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Evaluation of microbially induced desaturation and precipitation (MIDP) using semi-batch columns

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ABSTRACT

To evaluate the performance of Microbially Induced Desaturation and Precipitation (MIDP) via denitrification as a method to mitigate earthquake-induced soil liquefaction, four semi-batch columns filled with poorly graded sand were subjected to two substrate flushes of a 50 mmol L^{-1} nitrate and 50 mmol L^{-1} acetate treatment solution over a 120-day period. Two different water sources were used to prepare the treatment solution to simulate either freshwater or saline conditions. The chemical concentrations relevant to MIDP (e.g., nitrate, organic carbon, inorganic carbon) were measured from liquid and soil samples collected during and after the experimental period. Denitrification occurred for both settings, the saline condition generated sulfide and less N_2, and N_2 generation was not sufficient to mitigate liquefaction in either case. These findings provide insights into factors affecting the process kinetics of MIDP, including the role of inoculum size on the rate of denitrification, the impact of sulfate reduction on the degree of denitrification, and possible inhibition by high salt concentration or hydrogen sulfide is saline-water settings.

INTRODUCTION

Microbially Induced Desaturation and Precipitation (MIDP) via denitrification has been proposed as a mitigation method for earthquake-induced soil liquefaction (O'Donnell et al. 2017a; b). MIDP relies on native denitrifying bacteria to mitigate pore-pressure development through the biogenic production of di-nitrogen gas (N_2) and the precipitation of calcium carbonate ($CaCO_3$) (van Paassen et al. 2010). MIDP has shown the potential to mitigate liquefaction by mechanically strengthening soil and by decreasing the degree of saturation, which dampens the pore-pressure rise during cyclic loading (Hall et al. 2018; He et al. 2014; O'Donnell et al. 2017a; b; Pham 2017).

We investigated the performance of MIDP in semi-batch conditions using two separate water sources, simulated seawater and tap water (as a surrogate for freshwater). Semi-batch conditions offer the opportunity to observe the dynamic responses of microbial communities to changing conditions over time. This approach can reveal critical insights into nutrient consumption

and microbial competition, providing a more realistic picture of how MIDP might behave in field applications to better inform field trials and numerical modeling. These semi-batch reactor experiments enhance our understanding of MIDP kinetics and the potential scalability of MIDP.

A numerical model was developed by Hall et al. (2023) to quantify the MIDP processes. The Hall et al. (2023) model considers multi-phase speciation, microbial competition and inhibition, and chemical concentrations under batch conditions. Hall et al. (2023) optimized parameters such as nutrient concentrations, pH, and microbial activity. Although the Hall et al. (2023) modeling provided insight into the performance of MIDP under saline conditions, MIDP has not yet been fully explored experimentally for saline water. Specifically, the influences of microbial competition, inhibition, and chemical constituents lack experimental data to evaluate predictions from the modeling. By filling these knowledge gaps, our research will contribute to the development of more effective MIDP treatment techniques, providing valuable insights for future large-scale applications.

METHODS

We used four semi-batch column reactors to study the performance of MIDP when different waters were used for substrate addition. The columns used for this study, shown in Figure 1, were 110-cm tall Schedule 40 clear PVC pipes with an internal diameter of 10 cm. The columns were filled with Ottawa F65, a poorly graded silica sand with a mean grain size of 0.30 mm that was procured from U.S. Silica (Ottawa, IL, USA). The soil was placed into the reactors and densified by vibration. The soil height in these reactors was 90 centimeters, with 10 cm of headwater and 10 cm of headspace. The substrate inlet port was positioned at the bottom of the reactors to promote homogeneous substrate distribution throughout the soil column by displacing air upwards during injection. The outlet port was 15 cm from the top, in the headwater space. Each reactor had a TEROS-12 sensor (METER Group) placed 45 cm from the base of the column, denoted by the red star in Figure 1. These sensors assessed the MIDP process by measuring the volumetric water content (VWC, a measure of desaturation) and electrical conductivity (EC) of the soil.

The MIDP treatment recipe in our study was 50 mmol L^{-1} of nitrate and 50 mmol L^{-1} of acetate, following previous experimental studies on MIDP (Kwon et al. 2024; Pham et al. 2018; Stallings Young et al. 2020). Nitrate was added as calcium nitrate ($Ca(NO_3)_2$) (Sigma Aldrich), and acetate was added as calcium acetate monohydrate ($Ca(CH_3COO)_2 \cdot H_2O$) (Carolina Biological Supply Co.). A trace-element and salt solution was added to promote microbial growth (Stallings Young et al. 2020). Tap water (simulated fresh water) and simulated seawater were studied in duplicate reactors. Previous research has studied MIDP under batch or semi-batch conditions using only tap water (Kwon et al. 2024; O'Donnell et al. 2019; Stallings Young et al. 2020; Stallings Young 2021). However, Hall et al. (2023) modeled the impact of local biogeochemical characteristics on MIDP and concluded that source-water conditions and characteristics could have a significant impact on the performance of MIDP. Similar to field applications of MIDP, the tap water was used as a surrogate for local fresh water for substrate dissolution, whereas the saline water simulated using a water source in a coastal environment (Hall et al. 2023). The simulated seawater recipe used in Shiu et al. (2018), provided in Table 1, was selected based on the similar baseline water characteristics to the Hall et al. (2023) model.

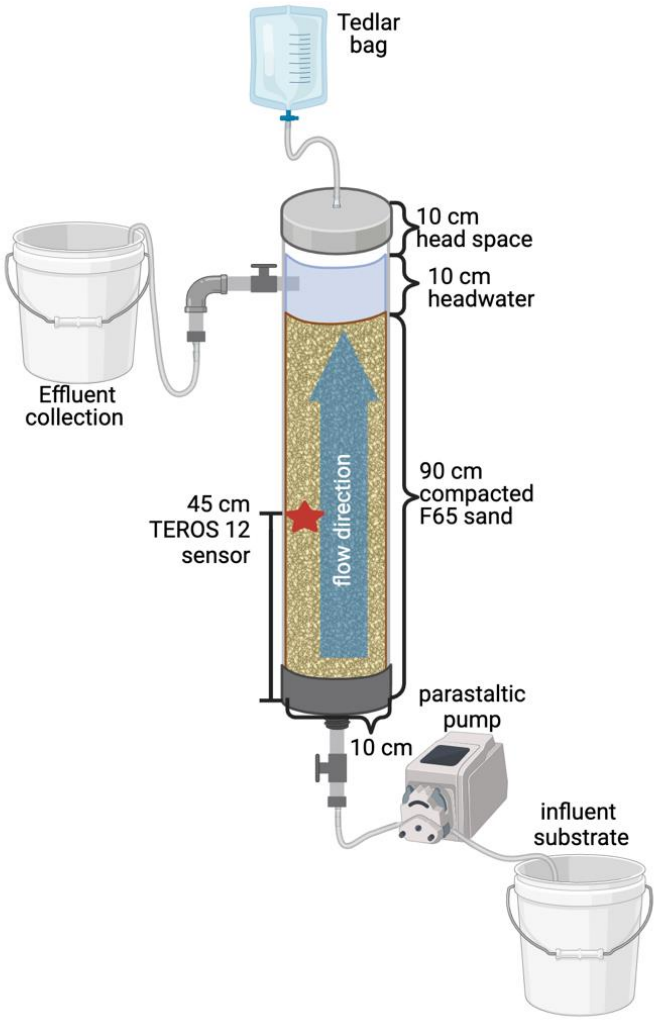

Figure 1. MIDP semi-batch column reactors, including dimensions and sensor location.

Table 1. Simulated saline water recipe (retrieved from Shiu et al. 2018)

Chemical Name	Concentration (mmol L^{-1})
sodium chloride	423
potassium chloride	9.00
calcium chloride	9.27
magnesium chloride	22.94
magnesium sulfate	25.5
sodium bicarbonate	2.14

During MIDP, denitrifying bacteria use organic carbon (i.e., acetate (CH_3COO^-) in this experiment) as the electron donor to reduce nitrate (NO_3^-) to form N_2 gas, dissolved inorganic carbon (DIC), and biomass ($CH_{1.8}O_{0.5}N_{0.2}$). The stoichiometry of the MIDP reaction can vary, depending on the metabolic status of the biomass. A typical overall reaction, including catabolic and anabolic processes, is shown in Eq. 1 (Rittmann and McCarty 2020):

$$1.2CH_3COO^- + NO_3^- + 0.2H_2O \rightarrow CH_{1.8}O_{0.6}N_{0.2} + 0.4N_{2(g)} + 1.4HCO_3^- + 0.8OH^- \quad \textit{Eq. 1}$$

Table 1 shows that sulfate (SO_4^{2-}) was present at 25.5 mmol L^{-1} in our simulated saline condition. Because we introduced organic carbon to the system to trigger denitrification, nitrate-reducing bacteria and sulfate-reducing bacteria competed for the organic substrate. A typical overall reaction for sulfate reduction is shown in Eq. 2, where biomass, hydrogen sulfide (H_2S), bisulfide (HS^-), and inorganic carbon (as CO_2 and HCO_3^-) are produced (Rittmann and McCarty 2020). For Eq. 1 and Eq. 2, we assumed that nitrate was the nitrogen source for biomass growth.

$$1.3CH_3COO^- + SO_4^{2-} + 0.1NO_3^- + 1.6H^+ \rightarrow 0.1C_5H_7O_2N + 0.5H_2S + 0.5HS^- + 0.9CO_2 + 1.3HCO_3^- + H_2O \quad \textit{Eq. 2}$$

The reactors underwent two separate 3.25-hour substrate flushes of the MIDP treatment solution: the first on day zero of the experiment and the second on day 60. A flow rate of 1.6 L/hr was employed, based on Darcy's law and Stoke's law, to reduce the potential for fluidizing the sand in the reactor. For each substrate flush, 5.2 L of substrate solution was prepared. This volume and flow rate allowed for complete pore water replacement over 3.25 hours. A saline bacterial culture was created and introduced to the duplicate saline reactors prior to the first substrate flush to simulate a representative bacterial culture in saline conditions. Additionally, a heterotrophic denitrifying bacterial culture was created. In both environments studied, the first substrate flush contained the previously mentioned treatment recipe and 52 mL of the nitrate-reducing bacterial culture. The second substrate flush contained only calcium acetate, calcium nitrate, and the trace element and salt solution at the above stated concentrations. On day 120 of the experiment, the reactors were flushed with either tap water or simulated seawater, as appropriate to each column.

During each of the 3.25-hour flush on days 60 and 120, six liquid samples were collected at 32-minute intervals to measure nitrate and sulfate concentrations and assess MIDP trends over each treatment period. pH, EC, nitrate, sulfate, dissolved organic carbon (DOC), and DIC were measured from the liquid samples. A HACH DR6000 Spectrophotometer and HACH TNTplus spectrophotometer kits (Loveland, CO, USA) were used for quantitative analysis of the chemical concentrations. A Shimadzu TOC-L SHP Total Carbon Analyzer (Columbia, MD, USA) was used to measure DOC and DIC concentrations.

RESULTS AND DISCUSSION

Figure 2 shows the average residual measured nitrate, sulfate, DIC, DOC, and total dissolved carbon (TDC) during the final flush on day 120 for both environments. Results from the duplicate reactors were consistent, with a maximum average standard deviation of 16% between duplicates for all parameters. Samples on the x-axis of Figure 2 are labeled according to their environment and sampling order. The environments are "tap" for tap water and "saline" for saline water. The sampling order represents liquid collected from the top at sequential 32-minute intervals during the flush on day 120. Given that the measured pH was 7.4, all DIC measured was bicarbonate, which was produced by the MIDP reactions represented by Eq. 1 or 2.

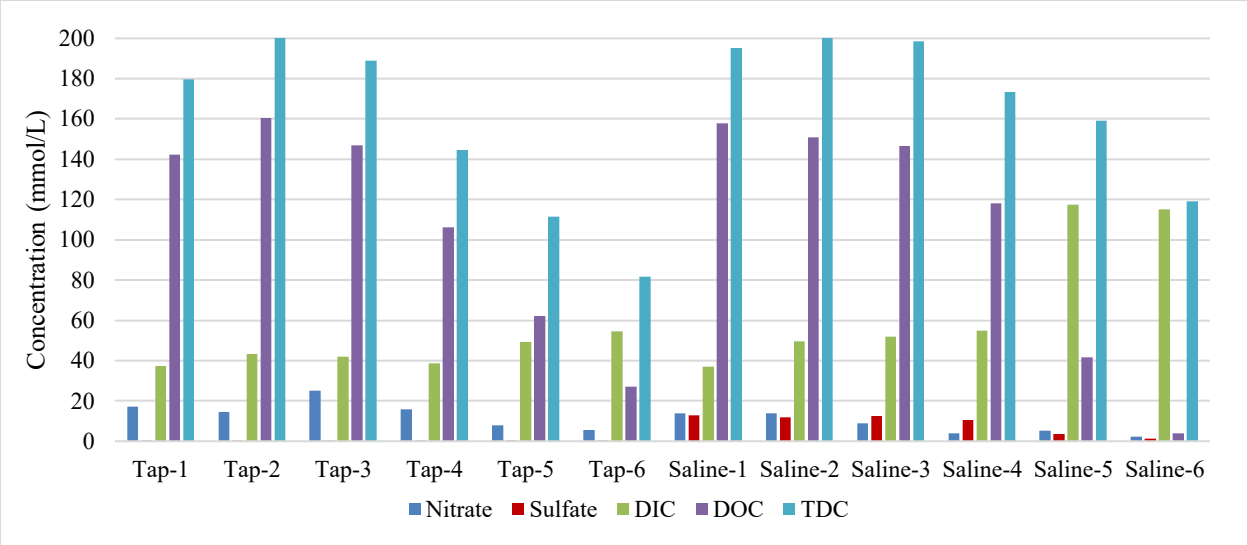

Figure 2. Measured chemical concentrations from liquid samples collected on day 120

The results in Figure 2 demonstrate that denitrification occurred in tap water and saline conditions. The initial NO_3^- concentration was 50 mmol L^{-1}, and the final measured NO_3^- concentrations ranged from 6 to 25 mmol L^{-1} in tap water and 2 to 15 mmol L^{-1} in saline water. The nitrate concentrations decreased with depth, indicating that denitrification was greater near the column inlet. DOC also decreased along the column but was never depleted. This trend is consistent with the work of Pham et al. (2018) and Stallings Young et al. (2022), who also observed residual organic carbon. DIC increased with the loss of DOC, although the TDC (sum of DIC + DOC) declined with distance in the column. The loss of total carbon could have been the result of biomass synthesis, CO_2 off-gassing, calcium carbonate precipitation, or a combination.

Although sulfate reduction was not detected in the tap water condition, substantial sulfate reduction occurred in the saline condition, and sulfate concentrations decreased with depth in the saline reactors. The high degree of sulfate reduction was consistent with the presence of DOC throughout the column.

Eq. 1 indicates that, for every 2.4 moles of organic carbon consumed, 1.4 moles of inorganic carbon are produced in denitrification. The results presented in Figure 2 support the stoichiometry presented by Eq. 1, which further indicates success with denitrification. Figure 2 also shows that DIC production was greater in saline conditions due to sulfate reduction. The initial DIC in the saline conditions did not contribute significantly to this higher DIC concentration observed in the flushed water because the initial inorganic carbon concentration in saline conditions (2.14 mmol L^{-1}) was a small fraction of the DIC produced as a result of denitrification (58 mmol L^{-1}).

Eq. 1 shows that, for every 1 mole of nitrate consumed, 0.4 moles of N_2 gas is produced. This means, for every 50 mmol L^{-1} of nitrate consumed, ~20 mmol L^{-1} of N_2 was produced. Integrating the results presented in Figure 2 gives an average of 14.3 mmol L^{-1} residual nitrate in tap water conditions, and an average 7.9 mmol L^{-1} residual nitrate in saline conditions. Based on the stoichiometry presented on Eq. 1, complete denitrification to N_2 of the lost NO_3 generated approximately 0.88 L N_2 gas in the tap-water columns and 1.06 L N_2 gas in the saline-water columns. The total pore volume was 2.8 L, which means that the average desaturation in tap water columns was 6% and 3% in saline columns. Hall et al. (2023) and Pham et al (2018) indicate that 10% desaturation is adequate for MIDP. Comparing the stoichiometric predictions from equation

1 to the goal desaturation level, we can conclude that sufficient desaturation for to reach 10% desaturation did not occur in either environment, although saline water had less N_2 generation, perhaps due to competition from sulfate reduction.

Our treatment took 40 days longer than expected based on modeling (Hall et al. 2023) and past experiments (Kwon et al. 2024; Pham et al. 2018; Stallings Young et al. 2022). The slowdown for the case of saline water might be attributable to inhibition of denitrification by high salinity and hydrogen sulfide generation (Hall et al., 2023). Salinity is known to inhibit denitrification by creating osmotic stress on denitrifying bacteria, which reduces their metabolic activity and impairs their ability to convert nitrate to nitrogen gas (Hall et al. 2023; Krishna Rao and Gnanam 1990). Hydrogen sulfide is a known inhibitor of denitrifying bacteria (Liang et al. 2020). However, neither of these inhibiting factors was present with tap water. Another possible cause for the long delay was that the inocula contained too few active denitrifiers, which led to an extended time to grow and accumulate sufficient denitrifying bacteria.

CONCLUSION

We conducted semi-batch column tests to compare the effectiveness of MIDP as a method for mitigating earthquake induced soil liquefaction in freshwater and saline conditions. Soil columns filled with poorly graded sand were subject to two substrate flushes over a 120-day period. Two different water sources were used to simulate freshwater versus saline conditions. Chemical concentrations relevant to MIDP measured from samples collected during and after the experiment documented denitrification in freshwater and saline conditions, but substrate utilization and MIDP production rates were affected by the source water. In saline conditions, the MIDP treatment led to sulfide production and less N_2-gas production. While a target desaturation level of 10% was not achieved in either environment, N_2 generation was calculated to increase desaturation by 3% for saline water and 6% for tap water. Further investigation is warranted to understand better the roles of the inoculum size on the rate of denitrification, electron-donor concentration on promoting sulfate reduction, competition between denitrification and sulfate reduction, and possible inhibition by high salt concentration or hydrogen sulfide in saline conditions.

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