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Applying MICP by denitrification in soils: a process analysis

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Applying MICP by denitrification in soils: a process analysis

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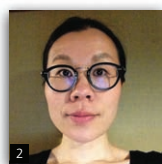
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The process of microbially induced carbonate precipitation (MICP) by denitrification was investigated in relation to its potential use as a ground improvement method. Liquid batch experiments indicated that the substrate solution had an optimum carbon–nitrogen ratio of 1-6 and confirmed that combining nitrate reduction and calcium carbonate precipitation leads to an efficient conversion, at which the pH is buffered slightly below 7 and the accumulation of toxic intermediate nitrogen compounds is limited. Sand column experiments confirmed that the volume and distribution of the gas phase strongly depend on the stress conditions. The produced gas volume is inversely related to the pore pressure and can be predicted based on a mass balance analysis, assuming conservation of mass and using theoretical laws of physics. At low pore pressure, the gas formed and accumulated at the top of the column, whereas calcium carbonate precipitation occurred mostly at the bottom near the substrate inlet; an excess amount of gas was produced, which vented from the sand columns and induced cracks in the sand at low confining pressures, which negatively affected the sand-stabilising effect of the calcium carbonate minerals.

Introduction

Microbially induced carbonate precipitation (MICP) has attracted increased interest in recent years for its potential in geotechnical and environmental applications (DeJong *et al.*, 2013). Most studies on MICP were based on the hydrolysis of urea. Hydrolysis of urea was one of the first processes associated with MICP in the late nineteenth century (Ehrlich and Newman, 1996). Due to the limited abundance of urea in nature, MICP by urea is now considered to be one of the least important sources of naturally occurring biogenic carbonate. However, it has been demonstrated at laboratory scale (Chu *et al.*, 2012; DeJong *et al.*, 2006; Harkes *et al.*, 2010; Montoya *et al.*, 2013; Whiffin *et al.*, 2007) and field scale (Burbank *et al.*, 2011; DeJong *et al.*, 2009; van Paassen, 2011; van Paassen *et al.*, 2010a) that MICP by urea hydrolysis can significantly strengthen granular soils and shows a wide range of potential applications (Phillips *et al.*, 2013). Commercial applications of MICP by urea hydrolysis are still limited, partly due to the costs of cultivation of ureolytic bacteria and the required

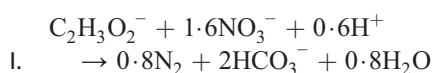
removal of ammonium chloride, which is formed as a by-product of the process. MICP by denitrification has been considered as a potential alternative biomediated ground improvement process, as it has several advantages over urea hydrolysis (Karatat *et al.*, 2008; Kavazanjian *et al.*, 2015; van Paassen *et al.*, 2010b). When nitrate (NO_3^-) is completely reduced to nitrogen gas, no adverse by-products are formed and removal effort is not required. The required substrates for denitrification are sufficiently soluble to limit the number of injections to reach the target amount of calcium carbonate (CaCO_3). Substrates can even be produced from waste streams, and the conversion does not require cultivation of very specific organisms. In fact, indigenous populations of denitrifying bacteria can be used (Martin *et al.*, 2013; van der Star *et al.*, 2009; van Paassen, 2009a; van Paassen *et al.*, 2010b).

Denitrification is one of the main biological processes in the global nitrogen cycle, in which a genetically diverse group of microorganisms is able to reduce nitrate through the intermediate

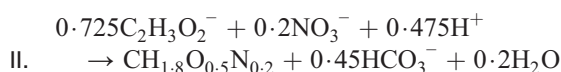
products of nitrite (NO_2^-), nitric oxide (NO) and nitrous oxide (N_2O) to nitrogen (N_2) gas (Robertson and Groffman, 2015). Denitrification has been intensively studied since the late nineteenth century, both in natural systems and for industrial applications (Archana *et al.*, 2012; Ferguson, 1994; Knowles, 1982; Kuenen and Robertson, 1988; Payne, 1981; Soares, 2000; Voorhees, 1902; Wang *et al.*, 1995).

The stoichiometry of the metabolic reactions involved in denitrification can be calculated using a method suggested by Heijnen *et al.* (1992) and Heijnen and Kleerebezem (2010). Metabolic reactions can be divided into an anabolic reaction, which describes the production of biomass, and a catabolic reaction, which generates the energy for the cells to produce new biomass in the anabolic reaction (Haynie, 2008). The stoichiometry of the anabolic and catabolic redox reactions are determined separately by solving the mass and electron balances for each reaction. The ratio between the catabolic and anabolic reactions is determined by solving the energy balance – that is, the produced energy from the catabolic reaction is equal to the energy required for biomass production and cell maintenance. The actual ratio depends on the growth rate of the microorganisms, which can range from maximum growth conditions, where microorganisms grow exponentially, to zero growth conditions, where the total amount of microorganisms does not increase, but maintaining the population still requires energy. The actual growth rate is controlled by the process and environmental conditions such as the availability of substrates and nutrients and the presence of inhibiting compounds (the condition of negative growth rate or cell decay, for example in the case where there are no substrates available, is not considered here).

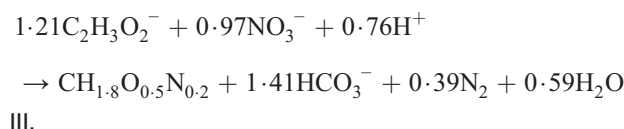
Using acetate ($\text{C}_2\text{H}_3\text{O}_2^-$) as the electron and carbon donor and nitrate as the electron acceptor leads to the following reaction stoichiometry for the catabolic reaction



Taking $\text{CH}_{1.8}\text{O}_{0.5}\text{N}_{0.2}$ as a representative molecular formula for bacterial biomass, the anabolic reaction equals

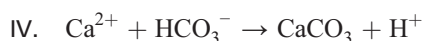


Solving the energy balance for maximum growth, the stoichiometry of the metabolic reaction (pH 7, temperature 298 K) becomes



For zero growth conditions, the overall metabolic stoichiometry is equal to catabolic Reaction I. When following this approach, the actual metabolic stoichiometry ranges between Reactions I and III. In other words, the amount of required acetate at zero growth is 0.6 mol/mol nitrate, while the acetate-to-nitrate (Ac/N) ratio at maximum growth is 1.25 (corresponding to a carbon-to-nitrogen (C/N) ratio range of 1.2–2.5). Similarly, the amount of the produced nitrogen gas ranges between 0.4 and 0.5 mol/mol nitrate and the inorganic carbon (carbonic acid (H_2CO_3), bicarbonate (HCO_3^-), carbon dioxide (CO_2)) ranges from 1.25 to 1.45 mol/mol nitrate.

If calcium ions (Ca^{2+}) are present, the inorganic carbon produced during the metabolism of the denitrifiers can precipitate to form calcium carbonate



At neutral pH, precipitation of calcium carbonate produces acid, which buffers the alkalinity production associated with denitrification. The complete set of coupled reactions related to MICP by denitrification, together with the potential consequences for the physical behaviour of granular soils, is illustrated in Figure 1. MICP by denitrification also results in gas production and biomass growth. Biofilms can also increase the strength and stiffness of soils and reduce hydraulic conductivity (e.g. Stal, 2010). Biogenic gas production in soil displaces water, which may decrease the hydraulic conductivity. The presence of gas in the pores can also increase shear strength by generating capillary suction forces (Fredlund and Rahardjo, 1993) and increase the resistance of the soil to cyclic loading (He *et al.*, 2013; Kavazanjian *et al.*, 2015; Yegian *et al.*, 2007). Unsaturated conditions may also improve the strengthening effect of MICP, as the precipitation is considered to take place more efficiently at the particle contacts (Cheng and Cord-Ruwisch, 2012; Cheng *et al.*, 2013).

Consequently, MICP by denitrification is considered to have significant potential as a ground improvement method, through biogenic gas production and MICP (Hamdan *et al.*, 2016; Kavazanjian *et al.*, 2015). However, the demonstrated reaction rate of this process is low compared with that of urea hydrolysis. Selecting the right substrate composition is essential. Too much nitrate may lead to accumulation of intermediate compounds, which can be toxic for the bacteria and inhibit growth (Almeida *et al.*, 1995), or increased emissions of nitrous oxide, which is a very strong greenhouse gas (GHG) (Chung and Chung, 2000; Hanaki *et al.*, 1992), while leaving a large excess of acetate would be inefficient. Finally, the formation, distribution and mobility of gas in porous media strongly depend on the soil characteristics and environmental conditions. Pore pressure underground strongly affects the produced gas volume and solubility (e.g. Rebata-Landa and Santamarina, 2011), and the accumulation and storage of gas is affected by the substrate supply regime (Soares *et al.*, 1991). If too

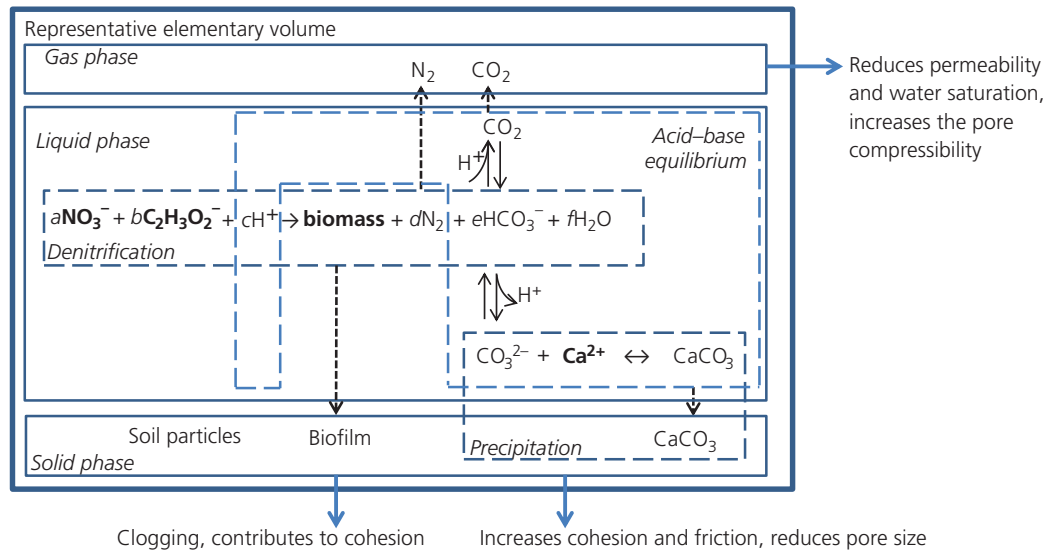


Figure 1. MICP induced by denitrification and potential effects in a representative elementary volume of soil or a soil specimen. Detailed stoichiometry of the denitrification reaction is given in the text

much gas is produced, it could trigger liquefaction on very loose sand instead of mitigating it (Grozic *et al.*, 1998).

For these reasons, this paper presents the results of the following three sets of experiments that were executed to evaluate the process performance of MICP through denitrification for a selection of process variables and test conditions related to its application as a ground improvement method.

- Batch experiments in a mixed liquid culture in which substrate composition was varied to study the preferable substrate ratio and understand the process mechanisms.
- Sand column experiments in a triaxial cell, operated at different pore and confining pressure conditions, to study the gas formation in different pressure conditions correspondent with different water levels and soil depths.
- Sand column experiments at ambient pressure and low confinement with multiple sequential batches of substrates to investigate the precipitation efficiency of the treatment.

Materials and methods

Substrate solutions

The substrate solutions used throughout the experiments contained calcium acetate ($\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$) and calcium nitrate ($\text{Ca}(\text{NO}_3)_2$) (Sigma-Aldrich) at varying concentrations (Figure 2). Besides those main substrates, all media further contained the following nutrients: 0.003 mM ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$), 0.0024 mM magnesium sulfate (MgSO_4), 0.006 mM monopotassium phosphate (KH_2PO_4), 0.014 mM dipotassium phosphate (K_2HPO_4) and 1 ml/l trace element solution SL12B (Overmann *et al.*, 1992) to avoid nutrient limitation during bacterial growth.

Liquid batch experiments

As an inoculum for the first batch experiment, a soil sample was taken from the Botanical Garden of the Delft University of Technology. Denitrifying organisms typically occur in soils that are wet and anaerobic and have sufficiently high organic content and a source of nitrate, such as wetlands or agricultural fields (Keddy, 2000). The sample was taken from a depth of 2 m using a hand auger. The black colour and wet condition of the sample indicated that favourable conditions for denitrifiers were met.

The experiments were performed in 250-ml glass bottles (Duran GLS80). Electrodes were inserted through the cap to measure the pH and electrical conductivity (C_E), and tubes were connected to the sample liquid to monitor the gas volume. In the first incubation, 30 g of soil was suspended in 250 ml of growth medium. After 3 d of incubation, all nitrate and nitrite were consumed and the bottle was shaken to stimulate the detachment of microorganisms from the soil particles. Afterwards, the liquid containing free denitrifying cells (without the solid fraction, which had been allowed to settle) was collected and transferred into a new bottle for further experiments. All five subsequent incubations used the inoculum from the previous incubation with the concentrations shown in Figure 2. The substrates used in these batches were 30 mM calcium acetate and 25 mM calcium nitrate, corresponding to calcium, acetate and nitrate (Ca–Ac–N) concentrations of 55, 60 and 50 mM respectively (55:60:50). In the fifth batch, calcium salts in one of the two incubations were replaced by sodium salts (110:60:50 sodium–acetate–nitrate (Na–Ac–N)). In the sixth batch, the Ac/N ratio was varied. Explicit concentrations of substrates and inoculums of these experiments are presented in Figure 2.

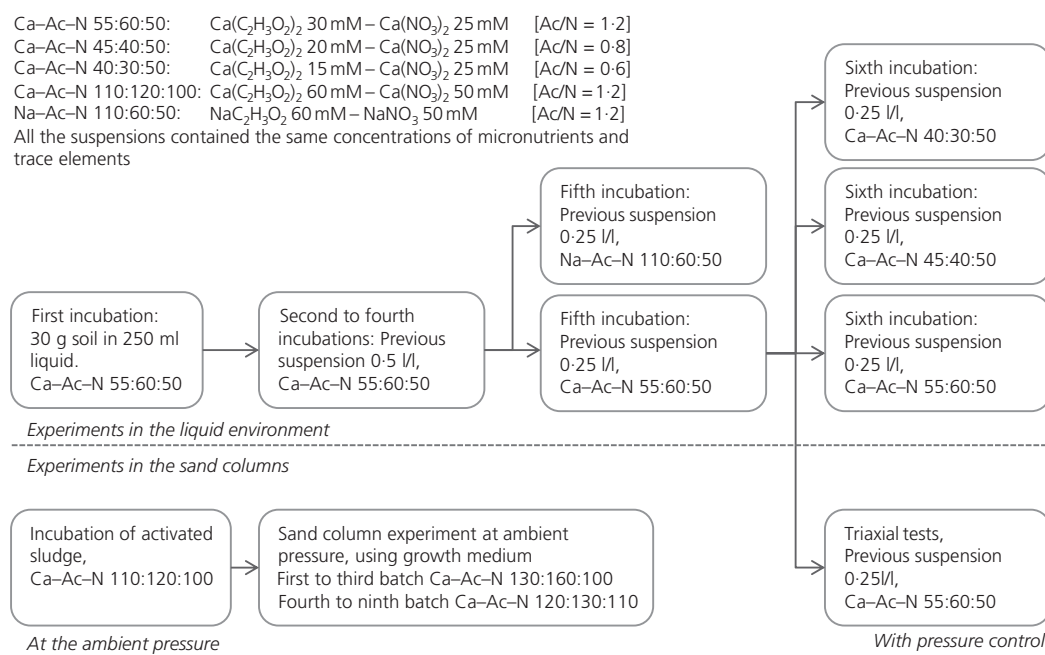


Figure 2. Schematic overview of the sequence of liquid batch experiments, the ambient pressure column experiment and triaxial cell experiments. Arrows illustrate the sequence of incubations and indicate the source of the inoculum for the test

Liquid from the bottles was regularly sampled to analyse the concentration of different solutes, the electrical conductivity (C_E) and the pH. All liquid samples were filtered through a 0·45- μ m membrane and subsequently diluted to the measurable range before measurements. Nitrate, nitrite and calcium were determined spectrophotometrically (Lasa 100, Hach Lange) with standard test kits LCK339, LCK341 and LCK327, respectively). Total carbon (C_T) was measured with a Dohrmann DC190 chromatograph. Acetate consumption was determined indirectly with total organic carbon (TOC), which is the result from the C_T measurements but pre-treated with hydrochloric acid (HCl) to remove the inorganic carbon (Schumacher, 2002). All incubations were performed in a climate room at 25°C. The pH and C_E were measured on all samples and continuously in the incubating bottles.

The gas produced during the experiment was captured using a water clock, made by placing a graduated cylinder upside down in a water bath. Before starting the incubation, nitrogen gas was flushed through the bottle to ensure anoxic conditions and the partial pressure of nitrogen in the gas phase was set at 100%. The pressure of the gas was kept constant at ambient pressure by manually adjusting the height of the cylinder. Liquid sampling and gas volume measurements were done on a daily basis. The amount of nitrogen gas (N_{N_2}) was calculated from the measured gas volume by using the ideal gas law at 1 atm and 298 K, assuming nitrogen gas was the only produced gas.

Sand column experiments in a triaxial cell at varying pressure conditions

Sand column experiments were performed at varying pressure conditions, using a triaxial test set-up described in the ISO/TS 17892-9:2004 standard (CEN, 2004). A uniform fine-grained siliceous sand (0·125–0·355 mm) was used (Sibelco, S60) with a silica content of 99·5% and a specific gravity of 2·65 g/cm³. The minimum and maximum dry bulk densities (ASTM, 2016a, 2016b) ranged from 1·57 ± 0·01 to 1·66 ± 0·02 g/cm³. Sand columns were prepared using a split mould with a rubber membrane inside. The mould was filled in three layers. For each layer, first, the suspension containing the bacteria and substrates was poured into the mould, followed by dry sand, ensuring that the sand always remained below the fluid level. The sample was not tamped, thus resulting in a very loosely packed sand with an average dry bulk density of 1·50 ± 0·1 g/cm³.

The suspension was prepared by mixing a 0·25 l/l inoculum harvested at the end of the fifth incubation of the liquid batch experiments, with concentrated substrate solution and demineralised water. The overall substrate concentration of the suspension was 30 mM calcium acetate and 25 mM calcium nitrate (Ca–Ac–N 55:60:50). This corresponds to an Ac/N ratio of 1·2, which was selected to ensure that there would be a sufficient carbon source in case the conditions in the sand would be favourable for bacterial growth, changing the metabolic stoichiometry towards maximum growth.

The tests were performed at three different pressure conditions. First, two back pressure conditions were selected to evaluate the effect of pore pressure on gas volume, while the effective confining pressure was kept constant: a low back pressure of 50 kPa with a cell pressure of 100 kPa and a high back pressure of 300 kPa with a cell pressure of 350 kPa. Next, a third test was performed in which the back pressure was set at 345 kPa, while the cell pressure was kept at 350 kPa to evaluate the process at a low confinement pressure. A control test was performed at a back pressure of 50 kPa and a cell pressure of 100 kPa by using the growth medium without bacteria.

Once the cell and back pressures were set at the designated stress levels, the sample was left to react while the volume change in the controllers was monitored. The pore pressure coefficient (or B factor), which is the ratio of the change in pore pressure over the change in cell pressure and which is related to the degree of water saturation of the sample (Skempton, 1954), was measured at the beginning and at the end of the treatment. At the end of each experiment, the sample was flushed from bottom to top with deaired water by using a third pressure controller, while the back pressure was lowered to obtain a constant head difference of 20 kPa. While flushing, the flow rate was measured to calculate the hydraulic conductivity of the sample.

After flushing the high-pressure samples, the volume of the expelled and flushed liquids was determined by emptying the back pressure controller. For the low-pressure conditions, the back pressure controller was emptied before flushing the sample to collect the ‘expelled’ water and after flushing it was emptied again to collect the ‘flushed’ water. Samples were taken from the expelled and flushed liquids to determine the concentrations of nitrate, nitrite and calcium ions and C_T . During the first experiment, biomass growth was observed in the cell water.

Consequently, for the other two tests, the volume and concentrations in the cell water were also determined.

Sand column experiments with multiple batches of substrate solution

Sand column experiments were performed at ambient pressure conditions in which multiple batches of substrate solution were flushed through the columns for the total period of 65 d.

The sand columns were prepared using plastic (polyvinyl chloride (PVC)) columns with a length of 180 mm and inner diameter of 65 mm, as described by Harkes *et al.* (2010). The column was filled with a uniform fine siliceous sand (d_{50} : 0.166 mm; Itterbeck fine, Smals IKW, SZI 0032). The top and bottom of the column were filled with 1 cm of fine gravel (d_{50} : 2.5 mm). The sand and gravel were packed by tamping under water to an average dry density of 1.57 g/cm³. Before treatment, the sand column was flushed with several pore volumes of distilled water.

The inoculum for this experiment was obtained from activated sludge collected at the Harnaschpolder municipal waste water treatment plant (Delft, the Netherlands). The sludge was suspended in substrate solution containing 60 mM calcium acetate and 50 mM calcium nitrate (110:120:100 Ca–Ac–N), and the suspension was incubated for 6 d, in which nitrate was fully consumed. The suspension containing the biomass, without the sludge (which was allowed to settle), was used as the inoculum for the column set-up. For the column experiment, 250 ml of the suspension containing the 0.5 l/l inoculum was mixed with substrate solutions, reaching concentrations as indicated in Figure 2, and flushed into the column. Subsequently, the column was flushed nine times with 250 ml of substrate solution, after which the column was flushed with one pore volume of distilled water to wash the remaining

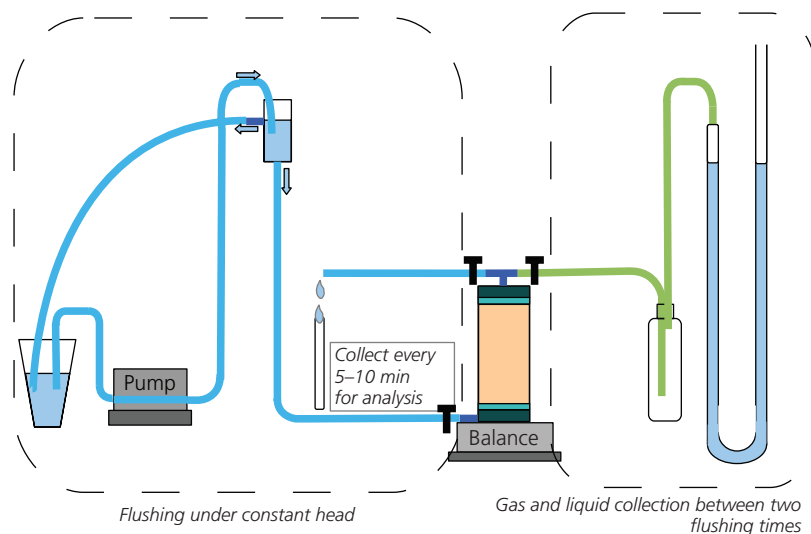


Figure 3. Set-up of the sand column with multiple substrate flushes at ambient pressure

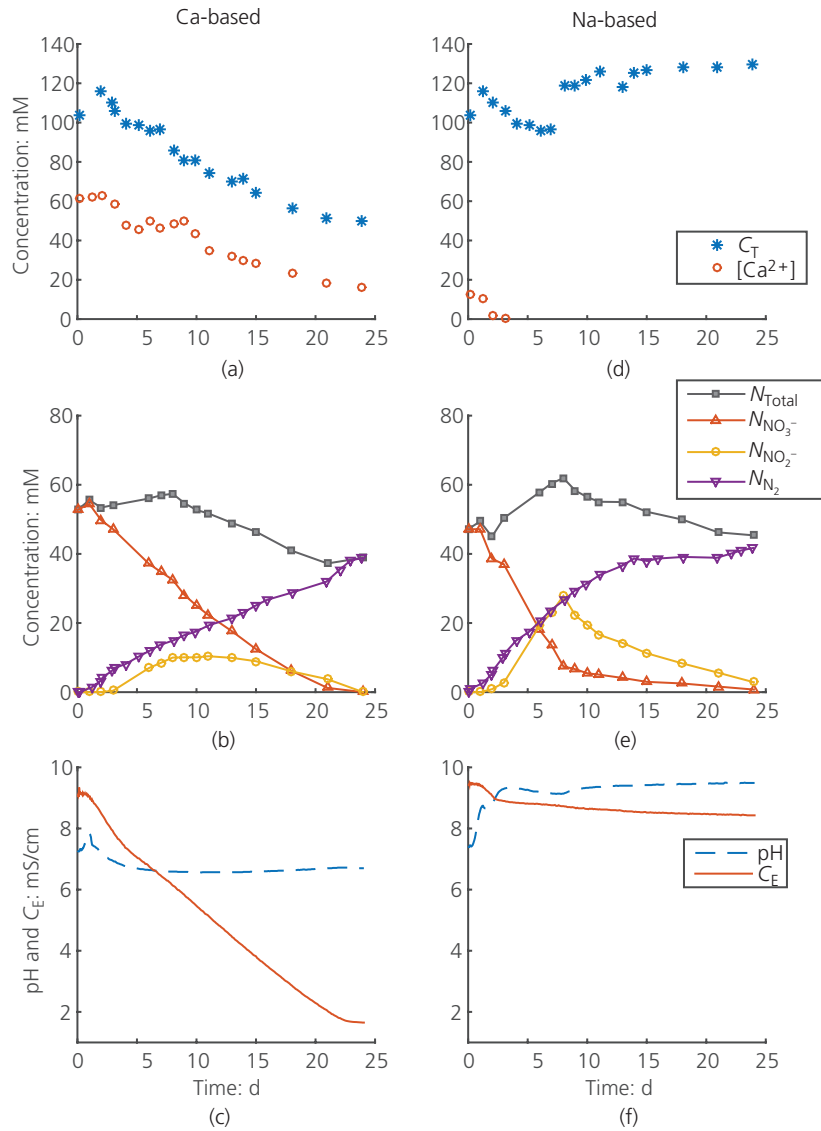


Figure 4. Comparison of changes in chemical composition during denitrification in the presence (left-hand column) and absence (right-hand column) of calcium ions during the fifth incubation. Legends indicate chemical compounds. (a and d) Results of total

carbon C_T and calcium concentration; (b and e) results of nitrate, nitrite, nitrogen gas and total nitrogen in a volumetric unit; (c and f) results of pH and electrical conductivity C_E

solutes from the column. For the first three flushes, the Ac/N ratio was kept high (about 1.6) in order to stimulate bacterial growth and prevent accumulation of toxic intermediate nitrogen compounds. For all subsequent flushes, the Ac/N ratio was about 1.2, which corresponds to the reaction stoichiometry at maximum growth (Reaction III). All liquids were flushed in upward flow under a constant head difference of 20 cm, and the effluent replaced by newly flushed medium was collected from the top during each flush. The set-up is presented in Figure 3.

During each flush, the flow rate was determined by collecting the effluent from the top of the set-up at regular time intervals and the hydraulic conductivity was determined using Darcy's law. The

pH, C_E and solute concentrations of these samples were measured. After each flush, the inlet at the bottom was closed and the column was left for 7–9 d to react. During this period, the produced gas and the expelled liquid were collected. The mass of the expelled liquid was measured after the reaction period before the next flush. After treatment, the sand column was first analysed using X-ray computed tomography (CT) scanning, then the PVC tube was cut open over the length of the sample and removed. The sample was sliced into nine parts and dried in an oven at 105°C. The dry samples before and after treatment were analysed with an environmental scanning electron microscope (ESEM) (Philips ESEM XL30). The calcium carbonate concentration was determined for 1.5–2.0 g of the dried samples at different

locations in the column by using the acid dissolution method described by Whiffin *et al.* (2007).

Results

Liquid batch experiments

Figure 4 shows the difference between incubation with calcium-based substrates and sodium-based substrates. The reduction in C_T and calcium ion concentrations in Figure 4(a) and the drop in C_E in Figure 4(c) indicate that calcium carbonate precipitation took place. Due to the precipitation of carbonate, the pH was buffered between 6 and 7, which reduced the accumulation of nitrite to a maximum level of 10 mM during the first week, compared with 30 mM of nitrite for the sodium-based substrate solution. In the sodium-based system (Figure 4(d)), no precipitation took place; hence, C_T remained constant. The small amount of calcium ions was due to the small fraction that was still present in the inoculum obtained from the fourth incubation. Due to MICP, this amount was quickly depleted. The decreases in nitrate and nitrite and the increase in nitrogen gas (Figures 4(b) and 4(e)) are evidence that denitrification still took place in both systems. In the absence of calcium ions, denitrification led to an alkaline environment, as shown in Figure 4(f), where the pH rose within 3 d to a level between 9 and 9.5.

Figure 5 shows the results of the incubations where the Ac/N ratio was varied. The experiment using the growth medium with an Ac/N ratio of 1.2, which is close to the reaction stoichiometry for maximum growth of the bacteria (Reaction III), resulted in an

excess of calcium and acetate at the end of the experiment. The Ac/N ratio of 0.6, which corresponds to the stoichiometry of the catabolic reaction (zero growth; Reaction I), resulted in an excess of nitrate and accumulation of nitrite. For the Ac/N ratio of 0.8, all substrates were converted most efficiently.

Triaxial tests at different pressure conditions

The volume change in the pressure controllers during the incubation experiments in the triaxial cell is shown in Figure 6. The total volume change is the sum of the volume change in the cell and back pressure controllers and represents the change in the sample volume. All tests showed that the volume in the back pressure controller increased rapidly during the first 3 d. At the same time, however, the volume of the cell pressure controller decreased in all experiments. As a result, the sample volume decreased before it started to rise. After 4 d, the volume in the back pressure controller of the experiment with a back pressure of 50 kPa continued to rise slowly, while for the two tests with higher back pressures, the volume started to decrease from the third day onwards, resulting in a significantly lower volume change at the end of the experiment. The change in sample volume even became negative at the end of the experiment. The resulting volume changes and B factors are presented in Table 1.

The volumes and solute concentrations of the different liquid fractions are presented in Table 2. At low pressure, the back pressure controller indicated that 55 ml was expelled from the sand column. However, the controller contained only 35 ml of liquid. Similarly, during flushing, 212 ml was flushed out, of

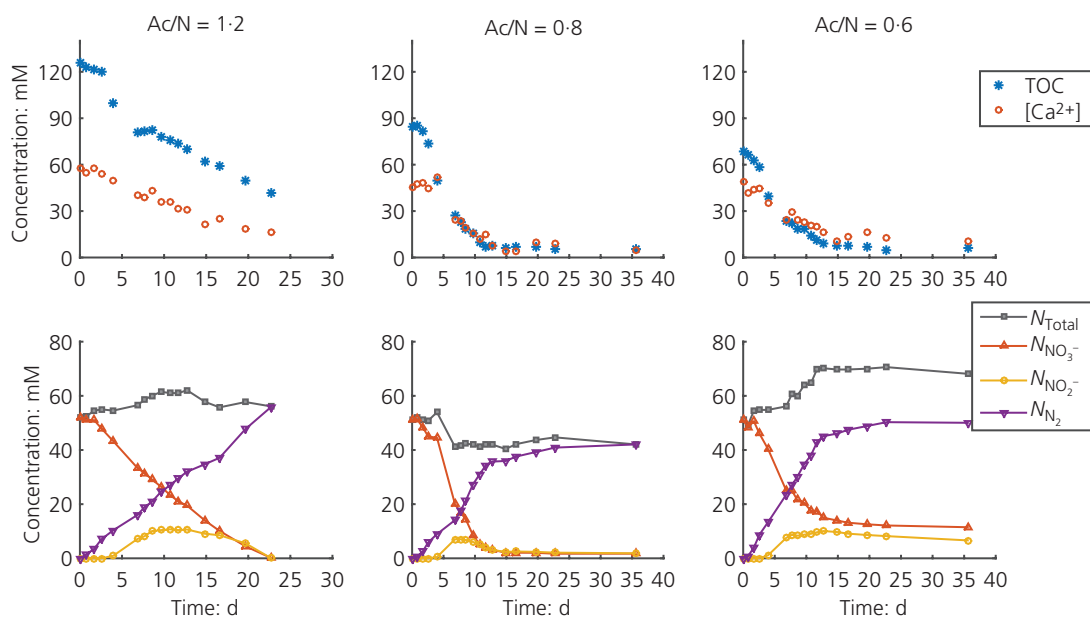


Figure 5. Comparison of changes in chemical composition during denitrification in the presence of calcium at three different Ac/N ratios in the sixth incubation. Left-hand column: Ca–Ac–N = 55:60:50, Ac/N = 1.2; middle column: Ca–Ac–N = 45:40:50,

Ac/N = 0.8; right-hand column: Ca–Ac–N = 40:30:50, Ac/N = 0.6. Legends indicate chemical compounds (TOC is total organic carbon)

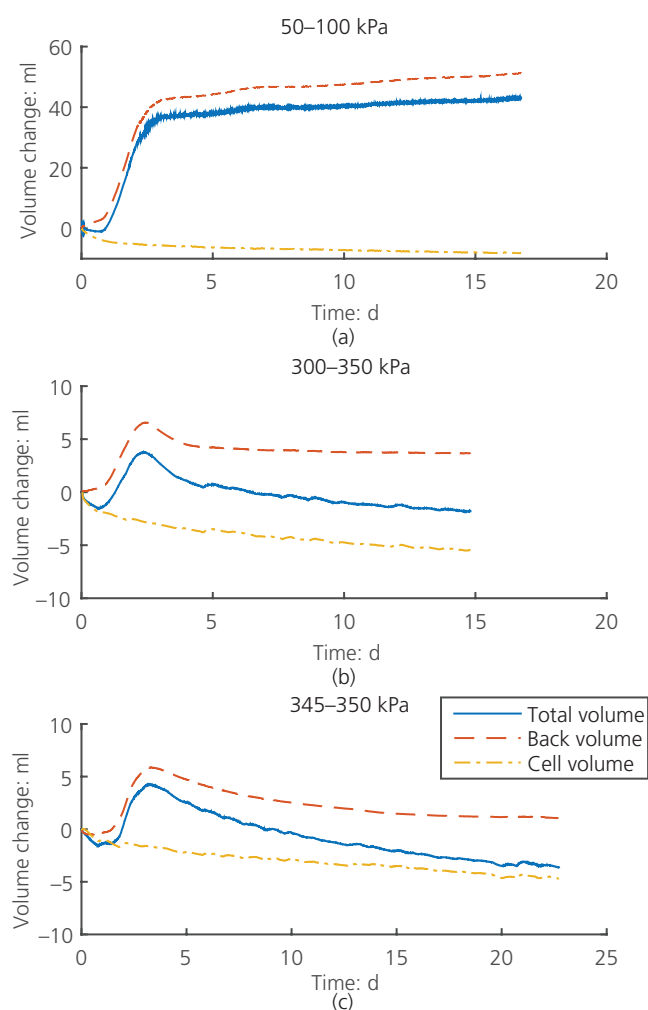


Figure 6. Volume changes in the triaxial tests at cell pressure – back pressure values of (a) 50–100, (b) 300–350 and (c) 345–350 kPa. The total volume change is the sum of volume changes recorded by back pressure and cell pressure controllers

which only 207 ml was collected. It was assumed that the rest of the controller volume was filled with gas.

Sand column experiment with multiple substrate flushes at ambient pressure

The breakthrough curves of C_E , pH and solute concentrations, which were measured during each flush, are shown in Figure 7. The initial part of each breakthrough curve can be considered as a vertical profile from the top to the bottom through the sand column. The flush was continued until complete breakthrough, indicated by sharp increases in C_E and substrate concentrations. In the first two flushes of the fresh medium, the C_E profile showed a relatively constant value of 4–5 mS/cm over the height of the column, indicating that there were no differences in solute concentration in the whole column. From the fourth flush onwards, the C_E profiles started to show a decrease from top to bottom, reaching maximum values ranging from about 8 mS/cm at the top

to about 3 mS/cm at the bottom, which indicates that, during the final flushes, a large part of the substrate in the upper half of the column was not yet converted when the next flush was applied.

The solute concentrations show a similar distribution. Nitrate was almost completely consumed over the full height of the column in the first four flushes, as only small concentrations of nitrite (<1.3 mM) were measured and about 20 mM calcium was still present. The gradient of the substrate distribution in the column was very clear in the last two flushes. During these flushes, the remaining nitrate at the top of the column was 20–30 mM (indicating a conversion of only about 50%), while at the bottom it was completely consumed. The calcium and acetate concentrations showed a similar distribution. Incomplete nitrate conversion in the last flushes caused a considerable amount of nitrite accumulation.

During the reaction period between each flush, 31–53 ml of fluid was expelled from the pores due to gas production inside the column. The hydraulic conductivity, which was calculated from the measured flow rate during flushing, showed a significant difference between the first flush and all other flushes. In the second and later flushes, initially no liquid flowed out of the column. The injected liquid first filled up the unsaturated pore space before flowing out of the column. During each flush, the flow rate gradually increased. The hydraulic conductivity at the end of the second and all later flushes was 50–70% lower than during the first flush. The increase in hydraulic conductivity can be explained by an increase in saturation as the trapped gas was gradually flushed out together with the liquid (increasing the relative permeability).

The images produced by X-ray CT scanning (Figure 8) show that the gas produced during the experiment led to the development of cracks and open voids, which remained present after the column was flushed with water. The constructed 3D image in Figure 8(b) shows that the air was mostly present in the pores in the gravel layers and in the voids, which were developed mostly at the top of the column by the air bubbles themselves. However, as the resolution of the CT scan was limited to approximately 200 μm , small gas bubbles could not be visualised.

Calcium carbonate measurements showed an average calcium carbonate content of about 1.1%. However, as shown in Figure 9, the calcium carbonate was not homogeneously distributed throughout the column. The distribution of calcium carbonate was largest at the bottom of the sand layer and decreased towards the top. Both gravel layers at the top and the bottom and the upper part of the column had a relatively low calcium carbonate content. The presence of calcium carbonate was also confirmed by ESEM analysis (Figure 10). The crystals showed a dendritic texture with a size of up to 200 μm .

Discussion

In order to assess the relevance of these results for ground improvement applications, first, the accuracy of the experiments is discussed by analysing the mass balance and, second, the effects

Experiment	Controller volume: ml						<i>B</i> factor		Hydraulic conductivity: 10 ⁻⁵ m/s
	Day 3			Day 15			Initial	End	
	Back	Cell	Total	Back	Cell	Total			
Control: 50–100 kPa	0	-2.2	-2.2	1.3	-4.5	-3.2	0.98	0.98	5.9
300–350 kPa	6.4	-3.0	3.4	3.7	-5.5	-1.8	0.98	0.60	5.9
345–350 kPa	7.4	-1.6	5.8	1.4	-3.5	-2.1	0.96	0.86	5.7
50–100 kPa	42.0	-5.5	36.5	50.0	-7.9	42.1	0.58	0.04	5.8

Table 1. Volume changes, *B* factors and hydraulic conductivities at the end of the reaction in the triaxial tests

of different process variables and test conditions on the process performance are evaluated.

Mass balance

Concentration measurements during and after the reaction in each of the experiments were used to evaluate the mass balance of the different nitrogen compounds. Figures 4 and 5 show the measured concentrations of the different nitrogen compounds. The nitrogen gas concentration was calculated using the ideal gas law assuming ambient pressure (1 atm) and temperature (298 K) and assuming that nitrogen gas was the only gas produced and all inorganic carbon either precipitated or remained in solution due to the relatively high solubility at neutral to high pH. The gas constant and Henry's constant of N₂ gas were taken from Kauzmann (2013) and Yaws (2012). Following these assumptions, the sum of all nitrogen compounds (N_{total}) should be constant and equal to the initial nitrate concentration. Nevertheless, during the liquid batch experiments, the sum of nitrogen compounds ranged between 40 and 70 mM,

whereas the initial nitrate concentration was 55 mM in each of the experiments, which corresponds to a deficiency in the mass balance of ±25%.

For the triaxial tests, the total amounts of nitrate and nitrite were calculated by multiplying the measured concentrations in each of the liquid fractions by the liquid volume. The total amount of nitrogen gas was calculated using the ideal gas law, assuming that the volume of nitrogen gas was equal to the maximum volume change in the back pressure controller and that the partial pressure of nitrogen gas was equal to the back pressure. Assuming that the gas phase was in equilibrium with the dissolved nitrogen gas, its concentration was calculated using Henry's law. The resulting nitrogen balance in the triaxial test is shown in Table 3.

The mass balance calculations in Table 3 show that for the two experiments under high-pressure conditions, 26 and 33% of the nitrogen compounds in the nitrate added were unaccounted for and

Experiment	Liquid	Liquid volume: ml	Nitrate: mM	Nitrite: mM	Calcium ions: mM	C _T : mM
300–350 kPa	Initial suspension	97	50.0	0	51.9	103.7
	Expelled liquid	ND	ND	ND	ND	ND
	Flushed liquid	206	2.9	0.04	0.69	9.25
	Cell water	ND	ND	ND	ND	ND
345–350 kPa	Initial suspension	97	52.7	0	61.6	104
	Expelled liquid	ND	ND	ND	ND	ND
	Flushed liquid	206	0	0	1.5	7.7
	Cell water	3630	0.15	0.15	0.59	1.73
50–100 kPa	Initial suspension	97	51.8	0	57.6	126
	Expelled liquid	35 (55) ^a	1.44	0.01	3.42	2.2
	Flushed liquid	207 (212) ^a	0.11	0	1.83	4.08
	Cell water	3633	0.1	0.158	0.66	0.92

^a The numbers in parentheses are the values recorded in the back pressure controller
ND, not determined

Table 2. Volumes and solute concentrations of different liquids in the triaxial tests

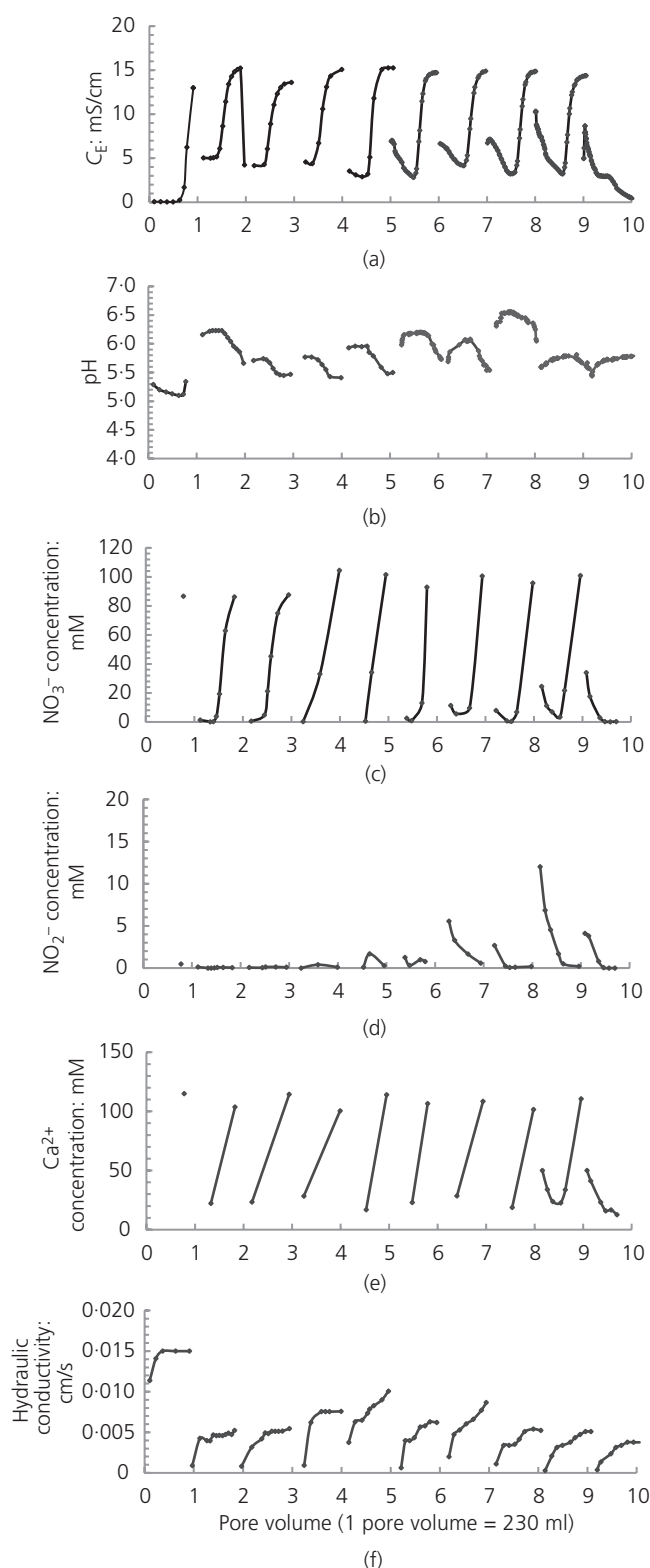


Figure 7. Measured values of (a) C_e , (b) pH, (c) nitrate concentration, (d) nitrite concentration, (e) calcium ion concentration and (f) hydraulic conductivity for the sand column experiment at ambient pressure

therefore lost, while for the experiment at (near) ambient pressure, the final nitrogen level was 25% higher than initially added. Further investigation is required to validate the assumptions and explain the observed discrepancies in the mass balance.

The effect of substrate composition on process efficiency

The results of the liquid batch experiments show that the substrate composition significantly affected the process performance. When denitrification was combined with MICP, the pH was buffered at values that are slightly lower than the optimal range for denitrification reported in literature (Knowles, 1982; Wang *et al.*, 1995). A similar pH buffering effect by MICP was observed by Burbank *et al.* (2011). In their field experiments, in which they stimulated MICP by a combination of urea hydrolysis and denitrification, Burbank *et al.* observed that the pH remained around neutral. The results of this study are partly in agreement with the observations of Glass and Silverstein (1998), showing that a high pH – which occurs when sodium-based media are used – favours the reduction of nitrate to nitrite but can cause nitrite to accumulate. On the other hand, the solutions containing calcium showed complete reduction of nitrate, with limited accumulation of nitrite, even though the pH was buffered at a value below 7. This contradicts the observations of Glass and Silverstein (1998), who found that nitrate reduction at pH 7 and below was completely inhibited. Still, the observed pH values are within the range at which active denitrification has been reported by others and far from the lower limit that could cause nitrous oxide accumulation (Knowles, 1982). Hence, the combined process of nitrate reduction and calcium carbonate precipitation promotes efficient conversion of nitrate to nitrogen gas and prevents the accumulation of toxic intermediates and potential GHGs.

Also, the C/N ratio in the growth medium significantly affected the conversion efficiency. As similarly observed by Chung and Chung (2000), this study found that high C/N ratios favoured the complete conversion of nitrate and limited nitrite accumulation but led to an excess of acetate and calcium at the end of the experiment. A low C/N ratio, on the other hand, led to full acetate consumption, but at the end of the experiment, significant concentrations of residual nitrate and accumulated nitrite remained. In this study's experiments, the optimum Ac/N ratio appears to be around 0.8, which is slightly lower than the optimum ratio found by Chung and Chung (2000), but is significantly lower than the ratio at maximum growth according to Reaction III.

The effect of pressure conditions on gas formation and distribution

When stimulating MICP by denitrification in sand, the pressure conditions significantly affected the formation and distribution of gas in the pore space. For a given amount of substrate, the volume of gas was much lower at high pore pressures than at low pore pressures. This is expected, considering that, according to the ideal gas law, the volume and pressure of gas are inversely proportional. Secondly, according to Henry's law, gas solubility is higher at high pressures. Thirdly, a higher gas solubility leads to

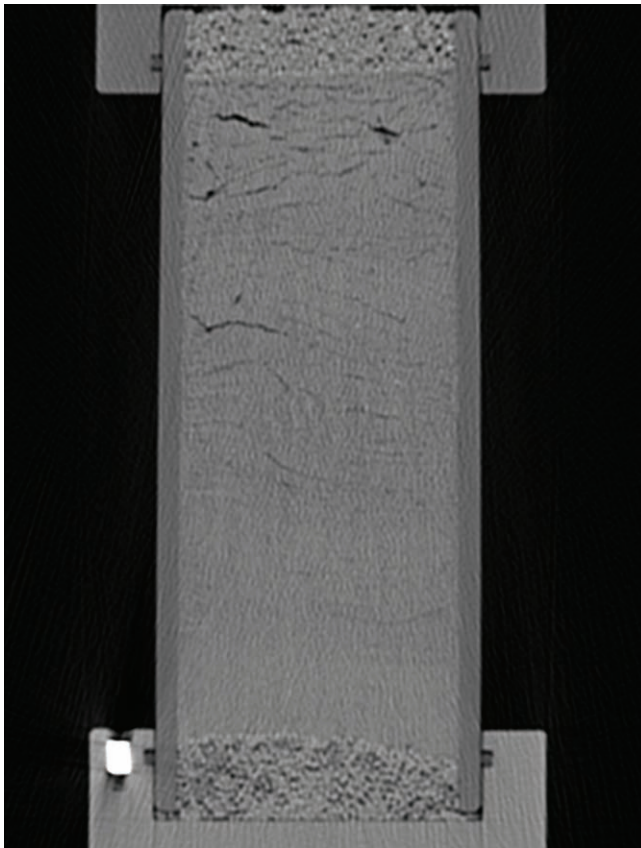
higher dissolved gas concentrations, which may lead to larger diffusive fluxes to surrounding water, as observed in the case of 300–350 and 345–350 kPa in Figure 6.

The total gas volume in the pore space significantly influences the geotechnical properties of sand, including strength, permeability and stiffness (DeJong *et al.*, 2013). After a specific amount of gas is produced, single gas bubbles will coagulate into gas pockets or form a continuous gas phase, which tends to migrate upwards in irregular patterns (Haines, 1930; van Paassen, 2010b). Also, the confinement pressure influences the potential of MICP by denitrification for ground reinforcement applications. At low confinement conditions

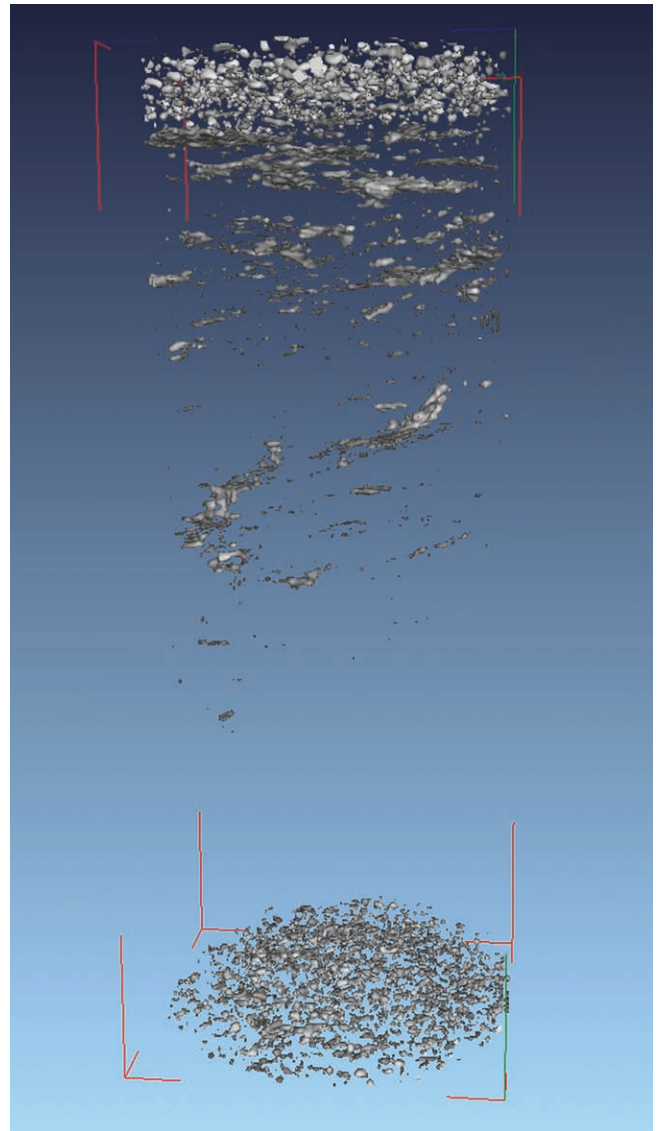
(shallow depth), the gas may lead to the formation of cracks, disturb the sand structure and reduce the stabilising effect of the calcium carbonate minerals, as shown in Figure 8.

The relationship between the distribution of gas and calcium carbonate

The average calcium carbonate content in the sand column which was flushed nine times with substrate solution reached 1.1%. Although this amount of calcium carbonate is relatively low compared with the results from other studies on MICP based on hydrolysis of urea, such as that of van Paassen *et al.* (2010a), it may still be sufficient to significantly increase cyclic shear



(a)



(b)

Figure 8. Result of the gas distribution in the sand column treated at ambient pressure with multiple substrate flushes: (a) X-ray CT scanned images; (b) constructed three-dimensional image

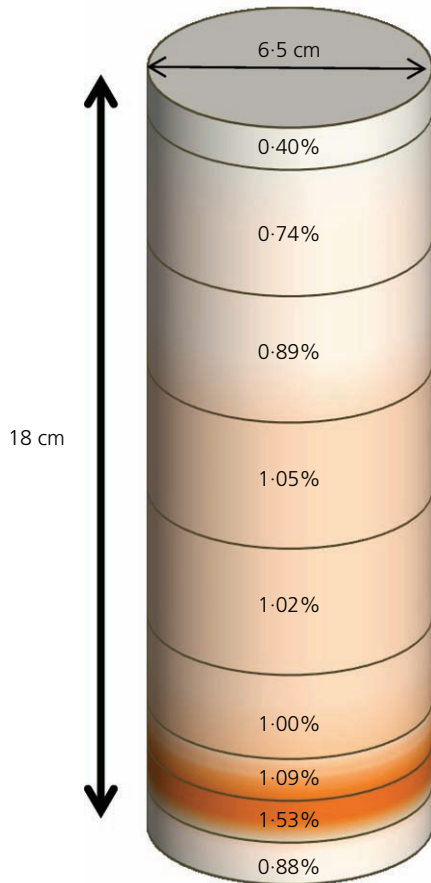


Figure 9. Calcium carbonate distribution in the sand column treated at ambient pressure

resistance to mitigate liquefaction of loose, sandy soils (DeJong *et al.*, 2014; Kavazanjian *et al.*, 2015). The relationship between strength and calcium carbonate content depends on many factors,

including the density, mineral type and grain size distribution of the treated sand (Cheng *et al.*, 2013; van Paassen *et al.*, 2010a) and substrate concentrations, reaction rate and environmental conditions of the treatment process (Al Qabany *et al.*, 2012; Mortensen *et al.*, 2011; van Paassen, 2009a).

Gas production can have a positive effect on the strength gain due to MICP. Cheng and Cord-Ruwisch (2013) showed that columns that have been treated under partially saturated conditions require lower amounts of calcium carbonate to obtain a similar increase in strength for fully saturated conditions. They considered that, in partially saturated conditions, substrate solutions preferably fill the pore throats due to suction effects, resulting in a more efficient distribution of cementing calcium carbonate minerals. Similarly, Kavazanjian *et al.* (2015) showed that MICP by denitrification results in more efficient cementation than MICP by urea hydrolysis. The present study showed that, within the sand column treated at ambient pressure, calcium carbonate was predominantly formed at the bottom of the column, while gas was predominantly formed at the top. This seems obvious because there is less substrate available to react when gas displaces the substrate solution. As a result, the potential improvement in the cementation efficiency due to desaturation is counteracted by a lower conversion efficiency. Secondly, the cracks that were formed at low confining pressure disturb the sand-stabilising effect of the calcium carbonate minerals. The low number, large size and dendritic structure of the calcium carbonate crystals could be due to the fact that the crystals grew in multiple phases at a relatively low reaction rate (van Paassen, 2009b) and in the presence of impurities such as organic polymers from the growing biomass.

Conversion rate

The overall reaction rate of denitrification and precipitation is an important process parameter, which depends on many factors, including the amount and type of denitrifying organisms, substrate and product concentrations and environmental effects, such as

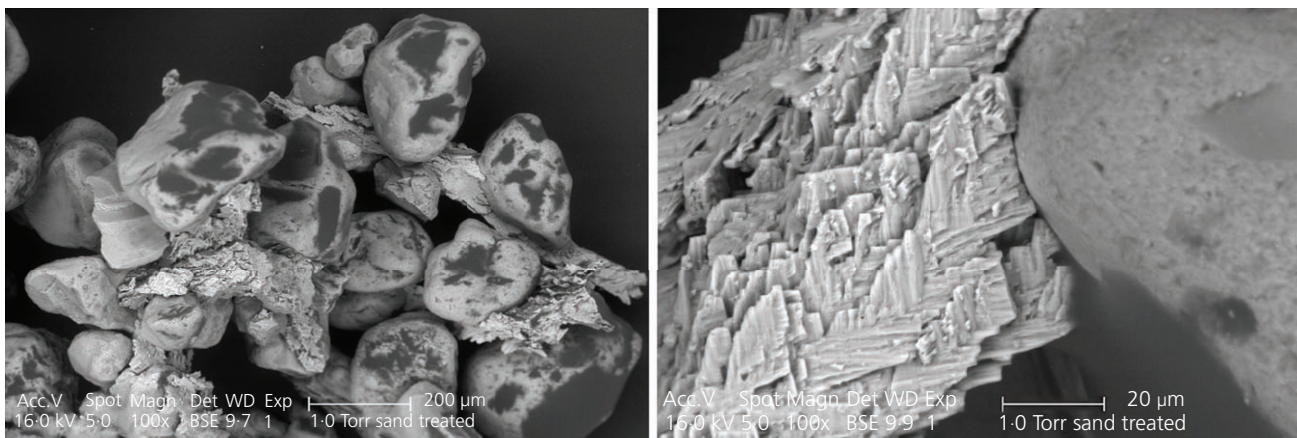


Figure 10. Presence of calcium carbonate in the sand treated with multiple substrate flushes at ambient pressure in ESEM images

		300–350 kPa	345–350 kPa	50–100 kPa
Initial: mmol nitrogen	Nitrate in the suspension	4.85	5.11	5.03
Final: mmol nitrogen	Nitrate in cell water	ND	0.55	0.36
	Nitrite in cell water	ND	0.54	0.57
	Nitrate in (flushed + expelled) water	0.6	0	0.1
	Nitrite in (flushed + expelled) water	0.01	0	0
	Nitrogen gas	2.14	2.13	5.18
	Nitrogen gas, aqueous	0.51	0.57	0.19
	Total	3.26	3.78	6.40
N_{gap} : mmol		1.59	1.33	-1.37
N_{gap} : %		33	26	-25

ND, not determined

Table 3. Nitrogen balance for triaxial tests

temperature, pH, salinity and the presence of other catalysing or inhibiting compounds (van Paassen, 2009a). Reported reaction rates range from a few millimoles of nitrate per litre per day up to 170 mmol nitrate/(l d) (Glass and Silverstein, 1998; Martin *et al.*, 2013; Soares *et al.*, 1991; van Paassen *et al.*, 2010b). This study showed that the reaction rate in the triaxial cell was about 10–15 mmol nitrate/(l d). The batch experiments in liquid environment in which the same substrate concentrations and inoculum size were used showed a rate of 1–2 mmol nitrate/(l d), about five to eight times lower than those for the triaxial cell. The average reaction rate in the sand column at ambient pressure was about 6 mmol nitrate/(l d). However, the remaining substrates in the final flushes indicated that the reaction rate in the unsaturated top part of the column was lower than that in the saturated bottom part. Considering that at least a small mass percentage of calcium carbonate needs to be formed, it is expected that, based on the current results, several weeks to several months of treatment are still required in order to obtain sufficient strength improvement when using MICP through denitrification. Further investigation is required to explain the observed differences in reaction rate.

Conclusions

This study has shown that the combined process of biological denitrification and MICP leads to a more efficient conversion than denitrification without precipitation. The precipitation of calcium carbonate buffers the pH and prevents the accumulation of toxic intermediates. The optimum acetate-to-nitrate (Ac/N) ratio, at which all substrates are consumed most efficiently, is about 0.8 (which corresponds to a carbon-to-nitrogen ratio of 1.6). Lower Ac/N ratios lead to the accumulation of toxic intermediate nitrogen compounds, while higher ratios leave significant amounts of residual calcium and acetate. Sand column experiments confirmed that the volume and distribution of the gas phase strongly depend on the pressure conditions. The produced gas volume is inversely related to the pore pressure and can be reasonably predicted using a generic thermodynamic approach, which solves the mass and charge balance to determine the stoichiometry of the metabolic

reaction, and making some simplifying assumptions to determine the distribution of gas over the dissolved and gas phases. Further investigation is required to explain the observed discrepancy between measured and expected values. Under high-pore pressure conditions in the triaxial cell, the produced gas initially expelled the liquid phase but later on seemed to dissipate from the sand sample, probably by diffusion through the latex membrane. At low pore pressures, the produced gas was higher than the gas storage capacity of the sand column and escaped to the back pressure controller column while expelling part of the liquid phase. In the sand column treated with multiple batches of substrate solution at ambient pressure, the conversion rate and cumulative amount of calcium carbonate appeared to be inversely related to the gas distribution because low confinement cracks were induced, which may negatively influence the sand-stabilising effect of the calcium carbonate minerals.

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