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Importance of the operating pH in maintaining the stability of anoxic ammonium oxidation (anammox) activity in moving bed biofilm reactors

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ABSTRACT

Two bench-scale parallel moving bed biofilm reactors (MBBR) were operated to assess pH-associated anammox activity changes during long term treatment of anaerobically digested sludge centrate pretreated in a suspended growth partial nitrification reactor. The pH was maintained at 6.5 in reactor R1, while it was allowed to vary naturally between 7.5 and 8.1 in reactor R2. At high nitrogen loads reactor R2 had a 61% lower volumetric specific nitrogen removal rate than reactor R1. The low pH and the associated low free ammonia (FA) concentrations were found to be critical to stable anammox activity in the MBBR. Nitrite enhanced the nitrogen removal rate in the conditions of low pH, all the way up to the investigated level of 50 mg NO_2-N/L. At low FA levels nitrite concentrations up to 250 mg NO_2-N/L did not cause inactivation of anammox consortia over a 2-days exposure time.

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1. Introduction

The discovery of anoxic ammonium oxidation (anammox) provided a cost effective nitrogen removal alternative for the treatment of high ammonium and low organic carbon wastewater (Fux and Siegrist, 2004). Anammox bacteria (related to *Planctomycetes*, Schmid et al., 2003) form a group of several genera of autotrophic organisms that convert ammonium and nitrite to dinitrogen gas and a small amount of nitrate. The anoxic nature of this reaction permits significant savings in aeration and organic carbon addition, yielding low excess biomass production (Eq. (1), Strous et al., 1998; van der Star et al., 2007). Overall, 60% of oxygen can be saved when about half of the ammonium is oxidized to nitrite and the nitrite route. Based on the Eq. (1), it should be noted that an increase of the pH in the anammox reactor may occur as hydrogen ions are consumed

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

The anammox process has been widely studied over the past two decades, however many inconsistencies appear among the obtained results. It was observed that the volumetric specific nitrogen removal rate (NRR) was highly variable for different and similar configurations (see Table 1). The nitrogen removal rate calculated per amount of volatile suspended solids (VSS) was also highly variable (Table 1). These inconsistencies in the specific anammox activity (SAA) were most likely related to such parameters as pH, substrate concentration, degree of enrichment and species of anammox organism enriched for, and inhibitory components, however these have not been investigated in significant detail.

The most widely accepted critical factor in the anammox system stability has been nitrite concentration, important to stability and responsible for severe inhibition under provided experimental conditions (Wett et al., 2007; Bettazzi et al., 2010). However, the effect of nitrite has not been clearly defined, with reported threshold concentrations varying between 5 and 274 mg N/L, under different experimental conditions and operating modes (Wett et al., 2007; Kimura et al., 2010).

Some researchers have suggested that NH_3 (or free ammonia – FA), may be one of the factors associated with deterioration of anammox activity deterioration, even at levels as low as 1.7 mg NH_3-N/L (Jung et al., 2007), however more recent literature does not consider FA to be an important parameter at FA concentrations below 13–15 mg NH_3-N/L (Fernández et al., 2008; Tang et al., 2010; Plaza et al., 2011). According to Martinelle et al. (1996), FA diffuses through the cell membrane into the cell and changes the inner pH, neutralizing the membrane potential, thereby causing, in the worst case scenario, cell death. In

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Table 1
Bench, pilot and full-scale maximum nitrogen removal rates (max NRR) with their maximum specific anammox activity (max SAA) in anammox systems.

Reactor type	Anammox system (values for anammox stage)		Reference
	Max NRR [kg N/ m^3 d]	Max SAA [kg N/ kg VSS d]	
Up-flow (granular sludge)	1.5–1.8	0.18	Strous et al. (1997b)
	15.4	–	Tang et al. (2009)
	43.7	1.8	Tang et al. (2010)
	6.5	1.0	van de Graaf et al. (1996)
MBBR	0.2	–	Szatkowska et al. (2007)
	1.0	–	Thole et al. (2005)
SBR	0.1	0.05	Bettazzi et al. (2010)
	0.6–2.4	0.3 per TSS	Fux et al. (2002)
	0.9	1.9	Strous et al. (1998)
	0.75	0.18 per TSS	van Dongen et al. (2001)

anammox cells, the riboplasm is alkaline and negatively charged relative to anammoxosome, thus giving a proton motive force (Fuerst et al., 2006). On the other hand, passive diffusion of protons across the biological membrane leads to high energy losses (van Niftrik et al., 2004). With a pKa of 9.24, the proportion of FA relative to ammonium (NH_4^+) is pH-dependent, and increases greatly (about 24 times) within the pH range (pH between 7 and 8.5) reported to be optimal for the anammox process (Strous et al., 1997b).

Biofilm processes have proved to be reliable for nitrogen removal having some advantages over suspended growth activated sludge processes (Yang et al., 2009). The moving bed biofilm reactor (MBBR) originated from Europe and was preliminary designed for cold climate operation, where slow growing organism were protected from wash-out (Ødegaard, 2006). The fundamental principle of the MBBR is to immobilize biomass on carrier media, eliminating the need for sludge settling and return in a continuous operation system.

MBBRs have been used in past research to investigate a variety of operational strategies for nutrient removal systems. Studies included the evaluation of energy recovery options through mechanical mixing (Phattaranawik and Leiknes, 2011) and assessing the effect of aeration on the concentration of extracellular polymeric substances (EPS) (Rahimia et al., 2011). Reactor stability under changing hydraulic residence time (HRT) (Li et al., 2011) or the presence of concentrated organic substrates (Wanga et al., 2009) was also investigated in literature.

There has been limited research utilizing MBBRs for anammox processes (Thole et al., 2005; Szatkowska et al., 2007). These studies focused on the overall feasibility of nitrogen removal in MBBR reactor systems using anammox organisms, without emphasis on process optimization. Important operating parameters which affect system performance and stability, such as pH, free ammonia concentration, and the nitrite concentration have not been studied.

Research targeting nitrite and pH associated FA threshold concentration separately, considering the possibility that their inhibitory effects might be overlapping. This current work aimed at elucidating the role of nitrite and pH in long term anammox reactor operation, under high pH (naturally occurring) and low (controlled) pH conditions.

2. Methods

2.1. Reactors set-up

A two stage configuration was used as shown in Fig. 1. The first part of the system consisted of one continuously-fed sequencing batch reactor (SBR) for the partial nitritation process, with a working volume of 20 L. A Masterflex peristaltic pump was used to feed anaerobic digester centrate from a local wastewater treatment plant (North End Water Pollution Centre NEWPCC, Winnipeg, MB, Canada) to the reactor during the reaction phase. Centrate had an average total ammonia concentration of 743 mg N/L (std. deviation 58). Centrate was delivered twice a week from the plant, settled to remove solids, and stored at a constant temperature of 5 °C. The effluent from the partial nitritation reactor was stored in a 5.5 L equalization tank where anoxic conditions were provided by sparging with nitrogen gas.

The second part of the system (where all tests were conducted) consisted of two moving bed biofilm reactors (MBBR), R1 and R2, with 3 L working volume, each. The fill ratio with media was

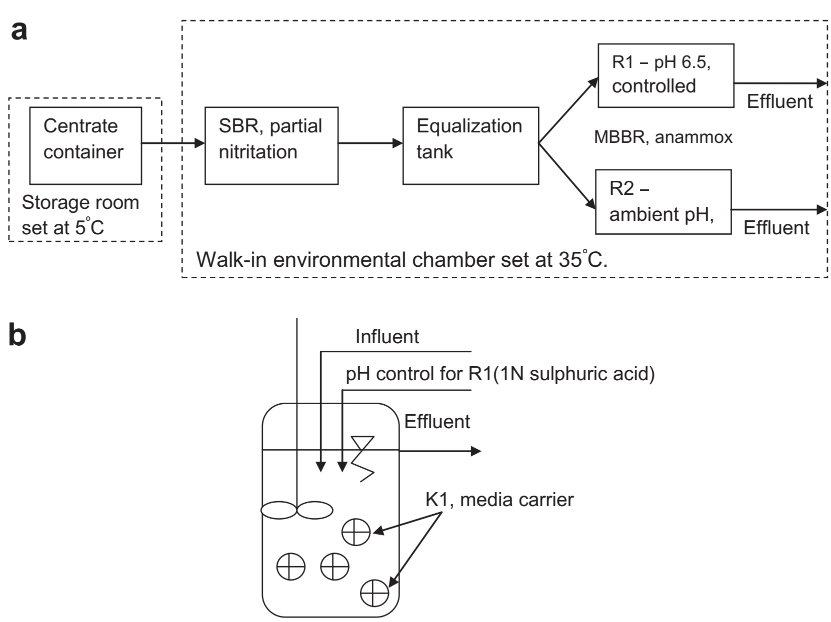

Fig. 1. Schematic of the partial nitritation and anammox reactor configuration: (a) system layout, and (b) anammox MBBR configuration.

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