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Studies on a Novel Actinobacteria Species Capable of Oxidizing Ammonium under Iron Reduction Conditions

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Ammonium (NH_4^+) oxidation coupled to iron reduction in the absence of oxygen and nitrate/nitrite (NO_3^-/NO_2^-) was noted in a forested riparian wetland in New Jersey [(1,2)], and in tropical rainforest soils [(3)], and was coined Feammox [(4)]. Through a 180-days anaerobic incubation of soil samples collected at the New Jersey site, and using 16S rDNA PCR-DGGE, 454-pyosequecing, and qPCR analysis, we have shown that an *Acidimicrobiaceae* bacterium A6, belonging to the phylum *Actinobacteria*, is responsible for this Feammox process, described previously [(1,2)]. We have enriched these Feammox bacteria in a high efficiency Feammox membrane reactor (with 85% NH_4^+removal per 48h), and isolated the pure *Acidimicrobiaceae* bacterium A6 strain [5,] in an autotrophic medium.

To determine if the Feammox bacteria found in this study are common, at least at the regional scale, we analyzed a series of local wetland-, upland-, as well as storm-water detention pond-sediments. Through anaerobic incubations and molecular biology analysis, the Feammox reaction and *Acidimicrobiaceae* bacterium A6 were found in three of twenty soil samples collected, indicating that the Feammox pathway might be widespread in selected soil environments. Results show that soil pH and Fe(III) content are key environmental factors controlling the distributions of Feammox bacteria, which require acidic conditions and the presence of iron oxides. Results from incubation experiments conducted at different temperatures have shown that, in contrast to another anaerobic ammonium oxidation pathways (e.g., anammox), the optimal temperature of the Feammox process is $\sim 20^\circ$ and that the organisms are still active when the temperature is around 10°.

An incubation experiment amended with acetylene gas (C_2H_2) as a selected inhibitor showed that in the Feammox reaction, Fe(III) is the electron acceptor, which is reduced to Fe(II), and $NH_4^+$is the electron donor, which is oxidized to NO_2^-. After this process, NO_2^- is converted to nitrogen gas (N_2) via conventional denitrification and/or anammox.

Based on the results obtained so far, we conclude that Feammox may be an important process for nitrogen loss in iron rich, acidic soil environments under oxygen-limited conditions. Our results from operating a membrane reactor with a high *Acidimicrobiaceae* bacterium A6 content ($\sim$ 50%) indicate that it might be possible to develop a novel anaerobic NH_4^+ removal technology from wastewater based on the Feammox process, which might be more robust at low temperatures than Anammox-based processes.

References

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