Effects of Pyrite Amended Media on Denitrification Rates in Constructed, Subsurface Wetlands

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Background

Denitrification is the process by which oxidized forms of reactive nitrogen (e.g., nitrate) act as a terminal electron acceptor for anaerobic respiration producing gaseous nitrogen products [5]. The removal of nitrate from wastewater is of increasing importance in order to prevent the potentially disastrous effects of algal blooms and eutrophication. It has generally been assumed that heterotrophic microorganisms that use organic carbon as a source of electrons perform the bulk of denitrification in wetlands. However, recent research suggests that chemoautotrophic denitrifiers (i.e. those that use alternative electron donors and synthesize their own organic carbon) may play an important role, even eclipsing the role of heterotrophs under certain conditions [2][1]. Sulfide minerals have been studied as the potential source of electrons for autotrophic microbes [4]. However, past studies have focused on aquifers, groundwater and/or a specific species of denitrifying bacteria [3][4]. Thus, the objective of this study is to investigate the effects of pyrite (FeS_2), a sulfide mineral, on the denitrification rates in constructed, subsurface wetlands.

Hypothesis

This study examined two main hypotheses:

1. Sulfide minerals, like pyrite, can be used as an inorganic electron source to fuel denitrification.
2. The addition of pyrite to subsurface wetlands will enhance the denitrification rate and overall nitrogen removal rates of wastewater.

Methods

To test our hypotheses, we performed batch and semi-batch microcosm experiments through which we tested the effect of adding pyrite to model wetland sediments on denitrification rates. The experiments were conducted in 125ml serum bottles sealed with septa and crimp caps. Three conditions were compared in triplicate, with a single control. The control serum bottle contained 30 mg of sand, 40 ml of wastewater and about 0.6 mg of inoculum. The inoculum consisted of sediments from the base of a vegetated wetland cell, which were collected from the Discovery Bay Wastewater Treatment facility. The first condition, henceforth designated as "A", contained the same contents of the control, with an additional 5.4g of woodchips. Condition B contained (in addition to the control contents): 2.7g of woodchips and 2g of pyrite. Finally, Condition C contained (in addition to the control) 4g of pyrite.

The wastewater was sparged with nitrogen gas (N_2) until the dissolved oxygen (DO) concentration was reduced to less than 0.5 mg/L. After sealing the serum bottles, the headspace was also sparged with N_2 to maintain anaerobic conditions.

Every 24 hours for 14 days, 6 ml of the wastewater in each serum bottle was removed for sampling purposes. 6 ml of wastewater, with a nitrate concentration of 30mg/L (warmed to 25°C and sparged to a DO < 0.5mg/L) was used to replenish each serum bottle.

Analysis

Samples were analyzed for concentrations of NO_3^-, NO_2^-, Cl^-, and SO_4^{2-} (in ppm) using Ion Chromatography (IC) on a Dionex ICS 1100, with non-purgeable organic carbon (NPOC) and total nitrogen (TN) concentrations (ppm) measured on a Shimadzu Total Organic Carbon Analyzer (TOC-V CSH), and spectral measurements (over the range of wavelengths between 200 and 800 nm) taken using a Shimadzu UV-Vis Spectrophotometer (UV-2600). Changes in sulfate concentrations and nitrate concentrations over time were used to evaluate the proportion of nitrate lost that could be attributed to pyrite-driven denitrification. This was achieved by comparing the ratio of sulfate produced over nitrate lost to the expected stoichiometric ratio for pyrite-driven denitrification. The [TN] was used alongside the [NO_3^-] and [NO_2^-] to determine if there was complete nitrogen removal or conversion of nitrate to organic nitrogen and or ammonia. Lastly, the absorbances at 254 nm along with [NPOC] were used to calculate $SUVA_{254}$ values, which provide information about the aromaticity and bioavailability of the dissolved organic carbon [6]**.**

Results

This study was run over the course of two weeks. The chart above shows results from the first few collection times. The duration of the study was two weeks. Unfortunately, the nitrate concentration in the influent wastewater had fallen to <0.5 mg/L. Thus, much of the remaining data doesn't add any viable information to this study, as there was not a continuous inflow of nitrate to the microcosms.

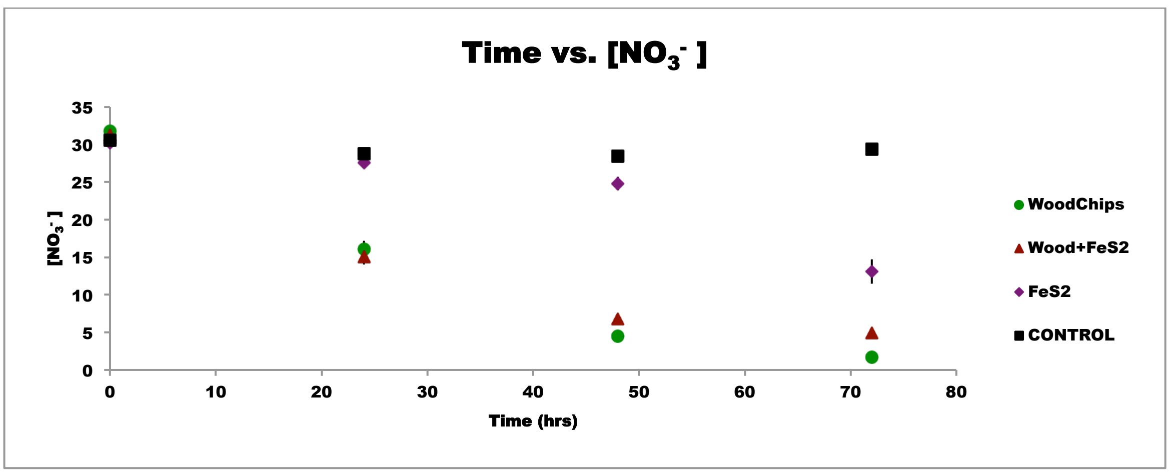

Chart 1: The nitrate concentration over time for the 4 conditions.

It seems from the chart that the condition with woodchips alone had the fastest denitrification rate, followed by the mixture of electron donors, and lastly pyrite alone.

Conclusion

This study shows that pyrite can in fact be used as the source of electrons to the denitrifying bacteria. This is drawn from the fact that denitrification takes place in microcosms with only pyrite as an electron source. No conclusions can be drawn regarding whether or not the addition of pyrite to subsurface wetlands increase the denitrification rates or overall nitrogen removal rates. The data available seems to support that woodchips have the highest denitrification rates compared to the other two cases, but this may have been related to a lag period in microbial growth. Lacking long-term data, we cannot state anything conclusively. However, further studies are required to test this further.

Future Work

Similar studies need to be conducted in flow through systems to model conditions in a subsurface wetland better. Literature states that whether autotrphic or heterotrophic denitrifcation occurs depend on whether the system studied is a flow through or a batch system. Future studies could also monitor the microbial communities. Autotrophic denitrification is said to dominate over heterotrophic denitrification even when a source of organic carbon is present [7]. Further studies into this can lead to advances in denitrification in constructed wetlands.

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