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Anaerobic ammonium oxidation coupled to iron reduction in constructed wetland mesocosms

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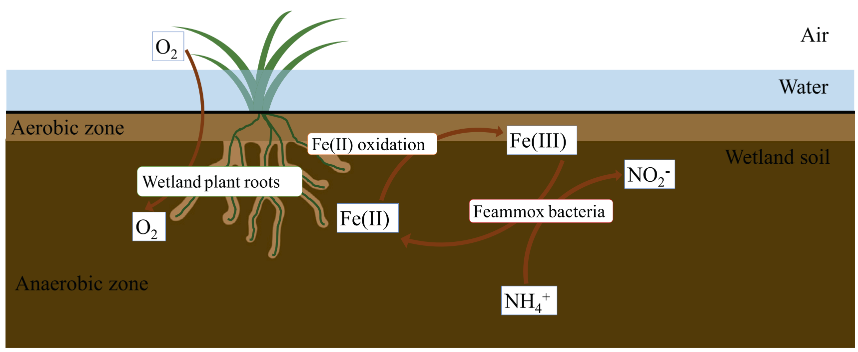
HIGHLIGHTS

- Constructed wetlands for ammonium oxidation coupled to iron reduction
- Effect of *Acidimicrobiaceae* A6 bioaugmentation on anaerobic NH_4^+ oxidation
- Feammox enhancement via supplementation of ferric iron to wetland sediments
- Phylogenetic analysis for Feammox, NH_4^+ oxidizing, and iron reducing bacteria
- Acidification by AOB facilitates NH_4^+ oxidation via Feammox.

GRAPHICAL ABSTRACT



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ABSTRACT

Acidimicrobiaceae sp. A6 (referred to as A6) was recently identified as playing a key role in the Feammox process (ammonium oxidation coupled to iron reduction). Two constructed wetlands (CW) were built and bioaugmented with A6 to determine if, under the right conditions, Feammox can be enhanced in CWs by having strata with higher iron content. Hence, the solid stratum in the CWs was sand, and one CW was augmented with ferrihydrite. Vertical ammonium (NH_4^+) concentration profiles in the CW mesocosms were monitored regularly. After four months of operation, when reducing conditions were established in the CWs, they were inoculated with an enrichment culture containing A6 and monitored for an additional four months, after which they were dismantled and analyzed. During the four-month period after the A6 enrichment culture injection, 25.0 ± 7.3% of NH_4^+ was removed from the CW with the high iron substrate whereas 11.0 ± 9.7% was removed from the CW with the low iron substrate on average. Since the CW with high NH_4^+ removal had the same plant density, same bacterial biomass, same fraction of ammonium oxidizing bacteria (AOB), a higher biomass of A6, and a higher pH (NH_4^+ oxidation by Feammox raises pH, whereas NH_4^+ oxidation by aerobic AOB decreases pH), this difference in NH_4^+ removal is attributed to the Feammox process, indicating that wetlands can be constructed to take advantage of the Feammox process for increased NH_4^+ removal.

1. Introduction

Ammonium (NH_4^+) is ubiquitous in industrial, domestic, and agricultural wastewaters and is transported easily to surface waters, groundwater, and soils via runoff because of its high solubility and mobility. Although NH_4^+ itself can serve as a nutrient for plants, the excessive

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load of NH_4^+ to natural systems from anthropogenic activities has led to a series of problems, including the toxicity of ammonia (NH_3) and nitrite (NO_2^-, which is formed from the oxidation of NH_4^+) (Berenzen et al., 2001; Daniels and Boyd, 1987), the acceleration of eutrophication in waterbodies when nitrogen is the limiting nutrient, and the increased emission of nitrous oxide (N_2O), a greenhouse gas.

The most common NH_4^+ removal process is nitrification during which NH_4^+ is oxidized by aerobic ammonium oxidizing bacteria (AOB) and/or archaea (AOA) to first NO_2^- and then nitrate (NO_3^-). Then NO_3^- is denitrified to N_2 anaerobically and released to the atmosphere. A novel anaerobic NH_4^+ oxidation process coupled to dissimilatory ferric iron reduction (also known as Feammox) has been described and studied in recent years (Clement et al., 2005; Ding et al., 2014; Huang et al., 2016; Huang and Jaffé, 2015; Li et al., 2015; Sawayama, 2006; Shresta et al., 2009; Yang et al., 2012). Though the Feammox process occurs under anaerobic condition, it is different from anammox, the other known anaerobic NH_4^+ oxidation process. The Feammox process oxidizes NH_4^+ with ferric iron (Fe(III)) as the electron acceptor, while the anammox process oxidizes NH_4^+ to N_2 with NO_2^- as the electron acceptor (Strous et al., 1999). Multiple species that belong to the phylum Planctomycetes have been identified as anammox bacteria (Kartal et al., 2007; Schmid et al., 2003; Strous et al., 1999), while so far only one microorganism capable to perform the Feammox process has been identified, namely *Acidimicrobiaceae* sp. A6 (ATCC, PTA-122488; from here on referred to as A6) (Huang and Jaffé, 2018). This lithoautotrophic bacterium A6 was obtained via enrichment cultures from soil samples collected at the Assunpink Wildlife Management Area, New Jersey, and has recently been isolated and grown as a pure culture. The stoichiometry of the reaction with ferrihydrite as the ferric iron source is expressed as follows (Huang and Jaffé, 2015; Huang and Jaffé, 2018):

$$3\ Fe_2O_3 \cdot 0.5H_2O + NH_4{}^+ + 10H^+ \rightarrow NO_2{}^- + 6\ Fe^{2+} + 8.5\ H_2O \qquad (1)$$

Other pathways reported by other authors for NH_4^+ oxidation under iron-reducing conditions include conversion of NH_4^+ to NO_3^- (Sawayama, 2006) or directly to nitrogen gas (Yang et al., 2012) and may be performed by different organisms. Thermodynamic calculation under typical conditions found in soil showed that Feammox to N_2 is more energetically favorable than Feammox to NO_2^- or NO_3^- (Yang et al., 2012). However, acetylene block experiments using A6 enrichment cultures showed that NH_4^+ consumed in the incubations was equal to NO_2^- plus N_2O produced, and isotope tracer incubations with ^{15}N-NH_4^+ as the NH_4^+ source showed ^{15}N-N_2O build-up (Huang and Jaffé, 2015). A6 pure strain, with isotope tracer, incubation experiments further confirmed that ^{15}N-NH_4^+ was oxidized to ^{15}N-NO_2^-, and the ratio of NH_4^+ removed to Fe(II) produced was 1:6 as shown by Eq. (1) (Huang and Jaffé, 2018). In this study, NH_4^+ oxidation to NO_2^-, as depicted in Eq. (1), was considered as the Feammox pathway, since an A6 enrichment culture developed from sediments obtained from the Assunpink site, NJ (Huang and Jaffé, 2015; Huang and Jaffé, 2018), was used to inoculate the CW mesocosms, and since we have never observed an oxidation to other N species than NO_2^- in incubations with cultures from that site.

Eq. (1) indicates that acidic conditions should be favorable for the Feammox process and that pH should increase as NH_4^+ is oxidized. In contrast, pH decreases when NH_4^+ is oxidized under aerobic conditions by ammonium oxidizing bacteria (AOB) or ammonium oxidizing archaea (AOA) as shown in Eq. (2):

$$2\ NH_4{}^+ + 3\ O_2 \rightarrow 2\ NO_2{}^- + 2\ H_2O + 4H^+ \qquad (2)$$

Huang et al. (2016) have analyzed and incubated a large set of soil samples from several locations in the US and China to determine under what conditions NH_4^+ oxidation was coupled to iron reduction. They showed that both A6 was present and that NH_4^+ oxidation occurred during iron reduction in samples from many locations, but only when the soils were acidic and rich in Fe(III). Calculations show that for Eq. (1), the change in Gibbs free energy (ΔG) becomes zero at a pH = 6.5 for typical soil conditions observed in our incubations (Huang and Jaffé, 2015), whereas incubations with the pure A6 strain under varying pH conditions (Huang and Jaffé, 2018) showed the highest NH_4^+ removal and iron production at a pH = 4, and they became negligible for pH ≥ 7.

Given that the Feammox process has been observed in multiple wetland environments such as forested riparian wetlands (Clement et al., 2005; Huang et al., 2016; Huang and Jaffé, 2015), paddy soils (Ding et al., 2014; Huang et al., 2016), and intertidal wetland sediments (Li et al., 2015), it is hypothesized here that this process can be induced to occur in constructed wetlands (CWs) as well. An estimate of nitrogen loss through Feammox in paddy soils (Li et al., 2015) concluded that the Feammox process is comparable to denitrification and anammox in terms of N transformation. Based on this estimate, the amount of NH_4^+ oxidized via Feammox in CWs could potentially be a considerable fraction of total NH_4^+ removal.

Since application of ammonium-based and urea fertilizers to agricultural land is a major non-point source of NH_4^+ to surface waters, CWs, which are designed to maximize NH_4^+ removal from runoff are an attractive option for controlling agricultural NH_4^+ discharges. As a passive treatment system, CWs have been used to treat suspended solids, organic matter, nitrogen, phosphorus, trace elements and microorganisms in wastewater (Lin et al., 2002). However, the feasibility of the Feammox process in CWs for NH_4^+ removal has not been investigated. Since NH_4^+ oxidation by AOB and AOA requires dissolved oxygen, most of the NH_4^+ removed in wetlands is oxidized in the free-standing water or in the direct vicinity of roots. Hence, enhancing the Feammox process in CWs might result in increased NH_4^+ removal under anaerobic conditions. Furthermore, NH_4^+ oxidation by AOB and AOA lowers the pH, which could aid the Feammox process, and conversely, the Feammox process might prevent the pH from becoming rather low.

The goal of this research was therefore to determine whether bioaugmentation of a CW with A6, especially when the strata of the CW is rich in Fe(III) results in an enhanced removal of NH_4^+. For this purpose, two CWs were built and operated. To enhance the Feammox activity, one of the CWs had its substrate supplemented with ferrihydrite. Both mesocosms were inoculated with A6, and NH_4^+ removal before and after inoculation was monitored via a mas balance. The microbial community was analyzed at the end of the experiment to contrast total bacteria, as well as abundance of AOB, Feammox, and iron reducers, and to gain new insights into the Feammox process.

2. Material and methods

2.1. Acidimicrobiaceae bacterium A6 enrichment culture

The A6 enrichment culture was developed from riparian wetland (Assunpink Wildlife Management Area, NJ, USA) sediment slurries as described previously (Huang and Jaffé, 2015). The enrichment culture was kept at room temperature (20–25 °C) in an anaerobic glove box. The medium for the enrichment culture consisted of: 2-line ferrihydrite 10 mM; NH_4Cl 3.8 mM; $(NH_4)_2SO_4$ 0.6 mM; $NaHCO_3$ 0.24 mM, $KHCO_3$ 0.71 mM, KH_2PO_4 0.052 mM, $MgSO_4 \cdot 7H_2O$ 0.41 mM; $CaCl_2$ 0.54 mM; AQDS (9,10-anthraquinone-2, 6-disulfonic acid) 0.05 mM; vitamin supplement (ATCC®) 0.1 μL/L (see Supplemental material Table S1) and 1 mL/L of a trace element solution (Supplemental material Table S2).

2.2. CW mesocosm design

The CW mesocosms were designed as shown in Fig. 1 with a 3-cm water level above the sediments. The inflow was pumped into the mesocosms from the bottom with a peristaltic pump and an opening was drilled at the top to allow the effluent to drain and to maintain a constant water level. PVC pipes with 6-inch inner diameter were used