ANAMMOX TREATMENT OF SWINE WASTEWATER USING IMMOBILIZED TECHNOLOGY

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1 INTRODUCTION

Appropriate land application of manure nitrogen at agronomic rates must be assured in order to preserve environmental quality of agricultural ecosystems, atmosphere, and water resources. When land is limiting, the combined bioprocess of nitrification-denitrification (NDN) has often been considered for the removal of nitrogen, implying the conversion of ammonium (NH_4^+) into innocuous dinitrogen gas (N_2) through nitrate (NO_3^-) as intermediate (Béline *et al.*, 2004; Vanotti *et al.*, 2009). However, significant savings in power requirements for the supplying of oxygen during nitrification are possible considering an alternative nitrogen pathway such as the anaerobic oxidation of ammonium (anammox) (Strous *et al.*, 1997). In addition, organic carbon is needed for heterotrophic denitrification, constraining pretreatments like anaerobic digestion for the production of biogas as renewable energy source (Magrí *et al.*, 2009).

Partial nitrification (PN) coupled with anammox has gained a lot of interest in recent years as a more sustainable alternative than conventional NDN. This PN-anammox process stands for a totally autotrophic strategy for the removal of nitrogen, particularly from wastewaters with high nitrogen and low organic contents (Paredes *et al.*, 2007). Some advantages of PN-anammox process are the reduction on oxygen requirements during nitrification (60% less than complete nitrification), a decrease on the sludge production and dioxide carbon emissions with respect to heterotrophic denitrification, and the possibility of working with higher nitrogen loading rates. Ammonium present in wastewater must be first partially oxidized to nitrite (NO_2^-) by ammonium oxidizing bacteria (AOB). Subsequently, anammox process results in the combination of ammonium and nitrite under absence of oxygen to form N_2. Optimal molar ratio NO_2^-/NH_4^+ of 1.32 was proposed for the influent targeting maximum N-removal efficiencies close to 90% (Strous *et al.*, 1998) (Eq. 1; $CH_2O_{0.5}N_{0.15}$ stands for anammox biomass).

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13\,H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \qquad (1)$$

A challenge for the implementation of the anammox process is to ensure biomass retention inside the reactor due to the low growth rates of these bacteria. Use of immobilization technologies may result in better biomass retention, minimizing risks of washout, and positively affect the treatment process capacity. The objective of this work was to develop applications of anammox bacteria for the treatment of swine wastewaters using microbial immobilization techniques such as surficial attachment and gel carrier entrapment.

2 MATERIALS AND METHODS

The research was conducted at the ARS Coastal Plains Research Center in Florence, South Carolina. Anammox biomass isolated in previous research (Vanotti *et al.*, 2006; Szogi *et al.*, 2007) was used in the immobilized reactors.

2.1 Molecular techniques

Enriched biomass was characterized using culture-independent techniques. Chromosomal DNA was extracted and 16S ribosomal DNA gene was amplified using the polymerase chain reaction (PCR) according to Fujii *et al.* (2002). Cloning techniques were applied, and finally cloned PCR products were sequenced. Such sequences were submitted to the blast search engine of the GenBank database (Altschul *et al.*, 1997) to obtain putative phylogenetic assignment.

2.2 Experimental set-ups

Two different strategies for immobilizing the anammox bacteria were used (Figure 1):

1) Attached biomass: A 10-L pilot reactor was operated in continuous flow configured so that influent wastewater was injected at the bottom-end and the treated effluent was discharged near the top after passing through a matrix of immobilized anammox. Such cylindrical bioreactor was made of glass being jacketed on the outside to control process temperature (30ºC). A biomass carrier was placed inside the reactor made of a polyester non-woven material coated with pyridinium type polymer (Japan Vilene Co., Japan), designed to enhance retention of microorganisms (Furukawa *et al.*, 2003). The reactor was continuously fed with synthetic substrate under a flow rate of 60 L d^{-1} during a 350-day time period.

2) Entrapped biomass: A 1-L bench reactor was operated in continuous flow containing anammox biomass entrapped in polyvinyl alcohol (PVA) pellets. Encapsulation procedure was performed according to the methodology described by Vanotti and Hunt (2000) using PVA-HC (100% saponification, 2000 polymerization) (Kuraray Co., Japan). Partially nitrified swine wastewater was continuously supplied as feed and process temperature was controlled at 30ºC.

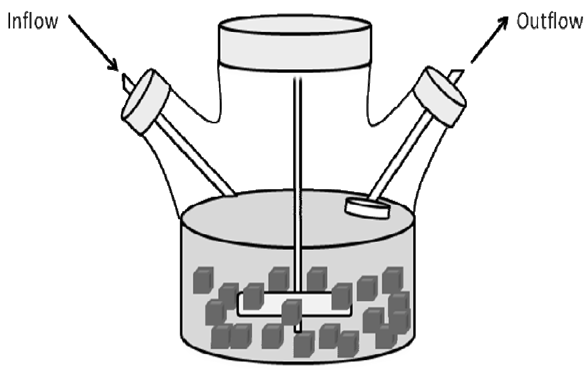

FIGURE 1 **View of bioreactors with immobilized anammox bacteria used in this research. *Left:* upflow reactor with attached biomass. *Right:* scheme of the reactor with encapsulated biomass.**

2.3 Analytical methods

Nitrogen concentrations of filtrated samples were measured using an auto-analyzer (Technicon Instruments Corp., USA) and according to APHA *et al.* (1998). NH_4-N was determined by the automated phenate method (4500-NH_3 G), NO_2-N was determined by the colorimetric method (4500-NO_2^- B), and NO_3-N was determined by the automated cadmium reduction method (4500-NO_3^- F). Total nitrogen (TN) was calculated as de sum of NH_4-N, NO_2-N and NO_3-N. Total alkalinity was determined by acid titration to an endpoint of pH 4.5 and expressed as mg $CaCO_3$ L^{-1}.

3 RESULTS AND DISCUSSION

3.1 Microbiology

Due to the relatively low DNA sequence homology and phylogenetic relationships, the dominant anammox bacteria was proposed as a novel species of the *Candidatus* “Brocadia” genera, being this novel anammox species named *Candidatus* “Brocadia caroliniensis” (Vanotti *et al.*, 2010) (Figure 2). A strain of B. caroliniensis was deposited under the provisions of the Budapest Treaty in Agricultural Research Culture Collection (NRRL) in Peoria, Ill., and has been assigned accession deposit number NRRL B-50286.

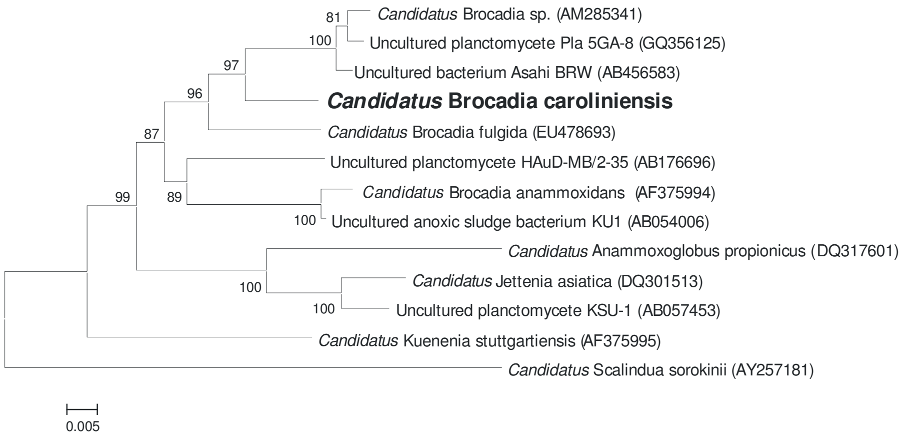

FIGURE 2 **Bootstrapped (n = 1000) neighbour joining phylogenetic tree of NRRL B-50286 identified as *Candidatus* "Brocadia caroliniensis" in relation to major genera of anammox bacteria found in the GenBank database (Vanotti *et al.*, 2010).**

3.2 Anammox attached biomass

Data in Table 1 summarizes stabilized performance obtained in the continuous flow pilot reactor. Average nitrogenous composition of substrate was of 283.4 ±22.4 mg N L^{-1} (131.4 ±13.0 mg NH_4-N L^{-1}, 152.0 ±13.0 mg NO_2-N L^{-1}, and 0.1 ±0.6 mg NO_3-N L^{-1}). Under hydraulic retention times (HRT) of 3.9 h, the average total nitrogen loading rate (NLR) was 1.74 kg N m^{-3} d^{-1} and the total nitrogen removal rate (NRR) was 1.48 kg N m^{-3} d^{-1}. At these high mass N loading, the average removal efficiency obtained was 85%. Similarly, the ammonium and nitrite were removed at efficiencies of 89 and 98%, respectively. Removal of NH_4^+ and NO_2^- was at stoichiometric ratios summarized as $NH_4^+ + 1.30\ NO_2^- \rightarrow 1.06\ N_2 + 0.18\ NO_3^-$ (Vanotti *et al.*, 2010).

TABLE 1 **Removal of nitrogen in upflow continuous pilot reactor**

	Mass Nitrogen			
	Influent	Effluent	Removal	Removal efficiency
	-------------------mg N L^{-1} d^{-1}	----------------------		------%-----
TN	1,735 ±137	260 ±122	1,477 ±168	85 ± 7
NH_4-N	804 ±80	92 ±71	709 ±88	89 ± 9
NO_2-N	930 ±80	22 ±47	915 ±90	98 ± 5
NH_4-N + NO_2-N	1,735 ±138	114 ±101	1,621 ±155	94 ± 6

3.3 Anammox entrapped biomass

Encapsulation of anammox bacteria in polymer gel carrier has been applied successfully for treatment of digester liquors using polyethylene glycol (PEG) as entrapment polymer (Isaka *et al.*, 2007; Furukawa *et al.* 2009). Under such reactor configuration, influent contents on total suspended solids up to 2000-3000 mg L^{-1} have been demonstrated as non-limitant for the activity of anammox bacteria. In this work, PVA was used for the immobilization of anammox bacteria (packing ratio of 25%). Partially nitrified wastewater was fed as influent being the NO_2-N/NH_4-N ratio adjusted to the desired value of 1.32 by adding raw wastewater (1,400 ±36 mg NH_4-N L^{-1}; 7,740 ±128 mg $CaCO_3$ L^{-1}).

4 CONCLUSIONS

New bacteria strain *Candidatus* "Brocadia caroliniensis" was identified in anammox enrichment started-up from animal waste sludge. The use of immobilization techniques enhanced anammox biomass retention inside the reactors and resulted in high nitrogen removal rates. These findings overall may lead to the development of more economical treatment systems for swine wastewater effluents containing high ammonium and low biodegradable organic carbon concentrations.

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