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To cite this article: Adharsh Rajasekar *et al* 2017 *IOP Conf. Ser.: Earth Environ. Sci.* **78** 012016

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MICP and Advances towards Eco-Friendly and Economical Applications

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Abstract. Biomineralization is a natural process aided by living organisms. Due to its applicability in ground improvement and bioremediation, Microbially Induced Calcite Precipitation (MICP) is an interdisciplinary field of study combining engineering, chemistry and microbiology. Bioremediation has been applied widely for contamination containment or removal, in this case it will be containment. MICP can also be applied to improve the efficiency of *insitu* bioremediation. Urease is an enzyme which can facilitate increased calcite precipitation. However the production of urease by bacteria and thus the resulting carbonate precipitation are inhibited by environmental factors including calcium concentration, bacterial concentration, pH and temperature. Under good conditions MICP can be used for heavy metal and radionuclide immobilization. However technologies such as bioconsolidation and biocementation require improvement such as time and cost. This paper highlights the application of MICP in addition to suggested improvements to make it more eco-friendly and sustainable.

1. Introduction

Microbial activity that alters the chemical environment favoring mineral formation is known as Biomineralization [1], [2]. It is a natural phenomenon that leads to precipitation of more than 60 different biological minerals that are formed through extracellular or intracellular pathways [3]. This occurs by a sequence of chemical reactions and physiological pathways which results in the precipitation of a range of different forms of solid mineral structure.

2. Microbially Induced Carbonate Precipitation

Microbially induced carbonate precipitation (MICP) is a biochemical mechanism which is driven by microorganism upon interacting with a chemical solution rich in calcium. Research in MICP has shown that microbially released CO₂ interacts with the biomineralization solution favoring carbonate formation. The carbonate combines with the calcium ion (Ca²⁺) leading to the precipitation of calcium carbonate. In the last two decades, multiple mechanisms have been identified for the precipitation of calcium carbonate. The mechanisms include photosynthesis [4], [5], urea hydrolysis [1], [6]-[9], sulfate reduction [10], [11] and extracellular polymeric substances [12], [13]. Each mechanism promotes a different chemical pathway (Figure 1), all of which may be effective for mineralization. However, the precipitation of calcium carbonate by bacteria via urea hydrolysis is the most commonly exploited mechanism.



Urease activity is found in a wide range of microorganisms, one of the most commonly studied bacteria is *Sporosarcina pasteurii*. It is a soil, non-pathogenic and endospore producing, bacteria with an optimum pH for growth of 9.0 that can tolerate extreme conditions. Multiple studies have been conducted with *Sporosarcina pasteurii* for MICP [6], [8], [14]-[16]. Additionally, Achal, Mukherjee [17] developed a mutant strain (BP-M-3) of *Sporosarcina pasteurii* MTCC 1761 which resulted in an enhanced level of urease activity and carbonate precipitation compared to the natural type. The most important criteria to consider for the selection of a bacterial strain for biomineralization is its ability to synthesize active urease. However, a further consideration is that there are many pathogens among urease producing bacteria. For example, active urease producers includes *Helicobacter pylori* which infects the human stomach, and the opportunistic human pathogens such as *Proteus vulgaris*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [18].

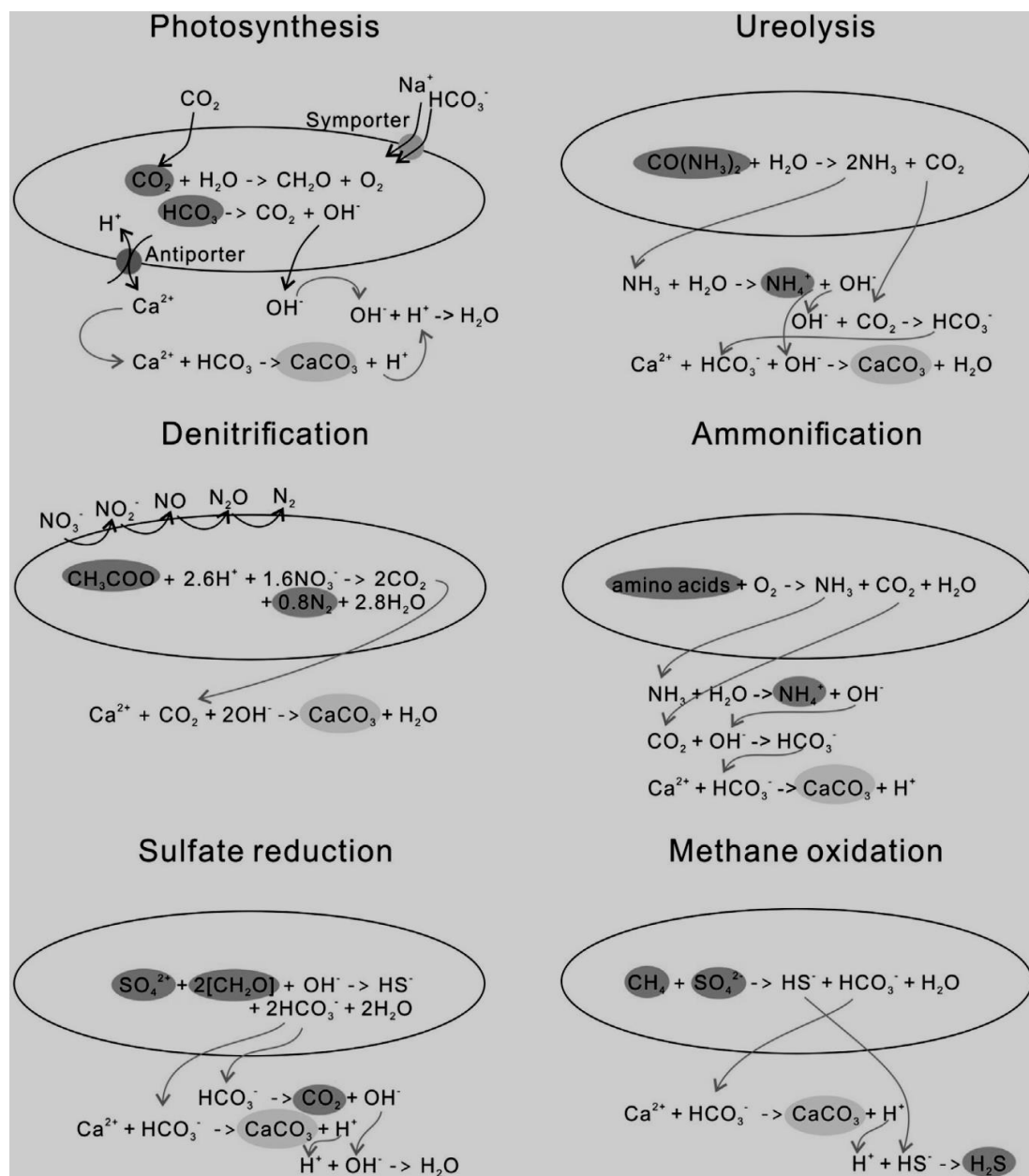


Figure 1. Processes that generate supersaturated environments essential for carbonate precipitation modified from [23].

Hammes and Verstraete [19] and Silva-Castro, Uad [20] reported that urease influences the chemical process associated with the formation of biominerals through four different factors; pH, dissolved inorganic carbon (DIC) concentrations, calcium concentrations and the availability of nucleation sites. The first three factors influence the carbonate ion concentration (CO_3^{2-}) while the last parameter promotes stable and continuous calcium carbonate formation [1], [21]. During the biomineralization process, bacteria commonly serve as nucleation sites for the precipitation of calcium carbonate. These four factors have a major influence on both ureolytic activity and calcium carbonate formation. Ca^{2+} ions bind to the negatively charged bacteria surfaces, creating a favorable environment for Ca^{2+} adsorption. Thus, Ca^{2+} ions bind more frequently onto the negatively charged cell surface of bacteria [22]. Bacterial cells are very important for the precipitation of CaCO_3 , because the bacteria both provide nucleation sites (heterogeneous nucleation) and affect the types of minerals being formed (Figure 2). Okwadha and Li [8] found that a high concentration of bacterial cells increases the amount of carbonate precipitation via MICP. This occurs because of the increase in the concentration of urease increasing the rate of urea hydrolysis.

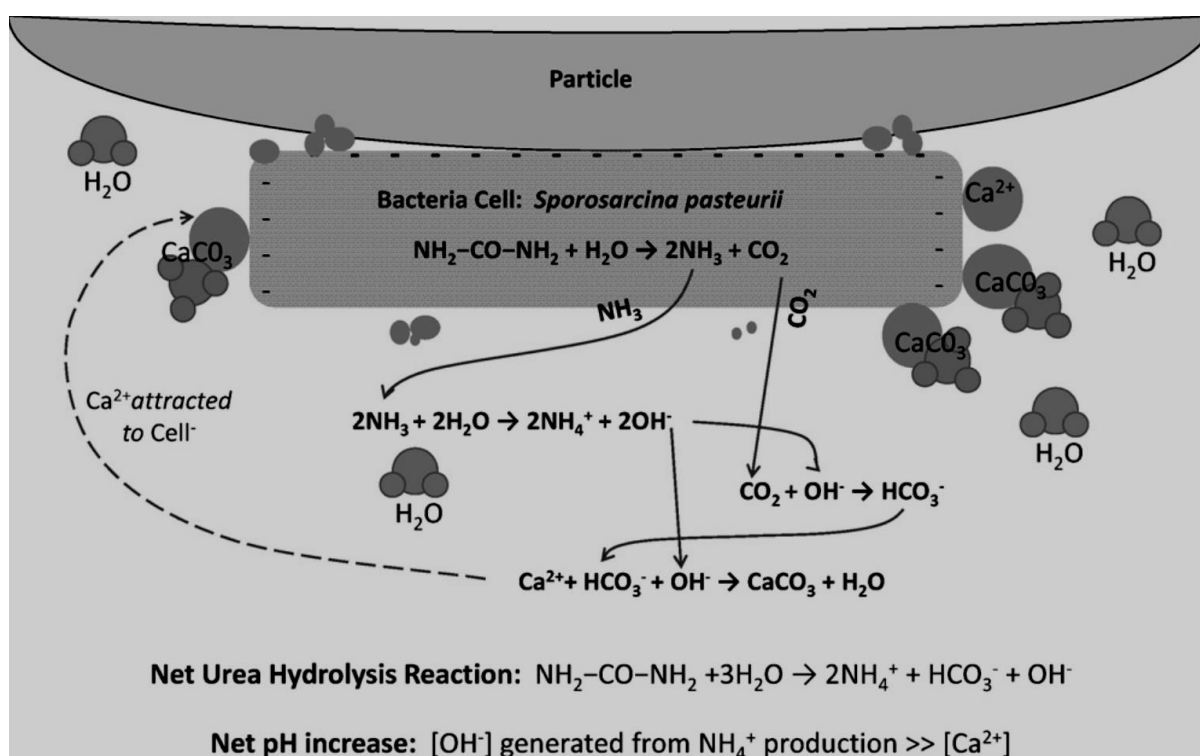


Figure 2. Schematic representation of Ureolysis in solution favoring MICP modified from [24].

3. Application towards Bioremediation

There have been a wide range of developments in bioremediation in recent years and these developments have their associated challenges which must be addressed for this technology to reach maturity as an engineering tool.

3.1. Removal of Heavy Metals and Radionuclides

Given the current rate of urbanization and industrialization, heavy metals and radioactive waste released both into the atmosphere and into soils due to industrial processes have been observed accumulating in both in landfills and residential environments [25]-[27]. These accumulated heavy metals and radionuclides pose serious health problems for humans and other living organisms within the environment. Some heavy metals in small dosages are beneficial to humans, but the rate of industrial release can be very toxic to humans [28]. The mobility of the released heavy metal ions may

increase the seriousness of the threat to the lives of humans and effective methods need to be implemented to impede their transportation especially through groundwater [29].

Heavy metals including arsenic, cadmium and lead are commonly identified in most landfills at medium to high concentrations [26], [30]. Fu and Wang [28] proposed that heavy metals can be immobilized from the environment using MICP. However, heavy metal toxicity will also affect microbial growth and thus efficiency of MICP may be reduced; several researchers have identified and isolated heavy metal tolerant microbes with ureolytic capability from diverse environments which could improve the efficiency of the MICP process in contaminated ground [31], [32]. During the MICP process, calcium ions are added to a solution to precipitate calcium carbonate, in the heavy metal containment MICP process, calcium carbonates can also incorporate heavy metals (e.g., Cd and Pb^{2+}) into their surfaces via substitution of suitable divalent cations (Ca^{2+}) in the carbonate lattice, which alters the chemical form of these carbonates and alters the heavy metals from soluble to insoluble forms reducing their potential for toxicity.

3.2. Removal of Radionuclides

The disposal of radionuclide wastewater from commercial nuclear plants is a major issues associated with nuclear waste management because it is highly toxic to the environment, particularly to human health. Fujita, Taylor [9] assessed a pump and treat method, but it was unsuccessful at radionuclides removal from the contaminated environment. In such scenarios, MICP can be applied to immobilize the radionuclides safely from the environment. The basic process behind MICP method involves ureolytic microorganisms to precipitate $CaCO_3$, this in turn leads to promote co-precipitation of radionuclides by substitution of Ca^{2+} ion and formation of radionuclide carbonate minerals [9], [33].

3.3. Improvements Needed to Make MICP more Economical and Eco-Friendly

MICP has a great potential for sustainable environmental remediation. However as MICP is still a new methodology in terms of engineering application, there are a few limitations which must be addressed prior to field implementation:

1) MICP is not 100% environmentally friendly, as ureolysis plays a major role in precipitation generating by-products including ammonium and nitrate. These compounds are toxic and thus hazardous both to human health and to indigenous microbial consortia especially at high concentrations [34]. This limits its application for biocementation as ammonium present inside building materials have the potential to be converted into nitric acid by bacteria, which might decrease the bio-deterioration of materials. Ganendra, Muynck [35] found that replacing calcium chloride with calcium formate did not result in the release the ammonia to the air or produce nitric acid. More investigation and optimization is required to advance the process such that the volume/concentration of unwanted byproducts is reduced. Reduction of these byproducts would greatly improve the validity of the assessment that MICP is an eco-friendly treatment.

2) MICP is a microbial process which greatly depends on temperature, pH, calcium concentration, DIC and the presence of nucleation sites [36]. This makes it a complex and time consuming process in comparison to the chemical process under standard environmental conditions. MICP has to be optimized for time effectiveness before it's used for *insitu* applications.

3) The economic limitations makes MICP less industrially friendly, as laboratory grade sources needs to be used. Since there's a potential of inefficient MICP when using non-laboratory grade chemical reagents. Although alternative inexpensive nutrient sources for MICP such as lactose mother liquor have been implemented, consideration of a wider range of alternative sources would provide a better assessment of its cost effectiveness [17]. In addition to this limitation, application of *insitu* MICP would require the generation of substantial volumes of chemical reagents and microbial solutions. Although recently indigenous bacteria capable of MICP are reported, more studies that are target specific need to be implemented to resolve this issue [9], [37], [38].

Given the discussion above, although studies of MICP have generated promising results, its application at the large scale is still challenging. This technology is however worth of further study, and the resolution of the issues outlined would promote its implementation as a replacement for less sustainable alternative methods.

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Acknowledgments

This work was supported by grant no. PGRS-12-02-06 and RDF-13-01-06 awarded by XJTU.