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Identification and quantification of anammox bacteria in eight nitrogen removal reactors

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ABSTRACT

Various studies have revealed anaerobic ammonium oxidation (anammox) as a very attractive alternative process suitable for nitrogen removal from wastewater. Here we investigated anammox bacteria in eight different nitrogen removal reactors. The diversity and abundance of anammox bacteria were determined by the 16S rRNA gene analysis, fluorescence in situ hybridization with specific probes and real-time quantitative PCR (qPCR). In these reactors, at least eight unique near full length anammox 16S rRNA gene sequences were detected, which were distributed over two genera; *Candidati Brocadia* and *Kuenenia*. FISH results confirmed that only one anammox bacterium dominated the community in each of the eight reactors investigated in this study. qPCR analysis revealed that anammox bacteria were present in seven of the reactors in the order of 10^9 cells/ml and 10^7 cells/ml in reactor A1. The dominant and divergent *Brocadia*-like anammox phylotype in one reactor represented a novel species for which we propose the name *Candidatus Brocadia sinica*. Taken together, these results indicated that a single seeding source could be used to seed anammox reactors designed to treat different types of wastewater, which could lead to a faster start-up of bioreactors.

1. Introduction

Anaerobic ammonium oxidizing (anammox) bacteria convert ammonium directly to nitrogen gas with nitrite as the electron acceptor under anoxic conditions. Since its discovery in mid-1990s (Mulder et al., 1995), the anammox process has been regarded as a cost-effective and environment-friendly way to treat wastewater containing high ammonium concentrations (Jetten et al., 1997). Through the use of the anammox process, the operational costs and the emission of greenhouse gasses could be reduced by 60% (no need for external COD, much less aeration, and much lower sludge production), and 90% (CO_2 consumption and no N_2O emission), respectively (Kartal et al., 2010). By smart application of anammox in municipal treatment, wastewater treatment plants could be converted from energy-consuming into energy-producing systems (Kartal et al., 2010).

The relatively high doubling time (11–20 days) of anammox bacteria was perceived as the major bottleneck for the successful implementation of this process to wastewater

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treatment. However, within the last decades this problem has been resolved in both laboratory and full scale reactor systems by configurations that enable very efficient sludge retention: sequencing batch reactor (SBR) (Hu et al., 2006; Strous et al., 1998; Kartal et al., 2008,), rotating biological contactor (RBC) (Pynaert et al., 2003,), trickling filter (Schmid et al., 2000), UBF reactor (Jin et al., 2008), granular sludge bed reactor (Zheng et al., 2004) and membrane bioreactor (van der Star et al., 2007; Trigo et al, 2006). Meanwhile, after the start-up the first full scale anammox reactor in Rotterdam (The Netherlands) more than sufficient biomass is available to inoculate new full scale anammox reactors which would reduce the start-up time considerably if one source of seeding sludge could be used for bioreactors treating different types of wastewater (van der Star et al., 2008; Abma et al., 2010).

When the biomass of laboratory and full scale reactors was studied with molecular techniques (PCR, clone libraries, FISH) it appeared that the anammox process is mediated by a group of bacteria within the phylum Planctomycetes. All five described anammox genera (*Candidatus Brocadia*, *Kuenenia*, *Scalindua*, *Anammoxoglobus*, *Jettenia*) have been detected in many different wastewater treatment systems (Strous et al., 1999; Schmid et al., 2000, 2003; Kartal et al., 2007, 2008; Quan et al., 2008); however, only one genus (*Candidatus Scalindua*) seems to be ubiquitous in marine ecosystems ranging from arctic to tropical regions (Kuypers et al., 2005; Schmid et al., 2007; Penton et al., 2006).

Seeding sludge, the type of influent wastewater and temperature have been regarded as the important factors that may control the community composition in anammox reactors (van de Vossenberg et al., 2008; Kartal et al., 2008; Zhang et al., 2008; Chen et al., 2010a; Tang et al., 2010a). Reactor scale, reactor type and reactor age could also influence the selection of the dominant anammox species and the quantity of anammox bacteria. Fully understanding the consequences of the seeding sludge on the enrichment of different anammox species is pivotal to a faster start-up of anammox bioreactors. It would ease the constraints of reactor design if several different anammox species could be enriched from one seed sludge and/or adapt to the same conditions.

In order to enhance our understanding of the diversity and abundance of anammox bacteria in different wastewater treatment systems in relation to nitrogen removal load, reactor configuration, sludge age and the source of seed sludge, we examined anammox bacteria in eight different scale wastewater treatment reactors. Using 16S rRNA gene clone libraries, FISH, and qPCR, the diversity and abundance of anammox bacteria in these eight wastewater treatment systems were investigated and compared to other studies.

2. Materials and methods

2.1. Sample collection

Sludge samples investigated in this study were collected from eight nitrogen removal reactors from China (Chen et al., 2010b; Ni et al., 2010; Tang et al., 2010b, 2010, see Table 1 for details).

2.2. DNA extraction

Biomass (2 ml) was harvested by centrifugation. Cells were re-suspended in sodium phosphate buffer and glass beads (~0.3 g, 0.25 mm diameter) were added to disrupt the cells by

Table 1 – Summary of different parameters of the reactors.

Sample	Reactor types	Wastewater	Temperature °C	Seeding sludge	Total nitrogen removed (kg N/m^3 d)	Activity pg N/cell/day	Reactor age
A1	CSTR	monosodium glutamate wastewater (COD:36–513 mg/L)	Not controlled (ambient)	Anaerobic digester sludge	1.5	42	30 months
A2	UBF	Inorganic medium	Not controlled (ambient)	mixed inoculation[a]	2.5	85	18 months
A3	Microbial attached expanded bed reactor	Inorganic medium	30	A1 reactor sludge	5.5	5	18 months
A4	UASB	Inorganic medium	30–35	Anaerobic digester sludge	16.5	4	14 months
A5	UASB	Inorganic medium + sucrose (COD:50–700 mg/L)	30–35	Anaerobic digester sludge	6	3	14 months
A6	UASB	Inorganic medium	30–35	Anaerobic digester sludge	42	5	14 months
A7	Microbial attached expanded bed reactor	Inorganic medium	30	Anaerobic digester sludge	3	0.5	20 months
A8	UASB	Inorganic medium	30	Nitrification sludge	2	1	12 months

a mixed inoculation:A1 reactor sludge + nitrification sludge + anaerobic digester sludge.