

# Microbially-Induced Desaturation and Carbonate Precipitation

Subjects: [Chemistry, Applied](#) | [Engineering, Civil](#) | [Biotechnology & Applied Microbiology](#)

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Microbially induced carbonate precipitation (MICP) has been proposed as a sustainable approach to solve various environmental, structural, geotechnical and architectural issues. In the last decade, a ubiquitous microbial metabolism, nitrate reduction (also known as denitrification) got attention in MICP research due to its unique added benefits such as simultaneous corrosion inhibition in concrete and desaturation of porous media. The latter even upgraded MICP into a more advanced concept called microbially induced desaturation and precipitation (MIDP) which is being investigated for liquefaction mitigation.

nitrate reduction

nitrogen gas

calcium carbonate

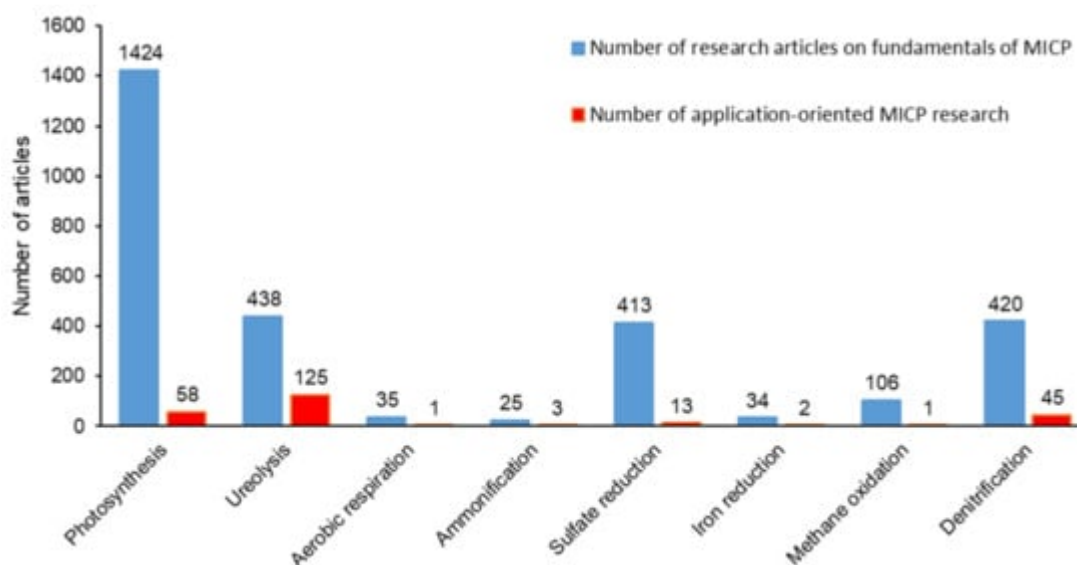
liquefaction mitigation

self-healing concrete

ground improvement

## 1. Introduction

**Figure 1** shows that among the metabolic pathways leading to MICP, urea hydrolysis drew significant attention not only in fundamental research describing the MICP mechanism, but it was also the most investigated metabolic pathway to develop new bio-based technologies. The ratio of application-oriented MICP research to fundamental research was considerably low for other metabolic pathways ( **Figure 1** ). Therefore, possible advantages of the other metabolic pathways in MICP applications were overlooked.



**Figure 1.** Number of research papers conducted on MICP through different microbial metabolic pathways based on the database of Web of Science from 2000 to 2021. The first column represents the search on the topic of “calcium carbonate” OR “calcite precipitation” OR “carbonate precipitation”, and the keyword of photosynth\*, ureoly\*, “aerobic respiration,”, ammonification, “sulfate reduction,” “iron reduction,” “methane oxidation,” and “denitrification”, respectively. The second column represents the number of articles searched on two additional keywords “technology” OR “biotechnology”.

In MICP through denitrification, the denitrifying bacteria are introduced into the soil together with a carbon source as an electron donor, nitrate ( $\text{NO}_3^-$ ) as a terminal electron acceptor, calcium as a precursor, and the general nutrients for bacterial growth and reproduction. In complete nitrate reduction process, so called denitrification, nitrate is reduced to nitrogen gas and the carbon source is oxidized to carbon dioxide. Since nitrate reduction process inherently removes  $\text{H}^+$  from the environment, the denitrification process leads to the production of alkalinity which further converts part of the produced  $\text{CO}_2$  gas into  $\text{CO}_3^{2-}$  ( **Table 1** ). Consequently, nitrogen ( $\text{N}_2$ ) and  $\text{CO}_2$  gases are generated, while  $\text{CaCO}_3$  is precipitated out of the solution in the presence of free  $\text{Ca}^{2+}$  ions ( **Table 1** ). If the aforementioned process occurs in a saturated porous environment, biogas production ( $\text{N}_2$  and  $\text{CO}_2$ ) and mineral ( $\text{CaCO}_3$ ) precipitation result in partial desaturation of the porous media and thus changes in its hydromechanical behavior. The process of simultaneous desaturation and  $\text{CaCO}_3$  precipitation is specific to denitrification pathway and thus MICP through denitrification becomes prominent among the other commonly investigated MICP pathways. In fact, this new process of simultaneous desaturation and  $\text{CaCO}_3$  precipitation occurring in porous environments upgraded MICP to a new level named as microbially induced desaturation and precipitation (MIDP) [\[1\]\[2\]](#).

**Table 1.** Reactions involved in different metabolic pathways leading to MICP.

Metabolisms	Microorganisms	Reactions	Author and Ref
Oxygenic photosynthesis	Cyanobacteria algae	$2 \text{HC O}_3^- + \text{Ca}^{2+} \rightarrow \text{CH}_2\text{O} + \text{CaCO}_3 + \text{O}_2$	Dupraz et al. [3]
Aerobic respiration	Aerobic heterotroph	$\text{Ca}(\text{C}_3\text{H}_5\text{O}_3)_2 + 6\text{O}_2 \rightarrow \text{Ca}^{2+} + 4\text{CO}_2 + 2 \text{HC O}_3^- + 4\text{H}_2\text{O}$ $4\text{CO}_2 + 2 \text{HC O}_3^- + 6 \text{Ca}(\text{OH})_2 \rightarrow 6\text{CaCO}_3 + 6\text{H}_2\text{O} + 2\text{OH}^-$	Ersan [2]
Ureolysis	Ureolytic bacteria	$\text{CO}(\text{NH}_2)_2 + 2\text{H}_2\text{O} \rightarrow 2\text{NH}_4^+ + \text{CO}_3^{2-}$ $\text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CaCO}_3$	Whiffin et al. [9]
Ammonification	Myxobacteria	Amino acids + $\text{O}_2 \rightarrow \text{NH}_3 + \text{CO}_2 + \text{H}_2\text{O}$ $\text{NH}_3 + \text{H}_2\text{O} \rightarrow \text{NH}_4^+ + \text{OH}^-$ $\text{OH}^- + \text{CO}_2 \rightarrow \text{HC O}_3^-$ $\text{Ca}^{2+} + \text{HC O}_3^- + \text{OH}^- \rightarrow \text{CaCO}_3 + \text{H}_2\text{O}$	González-Muñoz et al. [7]
Nitrate reduction/denitrification	Denitrifying bacteria	$\text{C}_2\text{H}_3\text{O}_2^- + 1.6\text{NO}_3^- + 2.6\text{H}^+ \rightarrow 0.8\text{N}_2 + 2.8\text{H}_2\text{O} + 2\text{CO}_2$ $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HC O}_3^- + \text{H}^+$ $\text{Ca}^{2+} + \text{HC O}_3^- + 2\text{OH}^- \rightarrow \text{CaCO}_3 + \text{H}_2\text{O}$	Van Paassen et al. [8]
Sulfate reduction	Sulfate-reducing bacteria	$\text{CaSO}_4 + 2\text{CH}_2\text{O} + \text{OH}^- \rightarrow \text{CaCO}_3 + \text{H}_2\text{O} + 2\text{CO}_2 + \text{HS}^-$	Baumgartner et al. [4]
Iron reduction	Iron-reducing bacteria	$\text{C}_2\text{H}_3\text{O}_2^- + 8\text{Fe}(\text{OH})_3 + 6 \text{HC O}_3^- + 7\text{H}^+ \rightarrow 8\text{FeCO}_3 + 20\text{H}_2\text{O}$	DeJong et al. [6]
Methane oxidation	Methanotroph	Methane Mono - Oxygenase $\text{CH}_4 + \text{O}_2 \xrightarrow{\text{NADH} + \text{H}^+ \rightarrow \text{NAD}^+} \text{CH}_3\text{OH} + \text{H}_2\text{O}$ Methanol Dehydrogenase $\text{CH}_3\text{OH} \xrightarrow{\text{PQQ} \rightarrow \text{PQQH}_2} \text{CHOH}$ Formaldehyde Dehydrogenase $\text{CHOH} + \text{H}_2\text{O} \xrightarrow{\text{NAD}^+ \rightarrow \text{NADH} + \text{H}^+} 2\text{HCOO}^- + \text{H}_2\text{O}$ $\text{HCOO}^- + \text{H}_2\text{O} \rightleftharpoons \text{HCOOH} + \text{OH}^-$ Formate Dehydrogenase $\text{HCOOH} \xrightarrow{\text{NAD}^+ \rightarrow \text{NADH} + \text{H}^+} \text{CO}_2$ $\text{Ca}^{2+} + \text{CO}_2 + 2\text{OH}^- \rightleftharpoons \text{CaCO}_3 + \text{H}_2\text{O}$	Ganendra et al. [5]

Currently, the potential of the denitrification pathway for MICP applications is overlooked ( **Figure 1** ). Both technical studies and review studies focus on MICP via ureolysis and the use of ureolytic pure cultures. Critical reviews on alternative MICP pathways are necessary to create a ground for detailed evaluation of advantages and disadvantages of various MICP pathways in different applications which will pave the way for tailored solutions. Therefore, this review study reveals the potential of denitrification pathway as an alternative to commonly proposed, less sustainable MICP pathways by covering the added benefits (i.e., corrosion inhibition, MIDP) offered by the stepwise occurrence of denitrification based MICP.

This review study consists of four major parts (i) denitrification mechanism and, the activities of denitrifying bacteria related to desaturation and  $\text{CaCO}_3$  precipitation, (ii) applications of MICP and MIDP through denitrification, (iii) the challenges involved in the practical applications and (iv) suggestions of future research to overcome those challenges and enable process upscaling and optimization of the novel applications.

## 2. The Denitrification Mechanism

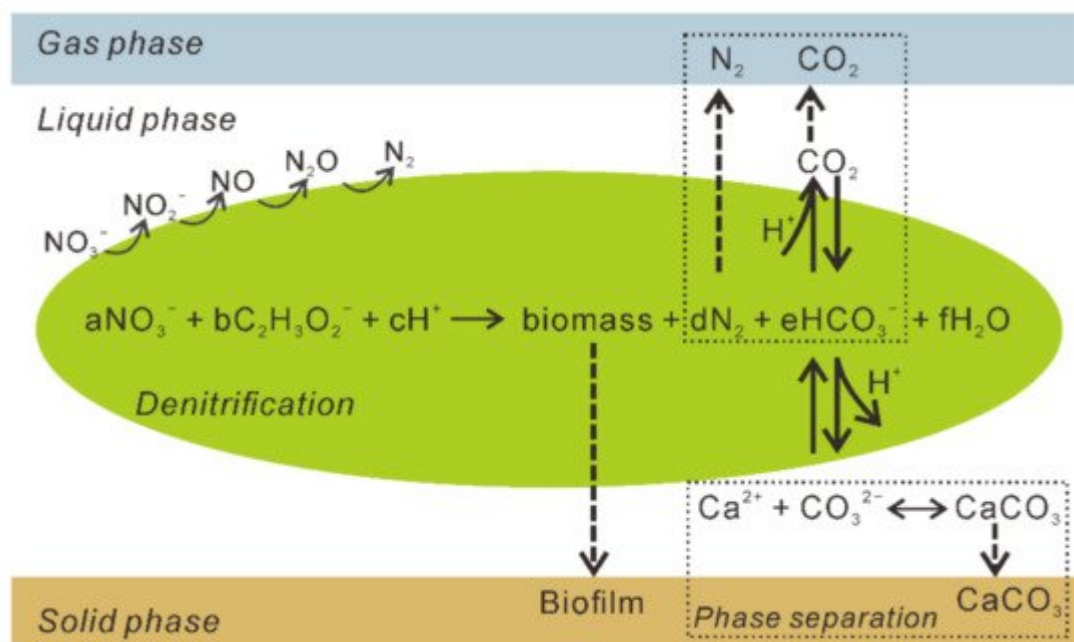
Organisms that are capable of denitrification, that is, denitrifying bacteria, are widely distributed with a high density in nature. These types of microorganisms are common in a variety of environments, and in agricultural soil they reach a population density of the order of  $10^6$  microorganisms/g of soil [3]. Typically, denitrifying bacteria constitute about 20% of the total microbial population that can grow under anaerobic conditions and their population corresponds to about 1% to 5% of the overall culturable soil microbiota [4]. More than 50 genera have been

identified, including *Bacillus*, *Alcaligenes*, *Diaphorobacter*, *Pseudomonas*, *Spirillum*, *Paracoccus*, *Thiobacillus*, and *Achromobacter* [5]. Thus, so far, many studies have used different denitrifying bacteria to link their nitrate reduction activity with either CaCO<sub>3</sub> precipitation or biogas generation or the combination of both (e.g., [6][7][2][3][8]).

Denitrification, or nitrate reduction, is an essential process in the global nitrogen cycle, in which the fixed nitrogen is cycled back into the atmosphere as N<sub>2</sub> gas. Thus, it closes the global nitrogen cycle and keeps the ecosystem in balance. Most denitrifying bacteria undertake denitrification in the presence of organic carbon and nitrate when oxygen is scarce or unavailable [9].

For growth-limited conditions the overall metabolic stoichiometry is equal to the catabolic reaction given in Equation (5).

Denitrifying bacteria can use a diverse range of electron donors in natural environments, including pure compounds (methanol, acetone, acetate, glucose, methane, and amino acids), sugars, wastewater, food industry waste, and sludge [5], which favor the generation of dissolved inorganic carbon (DIC). Inorganic carbon dissociates into CO<sub>2</sub>, bicarbonate HCO<sub>3</sub><sup>-</sup>, and CO<sub>3</sub><sup>2-</sup> in aqueous solutions. If a solution with a high pH and total inorganic carbon content contains excess dissolved Ca<sup>2+</sup>, CaCO<sub>3</sub> will precipitate [6], and the system will transfer to the solid phase ( **Figure 2** ). The denitrification process can be expressed by the equations using acetate (CH<sub>3</sub>COO<sup>-</sup>) as the electron donor ( **Table 1** ). Furthermore, the production of CaCO<sub>3</sub>, the generation of biogases, and the growth of bacteria result in biofilm and biomass accumulation, which favors the formation of a bio-barrier and decreases the permeability of the medium [10]. The full reaction system of the denitrification-based MICP is given in **Figure 2** .



**Figure 2.** The complete reactions for MICP through denitrification (modified from Pham et al. [11]).

### 3. Potential Applications of Denitrification-Based MICP Biotechnology

As nitrate reducing (denitrifying) bacteria are ubiquitous and could be isolated from various different environments, applications of MICP through denitrification is not limited by the regions or countries. Indeed, the variety of countries where MICP through denitrification was investigated for different purposes confirms the great potential of the technology for solution of certain problems worldwide (**Table 2**). As discussed above, depending on the application environment, denitrification-based MICP leads to a new concept which is a two-stage process, so called microbial induced desaturation and precipitation (MIDP). On the one hand, certain applications of denitrification-based MICP solely focus on the calcium carbonate precipitation, such as soil reinforcement, microbial self-healing concrete, calcium and metal removal from industrial waste streams, removal of undesirable compounds (organic matter, crusts, and mineral salts) from artwork and historical monuments (**Table 2**). On the other hand, some applications focus on both the desaturation effect due to biogas generation and the agglomerating effect due to mineral precipitation and thus they are considered as MIDP applications. Liquefaction mitigation can be mentioned among the MIDP applications (**Table 2**). In the following sections, MICP- and MIDP-driven applications will be discussed separately.

**Table 2.** Organisms involved in the applications of microbially induced desaturation and carbonate precipitation by denitrification.

Applications	Process	Microorganisms	Author and Ref	Country/Region
Soil reinforcement	MICP	<i>Pseudomonas denitrificans</i>	Karatas [8]; Hamdan [12]; O'Donnell [2]; Hamdan et al. [13]	Netherlands UK USA
		<i>Castellaniella denitrificans</i>	Van Paassen et al. [6]	
		<i>Halomonas halodenitrificans</i>	Martin et al. [14]	
		Denitrifiers from natural soil	Pham [1]; Pham et al. [11][15]	
Self-healing concrete	MICP	Nitrate reducing biogranules	Ersan et al. [16][17][18]; Ersan [19]	Belgium Turkey
		<i>Diaphorobacter nitroreducens</i>		
		<i>Pseudomonas aeruginosa</i>		
Sewer corrosion resistant concrete	MICP	Nitrate and sulfate reducing biogranules	Song et al. [20]	Australia

Applications	Process	Microorganisms	Author and Ref	Country/Region
Corrosion inhibition of steel in reinforced concrete	MICP	Nitrate reducing biogranules	Ersan et al. [17][21]	Belgium
		<i>Diaphorobacter nitroreducens</i>		
Treatment of industrial wastewater (calcium, nitrate, zinc, nickel, fluoride removal)	MICP	<i>Pseudomonas aeruginosa</i>		Belgium China Japan Spain
		<i>Diaphorobacter nitroreducens</i>	Ersan et al. [16]	
		<i>Pseudomonas aeruginosa</i>		
		Sludge from the biological treatment of leachate	Fernandez-Nava et al. [22]	
		Sludge from a Sewage Treatment Plant		
		<i>Acinetobacter</i> sp.	Aoki et al. [23], Fan et al. [24], Liu et al. [25], Su et al. [26]	
Remediation of artwork and historical monuments	MICP	<i>Bacillus cereus</i>	Castanier et al. [27]	France Greece Italy Spain
		<i>Pseudomonas stutzeri</i>	Ranalli et al. [28][29][30]; Bosch-Roig et al. [31]	
		<i>Pseudomonas aeruginosa</i>	Ranalli et al. [28][30]	
		<i>Pseudomonas pseudoalcaligenes</i>	Alfano et al. [32]	
		<i>Pseudomonas chlororaphis</i>	Daskalakis et al. [33]	
Liquefaction mitigation of soils	MIDP	<i>Paracoccus denitrificans</i>	Rebata-Landa et al. [34]	China USA
		<i>Acidovorax</i> sp.	He et al. [35][36]; He and Chu [37]	
		Mixed culture of bacteria from natural sand	O'Donnell [2]; O'Donnell et al. [38][39]	

## 4. Challenges in Denitrification-Based MICP/MIDP Biotechnology

Although denitrification-based MICP and MIDP biotechnology have been successfully demonstrated in many laboratory experiments and in several trials in the field, there are several challenges hindering the natural and commercial-scale applications of this technique. **Table 3** summarizes the up-to-date challenges in upscaling of the approach as: (i) including the generation of harmful intermediates, (ii) environmental impacts, (iii) monitoring the remediation process, (iv) control of gas generation, (v) the low rate of  $\text{CaCO}_3$  precipitation, and (vi) the homogeneous distribution of the treatment impact.

**Table 3.** Challenges faced in the real application of MICP and MIDP through denitrification, and the strategies to mitigate those challenges.

Challenges in In-Situ Applications	Strategies to Mitigate Those Challenges
Generation of harmful intermediates	Avoid by ensuring the completeness of reactions (i.e., proper substrate concentration) Use for other applications (nitrite can be utilized as a commercial anodic rebar corrosion inhibitor) Treat the harmful intermediates on site or collection after the application is done
Environmental factors	Stimulation of inactive cells in the field by providing appropriate nutritional conditions Incorporation of a functional isolate or a non-axenic microbial community into the application field to enumerate the number of functional microorganisms combined ureolysis and denitrification process
$\text{CaCO}_3$ precipitation rate	Proper substrate concentration Applying an optimized substrate regime and residence time Isolate and select more appropriate strains adding iron nanoparticles
Controlling of gas generation	Control the generation, distribution, and persistence of the gas applying an optimized substrate regime and residence time proper substrate concentration
Obtaining homogeneous treatment	Uniform distribution of microorganisms and solution chemistry Applying an optimized substrate regime and spatial distribution
Monitoring	Mathematical model

The first challenge of this biotechnology is to avoid the accumulation of harmful intermediates by ensuring a complete denitrification reaction. Although the end product of denitrification is harmless nitrogen gas, three toxic intermediates, that is, nitrite, nitric oxide, and nitrous oxide, can accumulate when incomplete microbial nitrate reduction occurs [6]. The only exception to this is that the intermediate nitrite is functional as a commercial anodic rebar corrosion inhibitor in microbial self-healing concrete applications [17][21].

Environmental factors, including pH, temperature, pressure, the concentrations of nutrients (electron donors/acceptors), and the abundance of operative microorganisms in the microbial community vary significantly in the natural soils. In contrast to laboratory experiments, in which most parameters can be controlled, these environmental factors are extremely complex and interfere with each other in natural soils. They affect the activities of the denitrifying bacteria and the generation and transportation of the denitrification reaction products. Thus, another challenge in the application of MICP and MIDP technologies is to design monitoring systems for field applications to quantify the influences of the complexities of these factors in natural soils and subsequent design of suitable microbial cultures for bioaugmentation of the relevant environment.

In terms of  $\text{CaCO}_3$  precipitation, denitrification-based MICP has a slower reaction rate than MICP through ureolysis, so it takes more time for the mechanical properties of soil to reach the desired values [6]. Ureolysis-based MICP has been reported to produce 6%  $\text{CaCO}_3$  ( w/w ) in a few days [40][41], whereas denitrification-based MICP only generates an average of 1–3%  $\text{CaCO}_3$  ( w/w ) within a few weeks to several months [6][2][11]. Although slow precipitation rates seem like a drawback of MICP through denitrification, they enable maintaining microbial activity without occlusion of microbial cells with the precipitated  $\text{CaCO}_3$  crystals. Therefore, applying an optimized substrate regime and residence time can make denitrification based MICP more advantageous over ureolysis in long-term. However, there is no valid optimized procedure for field applications of MICP through denitrification, which remains as an obstacle before the transition of the concept into real life examples.

## 5. Suggestions for Future Work

The findings evaluated in this paper demonstrate that microbial induced desaturation and/or precipitation through denitrification possesses a great potential to solve a wide range of environmental, geotechnical, architectural and structural problems under anoxic conditions in a sustainable, environmentally friendly, and cost-effective manner. Promising MICP-driven applications include microbial self-healing concrete with a corrosion inhibition property, bioremediation of artwork and monuments, treatment of high strength industrial wastewater and soil reinforcement. Most importantly, liquefaction mitigation is a novel and unique MIDP-driven application specific to denitrification pathway.

Along with other microbiological processes, such as urea hydrolysis, aerobic respiration and sulfate reduction, denitrification-based MICP has initiated a revolution in various civil engineering applications. However, there are still many challenges that are needed to be addressed before this biotechnology can be commercialized.

Further exploratory studies should be conducted to enhance the efficacy of the in-situ biogas and biomineral production at the microbial level and at the field scale ( **Table 3** ). Ureolytic bacteria ( *Sporosarcina pasteurii* ) is recognized as the most suitable microbe for MICP via ureolysis, but no specific denitrifying bacteria is widely accepted to be the most useful for denitrification-based MICP. Therefore, initial efforts should be made to isolate and select a model organism or develop a microbiome with superior carbonate precipitation yield (i.e., denitrification abilities). Furthermore, more tightly controlled experiments focusing on the key factors would be useful for understanding, optimizing, and successfully developing denitrification technologies. One key factor is the



substrate concentration, namely, of the electron acceptor (nitrate) and the organic carbon donor (e.g., formate, acetate, methanol, and ethanol), which affect the conversion rate of the denitrification reactions and the production of the intermediates. Other key factors include, but are not limited to, temperature, pH, pressure, grain size distribution, and salinity. Considering the complexity of natural soils and groundwater, a novel method, which may be helpful in future research, is a combination of metabolic pathways in a way that one process dominates the conditions in which nitrate and carbon source are present under anoxic conditions, and the latter process dominates when the environment is oxic. In addition, special efforts should be made to evaluate the long-term efficacy of denitrification-based MICP and MIDP in different applications. Currently, many studies are working on adding some environmentally friendly additives like nanoparticles and mainly iron nanoparticles for the removal of wastewater contamination [42][43][44]. The results are proving that these nanoparticles have a positive effect on the anaerobic digestion process and the bacterial growth [42], which in turn could have a positive effect on the denitrification process, thereby, efforts could be made to test the efficiency of MICP as well as MIDP by adding iron nanoparticles to the reaction systems. Finally, although a biogeochemical model (no-flow condition), has been developed to simulate the process of MIDP via denitrification by O'Donnell et al. [45], which is an upgraded version of the model created by O'Donnell et al. [2], mathematical models should be further studied to account for continuous flow.

The successful development and implementation of the denitrification-based MICP and MIDP processes described in this paper could also be used for other applications. Owing to their abundance in subsurface soils and groundwater, denitrifying bacteria and denitrification based MICP can be exploited for co-precipitation of minerals and metals enabling in-situ remediation of metal contaminants and radionuclides in anoxic conditions.

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