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Start up of anammox process with activated sludge treating high ammonium industrial wastewaters as a favorable seeding sludge source

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ABSTRACT

It is a big challenge to select suitable seeding sludge for the quick startup of anaerobic ammonium oxidation (anammox) systems. Six seeding sludge originating from three municipal wastewater treatment plants (WWTPs) and three industrial WWTPs with high influent ammonium concentrations (landfill, coking and antibiotic production wastewater) were selected to explore suitable seeding sludge for the startup of anammox systems. The abundances of anammox bacteria in the six seeding sludge were characterized by quantitative PCR targeting the hydrazine synthase β subunit (*hzs*B) genes, and industrial sludge samples exhibited relatively higher abundances of anammox bacteria than the municipal ones. The logarithm of anammox bacterial abundance in seeding sludge was significantly correlated with the influent ammonium concentration ($p < 0.05$). Start up performance was evaluated for five (three municipal and two industrial sources) of the six sludge samples in semi-batch mode for 175 d. It took 18–86 d for the five sludge to exhibit detectable anammox activity, with two industrial source sludge (coking wastewater and landfill leachate) exhibiting the shortest start up times (18–33 d). Cloning analysis showed that all of the five final anammox sludge were dominated by *Brocadia* or *Brocadia*-like species. This study showed that industrial WWTP sludge treating ammonium rich wastewater might be a good candidate seeding source for the quick startup of anammox process.

1. Introduction

The anaerobic ammonium oxidation (anammox) process was first described in a denitrifying pilot bioreactor (Mulder et al., 1995). In this process, anammox bacteria can convert NH_4^+ and NO_2^- into N_2 and NO_3^- under anaerobic conditions without carbon source (Anjali and Sabumon, 2017). The anammox process is an environment-friendly and cost-effective way to treat ammonium-rich and nitrite-rich wastewater. With this discovery, partial nitritation/anammox technologies have been developed to treat the ammonium-rich wastewater with low COD concentrations. As of 2014, more than 100 full-scale partial nitritation/anammox installations are in operation worldwide (Lackner et al., 2014). Though enriched anammox bacterial biomass has been found to be suitable seeding sludge for a quick start up in additional wastewater treatment plants (WWTPs) (Joss et al., 2009), anammox bacterial inocula are not readily available in most cases. It is necessary to find suitable seeding sludge sources for the rapid start up of the anammox process considering the relatively slow growth rates of anammox bacteria (Araujo et al., 2011).

Though diverse seeding sludge sources have been tried for the start up of the anammox process, including activated sludge from WWTPs (Bae et al., 2010; Date et al., 2009; Sànchez Melsió et al., 2009; Shen et al., 2012; Sun et al., 2011; Tang et al., 2013; Tao et al., 2013), anaerobic digester sludge (Date et al., 2009; Sànchez Melsió et al., 2009; Sun et al., 2011), flooded paddy soil (terrestrial ecosystems) (Hu et al., 2011), and marine sediment (Li et al., 2013; van de Vossenberg et al., 2008), activated sludge from municipal WWTPs has long been used as the main sludge source due to its availability in large quantities and similarity among different WWTPs (Azari et al., 2017; Dapena-Mora et al., 2004; Meng et al., 2017; Wang et al., 2012). The time required for the emergence of detectable anammox bacterial activity using the municipal sludge varied from 35 to 360 d (Bae et al., 2010; Dapena-Mora et al., 2004; Date et al., 2009; Sànchez Melsió et al., 2009; Shen et al., 2012; Sun et al., 2011; Tao et al., 2013), which is a relatively long time for the successful start up of anammox-based technologies (Ali and Okabe, 2015). Considering that high ammonium

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concentration (Guerrero et al., 2013; Shen et al., 2012), long sludge retention time (SRT) (Bae et al., 2010), and low COD/N ratio (Tsushima et al., 2007b) were speculated to be favorable for the growth of anammox bacteria, sludge from industrial WWTPs treating ammonium-rich wastewater and operating with a long SRT might be a suitable seeding sludge source for the rapid startup of anammox system.

The main purpose of this study was to evaluate the feasibility of sludge from industrial WWTPs for the quick startup of the anammox process. In total, six seeding sludge sources originating from three municipal and three industrial WWTPs with various influent characteristics and operational parameters were selected to test the key factors impacting the abundance of anammox bacteria. The startup performance was then evaluated for five of the six sludge sources in semi-batch mode for a period of 175 d. Both the stoichiometric and molecular analyses were adopted to follow the proliferation of anammox bacteria. The results of this study will provide scientific support for the selection of suitable inocula for the start up of anammox systems.

2. Materials and methods

2.1. Inoculum sources and characteristics

As shown in Table 1, activated sludge was collected in May 2014 from three full-scale municipal WWTPs, and two full-scale and one pilot-scale industrial WWTPs with varied influent qualities and operational parameters. The three municipal WWTPs included an anaerobic/anoxic/oxic activated sludge process (AAO-M), a membrane bio-reactor (MBR-M) and an oxidation ditch (OD-M). The industrial WWTPs were used for treatment of landfill leachate (MBR-L), coking wastewater (AO-C), and antibiotic production wastewater (AO-A). The abundances of anammox bacteria in the six seeding sludge samples were detected by quantitative polymerase chain reaction (qPCR).

The influent COD and ammonium concentrations of the six WWTPs varied from 459 to 10 000 mg l^{-1} and 46 to 2000 mg l^{-1}, respectively, and HRT and SRT varied from 11 to 72 h and 9–50 d, respectively. During the sampling period, all WWTPs were operated under conditions with a COD removal rate over 90% and ammonium removal rate over 88%. Among the six sludge sources, five (AAO-M, MBR-M, OD-M, MBR-L, and AO-C) were used to test start up in five anammox reactors.

2.2. Semi-batch anammox start up experiments

Five plexiglass sequencing batch reactors with working volume of 400 ml were used to test start up of anammox system. After being inoculated with each of the different seeding sludge to a concentration of 2000 mg VSS·l^{-1}, the reactor columns were sealed with rubber flange gaskets, flushed with high-purity gas mixture of Ar/CO_2 (95/5, v/v) for 20 min, and then incubated in an orbital shaker (125 rpm) at 35 ± 1 °C in the dark.

A synthetic mineral medium was prepared using deionized water as follows (mg·l^{-1}): $(NH_4)_2SO_4$ (378), $NaNO_2$ (522), KH_2PO_4 (27.2), $CaCl_2$ (136), $MgSO_4·7H_2O$ (300), $KHCO_3$ (5000), and 1.0 ml l^{-1} of two trace element solutions (Tang et al., 2013). Every three days, half of the culture (200 ml) was removed from the reactor using a syringe after standing for 30 min, with the same amount of fresh medium then introduced. The initial target concentrations of ammonium and nitrite were approximately 40 and 53 mg-N·l^{-1}, respectively. However, the real concentrations may differ in the beginning due to the occurrence of partial sludge dissolution, denitrification, or anammox system. To avoid the generation of H_2S by sulfate-reducing bacteria, $NaNO_3$ (60 mg l^{-1}) was added along with the medium in the early stage of the start up phase prior to the establishment of anammox system (Suneethi and Joseph, 2011).

Table 1
Water characteristics and parameters of WWTPs tested.

Seeding sludge code	Process type[a]	Influent type	Influent ammonium concentration (mg·l^{-1})	Ammonium removal efficiency (%)	Influent COD concentration (mg l^{-1})	COD removal efficiency (%)	COD/N ratio	SRT[b] (day)	HRT[c] (h)	Flow rate (10^3 m^3 d^{-1})	WWTP[d] locations
AAO-M	A/A/O	Municipal wastewater	46 ± 14	94 ± 3	576 ± 127	93 ± 3	12 ± 2	9 ± 3	15 ± 5	200 ± 60	Beijing, China
MBR-M	A/A/O-MBR	Municipal wastewater	48 ± 12	97 ± 2	459 ± 138	92 ± 4	10 ± 2	16 ± 5	13 ± 4	150 ± 45	Beijing, China
OD-M	OD	Municipal wastewater	67 ± 15	99 ± 1	544 ± 136	94 ± 2	8 ± 2	13 ± 3	11 ± 3	200 ± 60	Beijing, China
MBR-L	A/O-MBR	Landfill leachate	2000 ± 600	99 ± 1	10 000 ± 3300	97 ± 1	5 ± 1	30 ± 9	100 ± 50	2 ± 1	Beijing, China
AO-C	A/O	Coking wastewater	200 ± 52	88 ± 5	2000 ± 700	90 ± 4	10 ± 2	15 ± 5	72 ± 43	0.024 ± 0.014	Tangshan, China
AO-A	A/O	Antibiotic production wastewater	216 ± 71	95 ± 3	1390 ± 695	93 ± 4	6 ± 2	50 ± 13	72 ± 36	1 ± 0.5	Wuxi, China

[a] A/A/O, anaerobic/anoxic/oxic process; A/A/O-MBR, anaerobic/anoxic/oxic membrane bio-reactor; OD, oxidation ditch process; A/O-MBR, anoxic/oxic membrane bio-reactor; A/O, anoxic/oxic process.
[b] SRT, sludge retention time.
[c] HRT, hydraulic retention time.
[d] WWTPs-wastewater treatment plants.