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Rate determination and distribution of anammox activity in activated sludge treating swine wastewater

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ABSTRACT

This paper presents a quantitative investigation and analysis of anammox activity in sludge taken from biological swine wastewater treatment plants. An incubation experiment using a ^{15}N tracer technique showed anammox activity in sludge taken from 6 out of 13 plants with the rate ranging from 0.0036 μmol-N_2/g-VSS/h to 3.1 μmol-N_2/g-VSS/h, and in a biofilm with the highest activity at 25.8 μmol-N_2/g-VSS/h. It is notable that 9 out of 11 sludges in which the pH was maintained between 6.6 and 8.1 retained anammox activity, while those with either a lower or higher pH did not. Moreover, anammox-positive sludge had a significantly higher concentration of NO_2^-—N plus NO_3^-—N than did anammox-negative sludge. A significant difference was not observed between anammox-positive and -negative sludge regarding BOD/NH_4^+—N in the influent, DO concentration in aeration tanks, and the concentrations of NH_4^+—N, free nitric acid, and free ammonia.

1. Introduction

Wastes from livestock have a high potential of nutrient emissions in water systems in many regions (Steinfeld et al., 2006). Swine wastewater, which is a mixture of swine urine, some excrement and service water and contains an extremely high concentration of nitrogen, is one of the most difficult wastewaters from which to remove nitrogen among livestock wastes. Biological nitrogen removal has been mostly conducted by oxidizing NH_4^+ to NO_2^- and/or NO_3^- and subsequently reducing them to N_2 gas under an anoxic condition at the expense of electron donors such as organic matter (BOD). However, swine wastewater frequently does not have the required concentration of BOD to remove nitrogen, and therefore external addition of BOD is usually required for further complete denitrification, which consequently leads to an increase in the cost of the operation. Recently, a novel biological nitrogen removal process, anaerobic ammonium oxidation (Anammox), which oxidizes NH_4^+ to N_2 with NO_2^- as an electron acceptor under strictly anaerobic conditions, has been introduced (Mulder et al., 1995; Strous et al., 1997; van de Graaf et al., 1997, 1995). This process is advantageous over most commonly employed nitrification–denitrification processes mainly because, in principle, no external addition of BOD is required for anammox. Therefore, swine wastewater is expected to be a prime application of the anammox technique. From the standpoint of energy recovery from the biomass, some investigations of the application of anaerobic digestion of swine slurry, following partial nitrification to oxidize NH_4^+ to NO_2^-, and anammox treatment have been reported (Hwang et al., 2006; Karakashev et al., 2008; Molinuevo et al., 2009; Yamamoto et al., 2008). However, the technique has not yet been put into practical use on a farm.

One of the major obstacles in developing and disseminating a feasible anammox application technology for any type of wastewater is the long time required to establish an anammox reactor, ranging to several months or more (van der Star et al., 2007; Wett, 2006), due to the extremely slow growth rate of anammox microorganisms (Strous et al., 1998). In many cases, sludge taken from a well-established anammox reactor was inoculated as seed sludge for the prompt start-up of a next reactor (Wett, 2007). However,

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if it was difficult to obtain, sludge taken from a wastewater treatment plant would be inoculated as seed sludge for enrichment.

It is presumed that there is a high probability that anammox exists in the sludge of wastewater treatment processes even in non-accumulated sludge. The biomass obtained from upflow anaerobic sludge blankets, anaerobic digestion processes, activated sludge aeration tanks, nitrification tanks and denitrification tanks of landfill leachate, domestic, night soil, industrial or swine wastewater treatment plants has been employed as inocula for establishing anammox activity (Chamchoi and Nitisoravut, 2007; Date et al., 2009; Ike et al., 2001; Imajo et al., 2004; Tsushima et al., 2007a). These are evidence of the wide distribution of anammox microorganisms in various types of wastewater treatment processes. However, the selection of appropriate seed sludge is important for the rapid start-up of a reactor because the anammox potential for enrichment in unacclimatized sludge varies widely (Sanchez-Melsio et al., 2009; Toh et al., 2002; Tsushima et al., 2007b). Only Tsushima et al. (2007a) investigated anammox potential quantitatively in unacclimatized sludge using real-time PCR; every sludge sample taken from domestic, night soil, and landfill wastewater treatment plants was real-time PCR detectable but the copy numbers differed by two orders of magnitude. Since only scant attention has been paid to the quantitative analysis of anammox activity in sludge of wastewater treatment processes, systematic information on the distribution of an anammox population in various kinds of wastewater treatment sludge is lacking.

In the present study, a survey was conducted to find anammox activity in sludge taken from different types of processes under various conditions in swine wastewater treatment plants, using a ^{15}N tracer technique. Ultimately, the condition under which an anammox population is stably maintained was specified.

2. Materials and methods

2.1. Samples

Sludge samples were collected from 13 swine farms (Table 1) and stored at 4 °C in the dark until the experiment. The measurement of anammox activity was performed in 3 weeks except for D-2 and E-2 (6 weeks) and D-3 (12 weeks). Biofilm on attached growth media was removed and suspended in distilled water (D-4) or wastewater in an aeration tank (E-4); in the latter case, the biomass from the biofilm was about 50% of the total sludge biomass.

2.2. Measurement of anammox activity by batch incubation experiment with ^{15}N-labeled nitrogen compounds

Batch incubation with ^{15}N-labeled ammonium or nitrate followed by measurement using gas chromatography (GC)–mass spectrometry (MS) was performed according to the method of Amano et al. (2007). The sludge from the reactor was anaerobically incubated in 25-mL glass bottles with gas-tight butyl rubber stoppers along with 15 mL of inorganic synthetic medium and 100 mg/L of MLVSS with ^{15}N-labeled or non-labeled NH_4Cl (2.7 mmol/L) and $NaNO_3$ (2.7 mmol/L) at 25 °C with stirring in duplicate for each condition. The synthetic inorganic medium was a modified version of the medium proposed by van de Graaf et al. (1996) and contained the following compounds (per L): KH_2PO_4, 27.2 mg; $MgSO_4{\cdot}7H_2O$, 300 mg; $CaCl_2{\cdot}2H_2O$, 180 mg; $NaHCO_3$, 420 mg; HEPES 475 mg; trace element solution I, 1 mL; and trace element solution II, 1 mL. The pH was adjusted to 7.6 before incubation. Trace element solution I contained (per L) 5.71 g of EDTA-2Na and 9.15 g of $FeSO_4{\cdot}7H_2O$. Trace element solution II contained (per L) EDTA-2Na, 15 g; $ZnSO_4{\cdot}7H_2O$, 0.43 g; $CoCl_2{\cdot}6H_2O$, 0.24 g; $MnCl_2{\cdot}4H_2O$, 0.99 g; $CuSO_4{\cdot}5H_2O$, 0.25 g; $Na_2MoO_4{\cdot}2H_2O$, 0.22 g; $NiCl_2{\cdot}6H_2O$, 0.19 g; Na_2SeO_4, 0.11 g; and H_3BO_3, 0.014 g. Net N_2 production by anammox was calculated from gross $^{29}N_2$ concentration by deducting (1) contamination from $^{29}N_2$ in ambient air and (2) $^{29}N_2$ production via denitrification using $^{15}NO_3^-$ and $^{14}NO_3^-$ contamination in the reagent of $^{15}NO_3^-$.

2.3. Analytical method

Concentrations of N_2 isotopomers ($^{28}N_2$, $^{29}N_2$, $^{30}N_2$) produced by batch incubation with ^{15}N-labeled nitrogen compounds were detected using a GC (6890 N, Agilent Technologies, Inc., Japan) equipped with a 2-m stainless column packed with a molecular sieve (80/100 mesh) and a quadrupole MS (5973, Agilent Technologies, Inc., Japan) (Amano et al., 2007) or GC (8A, Shimadzu, Japan) equipped with a 25-m capillary column (CP-pora BOND Q Fused Silica, Varian Inc., USA) and magnetic sector mass spectrometer (DELTA plus N, Thermo Fisher Scientific Corporation, Japan). The former was used when anammox activity was higher (samples D-1 and D-4), and the latter was used in other cases. When the latter was used, $^{15}NO_3^-$ was not employed for incubations, because it could not detect high concentrations of $^{30}N_2$ quantitatively.

The concentrations of NO_3^- and NO_2^- in sludge and wastewater samples were determined with an HIC-VP super ion-exchange chromatograph (Shimadzu, Japan), and the concentrations of NH_4^+

Table 1
Characteristics of biomass sample and treatment facility.

Treatment method	Farm/sample name	Aeration condition	Sampling date	Sampling point	Farm location
Activated sludge process	A	Continuous	June 08	Aeration tank (A-1) Sedimentation tank (A-2)	Aichi Pref.
	B	Continuous	July 08	Aeration tank	Aichi Pref.
	C	Intermittent	November 08	Aeration tank	Miyazaki Pref.
Activated sludge process with attached growth material	D	Continuous	October 07 (D-1) January 08 (D-3,4) March 08 (D-2)	Aeration tank (D-3) Sedimentation tank (D-1 D-2) Attached growth material (D-4)	Saga Pref.
	E	Continuous	November 07 (E-1) March 08 (E-2,3,4)	Aeration tank (E-1, E-3) Sedimentation tank (E-2) Attached growth material (E-4)	Saga Pref.
Sequencing batch reactor	F	Intermittent	November 08	Aeration tank	Miyazaki Pref.
	G	Intermittent	July 08	Sedimentation tank	Aichi Pref.
	H	Intermittent	May 08	Aeration tank	Okinawa Pref.
Membrane-separation activated sludge process	I	Continuous	June 08	Aeration tank	Aichi Pref.
	J	Continuous	November 08	Aeration tank	Miyazaki Pref.
	K	Intermittent	November 07	Aeration tank	Saga Pref.
	L	Continuous	June 08	Aeration tank	Aichi Pref.
	M	Intermittent	June 08	Aeration tank	Gunma Pref.