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Characterization of Bacterial Populations Capable of Nitrification at Cold Temperatures and High Ammonia Levels

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Abstract

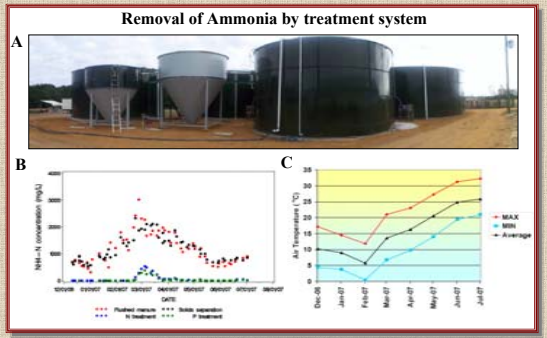
With the growth of large-scale, confined, commercial animal systems in the United States, management of the ensuing livestock waste has become a major source of environmental concern. For the swine industry, this waste is typically treated in large anaerobic lagoons. However, high levels of ammonia (NH_3) may escape into the atmosphere by volatilization. Its subsequent deposition across the surrounding landscape can be a significant source of nitrogen non-point pollution in these areas. Biological removal of ammonia via nitrification is a therefore a critical and often rate-limiting step in the removal of nitrogen as N_2 through biological nitrification/denitrification systems. The discovery of bacterial populations capable of efficiently nitrifying high levels of ammonia at cold temperature represents a tremendous benefit to on-farm wastewater treatment systems. This study describes the identification and characterization of such bacterial populations.

Materials and Methods

Nitrification Experiments. Full scale studies on a second generation swine wastewater plant were performed at the Super Soil System USA, Inc. swine waste treatment system at Tyndall Farm, Clinton, NC. (See Vanotti and Szogi, Second Generation Super Soil Technology, Final Report for NC Department of Justice Office of the Attorney General Environmental Enhancement Fund Program, 2007). Bench fluidized reactors (1.2L) were operated under continuous flow using swine lagoon wastewater from Goshen Ridge farm containing 330 to 450 mg NH_4-N/L (Vanotti et al., ASABE International Symposium on Air Quality and Waste Management for Agriculture, September 16-19 2007, Broomfield, CO). Water temperature was controlled using a submerged temperature probe and a refrigerated circulating bath. Influent wastewater was kept at 4°C before treatment. Experiments were started at 15°C and a hydraulic retention time of 18 hours, with wastewater process temperatures decreased 2.5-3°C every three weeks to a low of 3°C. For continuous flow, water samples were taken twice per week for analysis. All water analyses were performed according to Standard Methods for the Examination of Water and Wastewater (APHA, AWWA, WEF, 1998).

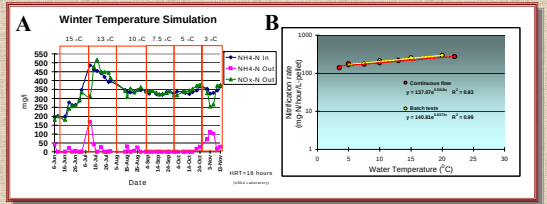
DNA Analysis. DNA was isolated from the centrifuged sediment of a nitrification reactor containing flocculated organism. A bead-beater, 10% SDS lysis protocol was followed, with DNA being isolated from the lysate by successive rounds of phenol/chloroform extraction. The 16S rDNA was amplified using universal bacterial primers Eubac27f and 1492r, and cloned into a TOPO-TA vector (Invitrogen). Inserts were amplified using M13f/M13r primers, and properly sized fragments were restriction digested with *Hae*III, *Msp*I and *Rsa*I. Clones with different banding patterns (signifying a single operational taxonomical unit [OTU]) were selected for sequencing on an ABI 3730*xl*. Sequences were examined and analyzed using Geneious v3.0 (Biomatters, Ltd).

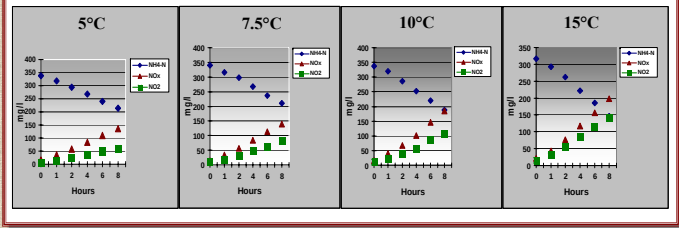
Fluorescent In Situ Hybridization (FISH). FISH was performed as previously described using probe Nso190 (50% formamide, 0.028M NaCl) which hybridizes to most ammonia-oxidizing β-proteobacteria (Egli et al., 2003. AEM. 69(6):3213), with a Nikon Eclipse C1 Plus confocal microscope (Nikon Instruments).



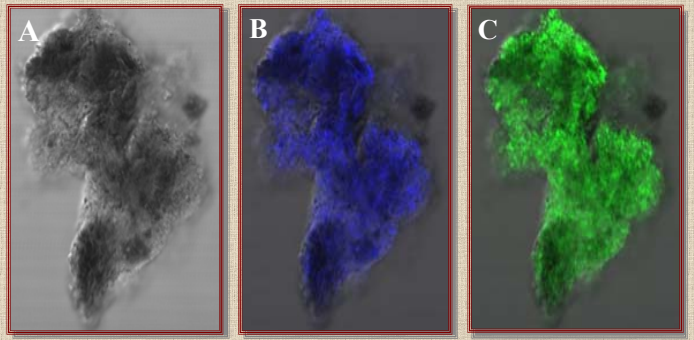
↑Removal of ammonia by treatment system. A. Super Soil Systems USA, Inc. swine waste treatment system at Tyndall Farm, Clinton, N.C. **B.** NH_3 concentrations from December 2006 through August 2007 at various stages of waste treatment (of particular interest is difference between solids separation [●●●] and N effluent [●●●]). **C.** Outside temperatures at Tyndall Farm from December 2006 through August 2007.

↓Continuous culture nitrification. A. Examination of nitrification from 15°C to 3°C. **B.** Nitrification rate of both continuous culture and batch reactor nitrification experiments. The *Q*10 of these tests was 1.41 as compared to 2.95 to 3.25 for other studies (Knowles et al. 1965, Characklis and Gujer, 1979).

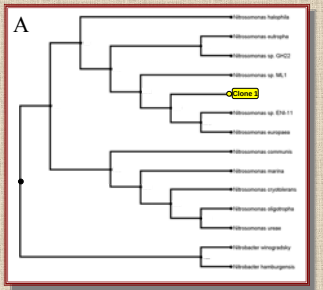


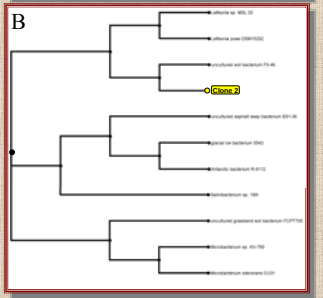


Batch Reactor Nitrification Experiments. After 2 weeks of equilibration at the above mentioned temperatures in continuous flow, 8 hour batch experiments examining nitrification were performed. Batch experiments were done in triplicate.



Fluorescent In Situ Hybridization of low temperature ammonia oxidizing bacteria identified in panel 3 (Clone 1). Image is of a single floccule as seen under confocal microscopy. **A.** Nomarski image. **B**. Nomarski with DAPI staining overlay. **C.** Nomarski with 5' 6-FAM labeled FISH probe (Nso190) overlay.





Phylogenetic Analysis. Phylogenetic trees displaying relationships of the two most abundant OTUs to previously identified organisms. **A.** Relationship of Clone 1 to organisms of the family *Nitrosomonas*, responsible for nitrification. **B.** Relationship of Clone 2 to other organisms, including the psychrophilic family *Leifsonia*.