporated in the lattice structure of calcite via substitution of  $Ca^{2+}$  (Torres-Aravena et al. 2018). Achal et al. (Achal et al. 2011d) tested the copper bioremediation capacity of *Kocuria flava* for cleaning up the copper-contaminated soil. They found that the isolated bacteria produce a significant amount of urease (472 U m/l) and are able to remove 95% of copper after 120 h from the nutrient broth medium supplemented with urea- $CaCl_2$ . In a similar investigation, Li et al. (Li et al. 2013) used different isolates to assess their capability for removal of nickel, copper, lead, cobalt, zinc, and cadmium. It was found that the isolates could successfully remove the contaminations ranging from 88% to 99% in a short period of time (24 h). The results show that *S. koreensis* had the highest removal rates for copper and lead. *Sporosarcina* sp. and *Terrabacter tumescens* showed the highest removal for cobalt, and zinc, nickel and cadmium, respectively. Similarly, Kang et al. (Kang et al. 2014b) investigated the capability of bacteria to remediate cadmium-contaminated soil in laboratory-scale experiments. CH-5 and CH-11 (*L. sphaericus*) showed the highest rate of calcite and urease production, respectively. It was also shown that *L. sphaericus* could remove 99.95% of cadmium at 2 g/L in 48 h. The literature also indicates the successful removal of other heavy metals such as chromium (Hua et al. 2007), arsenic (Achal et al. 2012b; Dey et al. 2016), and lead (Kang et al. 2015) from contaminated environments. Moreover, the application of bioremediation can be a promising tool for remediation of highly toxic materials such as strontium. It has been reported that strontium is capable of exerting long term health impacts as it has a long half-life of 28.8 years (Singh et al. 2008). Its solubility facilitates the mobility and transportation into the groundwater and soil, and it can be readily passed through the food chain. Warren et al. (Warren et al. 2001b) employed *S. pasteurii* to remove strontium through a solid phase capture. Associated solid phase capture of strontium was found to be highly effective, capturing 95% of the 1 mM strontium only in 24 h. In another investigation, the successful sequestration of strontium by *S. pasteurii* WJ-2 was reported (Kang et al. 2014a). It was noted that approximately 80% of the strontium from the soluble fraction of the sand was sequestered. A similar finding was observed by Achal et al. (Achal et al. 2012c) when they used strontium resistant bacteria to remediate strontium from aquifer quartz sand. It was found that *Halomonas* sp. removed 80% of strontium from soluble–exchangeable fraction of aquifer quartz sand.

Bioremediation technology shows a promising result and has been proven to be effective in laboratory scale. However, further research is required to understand the fundamentals behind the microbial mechanisms in the

degradation process before *in situ* application for biorecovery of heavy metals and radionuclides. For example, oxygen limitation is one of the main barriers against the MICP in deeper parts of soils. Although in many types of soils the effective oxygen diffusion for desirable rates of bioremediation extends to ranges less than 30 cm, Vidali (Vidali 2001) reported the successful remediation at a depth of 60 cm and greater. The utilization of oxygen releasing compounds could be a potential solution to further increase the availability of oxygen and consequently bioremediation efficiency.

### ***Removal of calcium from industrial waste***

Calcium-rich effluents are associated with landfill leachates, reverse osmosis concentrates, and industrial processes (Van Langerak et al. 1997). It is reported that such high concentrations of  $\text{Ca}^{2+}$  are a serious hazard for the environment or, in some cases, may negatively affect the processes. For example, in aerobic or anaerobic reactors,  $\text{Ca}^{2+}$  tends to clog the pipelines, boilers, and heat exchangers and therefore causes scaling or malfunctioning of instrumentations (Hammes et al. 2003). As a result of this,  $\text{Ca}^{2+}$  needs to be captured and MICP serves as a new emerging solution to address this problem. Hammes, et al. (Hammes et al. 2003) reported the positive effect of the ureolytic microbial community on removing excess calcium from industrial effluents. They noted that 85–90% of the soluble calcium was precipitated in the form of calcium carbonate sedimentation in the treatment reactor. However, to be widely implemented some challenges need to be overcome, such as pH adjustment, ammonium release, and calcium/urea source. As a result of this, future research should be focused on such challenges.

### ***Carbon dioxide sequestration***

Increasing greenhouse gas emissions and mounting their concentrations in the atmosphere result in major environmental issues such as global warming. Among these gases,  $\text{CO}_2$  is the most abundant greenhouse gas which is emitted in the atmosphere by anthropogenic activity and has a significant impact on the Earth's climate (Drake 2014; Srivastava et al. 2014). So far, different approaches have been proposed for carbon capture and storage (CCS) and carbon capture and utilization (CCU) (Cuéllar-Franca and Azapagic 2015). In CCS, the captured  $\text{CO}_2$  is transferred to a suitable site, such as geological or ocean sites for long-term storage (Markewitz et al. 2012; Zapp et al. 2012), while in CCU, the captured  $\text{CO}_2$  is converted into commercial products such as chemical feedstock, fuels, and mineral carbonation (Styring et al. 2011). However, there are serious concerns regarding the implementation of these techniques. For example, the leakage and escaping of stored concentrated  $\text{CO}_2$  negatively affect the environment. Based on the permeability of the geological structure of storage site and its faults or defects, it is estimated that between 0.00001% to 1% leakage is happening every year (Pehnt and Henkel 2009; Singh et al. 2011). Therefore, these approaches can have long term effects on the ecosystem and their processes are not economically viable or energy efficient. On the other hand, the sequestration of  $\text{CO}_2$  in a form of stable and environmentally friendly solid carbonate offers a great solution for long term storage of  $\text{CO}_2$  to lessen the environmental concerns. Mineral carbonation is achieved in a chemical process that  $\text{CO}_2$  reacts with a metal oxide such as calcium to form carbonates. Carbonation has the potential to be an effective tool for capturing  $\text{CO}_2$  as it is a single quick process and, more importantly, it does not require any  $\text{CO}_2$  transport which

can substantially reduce the costs and risks of leakage. However, it has been reported that the biochemical fixation of CO<sub>2</sub> to carbonate minerals is a slow process in nature. Therefore, utilization of biological catalysts, such as carbonic anhydrase (CA), which is ubiquitously distributed in organisms and involved in many biochemical and physiological processes, can catalyze the reverse hydration of CO<sub>2</sub> (Tripp et al. 2001; Zhang et al. 2011). In this context, Ramanan et al. (Ramanan et al. 2009) investigated the effect of six different bacteria with high CA activity for removal of CO<sub>2</sub>. They observed calcium carbonate deposition when CaCl<sub>2</sub> solution was saturated with CO<sub>2</sub> in the presence of CA enzyme. Authors also found that the purified enzyme has a higher capability of carbonate precipitation (15 times) than crude enzyme. Likewise, the biomimetic sequestration of CO<sub>2</sub> into calcium carbonate using CA purified from *Pseudomonas fragi*, *Micrococcus lylae* and *Micrococcus luteus* has been successfully demonstrated (Sharma and Bhattacharya 2010).

In addition to the above mentioned applications, MICP can be a promising solution for other environmental issues. Conventionally, the intrusion of salt water into freshwater aquifers during groundwater extraction is being overcome by creating underground dams or increasing artificial recharge of fresh water (Phillips et al. 2013). Subsurface MICP barriers can be an alternative to prevent mitigation of salt-laden water into freshwater aquifers (Rusu et al. 2011; Tobler et al. 2011). To achieve this, the selected microorganisms must be able to tolerate high saline conditions and induce calcium carbonate precipitation under such an environment. In continuation of MICP for environmental engineering applications, Anbu et al. (Anbu et al. 2016) proposed that the precipitated calcium carbonate can be used as a coating agent to immobilize and subsequently remove polychlorinated biphenyls contaminated oil from the environment.

### **Conclusions and future perspective**

Due to the rapid growth of MICP technology, a vast range of opportunities continue to expand. The MICP processes can be used for the production of multifunctional materials. This technology can also help to increase the efficiency of crude oil extraction by a reduction in permeability and strengthening the loosely cemented layers. Moreover, it may minimize the risk of oil leakage and contamination at the top layers of soil where the majority of soil microorganisms are present. As compared to conventional techniques, MICP can be a promising solution for consolidation of particles and suppression of dust. For environmentalists, the leakage from ponds or reservoirs has always been a serious concern. The leakage from ponds/reservoirs not only causes the loss of fluid, but also results in seepage into underlying foundation soil or sand. For example, this phenomenon in aquaculture ponds causes the contamination of groundwater with nutrients and organic aquacultural wastes. This challenge may be overcome by reducing the seepage rate and permeation of reservoir through MICP process in a sustainable way.

Despite the positive effect of MICP technology, there are still shortcomings associated with its industrial application. The first challenge is related to upscaling and the ability of MICP to uniformly treat a large area. The treatment homogeneity is another factor that needs to be investigated as it influences the mechanical properties of the treated area. The next challenge lies in the duration of microbial treatment. As compared to chemical methods, the microbial process is usually much slower, which affects the performance of MICP. Moreover, from the economical point of view, the cost of the MICP process needs to be further reduced to make it a much more



feasible option for a wide range of applications. This could happen by the utilization of nutrients from the waste streams and/or modification of microbial preparation procedure.

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### **Conflict of interest**

The authors declare that they have no competing interests.

### **Ethical approval**

This study does not contain any studies with human participants or animals performed by any of the authors.

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