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Development of Anammox Process for Animal Waste Treatment: Experiences in the USA

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Abstract. Research was conducted to develop process applications for anaerobic ammonium oxidation (anammox) bacteria acclimated to animal wastewater conditions using microbial immobilization techniques. In the anammox reaction, under anaerobic and autotrophic conditions, ammonium (NH_4^+) serves as the electron donor using nitrite (NO_2^-) as the electron acceptor resulting in production of harmless di-nitrogen (N_2) gas. Compared to conventional nitrification-denitrification, this combined pathway saves more than 50% of the oxygen supply for nitrification and 100% of the external organic source for denitrification. This leads to a significant reduction in energy needs of treatment, and a decrease in operational costs. In addition, by-products do not include greenhouse gases. The isolation of anammox adapted to animal wastewater environments can be of significant importance to farming systems because excess ammonia in modern, industrial-type livestock production is a global problem and the use of conventional biological N removal methods is usually hindered by cost. Thus, we think that the more economical anammox based treatment can greatly facilitate adoption of advanced wastewater treatment technologies by farmers. The anammox cultures were successfully established using continuous-flow unit processes and biomass carriers seeded with sludges of manure origin. Anammox removal rates obtained were in the range of industrial N removal applications.These findings overall may lead to development of more economical treatment systems for livestock wastewater and other effluents containing high ammonia concentration.

Keywords: manure, swine lagoons, ammonia treatment, anammox, anaerobic ammonium oxidation

Introduction

Traditionally, removal of nitrogen (N) from wastewaters uses a combination of nitrification and denitrification. Nitrification consumes large amounts of oxygen to convert NH_4^+ into nitrate (NO_3^-), while denitrification requires addition of organic carbon to convert NO_3^- into N_2 gas. In the anammox reaction, under anaerobic and autotrophic conditions (without organic carbon), ammonium (NH_4^+) serves as the electron donor using nitrite (NO_2^-) as the electron acceptor resulting in production of harmless di-nitrogen (N_2) gas (Strous et al., 1998; Jetten et al., 1999).

In the anammox alternative pathway, NH_4^+ is converted to N_2 gas in a totally autotrophic process by two sequential reactions: partial nitritation (eq. 1) and anammox (eq. 2, Strous et al., 1998). Although equation 2 does not consider other reactants related to cell synthesis (Dongen et al., 2001), it has been used to describe the basic anammox process.

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_2O + 2\ H^+ \qquad (1)$$
$$NH_4^+ + 1.32\ NO_2^- \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 2\ H_2O \qquad (2)$$

Compared to conventional nitrification-denitrification, these combined reactions save more than 50% of the oxygen supply for nitrification and 100% of the external organic source for denitrification. This leads to a significant reduction in energy needs of treatment and a decrease in operational costs. In addition, by-products do not include greenhouse gases.

Our goal was to develop process applications for anaerobic ammonium oxidation (anammox) bacteria acclimated to animal wastewater conditions using microbial immobilization techniques. This paper reports the advances made on development of the anammox process isolated from sludges of swine manure origin in the USA. Similar research was conducted in Brazil; its results are reported by Kunz et al. (2007; this symposium proceedings)

Materials and Methods

The anammox cultures were successfully established in three consecutive experiments using continuous-flow unit processes and biomass carriers seeded with sludges of manure origin from two swine farms located in North Carolina, USA. The sludges containing anammox bacteria were obtained from the two following sources: an aerobic nitrification tank treating anaerobically-digested swine manure, and an anoxic denitrification tank treating liquid swine manure after solid-liquid separation. Laboratory bioreactors were seeded with the manure sludges after acclimation with nitrate solution to remove endogenous carbon.

In a first phase of the research, two sequential experiments were conducted over a 5-year period (2000-2005) to investigate conditions to 1) isolate anammox bacteria from the swine sludges (3 years), and 2) optimize anammox treatment in bioreactors (2 years). The bioreactors were operated in continuous flow and contained polyvinyl alcohol (PVA) hydrogel biomass carrier beads for immobilization and enrichment of the slow growth microorganisms (Figure 1). These PVA beads had an average diameter of 4.0 mm, specific gravity of 1.025, and hydrous ratio (void %) of 90% (Kuraray Co., Osaka, Japan). The reactors had a volume of 1.0 L (6.5 cm diameter glass cylinder). The reactors received a continuous flow of synthetic enrichment medium containing NH_4^+ and NO_2^-. In experiment 1, the bioreactor was operated at constant hydraulic retention time (HRT) of 28 hours and a variable influent total N concentration (20 – 250 mg N/L). In experiment 2, the HRT was variable (24 to 12 hours), and influent concentration was constant (300 mg N/L). The bacterial community structure was determined by FISH (fluorescence *in situ* hybridization) using the 16S rDNA-specific oligonucleotide probes Amx 820 (Schmid et al., 2000) and Ana-1 (Fujii et al., 2002).

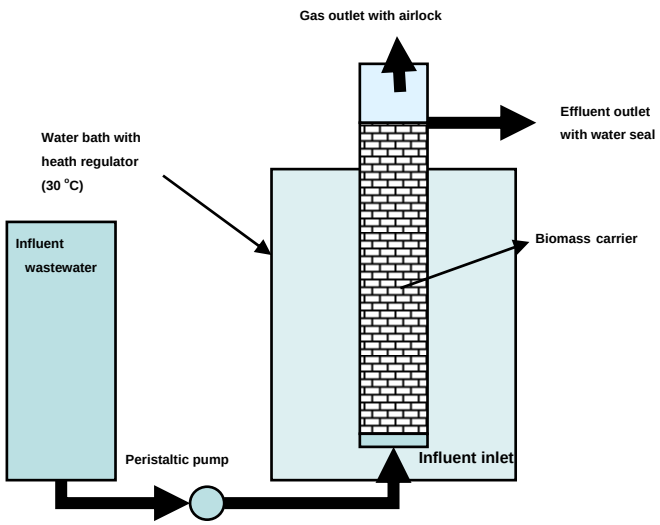

Figure 1. Schematic diagram of the bioreactor containing polyvinyl alcohol hydrogel biomass carrier beads for immobilization and enrichment of the slow growth microorganisms

In experiment 3 (second phase of the research), a 10-L pilot reactor was seeded directly with the anammox red sludge biomass produced in the first phase (Figure 2). As biomass carrier, the reactor used a polyester non-woven material coated with pyridinium type polymer (Japan Vilene, Tokyo, Japan) designed to enhance retention of microorganisms (Furukawa et al., 2003). The bioreactor was operated with a HRT of 12 h and an inflow concentration of 200 mg N/L.

All water analyses in each of the three experiments were performed according to Standard Methods for the Examination of Water and Wastewater (APHA, AWWA, WEF, 1998).

Figure 2. Anammox pilot reactor

Results and Discussion

In experiment 1 (isolation), the anammox reaction was first noticeable at day 490 with simultaneous removal of NO_2^- and NH_4^+ (Figure 3). At the same time, a distinct red biomass growth, which is typical of the anammox planctomycetes bacteria, colonized the PVA hydrogel biomass carrier beads. These developments occurred only when high N (>200 mg/L) and adequate salt strength was used in the synthetic media. Presence of anammox bacteria was confirmed by FISH analysis of the biofilm using 16S rDNA oligonucleotides Ana-1 and Amx 820; imaging revealed a high density of cells growing in clusters (Figure 4).

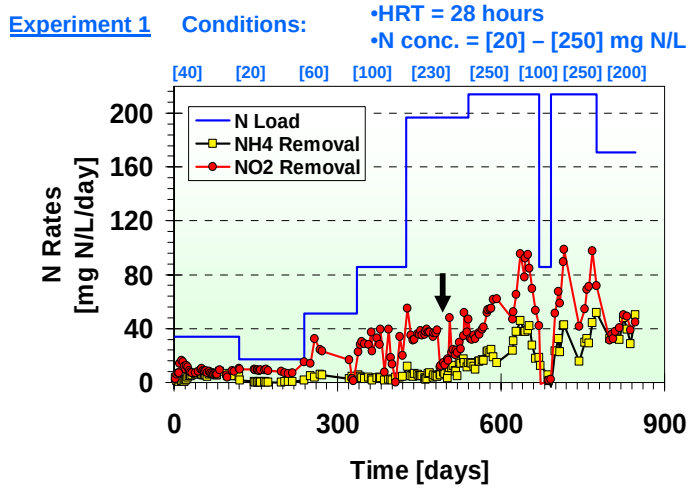

Figure 3. Anammox reaction with simultaneous removal of ammonium (NH_4) and nitrite (NO_2). The black arrow indicates when anammox reaction was first noticed after day 490 when total N loading rates were > 200 mg N/L.

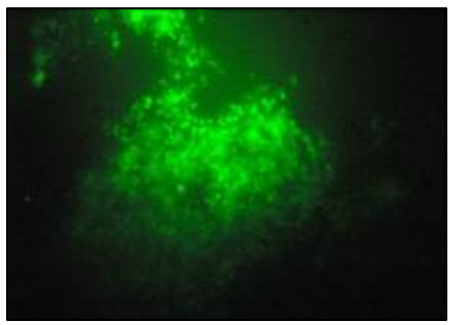

Figure 4. Anammox bacterial community identification determined by FISH (fluorescence *in situ* hybridization) using 16S rDNA-specific oligonucleotide probe Ana-1.

Under the conditions and protocol determined in experiment 1, it took about 100 days in experiment 2 to develop the anammox reaction from sludges obtained from swine farms in USA. Nitrogen removal rates obtained during first-phase development were 0.7 to 0.8 kg N/m^3/day, which are already in the range of industrial bio-treatment applications. These rates compare to reported removal rates for conventional nitrification/denitrification for industrial applications (e.g. treatment of effluents from anaerobic digestors, rendering plants or landfill leachate) is within the range of 0.05 to 4.0 kg N/m^3/day (Jetten et al., 2002). Removal of NO_2^- and NH_4^+ was simultaneous at the stoichiometric ratios shown in figure 5 and summarized in eq. 3.

$$NH_4^+ + 1.24\ NO_2^- \rightarrow 1.00\ N_2 + 0.24\ NO_3^- \qquad (3)$$

Figure 5. Stoichiometric ratio of the anammox isolated from swine manure (Experiment 2).

In experiment 3, removal of NO_2^- and NH_4^+ at typical stoichiometric ratios of the anammox reaction occurred from day 1 of operation of the pilot reactor seeded with an anammox rich sludge isolated from previous experiments. Nitrogen removal rates increased quickly with time in the pilot reactor seeded with the anammox enriched sludge (Experiment 3, Figure 6).

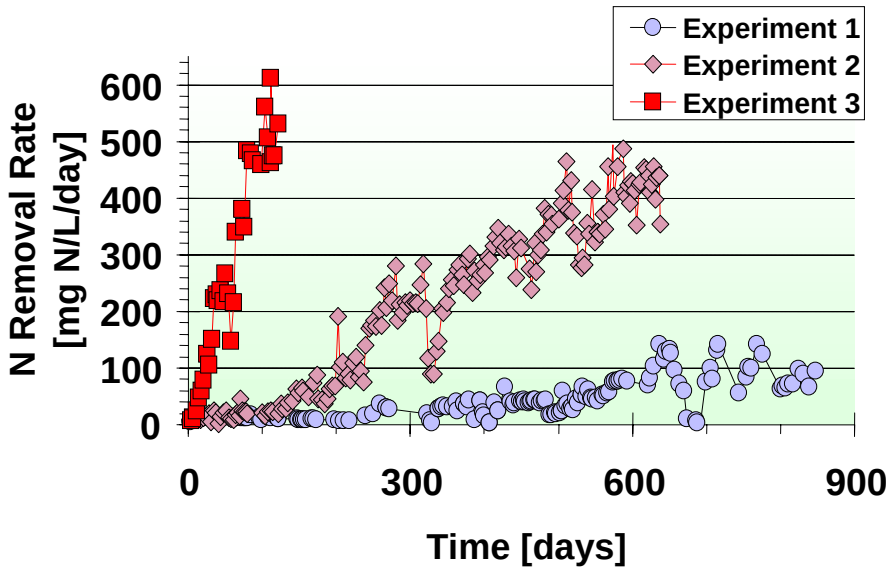

Figure 6. Nitrogen removal rates in anammox bioreactors: Exp. 1 – bacteria isolation; Exp. 2 – anammox reaction optimization; Exp. 3 – pilot reactor seeded with anammox sludge.

Conclusions

Anammox bacteria were successfully isolated from animal waste sludge using immobilizing techniques and continuous flow reactors. Anammox reaction startup was favored by high nitrogen influent concentrations. Anammox removal rates obtained were in the range of industrial N removal applications. These findings overall may lead to development of more economical treatment systems for livestock wastewater and other effluents containing high ammonia concentration.

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