Preventing coastal erosion by biological denitrification

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ABSTRACT
Coastal erosion is a major problem along sandy coastlines. Increasing the strength and stiffness of sand could be a possible strategy to prevent land loss. Recent studies have found that denitrifying bacteria may be able to increase erosion resistance by inducing precipitation of calcium carbonate. In this study bacteria obtained from different sites have been evaluated on their activity and efficiency in seawater conditions with different substrate concentrations. Both bacteria, one obtained from freshwater the other from a seawater environment, were found to be active and efficient in seawater. The most active and efficient combination turned out to be bacteria obtained from the North Sea at low substrate concentrations.

Keywords
Coastal erosion, biological denitrification

INTRODUCTION
Sandy coastlines can be subject to erosion as the loose sand grains can get carried away by wind, waves or currents. A possible strategy to prevent the erosion is to increase the erosion resistance. In this framework, research has been conducted using bacteria, which are able to precipitate calcium carbonate crystals. These crystals form bridges between the sand grains that result in an increase in the strength and stiffness of the sand. (Phillips et al., 2013; van Paassen et al., 2010). There are multiple biological processes which can lead to the production of calcium carbonate (van Paassen et al., 2010). Among these processes biological denitrification is considered potentially suitable for ground improvement applications as it has been shown to generate precipitation, while it does not lead to harmful by-products and can be more sustainable and cost efficient than common ground improvement methods like jet-grouting (van Paassen et al. 2010).
Denitrification is an anaerobic biological process that can be performed by multiple bacteria (Zumft, 1997).

The reduction of nitrate to nitrogen gas process takes place in 5 steps, but for simplification and considering the main accumulated intermediate products in the liquid phase, it is divided into two steps.
First, the bacteria consume nitrate (NO_3^-) and acetate ($C_2H_3O_2^-$) and convert this nitrate in to nitrite (NO_2^-).

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$$NO_3^- \rightarrow NO_2^-$$
$$NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Second, they reduce nitrite into nitric oxide (NO), nitrous oxide (N_2O) and finally into nitrogen gas (N_2). (Zumft, 1997) The intermediate compounds, nitrite and nitric oxide, are toxic when accumulated and nitrous oxide is a greenhouse gas.
Nitrite accumulation is not only toxic for the bacteria, but also for the coastal environment. (Almeida, Julio, Reis, & Carrondo, 1995; Glass & Silverstein, 1998) For this reason, the nitrite concentration should be kept as low as possible. However, the end product nitrogen gas is harmless (Pham, 2015; van Paassen et al., 2010) So it's important that both steps of the denitrifying process are in balance with each other, so no accumulation of the intermediate products can take place.
The acetate is the energy source for the bacteria and it functions as an electron- and carbon donor. The nitrate receives these electrons and plays the role of the electron acceptor (Pham, 2015).

$$C_2H_3O_2^- + 1.6\ NO_3^- + 0.6\ H^+ \rightarrow 2\ HCO_3^- + 0.8\ N_2 + 0.8\ H_2O$$

To form the calcium carbonate crystals, calcium ions (Ca^{2+}) should be added to the bacteria solution and or substrate solution. These ions will react with the newly produced inorganic carbon resulting in calcium carbonate and acid (H^+), which will help balance out the alkaline pH resulting from the denitrifying process. (Pham, 2015)

$$Ca^{2+} + HCO_3^- \leftrightarrow CaCO_3 + H^+$$

Research question
Denitrifying bacteria can be found in different environments, but their favourable environment is nutrient rich soil with high humidity. In the laboratory an enrichment culture of denitrifying bacteria, obtained at the Botanical Garden of the Delft University of Technology, was available. This culture has been used to investigate the denitrifying process for the purpose of ground treatment. It was questioned whether these bacteria would be able to survive and be active under seawater conditions, because a difference in salinity between the cell interior and the surrounding environment may generate an osmotic gradient over the cell wall, causing the bacteria to go into survival mode or die. (Wood, 2015).
Another soil sample was obtained from the North Sea at the estuary near Oostvoorne. It was expected this soil contained less denitrifying bacteria compared to the botanical garden soil, because nutrient rich soil contains more nitrate components than marine sands where there is little plant growth. (Seeds, 2014), so the environment has naturally selected for different types of bacteria present in the two soils.

So for the interest of applying the biological denitrification in a coastal area, the research question was formulated: "**Are the denitrifying bacteria from the different environments able to survive and operate under seawater conditions and under which conditions are they most efficient?"** To answer this question a series of experiments has been performed testing liquid batch cultures for their activity and efficiency.

Material and methods

Experiment variations
Six different liquid batch experiments were performed, varying the type of bacteria and solvent and substrate concentrations. The bacteria were either obtained from a freshwater (FWb) or seawater (SWb) environment. The solvent was varied between demineralized water (FW) and seawater (SW) from the North Sea; substrate concentrations were either high or low. High concentrated substrate solutions contained 50 mM $Ca(NO_3)_2$ and 60 mM $Ca(C_2H_3O_2)_2{\cdot}2H_2O$. The low concentrated solutions contained 10 mM $Ca(NO_3)_2$ and 12 mM $Ca(C_2H_3O_2)_2{\cdot}2H_2O$. Overview of the performed experiments is presented in *table 1*. Additional Nutrients and trace elements were added in the following amount for all tests: 0.003 mM $(NH_4)_2SO_4$, 0.0024 mM $MgSO_4$, 0.006 mM KH_2PO_4, 0.014 mM K_2HPO_4 and 1ml/L of trace element solution suggested by Overmann et al. (1992). The substrate ratio used in the experiments was based on the stoichiometry for maximum growth conditions as explained by Pham et al. (2015).

Table 1: Overview performed tests

Solvent	Inoculum	Substrate concentration	
		High	Low
FW	FWb	1.1	1.4
SW		1.2	1.5
	SWb	1.3	1.6

Inoculation
Experiments were performed in 250-ml glass bottles (Duran GLS80), which were filled up to the rim with 380 ml. The incubations containing freshwater bacteria were inoculated with 25 mL/L of stock inoculum. The incubations with seawater bacteria were inoculated with approximately 50 gr of wet soil sample.

Test set-up
After the bottles were filled they were closed using a cap with four ports and they were flushed with nitrogen gas to ensure anoxic conditions in the bottle and the connecting tubes. One port was used to take liquid samples, another one makes sure the gas, which is produced during the denitrifying process, can leave the bottle. The gas is collected in a measuring cylinder, which is placed upside down in a water bath. Electrodes were installed in the remaining two ports in order to measure the pH, electrical conductivity and the temperature.

Data collection
The produced gas volume was measured. The amount of produced N_2 gas was calculated according to the ideal gas law under ambient pressure (1 atm) and the temperature of 298 K, assuming that there was only N_2 in the gas phase. The pH, electrical conductivity and temperature were measured with the consort multi-parameter analyser C3010 and C3060. Samples of the liquid from the bottles were taken on a regular basis and were filtered through a 25 mm Syringe filter with a 0.45 µm Polyethersulfone membrane.
The obtained filtrates were diluted with demi water so the nitrate, nitrite, calcium and acetate concentrations were in the measurable range for the test kits. Nitrate, nitrite, calcium, acetate and ammonium were measured using Hach Lange test kits: LCK339, LCK341, LCL327, LCK365 and LCK303 respectively, according to the manufacturer's protocol. Measurements were performed using spectrophotometry, Hach Lange DR6000; Hach Lange LT200 was also used to heat up the samples as needed for test kit LCK365.

Data analysis
The activity of the bacteria during every test was determined by the conversion rates of nitrate and nitrogen gas and the maximum amount of nitrite accumulation. The efficiency of the bacteria during every test was determined by the percentage of calcium ions converted to calcium carbonate.

Results

Figure 1 shows the measured concentrations of nitrate, nitrite and nitrogen gas for all tests.

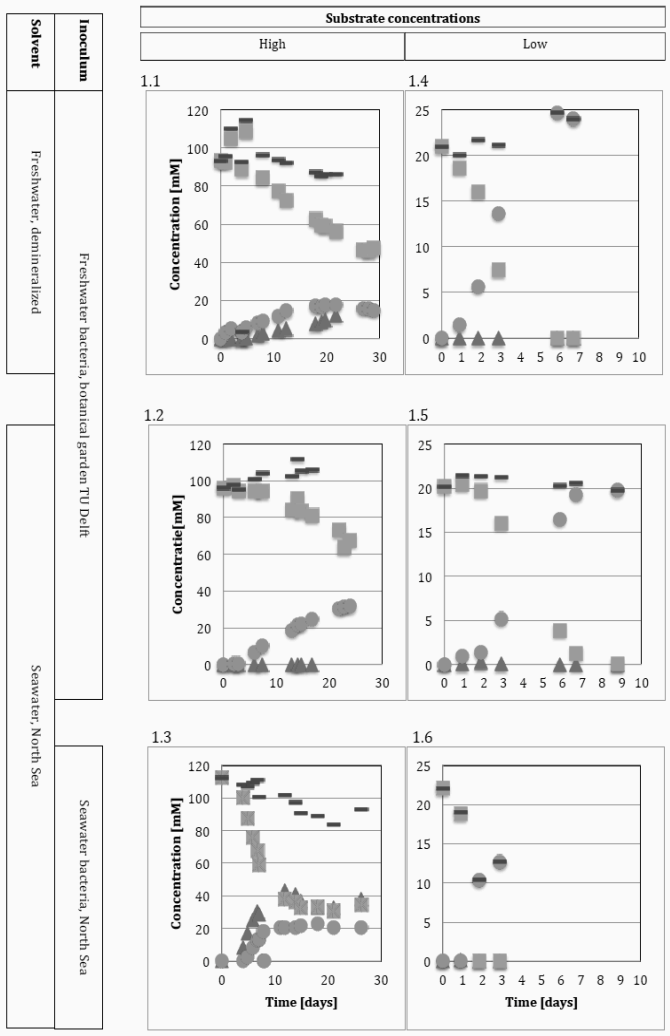

Figure 1: Nitrite (▲), nitrate (■), nitrogen gas (●) and total nitrogen concentration − in time for all tests

As can be seen from these figures in all low concentrated solutions, all nitrate is consumed within 7 days, without

accumulation of nitrite. All the high concentrated solutions show still a lot of nitrate remaining even after a period of 30 days and in most tests high amounts of nitrite accumulation is observed.

Table 2: Overview of substrate conversion rates and nitrogen gas production rate of all performed tests

Test	Solvent	Inoculum	Substrate concentration	Lag phase [days]	Nitrate [mM/day]	Acetate [mM/day]	Nitrogen gas [mM/day]
1.1	FW	FWb	High	5	3.1	1.4	0.40
1.2	SW			8	1.6	0.95	0.66
1.3		SWb		0	6.3	2.6	0.90
1.4	FW	FWb	Low	0	3.6	2.9	2.0
1.5	SW			3	2.8	2.7	1.3
1.6		SWb		3	**20**	**12**	**3.2**

Table 2 provides an overview of nitrate and acetate conversion rates and nitrogen gas production rate of all the performed tests. It is clear based on nitrogen gas production rates that low concentrations result in higher bacterial activity. Seawater bacteria with low substrate concentrations have the highest rates. In *figure 2* the maximum observed nitrite concentrations during the tests are presented. Seawater bacteria with high substrate concentrations produced the highest nitrite concentration.

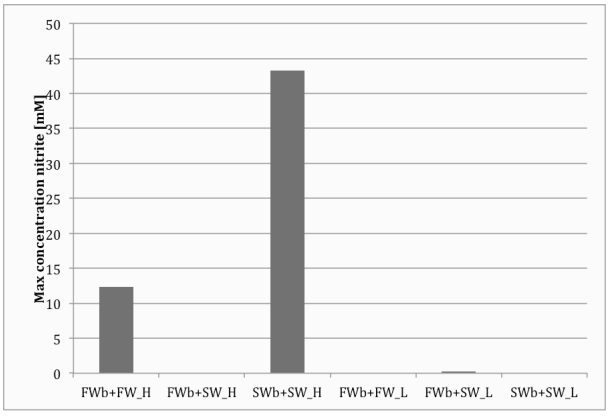

Figure 2: Maximum observed nitrite concentrations of all tests

The percentage of precipitated calcium ions is presented in *figure 3*. The highest percentage of consumed calcium was measured for the low substrate concentration tests.

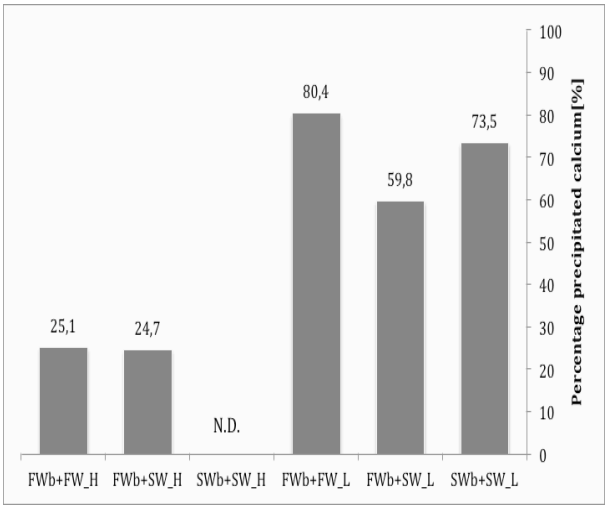

Figure 3: Percentages of precipitated calcium ions

CONCLUSION

The experiments using liquid batch cultivations have shown that denitrifying bacteria obtained from the North Sea and from the botanical garden at TU Delft are both able to survive and operate under seawater conditions. From the results it's concluded that the test with seawater bacteria in seawater conditions with low substrate concentrations has the highest activity. This test is also nearly as efficient in the precipitation of calcium ions as the reference tests in which freshwater was used as a solvent. The freshwater bacteria are less active and efficient in precipitating calcium ions in seawater than in demineralized freshwater, but the difference is not immense. For the practical purposes of preventing coastal erosion by denitrification, these results suggest that both freshwater bacteria and seawater bacteria are able to actively use the denitrification process under seawater conditions that are present at the coast. Both bacteria perform best when low amounts of substrate concentrations are added, which means that they need to be fed frequently to keep them alive and active and the right amount of substrates should be added to prevent a toxic environment. Therefore, these bacteria might be a useful approach in ground improvement and the prevention of coastal erosion.

ROLE OF THE STUDENT

This work is part of a bachelor thesis of civil engineering in the field of geo engineering and within the STW sponsored research program BioGeoCivil at the Delft University of Technology. Manon Ligeon was an undergraduate student working under the supervision of Leon van Paassen, Vinh Pham and Dianne den Hamer when the research in this report was performed. The experiments, design of the questionnaire, the processing of the results as well formulation of the conclusions and the writing were done by the student with the help of the supervisors.

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