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Ammonia Removal Using Nitrification and Anammox in a Single Reactor

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Abstract. *In this work we evaluated the combination of nitrification and anammox bacteria in a single tank to remove ammonia by deammonification process. The deammonification process is a completely autotrophic nitrogen removal approach that eliminates the carbon needs for denitrification. Thus, it can be a promising approach for the biological removal of ammonia (NH₄⁺) from anaerobic digester effluents that are low in carbon and high in ammonia concentration. A high performance nitrifying sludge, HPNS (NRRL B-50298), was mixed with anammox bacterial sludge, Brocadia caroliniensis (NRRL B-50286), in a single reactor. The reactor was an aerated vessel operated under continuous flow. It contained biofilm plastic carriers that were fluidized by the aeration. The process water temperature was 22°C and DO <0.5 mg/L. It was tested using inorganic synthetic wastewater and anaerobically digested swine wastewater. Ammonia removal rates of 1.2 kg N/m³-reactor/day were obtained with influent wastewater concentration of 440 mg/L NH₄-N and a removal efficiency of 87%. The stoichiometry of the reaction obtained was consistent with deammonification process combining partial nitrification and anammox. Results obtained with the deammonification process showed several advantages over nitrification/denitrification: 1) it reduced 56% of the oxygen requirements to remove the ammonia; 2) it did not require carbon; and 3) it removed nitrogen at higher rates in a single-tank, further reducing equipment costs. Therefore, deammonification can be a key technology for development of more economical and energy efficient biological ammonia removal systems in the near future.*

Keywords. Deammonification, anammox, wastewater treatment, swine manure, ammonia emissions abatement.

Introduction

One of the largest environmental concerns associated with livestock production is the loss of ammonia gas (NH_3) from the manure (Aneja et al., 2000). The Research Triangle Institute International (RTI, 2003) estimated the monetized economic benefits to North Carolina households of changes in environmental quality resulting from the generalized adoption of alternative manure treatment technologies. Their results indicated that adoption of technologies that provide a 50% reduction of NH_3 emissions accounts for an estimated benefit of \$190 million/year in avoided human health impacts (RTI, 2003).

While various systems have been developed for removing NH_3 from wastewater, there still remains a need for improved systems that remove the NH_3 more economically. The combined bioprocess of nitrification-denitrification (NDN) has been successfully used for the removal of ammonia nitrogen from animal wastewater through the conversion of ammonium (NH_4^+) into dinitrogen gas (N_2) via nitrate (NO_3^-) as intermediate (Béline et al., 2004; Vanotti et al. 2009). Removing ammonia from wastewater has many environmental benefits on animal production. In a full-scale demonstration of a second-generation environmentally superior technology (EST) for treatment of swine manure in the USA that used NDN, Vanotti et al. (2009) found that animal health and productivity were significantly enhanced as a result of recycling mostly ammonia-free treated wastewater into the barns. However, the operational cost of NDN was an important consideration because about 70% of the total electrical power used by the system (which included solids separation, NDN, and phosphorus removal/disinfection unit processes) was to power the air blower for the nitrification process. They concluded that significant savings in power requirements by the EST system in the future will come from changes in the N treatment, such as the incorporation of anaerobic ammonium oxidation (anammox) that requires about half the aeration required by NDN (Fig. 1).

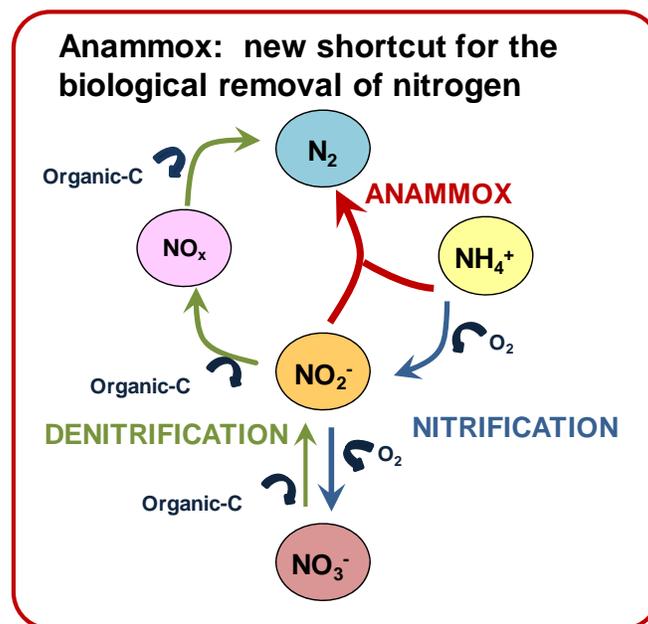


Figure 1. Deammonification involves two steps: ammonia oxidizing bacteria that convert about half of the wastewater ammonia (NH_4^+) into nitrite (NO_2^-) (partial nitrification), and anammox bacteria that utilize the remaining ammonia (NH_4^+) and nitrite (NO_2^-) to produce N_2 .

Farmers that would like to use NDN to remove N from the effluent of anaerobic digesters (AD) – for example to comply with surplus nitrogen regulations or to take advantage of environmental nutrient credit programs – are often limited by the low amount of endogenous carbon available for traditional denitrification, since the carbon is consumed in the biogas production. Two solutions could be implemented: 1) to add supplemental carbon (i.e., MeOH), or 2) to use some of the raw manure directly into NDN. In the first case, the cost increases because chemicals need to be purchased, and in the second case, the benefit decreases because less manure is being used to produce biogas. For example, in an on-farm treatment plant in a swine operation in Brittany (France) that used AD/NDN, as much as 30% of the raw manure bypassed AD directly into NDN to obtain optimal denitrification (F. Beline, pers. comm., 5/22/2012). A better approach would be to use deammonification pathway, which is the focus of this research.

Deammonification Process

Deammonification is a completely autotrophic nitrogen removal approach that combines partial nitrification (PN) and anammox (Fig. 1). Partial nitrification is the biological conversion of about 50% of the ammonia (NH_4^+) to nitrite (NO_2^-) (Magri et al., 2012). Anammox is a biologically mediated reaction that oxidizes NH_4^+ and releases di-nitrogen gas (N_2) under anaerobic conditions using nitrite (NO_2^-) as the electron acceptor (Strous et al., 1998). According to the anammox reaction ratio, 1.32 g NO_2^- -N are consumed per g NH_4^+ -N removed. Since no carbon is needed, it is especially suited for the biological removal of ammonia in systems that include the anaerobic digestion process with effluents that are high in ammonia concentration but low in carbon. Compared to traditional nitrification/denitrification, the PN/anammox process has several potential advantages: 1) Lower oxygen requirements; 2) Elimination of carbon needs for denitrification; and 3) High nitrogen removal rates. Therefore, deammonification could be key technology for development of more economical and energy-efficient biological N removal systems in the future. Our objective was to test the deammonification concept for swine wastewater using a one-stage deammonification process (PN and anammox in a single tank).

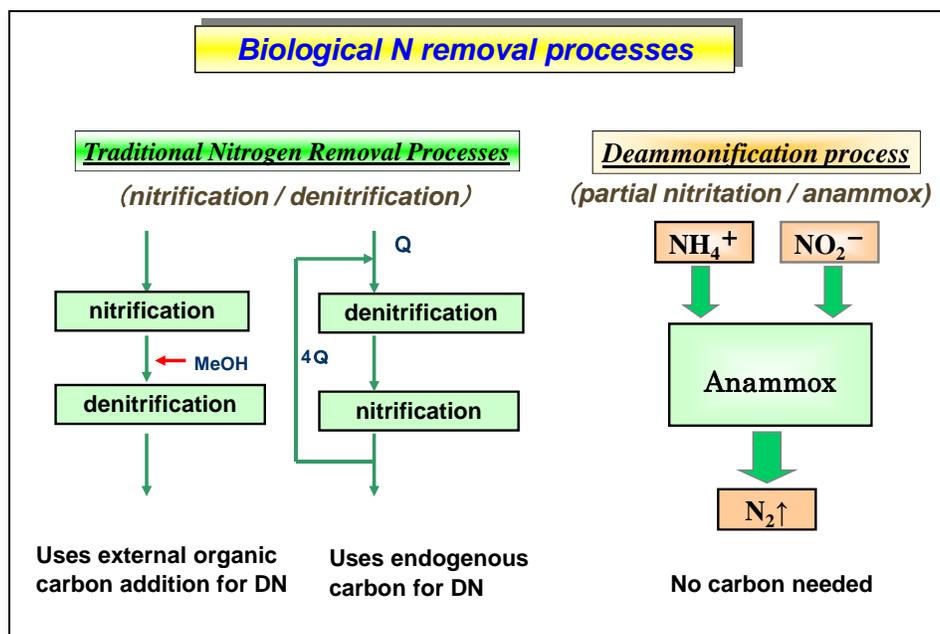


Figure 2. Biological processes to remove nitrogen from wastewater.

Bacterial Cultures Used

Nitrifying Bacteria

For partial nitrification, we used a high performance nitrifying sludge (HPNS) developed for treatment of high ammonium concentration and low temperature wastewater (Vanotti et al., 2011a). The HPNS was deposited under the provisions of the Budapest Treaty of the United Nations in the Agricultural Research Service Culture Collection in Peoria, IL, on June 26, 2009, with deposit accession number NRRL B-50298. The HPNS is a composition of bacteria comprised of 35 strains or populations of isolated bacteria; 26 of the bacteria are affiliated with Proteobacteria, 7 with Bacteroidetes, and 2 with Actinobacteria (HPNS.1 through HPNS.35 with GenBank accession GQ223345 through GQ223379, respectively). The HPNS was derived from swine lagoon nitrifying sludge after prolonged cultivation in a suspended biomass reactor at low temperature (10°C) at the USDA-ARS laboratory in Florence, SC. The HPNS was maintained in an aeration tank with fine bubble aeration at 10°C water temperature using the fill-and-draw cultivation method and an inorganic salts medium (Vanotti et al., 2011a). The HPNS had the following characteristics (Vanotti et al., 2011a): (i) a specific nitrification activity of 51.0 mg N per g total suspended solids (TSS) per hour [62.1 mg N per g volatile suspended solids (VSS) per hour] obtained at 30°C using inorganic salts medium with 300 mg NH₄⁺-N/L, biomass concentration 2.01 g VSS/L and process DO 5.0 ±0.6 mg/L; and (ii) a sludge volume index (SVI) of 62 mL/g TSS.

Anammox Bacteria

The anammox bacteria used was Candidatus *Brocadia caroliniensis* deposited under the provisions of the Budapest Treaty in the Agricultural Research Service Culture Collection (NRRL) at Peoria, IL, with accession number NRRL B-50286 (Vanotti et al., 2011b). It was maintained at the USDA-ARS laboratory (Florence, SC) in a 10-L jacketed, up-flow continuous reactor (120 cm), packed with a biomass carrier to enhance retention of microorganisms (parent reactor). At the time of sludge harvesting, the parent reactor was being fed with synthetic wastewater containing 153 mg NH₄⁺-N/L and 153 mg NO₂⁻-N/L and operated with a flow rate of 60 L d⁻¹, an N-loading rate (NLR) of 1735 mg N/L/d, and a water temperature of 30°C. Under these conditions, the N-conversion efficiency (NCE) obtained was 94% and the total N-removal efficiency (NRE) was 85% (Vanotti et al., 2011b). The anammox sludge had the following characteristics: (i) a granular structure with average granule size of 1.5 mm, (ii) a reddish color, and (iii) a specific conversion rate (nitrite + ammonia) was 0.506 mg N/mg VSS/day. Start-up of the single-tank reactor was done using 800 mL of the anammox sludge.

Single-tank Deammonification Experiment

The single reactor was a 5-L aerated vessel operated under continuous flow. It contained biofilm plastic carriers (1200 m²/m³) at 30% v/v packing ratio that were fluidized by the aeration. The reactor was started by adding 800 mL of anammox bacterial sludge *Brocadia caroliniensis* (NRRL B-50286) and 1.5-L of the biofilm plastic carriers into the reactor. During the first week, the reactor was operated as an anammox reactor -without air being applied- and fed continuously a synthetic solution containing equal amounts of ammonia and nitrite (140 mg/L). Then aeration was started and 400 mL of nitrifying sludge HPNS (NRRL B-50298) was added at once into the reactor, which already contained the active anammox bacteria (Fig. 3).

During the first five weeks of operation of the single-tank deammonification reactor, the influent contained 140 mg/L $\text{NH}_4\text{-N}$ and 20 mg/L $\text{NO}_2\text{-N}$. Thereafter, the $\text{NO}_2\text{-N}$ was eliminated from the influent leaving only ammonia as the N source. We tested different influent wastewaters (synthetic and swine wastewater) containing varied ammonia (150 to 440 mg N/L) and alkalinity (750 to 2000 mg/L carbonate alkalinity). The process water temperature was 22°C. The aeration rate applied varied from 300 to 850 mL/min as N loading rate increased from 0.8 to 1.4 kg N/m³-reactor/day. The process was optimized with interrupted aeration cycles (23 min ON/7 min OFF). During aeration, the DO was generally below 0.5 mg/L. Under these conditions, NH_4^+ removal rates of about 0.7 to 1.2 kg N/m³-reactor/day were obtained using a single tank (Fig. 5).



Figure 3: Nitrifying sludge HPNS (in cylinder) prior to mixing with the anammox sludge.

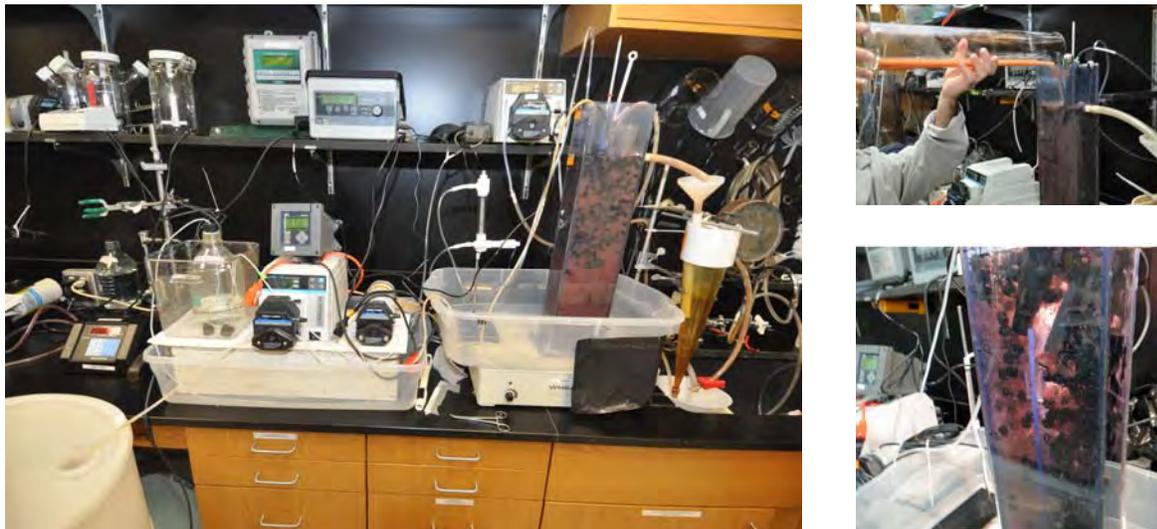


Figure 4: Deammonification treatment using single-tank to perform partial nitrification and anammox in a fluidized continuous flow aerated reactor. A) Reactor set-up, B) Addition of HPNS to reactor, C) Reactor detail with biofilm carriers inside.

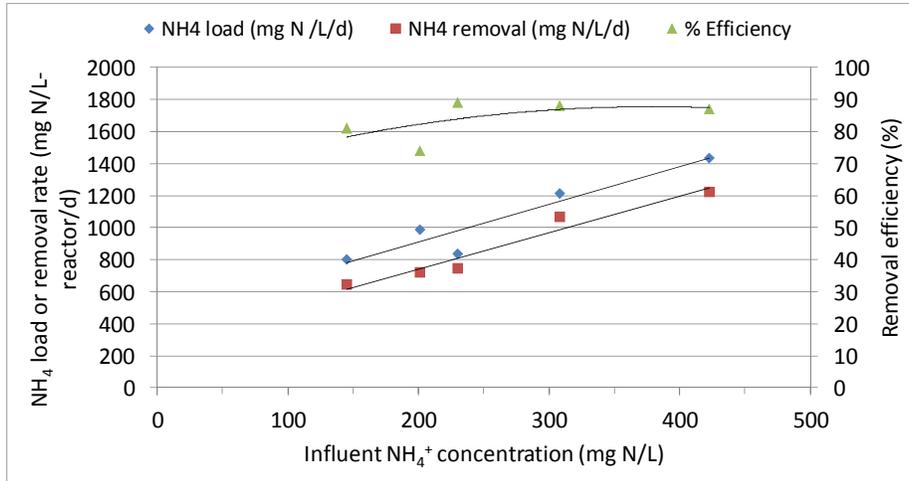


Figure 5: Ammonia removal in the single-tank reactor at various influent N concentrations.

Batch tests were conducted to determine the stoichiometry of the single-tank deammonification process (Figs. 6 and 7). The stoichiometry was derived from the concentration profiles of ammonia, nitrite, nitrate, and carbonate alkalinity obtained in the batch tests using synthetic wastewater (Fig. 6) and digested swine wastewater (Fig. 7). The rate of ammonia removal in the swine wastewater test was 1.03 kg N/m³-reactor/day with ammonia removal efficiency of 100% and total N removal efficiency of 89%. The results are summarized in the single-stage equations 4 and 5 shown in table 1 and compared with the theory represented by equation 3 (after combining equations 1 and 2). The results indicate that the stoichiometry of the reaction obtained in the single-tank process was consistent with the theory of the deammonification process combining partial nitrification and anammox. The results also indicate that the biological N removal in the single-tank was completely autotrophic because no carbon was added in the composition of the synthetic wastewater used in the first batch (Fig. 6). Compared with nitrification/denitrification (that requires about 2 mol of O₂ per mol of NH₄⁺ removed), our results obtained with the deammonification process reduced the oxygen required to remove the ammonia by 56% (0.87-0.88 mol of O₂ per mol of NH₄⁺ removed, table 1).

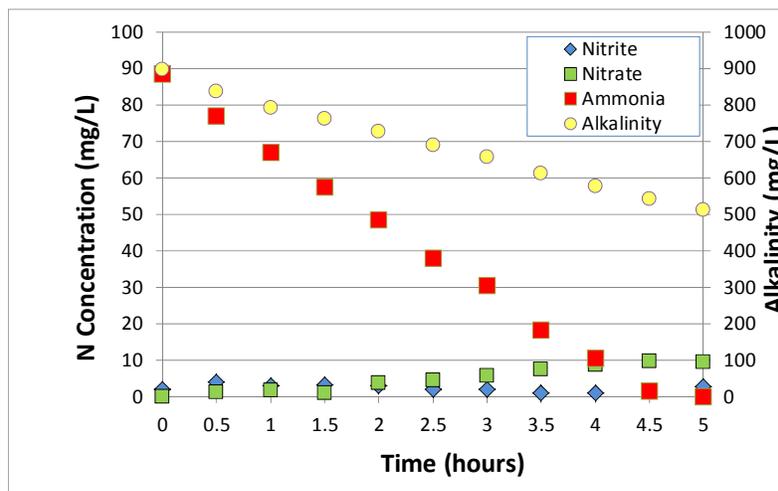


Figure 6: Experimental data used to estimate single-tank stoichiometry using synthetic wastewater (Equation 4, Table 1).

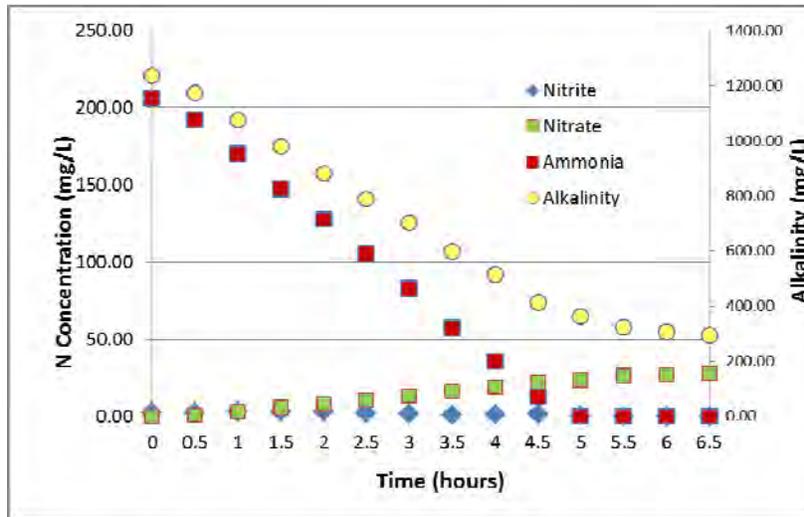


Figure 7: Experimental data used to estimate single-tank stoichiometry using digested swine wastewater (Equation 5, Table 1).

Based in our previous experience with deammonification using separate reactors (i.e., one for PN and another for anammox, Magri et al., 2012), the single-tank approach was much easier to manage. There was no need to use controls to ensure a balanced partial nitritation, nor to keep closed reactors and anaerobic conditions for anammox. The two bacteria groups were able to associate effectively in a single tank providing a streamlined ammonia removal process. Thus, the single tank configuration offers the potential to further reduce the cost of treatment of ammonia in livestock wastewaters containing high ammonia nitrogen.

TABLE 1: Comparison of the theoretical stoichiometry of deammonification with experimental reaction data (Figures 6 and 7)

<u>Deammonification stoichiometry theory:</u>	
(1) Partial nitritation:	$2 \text{NH}_4^+ + 1.5 \text{O}_2 \rightarrow \text{NH}_4^+ + \text{NO}_2^- + \text{H}_2\text{O} + 2 \text{H}^+$
(2) Anammox:	$\text{NH}_4^+ + 1.32 \text{NO}_2^- \rightarrow 1.02 \text{N}_2 + 0.26 \text{NO}_3^- + 2 \text{H}_2\text{O}$
(3) Single-stage theory (1+2):	$\text{NH}_4^+ + 0.85 \text{O}_2 \rightarrow 0.44 \text{N}_2 + 0.11 \text{NO}_3^- + 1.43 \text{H}_2\text{O} + 1.14 \text{H}^+$
<u>Single-stage stoichiometry obtained in this research (Figures 6 and 7):</u>	
(4) Synthetic wastewater	$\text{NH}_4^+ + 0.88 \text{O}_2 \rightarrow 0.44 \text{N}_2 + 0.11 \text{NO}_3^- + 1.43 \text{H}_2\text{O} + 1.14 \text{H}^+$
(5) Swine wastewater	$\text{NH}_4^+ + 0.87 \text{O}_2 \rightarrow 0.45 \text{N}_2 + 0.11 \text{NO}_3^- + 1.41 \text{H}_2\text{O} + 1.18 \text{H}^+$

Conclusion

We evaluated deammonification treatment using a single, fluidized, aerobic tank by mixing a high performance nitrifying sludge and anammox bacteria. Compared to traditional

nitrification/denitrification, the PN/anammox process in a single tank showed several advantages: 1) it reduced 56% of the oxygen requirements to remove the ammonia; 2) it eliminated carbon needs for denitrification; and 3) it removed nitrogen at higher rates, further reducing equipment costs. Therefore, deammonification can be a key technology for development of more economical and energy-efficient biological ammonia removal systems in the near future.

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