

Review

Microbially Induced Desaturation and Carbonate Precipitation through Denitrification: A Review

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Abstract: 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. In this paper, we present the findings on MICP through denitrification by covering applications under two main titles: (i) applications solely based on MICP, such as soil reinforcement, development of microbial self-healing concrete, restoration of artwork and historical monuments, and industrial wastewater treatment, (ii) an application based on MIDP: liquefaction mitigation. After explaining the denitrification process in detail and describing the MICP and MIDP reaction system occurring through denitrification metabolism, the most recent advances in each potential field of application are collected, addressing the novel findings and limitations, to provide insights toward the practical applications in situ. Finally, the research needs required to deal with the defined challenges in application-oriented upscaling and optimization of MICP through denitrification are suggested. Overall, collected research findings revealed that MICP through denitrification possesses a great potential to replace conventionally used petrochemical-based, labour intensive, destructive and economically unfeasible techniques used in construction industry with a bio-based, labourless, low-carbon technology. This worldwide applicable bio-based technology will facilitate the sustainable development and contribute to the carbon-emission-reduction.

Keywords: nitrate reduction; nitrogen gas; calcium carbonate; liquefaction mitigation; self-healing concrete; ground improvement



Citation: Lin, W.; Lin, W.; Cheng, X.; Chen, G.; Ersan, Y.C. Microbially Induced Desaturation and Carbonate Precipitation through Denitrification: A Review. *Appl. Sci.* **2021**, *11*, 7842. <https://doi.org/10.3390/app11177842>

Academic Editor: Daniel Dias

Received: 19 July 2021

Accepted: 19 August 2021

Published: 25 August 2021

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1. Introduction

In nature, under a wide variety of conditions, organisms have been reported to directly or indirectly mediate the formation of over 60 different mineral types (e.g., carbonates, oxides, silicates, and sulfides) through a process called biomineralization [1]. Carbonates are perhaps the most studied minerals formed by microbes. Microbially induced carbonate precipitation (MICP) can occur as a result of the conventional metabolic pathways, including oxygenic photosynthesis, aerobic respiration, ureolysis, ammonification, nitrate reduction (denitrification), sulfate reduction, iron reduction and methane oxidation [2–8]. In Table 1, MICP studies exploiting different microbial metabolisms and metabolism-specific biochemical reactions that lead to the CaCO₃ precipitation were given. As seen in the reactions, each

of the metabolisms generates dissolved inorganic carbon and except aerobic respiration all microbial pathways generate alkalinity in the microenvironment of the bacteria.

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

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

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.

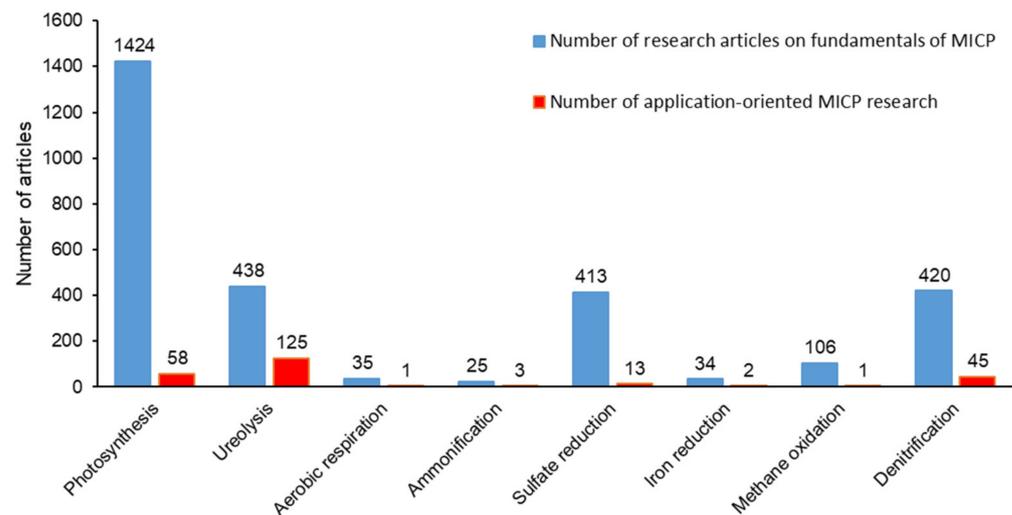


Figure 1. Number of research papers conducted on MICP through different microbial metabolic pathways based on the database of Web of Science from 2000 to 2021. The first column represents the search on the topic of “calcium carbonate” OR “calcite precipitation” OR “carbonate precipitation”, and the keyword 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 urea hydrolysis, ureolytic bacteria produces urease enzyme which catalyzes the hydrolysis of urea into carbonate and ammonium, and thus favors an increase in the pH leading to the precipitation of CaCO_3 in the presence of free calcium ions. This approach has been demonstrated in many laboratory studies and several field technical applications, especially in enhanced oil recovery (EOR), heavy metal removal, atmospheric CO_2 sequestration, soil improvement, and construction restoration [9–15].

Most of the ureolytic bacteria are strictly aerobic organisms live in soil deposits, and they were used as model organisms in a majority of the studies [16]. However, ureolytic bacteria are not ubiquitous in extreme environmental conditions, such as nutrient and O_2 deficiencies, desiccation, high pressures, high pH, and high salt concentrations [13,17]. For instance, their growth, and the production of urease are inhibited by anoxic conditions. This results in gradually decreasing urease activity and survival rate of the bacteria over time in different MICP applications such as self-healing concrete, soil reinforcement and biogrouting, due to the lack of oxygen in the deeper zones of the concrete crack and sand/soil layers, respectively [18]. Moreover, the utilization of ureolysis for soil reinforcement under the groundwater table and in oil reservoirs (i.e., in an anaerobic environment) becomes unlikely. Furthermore, the side product of ureolysis is ammonia, which is known for its toxicity in aquatic environment [19]. In addition, there are extra costs related to the removal of ammonium in groundwater [8]. Therefore, alternative microbiological processes capable of inducing CaCO_3 precipitation should be tested and considered to determine the most sustainable option for use in different practical applications.

Van Paassen et al. [8] evaluated the theoretical feasibility of ureolysis and three alternative MICP processes (i.e., denitrification, aerobic oxidation, and sulfate reduction) for soil reinforcement based on four factors: (i) substrate solubility, (ii) the calcium carbonate precipitation rate, (iii) calcium carbonate yield and (iv) the amount and type of the by-product. Overall, denitrification was determined to be the most suitable MICP method.

In MICP through denitrification, the denitrifying bacteria are introduced into the soil together with a carbon source as an electron donor, nitrate (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 H^+ from the environment, the denitrification process leads to the production of alkalinity which further converts part of the produced CO_2 gas into CO_3^{2-} (Table 1). Consequently, nitrogen (N_2) and CO_2 gases are generated, while $CaCO_3$ is precipitated out of the solution in the presence of free Ca^{2+} ions (Table 1). If the aforementioned process occurs in a saturated porous environment, biogas production (N_2 and CO_2) and mineral ($CaCO_3$) precipitation result in partial desaturation of the porous media and thus changes in its hydromechanical behavior. The process of simultaneous desaturation and $CaCO_3$ 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 $CaCO_3$ precipitation occurring in porous environments upgraded MICP to a new level named as microbially induced desaturation and precipitation (MIDP) [17,20].

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 $CaCO_3$ 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.

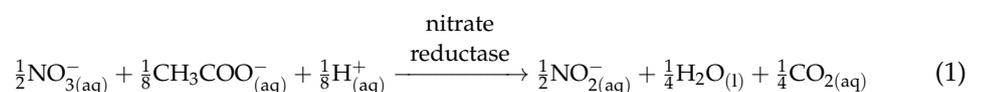
2. The Denitrification Mechanism

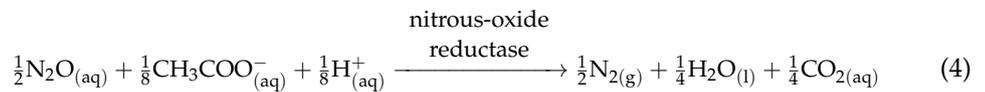
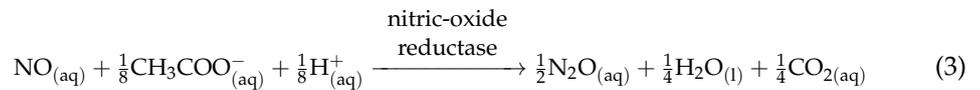
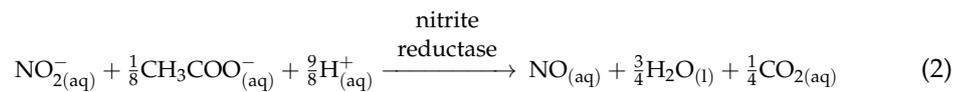
Organisms that are capable of denitrification, that is, denitrifying bacteria, are widely distributed with a high density in nature. These types of microorganisms are common in a variety of environments, and in agricultural soil they reach a population density of the order of 10^6 microorganisms/g of soil [21]. 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 [22]. More than 50 genera have been identified, including *Bacillus*, *Alcaligenes*, *Diaphorobacter*, *Pseudomonas*, *Spirillum*, *Paracoccus*, *Thiobacillus*, and *Achromobacter* [23]. Thus, so far, many studies have used different denitrifying bacteria to link their nitrate reduction activity with either $CaCO_3$ precipitation or biogas generation or the combination of both (e.g., [8,13,20,21,24]).

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_2 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 [2].

2.1. The Intermediates of Denitrification

Denitrification involves a series of dissimilatory microbial reductions of nitrogen, beginning with NO_3^- , through NO_2^- , NO , and N_2O and ending up with the production of N_2 gas Equations (1)–(4):





Each reduction step is driven by a different microbial enzyme in the relevant environment. Denitrifying bacteria use a consecutive enzymatic pathway that is composed of four steps, driven by four enzymes (Equations (1)–(4)) in the periplasmic and/or inner membrane [23]. These reactions are essentially anaerobic respiration pathways that can be used by a great variety of microorganisms, mostly heterotrophic bacteria, which have the ability of using nitrate as an electron acceptor. The above biological denitrification process is an irreversible reaction that produces OH^- , which increase the pH of the surrounding medium (Figure 2).

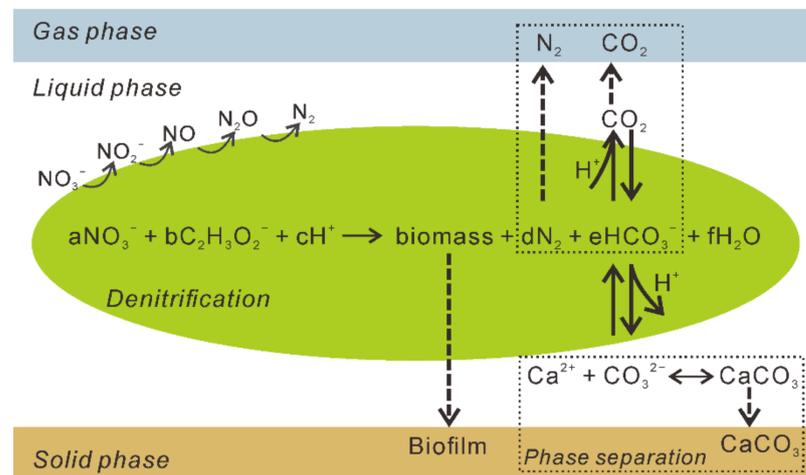
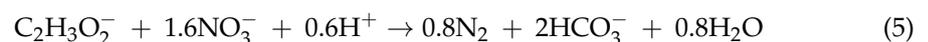


Figure 2. The complete reactions for MICP through denitrification (modified from Pham et al. [25]).

2.2. Stoichiometry of Complete Denitrification

Following the methodology of Heijnen et al. [26] and Heijnen and Kleerebezem [27], the metabolic reactions used for modeling include the stoichiometry of complete denitrification which can be divided into an anabolic reaction and a catabolic reaction. The stoichiometry of the two reactions is calculated separately by figuring out the mass and electron balances for each reaction. The ratio between the catabolic and anabolic reactions is determined by solving the energy balance.

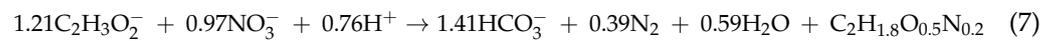
The catabolic reaction produces the required energy for the cells to convert nutrients into new biomass. Using acetate (CH_3COO^-) as the electron donor and nitrate as the electron acceptor in catabolism, the following redox reaction occurs (Equation (5)):



The stoichiometry of the anabolic reaction given in Equation (6) indicates the production of 1 C-mol biomass from the supplied carbon source and nitrogen source.



Solving the energy balance for maximum growth, the stoichiometry of the overall metabolism (pH 7, temperature 298 K) becomes:



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

2.3. Inducing Calcium Carbonate Precipitation through Denitrification

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 [23], which favor the generation of dissolved inorganic carbon (DIC). Inorganic carbon dissociates into CO_2 , bicarbonate HCO_3^- , and CO_3^{2-} in aqueous solutions. If a solution with a high pH and total inorganic carbon content contains excess dissolved Ca^{2+} , CaCO_3 will precipitate [8], and the system will transfer to the solid phase (Figure 2). The denitrification process can be expressed by the equations using acetate (CH_3COO^-) as the electron donor (Table 1). Furthermore, the production of CaCO_3 , 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 [28]. The full reaction system of the denitrification-based MICP is given in Figure 2.

3. Advantages of Denitrification Metabolism in MICP

Denitrification has been proposed as a promising alternative metabolic pathway for biomineralization applications in subsurface environments because it possesses several advantages over other microbial metabolisms when MICP process is considered [8]. Denitrifying bacteria are widespread in subterranean environments, and as such, MICP through denitrification can be achieved using the indigenous populations of denitrifying bacteria in the relevant application area [29]. Different from the oxygen dependent microbial metabolisms such as ureolysis and aerobic respiration, denitrification occurs in the presence of nitrate in oxygen-deficient subsurface environments. When specific strains are used, denitrification can even occur in the absence of micronutrients. Ersan et al. [30] revealed that significant CaCO_3 can be precipitated in minimal nutrient conditions (only in the presence of macronutrients) by either *Diaphorobacter nitroreducens* or *Pseudomonas aeruginosa* culture. Moreover, compared to other microbial metabolic activities (except aerobic respiration), denitrification has a negative change in Gibbs free energy (-785 kJ/mol acetate) under standard conditions and in fact it surpasses the free energy of ureolysis (-27 kJ/mol acetate) and the other anaerobic metabolic pathways [6,8]. Therefore, denitrification is thermodynamically more suitable than all the other metabolic pathways except aerobic respiration, and thus, in various MICP applications, it can be expected to dominate at greater depths where presence of oxygen is unlikely.

Unlike aerobic respiration, MICP through denitrification does not rely on external alkalinity, as the process itself generates enough alkalinity to raise the pH to levels favoring calcium carbonate precipitation [24]. Furthermore, when complete nitrate reduction (denitrification) occurs, the side products of denitrification (i.e., N_2 gas and possibly small amounts of unprecipitated CO_2) are non-toxic and chemically inert, whereas the gases produced in other metabolic pathways such as, ammonia (from ureolysis), and hydrogen sulfide (from sulfate reduction), may pose risks for environment, structures and human health [11,24]. In addition, denitrification yields a larger calcium carbonate precipitation per mole of electron donor (i.e., acetate) than other MICP pathways (Table 1). This greater production of carbonate promotes more precipitation of CaCO_3 per mole of external substance. Overall, MICP through denitrification appears to be more sustainable for applications in situ.

4. 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 [24]; Hamdan [31]; O'Donnell [20]; Hamdan et al. [32]	Netherlands
		<i>Castellaniella denitrificans</i>	Van Paassen et al. [8]	UK
		<i>Halomonas halodenitrificans</i>	Martin et al. [29]	USA
Self-healing concrete	MICP	Denitrifiers from natural soil	Pham [17]; Pham et al. [25,33]	Belgium
		Nitrate reducing biogranules	Ersan et al. [30,34,35]; Ersan [36]	
Sewer corrosion resistant concrete	MICP	<i>Diaphorobacter nitroreducens</i> <i>Pseudomonas aeruginosa</i>	Song et al. [37]	Australia
Corrosion inhibition of steel in reinforced concrete	MICP	Nitrate reducing biogranules <i>Diaphorobacter nitroreducens</i> <i>Pseudomonas aeruginosa</i>	Ersan et al. [34,38]	Belgium
Treatment of industrial wastewater (calcium, nitrate, zinc, nickel, fluoride removal)	MICP	<i>Diaphorobacter nitroreducens</i> <i>Pseudomonas aeruginosa</i>	Ersan et al. [30]	Belgium
		Sludge from the biological treatment of leachate Sludge from a Sewage Treatment Plant	Fernandez-Nava et al. [39]	China Japan Spain
Remediation of artwork and historical monuments	MICP	<i>Acinetobacter</i> sp.	Aoki et al. [40], Fan et al. [41], Liu et al. [42], Su et al. [43]	France Greece Italy Spain
		<i>Bacillus cereus</i>	Castanier et al. [44]	
		<i>Pseudomonas stutzeri</i>	Ranalli et al. [45–47]; Bosch-Roig et al. [48]	
		<i>Pseudomonas aeruginosa</i>	Ranalli et al. [45,47]	
		<i>Pseudomonas pseudoalcaligenes</i>	Alfano et al. [49]	
Liquefaction mitigation of soils	MIDP	<i>Pseudomonas chlororaphis</i> <i>Paracoccus denitrificans</i>	Daskalakis et al. [50]	China USA
		<i>Acidovorax</i> sp.	Rebata-Landa et al. [51] He et al. [52,53]; He and Chu [54]	
		Mixed culture of bacteria from natural sand	O'Donnell [20]; O'Donnell et al. [55,56]	

4.1. MICP-Driven Applications

4.1.1. Soil Reinforcement

In many areas of the world, the in situ physical properties of soil (e.g., shear strength, stiffness, compressibility, permeability, and hydraulic conductivity) are often insufficient to meet the demands of human being. There are three methods of improving these soil properties, namely, mechanical compaction, chemical grouting, and biological techniques, such as biogrouting [57]. Among them, biogrouting based on MICP is considered to be a promising new technique because it is more environmentally friendly and cost-effective, and it does not mechanically/chemically disrupt the infrastructure [9,20]. Over the past decade, many studies have evaluated the exploitation of MICP through denitrification for biomediated soil improvement under anaerobic conditions at the laboratory scale.

Karatas [24] conducted soil column experiments and reported the CaCO_3 precipitation yield in the range of 1.3–10.6 g CaCO_3 (s)/g NO_3^- , in 2–40 days which resulted in weak cementation of sand particles (Table 3). This was the first study to demonstrate that microbial nitrate reduction may be a feasible mechanism for soil improvement. Van Paassen et al. [8] also reported the successful application of denitrification-based MICP for soil improvement by using *Castellaniella denitrificans*. They reported that about 6 g CaCO_3 (s)/g NO_3^- was precipitated in seven days. After 100 days, the CaCO_3 content (by mass) at the top of the sand column was ~10%, and it gradually decreased with depth to less than 1% at the bottom side. Furthermore, based on the observed conversion rate in their study, it was claimed that about 60 days would be enough to reach a target amount of 100 kg of CaCO_3 per cubic meter of soil, which was still far from the amount required for practical engineering applications. Martin et al. [29] demonstrated that denitrification could be used to induce CaCO_3 precipitation in subsurface environments under anoxic and highly saline conditions at pressures of up to 8 MPa. In addition, they revealed that denitrification based MICP could be used for biocementation of fine sand particles and coarse gravel. After that, some attempts were conducted to improve the efficiency of MICP through denitrification. Pham et al. [25] used multiple substrate flushes for the total period of 65 days to obtain an average CaCO_3 content of approximately 1.1% in a sand column, and reported that regardless of the substrate type the most efficient microbial activity was achieved when the carbon to nitrogen ratio was about 1.6 (Table 3). Subsequently, Pham et al. [33] further optimized the experimental process by increasing the number of flushes, lowering the substrate concentrations (e.g., 12 mmol/L $\text{Ca}(\text{CH}_3\text{COO})_2$ and 10 mmol/L $\text{Ca}(\text{NO}_3)_2$), and decreasing the hydraulic residence time (2–3 days). They reported that under the optimized conditions, an average CaCO_3 content (by mass) of 0.65% could be achieved after five weeks of treatment, and precipitation rate could reach up to 0.26% CaCO_3 by weight/day. The treatment resulted in a distinct increase in the small strain stiffness, which indicated that denitrification-based MICP may be sufficient for practical soil improvement applications. However, a field application of biocementation through denitrification has not yet been reported.

Table 3. Overview of key findings reported in the literature for soil improvement by denitrification-based MICP.

Author and Ref	Electron Donor (Concentration)	NO ₃ ⁻ Consumed (mM)	Initial Ca ²⁺ Concentrate (mM)	CaCO ₃ (by Weight%) Range Reported	Time (Days)
Karatas [24]	NB* (16.7 g/L)	8	20–100	ND*	2–40
	Acetate (75 mM)	12			
	L-glutamic acid (50/75 mM)	15			
		25			
Van Paassen et al. [8]	Acetate (24 mM)	16	20	Less than 1% at the bottom to 10% at the top of the sand column	100
	Acetate (60 mM)	40	50		
	Acetate (120 mM)	80	100		
	Acetate (240 mM)	160	200		
Martin et al. [29]	Acetate (45 mM)	50	100	6% at the top to 42% at the bottom	1.5
Hamdan et al. [32]	NB* (20 g/L)	17.6	20	≈0.01%	7
	L-glutamic acid (75 mM)				
Pham et al. [25]	Acetate (160 mM, first three flushes)	100 (first three flushes)–120 (fourth to ninth flush)	130 (first three flushes)–120 (fourth to ninth flush)	Average 1.1%	65
	Acetate (130 mM, fourth to ninth flush)				
Pham et al. [33]	Acetate (120 mM, three flushes)	100 (three flushes)	110 (three flushes)	0.28% by weight 0.65% by weight	35
	Acetate (24 mM, 15 flushes)	20 (15 flushes)	22 (15 flushes)		

ND: Not Determined, NB: Nutrient Broth

Considering the scope of sustainable development and the transition towards a low carbon economy, in the field of geotechnical engineering, it is necessary to minimize the use of petrochemical-based products and/or environmentally hazardous chemicals, decrease the carbon emissions and avoid energy intensive treatment processes. These requirements created a great challenge in the field and provoked experts to reconsider the conventionally used ground improvement techniques in terms of their sustainability. The aforementioned promising findings revealed that MICP through denitrification possesses a great potential as a sustainable ground improvement technique and thus may find itself a solid place in the field in the near future.

4.1.2. Remediation of Cracks and Inhibition of Steel Corrosion in Reinforced Concrete

Denitrification occurs when bacteria uses nitrate as an electron acceptor to oxidize organic matter under anoxic conditions. Therefore, it has the benefit that precipitation can occur in oxygen-limited environments, such as the inner part of a concrete crack. Application of the MICP technique via denitrification has recently been reported to provide crack healing by inducing CaCO₃ precipitation within the crack [34,35,58–60]. Ersan et al. [58] was the first to use axenic denitrifying strains of *Pseudomonas aeruginosa* and *Diaphorobacter nitroreducens* protected within either granular activated carbon particles or expanded clay particles as microbial healing agents, in order to test the feasibility of repairing concrete cracks using the MICP through denitrification. Their results showed that these denitrifying axenic strains induced the healing of concrete cracks up to 400 µm crack width upon four weeks (Figure 3a,b), and cracks up to 470 µm crack width upon seven weeks (Figure 3c,d), making their use as effective as commonly proposed microbial methods (e.g., MICP through lactate oxidation or ureolysis), but more environment-friendly [58].

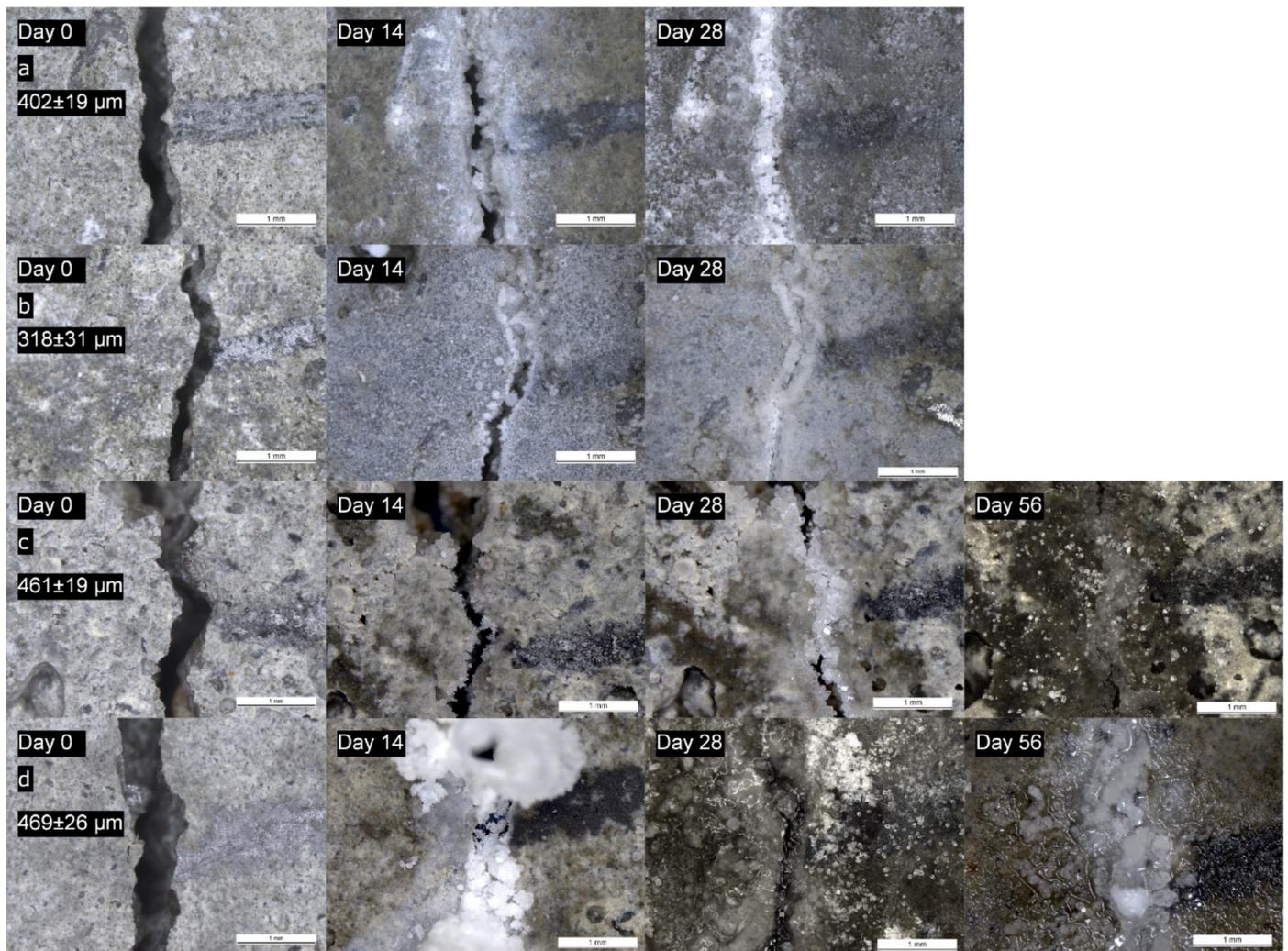
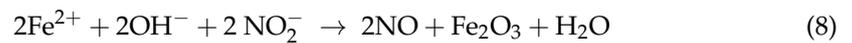


Figure 3. Representative photomicrographs of crack healing in microbial nitrate reduction based self-healing concrete containing (a) *Diaphorobacter nitroreducens* and (b) *Pseudomonas aeruginosa* loaded expanded clay particles; (c) *Diaphorobacter nitroreducens* and (d) *Pseudomonas aeruginosa* loaded granular activated carbon particles (redrafted after Ersan et al. [58]).

Ersan et al. [30] also replaced the protected axenic cultures with self-protected non-axenic biogranules called activated compact denitrifying core (ACDC). ACDC biogranules were compatible with concrete up to 3% *w/w* cement incorporation dose [61] and they could survive in mortar [38], inhibit steel corrosion [34], and induce complete healing of 500 μm -wide cracks upon four weeks of tap water treatment [60]. Additionally, it was recently reported that even under wet/dry cycles, this nitrate reducing biogranule containing bioconcrete can self-heal cracks as wide as 400 μm [36]. They also tested the crack healing performance in biogranule (ACDC) containing aged concrete and found out that the developed bioconcrete was effectively self-healing the cracks in mature specimens [60]. Further investigations via X-ray μCT scanning of healed and unhealed mortar specimens with a 400 μm wide initial crack width revealed that after four weeks of healing period, the healed crack volume was 6% and the thickness of the calcite layer was 10 mm which decreased the water permeability of cracked concrete by 83% [35].

A significant added benefit of using denitrifying cultures in self-healing concrete was reported as the simultaneous corrosion inhibition of steel rebar during autonomous crack healing process. In their study, Ersan et al. [34] benefit from the production and accumulation of NO_2^- , a well-known anodic corrosion inhibitor, as a metabolite during MICP through denitrification in a microbial self-healing concrete. In the construction sector, chemical compounds of NO_2^- , such as $\text{Ca}(\text{NO}_2)_2$, are used as corrosion inhibiting

admixtures, as NO_2^- rapidly stabilizes the mobile ferrous compounds formed in anodic reactions in the form of ferric compounds (Equation (8)), which hinders the migration of the Fe^{2+} from the anodic zones of the rebar, and thus, inhibit steel rebar corrosion. In this process, the precipitation of ferric oxides locally creates a new passive layer which prevents material loss and inhibits corrosion. Microbially produced NO_2^- inside a concrete crack was found to be as effective as commercially available NO_2^- containing chemical admixtures in terms of rebar corrosion inhibition [34].



Ersan et al. [34] monitored the open circuit potentials of steel rebars either placed in ureolysis or denitrification based self-healing concrete which were immersed in a 0.5 M Cl^- solution for 17 weeks upon crack formation (Figure 4a,b). It was reported that although both of the self-healing concretes completely healed cracks of 300 μm crack width in four weeks, only in denitrification based self-healing concrete, the open circuit potential stayed above the critical value, and steel corrosion was successfully inhibited. It was emphasized that sole crack healing was not enough to protect the steel in aggressive environments which compromised the application of ureolysis based self-healing concrete (Figure 4c,d) in aggressive environments and promoted denitrification based self-healing concrete as a better alternative. Overall, MICP through denitrification is a promising process to develop a multifunctional microbial self-healing concrete that is suitable for structures exposed to marine conditions (wet/dry cycles, aggressive ions, etc.).

Regular monitoring of cracks and the state of the reinforcement bars in immersed or underground concrete structures are grueling. Moreover, maintenance works on structures such as tunnels (underground or underwater), bridges and car parks cause significant pause in the provided service and thus people using these structures are also affected negatively. Additionally, maintenance works to stop corrosion and protect the healthy state of steel reinforcement bars are both labor and cost-intensive. Furthermore, in conventional concrete structures, crack and corrosion related durability issues also cause renewal of these structures long before their actual service life and thus almost double their carbon footprint. As studies on microbial self-healing of concrete cracks revealed that application of MICP through denitrification can avoid the need for external crack repair, similar to other internal self-healing mechanisms, it will avoid the disruptions in the service provided by concrete structures. Additionally, continuous self-healing of microcracks will avoid the need for regular monitoring of concrete structures for cracks. Lastly, recovery of water tightness and microbial corrosion inhibition mechanism will avoid most of the durability issues and prolong the service life of the structures. Overall, with these benefits, novel microbial self-healing concrete can find a solid place in the approaching low-carbon economy era.

4.1.3. Nitrate and Calcium Removal from Industrial Streams

Various industrial wastewaters contain significant amount of nitrate, and unless properly handled, these streams possess a considerable threat against the environment [39,62]. Numerous methods of removing nitrate from wastewater have been performed, such as microbial denitrification, catalytic denitrification, reverse osmosis, electro dialysis, ion exchange and chemical treatment [63]. Among them, microbial denitrification is a promising, cost-effective, and environment-friendly method because it is capable of practically and permanently removing nitrate from water, forming harmless end products [64,65].

The application of microbial denitrification to remove nitrate from wastewater has been studied and reviewed in detail by many researchers [64–66]. In this paper, we focused only on high nitrate and high calcium (generated in the stainless-steel pickling process) containing industrial streams, and the removal of these two ions through denitrification-based MICP. Fernández-Nava et al. [39] investigated the use of seed sludge from the leachate treatment plant and from the sewage treatment plant for nitrate removal from calcium rich industrial streams. Microbial denitrification generates alkalinity (Table 1, Figure 2) in the form of CO_3^{2-} and HCO_3^- ions. When the wastewater is rich in Ca^{2+} , the

generated alkalinity leads to the precipitation of CaCO_3 minerals, as well as the other calcium compounds (i.e., CaF_2 , $\text{Ca}_3(\text{PO}_4)_2$) by increasing the pH of the media. The process removes 90% to 96% of the calcium ions in the wastewater and enables reuse of the treated water as process water without causing significant crust formation in the pipeline network [39].

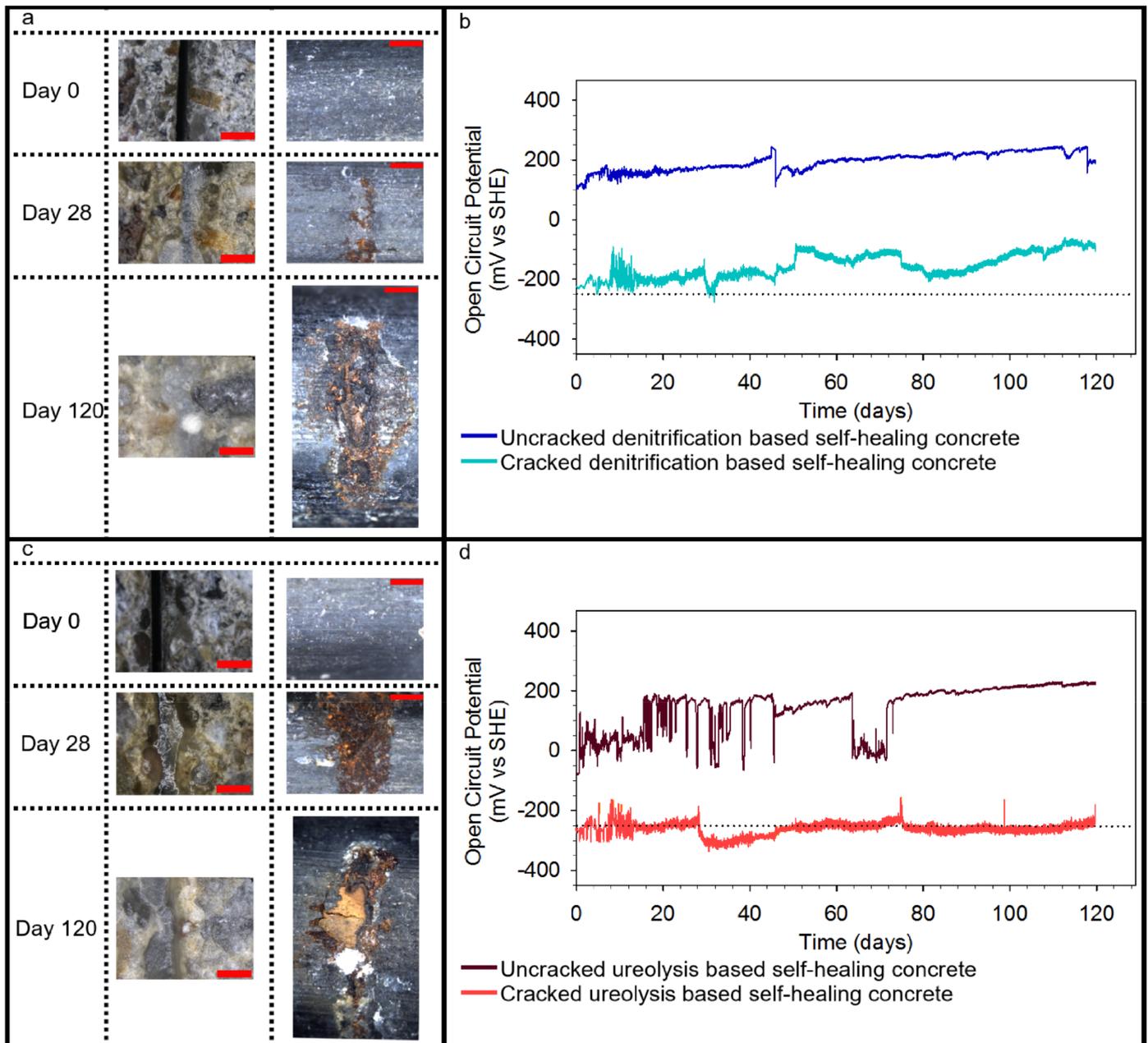


Figure 4. Revealing the simultaneous corrosion inhibition function of denitrification-based microbial self-healing concrete by comparing the evolution of 300 μm wide cracks, embedded steel rebar surfaces and the open circuit potentials (OCP) of the rebars (a,b) in denitrification based microbial self-healing concrete; and (c,d) in ureolysis based microbial self-healing concrete. The dashed horizontal line (···) represents the critical OCP value (−250 mV) for Fe in the tested Cl[−] solution. Scale bars represent 1 mm (based on Ersan et al. [34]).

Ersan et al. [30] used two types of denitrifying bacteria, *Pseudomonas aeruginosa* and *D. nitroreducens*, as well as the indigenous non-axenic microbial community of paper mill wastewater treatment plant, to evaluate the feasibility of exploiting denitrification-based MICP to remove Ca^{2+} from paper mill wastewater. They reported that both strains and

the indigenous microbial community in the paper mill wastewater performed similarly with a CaCO_3 precipitation yield of 12.7 g CaCO_3 /g $\text{NO}_3\text{-N}$ at the end of four days of incubation. Those findings showed that denitrification based MICP can be an effective strategy to remove Ca^{2+} from paper mill wastewater and the indigenous microbial species in already operating paper mill wastewater treatment plant can be exploited for such application. However, the addition of external nitrate was reported to be the drawback of this approach as the wastewater generated in the paper mill industry does not contain enough nitrate for effective removal of calcium. In order to optimize calcium and nitrate removal from industrial streams, and maximize microbial activity for generation of reusable treated water, one might consider combining high nitrate and high calcium wastewaters of different industries.

Industrial wastewaters mostly contain hazardous contaminants such as heavy metals in high concentrations. Most of the conventional treatment technologies either use chemicals to trigger chemical precipitation of those pollutants or high-cost and energy intensive technologies such as electrolysis, ion exchange and membrane separation. The aforementioned results indicated that MICP through denitrification can be a more sustainable treatment alternative, as removal of unwanted toxic heavy metals by immobilizing them inside the CaCO_3 precipitates is also possible. In MICP, it might also be possible to precipitate heavy metals in the form of metal carbonates. The latter was also discussed in a separate section in Section 4.1.5.

4.1.4. Remediation of Artwork and Historical Monuments

Physical, chemical and biological weathering is a common deterioration mechanism in various types of artwork, such as stone materials, lithoid materials, paintings, and frescoes [67,68]. As a result, the surfaces of artwork and monuments are damaged and altered by nitration, sulfation, black crust, and the accumulation of dust and residual hydrocarbons [67]. Moreover, the mineral matrix of stonework can dissolve causing an increase in the material porosity and a decrease in mechanical properties [69]. Compared to the traditional physical and chemical approaches, biobased solutions are considered to be more sustainable for the remediation and restoration of artwork and historical monuments because they provide powerful, inexpensive, gentle and environmentally friendly solutions and pose a low risk to human health [44]. Castanier et al. [44] successfully used a denitrifying bacteria (*Bacillus cereus*) to induce carbonate precipitation to protect and regenerate the limestone, which is the base material used in statuary, and also in buildings of historic patrimony.

Some other strains of denitrifying bacteria, such as *Pseudomonas stutzeri* and *Pseudomonas aeruginosa*, have also been employed in the removal of nitrates, mineral salts, and organic matter from the stones, frescoes and wall paintings of cultural heritage monuments under laboratory conditions [45–47,49]. Daskalakis et al. [50] reported the potential of MICP for ornamental stone biorestoration and protection by using the isolated microorganism (i.e., *Pseudomonas chlororaphis*) from marble stones. The results demonstrated that the entire surfaces of the marble were covered with vaterite after 10 days and they were stable throughout the experimental runs.

4.1.5. Heavy Metal and Metalloid Immobilization

MICP revealed as a great potential for the immobilization of metals and metalloids as well as their recovery from wastes, thus protecting human health and the entire natural environment. Several microorganisms, such as fungi and ureolytic bacteria, with the carbonate precipitation abilities were reported for the removal of various heavy metals and metalloids [12,70–72]. In immobilization of heavy metals and metalloids, the adequate divalent metals (e.g., Cu^{2+} , Cd^{2+} , Co^{2+} , Zn^{2+} and Pb^{2+}) are integrated into the calcium carbonate lattice during a competitive co-precipitation mechanism given in (Equation (9)) and thus removed from the environment.



where, M^{2+} represents a divalent metal ion.

The removal percentages could reach up to 100%, confirming the applicability of MICP based metal removal and recovery [13,70,72,73]. As discussed above, denitrification was proven as an effective and environment friendly metabolic pathway for a wide range of MICP applications [24,25,30], which paves the way for further investigation of denitrification based MICP for metal and metalloid immobilization, particularly in anoxic environments.

4.2. MIDP-Driven Applications

Liquefaction Mitigation

When the soil is saturated, cyclic loading induces an undrained response, leading to the generation of excess pore pressures. These excess pore pressures may rise to the extent that the soil loses all shear strength and thus behaves like a fluid, and undergoes a large deformation (Figure 5a–c). Soil liquefaction can cause disastrous consequences to buildings and loss of human lives. Conventional liquefaction mitigation methods include densification, solidification, drainage and reinforcement of the soil. In Table 4, the mechanism of each conventional liquefaction mitigation method was described. As described in detail in Table 4, some of these methods are not suitable for mitigating the liquefaction potential beneath or near the existing structures because of their disruptive nature, environmental impacts or high cost [74]. Additionally, nondisruptive methods to mitigate liquefaction beneath or near existing structures appeared to be economically unfeasible (Table 4). Disadvantages and limitations of each method which arise the need for a more sustainable and non-disruptive liquefaction mitigation methods were also described in Table 4.

Table 4. Comparisons of the existing liquefaction mitigation methods.

Liquefaction Mitigation Methods	Mechanisms	Costs	Negative Effects	Limitations	Author and Ref
Densification	Densifying the existing soil, increasing the strength and stiffness of soil	The cost of labor and grout materials start at about 20/m ³ of improved soil	Making the soil more dilatant, producing ground settlement, and disrupting nearby existing structures and utilities	Limited in finer grained liquefiable soils	O'Donnell [20]; Andrus and Chung [74];
Solidification	Injecting or mixing cementitious materials (e.g., Portland cement or polymers) into the soil to solidify the soil mass.	The cost of injection labor and grout materials varies from 100/m ³ to 320/m ³ of improved soil	Environmentally unfriendly	Limited by the ability of grout to pass through pore spaces and relatively uniformly permeate the soil	O'Donnell [20]; Andrus and Chung [74];
Drainage	Installation of free draining materials to mitigate the buildup of excess pore pressures during cyclic loading	2.5/m for prefabricated vertical drains and 10/m ³ for preloading	Excessive vibrations leading to producing ground settlement, and disrupting nearby existing structures and utilities	When using gravel drains and prefabricated vertical drains, they do nothing to mitigate seismic settlement	O'Donnell [20]; Andrus and Chung [74]; Kim et al. [75]
Reinforcement	Installation of reinforcing elements to improve the strength and stiffness of a soil mass	118/m ² to 134/m ² for geotextile reinforced soil	Environmentally unfriendly, and excessive vibrations leading to producing ground settlement, and disrupting nearby existing structures and utilities	Can be affected by a variety of factors, including the soil type, ground water conditions, grout mix, injection rate, jet pressure, withdrawal rates, etc.	O'Donnell [20]; Andrus and Chung [74]; Durukan and Tezcan [76]

Table 4. Cont.

Liquefaction Mitigation Methods	Mechanisms	Costs	Negative Effects	Limitations	Author and Ref
Desaturation	Inclusion of a small amounts of gas in the soil can add considerable compressibility to the pore fluid, mitigating the buildup of excess pore pressures	The estimated cost of electron donor and acceptor for 10% (volume of gas/volume of water) desaturation of soil with porosity 50% is from 0.25 to 0.31/m ³ of saturated soil.	Traditional desaturation methods, such as dewatering or lowering of the groundwater table through continuous pumping may causing slightly settlement	May not lead to long term desaturation in clean sands or gravel	Tsakamoto et al. [77]; He et al. [52]; Li et al. [78]

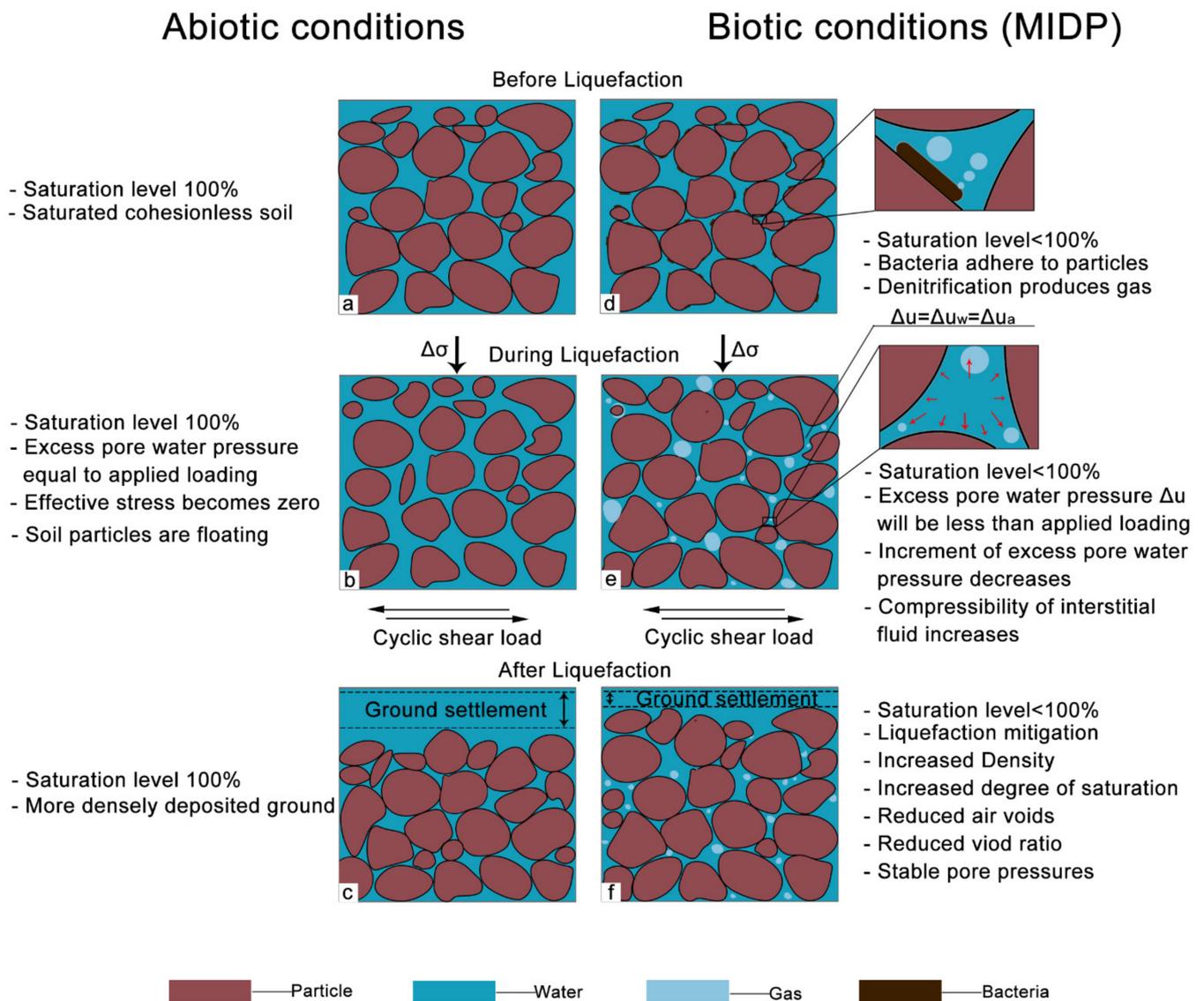


Figure 5. Schematic representation of the comparison between the liquefaction behavior of untreated saturated soil (a–c) and upon denitrification based MIDP (d–f), (a) fully saturated soil; (b) liquefaction of fully saturated soil after cyclic loading; (c) ground settlement due to liquefaction; (d) production of gas bubbles through microbial denitrification upon bioaugmentation or biostimulation of soil; (e) the decrease in excess pore water pressure of gas entrapped soils during cyclic loading; (f) ground settlement of partially saturated soil after cyclic loading.

Many previous studies have demonstrated that inclusion of small amounts of gas bubbles in soil results in a decrease in the degree of saturation which significantly increases

the compressibility of the pore fluid, and hence the cyclic resistance of the soil can be enhanced [77,79–83]. As shown in Figure 5e, a section of soil is under overall confining effective stress σ'_3 . When an external load ($\Delta\sigma$) is applied to the soil, by Terzaghi's effective stress principle, the change in effective stress is:

$$\Delta\sigma' = \Delta\sigma - \Delta u \quad (10)$$

where, $\Delta\sigma'$: change in effective stress, $\Delta\sigma$: external load, Δu : excess pressure.

The excess air pressures (Δu_a) and water pressures (Δu_w) generated in the voids are equal if the surface tension between air and water is neglected due to air being in bubble form [84]. That means when an external load is applied, air and water in the pores experience the same excess pressure Δu .

$$\Delta u_a = \Delta u_w = \Delta u \quad (11)$$

where, Δu_a : excess air pressure, Δu_w : excess water pressure.

Based on the above equations, the relationship between the excess pore water pressure (Δu) and the external load ($\Delta\sigma$) is deduced by Eseller-Bayat [85] from the formula given below:

$$\Delta u = \frac{1}{1 + \frac{n \left[SC_w + \frac{(1-S)}{u_a} \right]}{C_s}} \quad (12)$$

where, Δu : excess pressure, n : porosity, C_w : compressibility of water (~ 0), C_s : compressibility of soil, S : the degree of saturation.

Considering Equation (12), when $S = 1.0$, since C_w is almost 0, the excess pore water pressure Δu becomes equal to the applied load $\Delta\sigma$. However, when $S < 1.0$, the excess pore water pressure Δu is less than the applied load ($\Delta\sigma$). This means that the inclusion of gas in the voids can reduce the excess pore water, thereby providing mitigation of liquefaction.

Based on the aforementioned theoretical approach, and the results of previous studies reporting liquefaction mitigation, upon injecting bubbles to the saturated soil, another potential application of microbial denitrification has appeared. In this novel approach, microbial denitrification was considered as a novel bio-based method to mitigate the liquefaction of saturated sand through the microbial production of N_2 and CO_2 gases (Figure 5d–f). MICP through denitrification generates N_2 gas which in the soil environment is entrapped between the precipitated crystals or between the bound soil grains. Occupancy of even a small volume of pore space by gas bubbles creates unsaturated conditions which significantly affect the hydromechanical behavior of the soil. Furthermore, the water flow in the unsaturated soil is affected by the growing crystals and varying pore size distribution [86,87] and thus leads to a significant decrease in the hydraulic conductivity of soil [88,89].

The reduction in bulk stiffness of the pore fluid, the Skempton's B parameter, and the P -wave velocity due to the gases generated during microbial denitrification was first demonstrated by Rebata-Landa and Santamarina [51] who brought out microbial denitrification as a novel alternative for mitigating liquefaction of soils. He et al. [52] reported that the in-situ formation of nitrogen gas bubbles through denitrification could reduce the saturation degree of sand to 80–95%. Additionally, their shaking table model tests revealed that upon desaturation of the soil through denitrification, the pore water pressure became lower, and the ground volumetric strain and the amount of settling were smaller than those of saturated soil. The results of He and Chu [54] also revealed that for 88–95% saturated soil, under compression and extension the recorded undrained shear strength ratio was more than two folds higher when compared to those recorded for fully saturated soil. Moreover, a small reduction in the saturation degree ($S < 95\%$) leads to a transition in the undrained stress–strain behavior of the loose sand from static liquefaction to non-liquefaction. Later, He et al. [53] detected the existence of biogas bubbles in microbially desaturated sand using computed tomography. The computed tomography images revealed that in treated sand,

the gas was present as small pockets of pores and these pores were slightly bigger than the average size of the grains.

In the studies of O'Donnell et al. [55,56], MIDP by denitrification were used as a two-stage process to mitigate liquefaction by stimulation of denitrifying bacteria from natural sand. In Stage 1, the carbon dioxide and nitrogen gases produced by the denitrifying bacteria desaturated the saturated soil, thereby increased the cyclic strength of the soil (upwards of 40% improvement) and provided temporary mitigation [55]. Then, in Stage 2, approximately 1.5–2.0% (*w/w*) calcium carbonate precipitated over a period of one year, which increased the physical and mechanical properties (e.g., the strength, stiffness, dilatant behavior, and resistance to cyclic loading) of the soil resulting in a long-term mitigation due to the provided interparticle cementation, void ratio reduction, and roughening of soil particles induced by MICP. These results indicated that desaturation and calcium carbonate precipitation via denitrification could notably mitigate the earthquake-triggered soil liquefaction [56].

Conventional ground improvements (e.g., solidification and reinforcement) for mitigation of soil liquefaction are energy-consuming and expensive, or not suitable for mitigating liquefaction potential beneath or near existing structures (e.g., densification, drainage and reinforcement). An alternative for mitigating liquefaction of soils is induced-partial saturation (i.e., desaturation) that can be conducted by injection of gas in saturated soil and entrapment of gas bubbles there. The advantage of desaturation over other mitigation methods will be its cost and energy effective implementation for new as well as existing structures. Microbe-induced desaturation by denitrification is a novel method, with desaturation via biogas generation providing short term mitigation and interparticle cementation via MICP providing long term mitigation of liquefaction. Overall, MIDP through denitrification shows promise as an environmentally friendly, and cost-effective ground improvement technique for liquefaction mitigation through desaturation via biogas generation and solidification via MICP.

5. 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 5 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 CaCO₃ precipitation, and (vi) the homogeneous distribution of the treatment impact.

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 [8]. 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 [34,38].

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.

Table 5. 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
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

Using nitrogen gas production for soil improvement such as liquefaction mitigation also involves potential challenges, including how to control the generation, distribution, and persistence of the nitrogen gas. Rebata-Landa and Santamarina [51] reported that the gas bubbles formed during denitrification were not all retained in sand with a lower content of fines, resulting in the partial recovery of the degree of saturation without a continuous supply of nutrients. He et al. [52] reported that gas bubbles were unstable in a 1 m high sand column under vertical and/or horizontal flow of groundwater. Moreover, an excess production of gas may induce cracks in the sand under low confinement conditions (e.g., shallow depths), which damage the sand structure and affect the sand column stability [25].

In terms of CaCO₃ 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 [8]. Ureolysis-based MICP has been reported to produce 6% CaCO₃ (*w/w*) in a few days [90,91], whereas denitrification-based MICP only generates an average of 1–3% CaCO₃ (*w/w*) within a few weeks to several months [8,20,25]. 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 CaCO₃ 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.

The final challenge of using MICP technologies for practical applications is the struggle on obtaining a uniform treatment distribution. This is also valid for the other metabolic pathways such as ureolysis. The generation and distribution of nitrogen gas and carbonates are highly influenced by the movement and transport of fluid, which are affected by the solution's chemistry and the existing microbial community [6]. As for groundwater, the chemical content of the injected solution is diluted along the flow direction. In the case of soil, CaCO₃ precipitates quickly form around the injection site, and the carbonate buildup blocks the further transport of the solution and occludes the neighboring pores as well. Thus, in both groundwater and soil, spatial heterogeneity is a common issue. Therefore, more efforts are needed to improve the current approaches to create a homogeneous distribution of both microbes and the available precursors in groundwater and soil. Among the different MICP pathways, MICP through denitrification seems promising as it is not a rapid process, does not rely on presence of oxygen and external alkalinity.

The above-mentioned challenges limit the usage of MICP technologies in real applications. These challenges need to be overcome before the method is upscaled from the laboratory-scale to field-scale applications.

6. 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 5). 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 [92–94]. The results are proving that these nanoparticles have a positive effect on the anaerobic digestion process and the bacterial growth [92], 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. [95], which is an upgraded version of the model created by O'Donnell et al. [20], 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.

The application of biotechnology in different fields of engineering is getting more and more popular, therefore, additional interdisciplinary research, including microbiology, chemistry, geology, and geotechnical engineering, should be conducted by experts worldwide to realize the potential of the current MICP biotechnology.

Author Contributions: Conceptualization, W.L. (Wenbin Lin) and Y.C.E.; methodology, G.C.; investigation, W.L. (Wei Lin); resources, W.L. (Wenbin Lin) and Y.C.E.; data curation, W.L. (Wei Lin); writing—original draft preparation, W.L. (Wenbin Lin) and Y.C.E.; visualization, W.L. (Wei Lin); writing—review and editing, X.C., and G.C.; project administration, W.L. (Wenbin Lin) and Y.C.E.; funding acquisition, W.L. (Wenbin Lin) and Y.C.E. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Fujian University of Technology (NO. GY-Z18146 and NO. GY-Z20091), the Scientific and Technological Research Council of Turkey (NO. 118M768), and National Defense Science and Technology Innovation Zone of China [Grant Number 18-163-13-ZT-007-021-01].

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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