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Ammonium Oxidation Under Iron Reducing Conditions: Environmental Factors Characterization and Process Optimization

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Ammonium (NH4+) oxidation coupled to iron (Fe) reduction in the absence of oxygen and nitrate/nitrite (NO_3-/NO_2-) has been reported by several investigators and is referred to as Feammox. Feammox is a biological reaction, where Fe(III) is the electron acceptor, which is reduced to Fe(II), and NH4+ is the electron donor, which is oxidized to NO_2-. An Acidimicrobiaceae bacterium named A6, a previously unreported species in the Acidimicrobiaceae family, has been identified as being responsible for the Feammox process(1, 2)

Feammox process was noted in riparian wetland soils in New Jersey(1,3), in tropical rainforest soils in Puerto Rico (4) and in paddy soils in China (5). In addition to these published locations, Feammox process was also found in samples collected from a series of local wetland-, upland-, as well as storm-water detention pond-sediments in New Jersey, river sediments from South Carolina, and forested soils near an acid mine drainage (Dabaoshan, Guangdong province) in China. Using primers acm342f - 439r (2), Acidimicrobiaceae bacterium A6 was detected in samples where Feammox was observed, after strictly anaerobic incubations. According to a canonical correspondence analysis with environmental characteristics and soil microbial communities, the species-environment relationship indicated that pH and Fe oxides content were the primary factors controlling Feammox process. Anaerobic incubations of Feammox enrichment cultures adjusted to different pH, revealed that the optimal pH for Feammox is $4 \sim 5$, and the reaction does not proceed when pH > 7. No correlation was found between the distributions of Feammox bacteria and other NH4+ oxidation bacteria.

Pure Acidimicrobiaceae bacterium A6 strain was isolated in an autotrophic medium, from an active Feammox membrane reactor (A6 was enriched to 65.8% of the total bacteria). A 13C labeled CO_2 amendment was conducted, and the 13C in cells of A6 increased from 1.80% to 10.3% after 14 days incubation. In a separate incubation, 15NH4Cl was added with a final concentration of 0.5 mmol L-1, and 0.133 mmol L-1 of 15NO_2- was detected, while no 15NO_3- was produced.

In Feammox culture amended with different Fe(III) sources, Feammox reaction proceeded only when Fe oxides (ferrihydrite or goethite) were supplied, whereas samples incubated with ferric chloride or ferric citrate showed no measurable NH4+ oxidation. Acidimicrobiaceae bacterium A6 were then grown in Microbial Electrolysis Cells (MECs). While being gently shaken, with a voltage input of 0.7V and a 10Ω resistance between the anode and cathode, the MECs produce currents, increasing from 0.1μA up to $\sim 35\mu$A while NH4+ was available in the medium. Up to 105 copies of DNA/ml have been detected in liquid medium after 3 weeks of operation. Hence, MECs represent an alternative, iron-free form, for optimized biomass production of pure Acidimicrobiaceae bacterium A6.

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