Impact of carbon to nitrogen ratio and aeration regime on mainstream deammonification

M. Han, H. De Clippeleir, A. Al-Omari, B. Wett, S. E. Vlaeminck, C. Bott and S. Murthy

ABSTRACT

While deammonification of high-strength wastewater in the sludge line of sewage treatment plants has become well established, the potential cost savings spur the development of this technology for mainstream applications. This study aimed at identifying the effect of aeration and organic carbon on the deammonification process. Two 10 L sequencing bath reactors with different aeration frequencies were operated at 25 °C. Real wastewater effluents from chemically enhanced primary treatment and high-rate activated sludge process were fed into the reactors with biodegradable chemical oxygen demand/nitrogen (bCOD/N) of 2.0 and 0.6, respectively. It was found that shorter aerobic solids retention time (SRT) and higher aeration frequency gave more advantages for aerobic ammonium-oxidizing bacteria (AerAOB) than nitrite oxidizing bacteria (NOB) in the system. From the kinetics study, it is shown that the affinity for oxygen is higher for NOB than for AerAOB, and higher dissolved oxygen set-point could decrease the affinity of both AerAOB and NOB communities. After 514 days of operation, it was concluded that lower organic carbon levels enhanced the activity of anoxic ammonium-oxidizing bacteria (AnAOB) over denitrifiers. As a result, the contribution of AnAOB to nitrogen removal increased from 40 to 70%. Overall, a reasonably good total removal efficiency of 66% was reached under a low bCOD/N ratio of 2.0 after adaptation.

Key words | denitrification, energy-positive sewage treatment, nitrification, resource recovery, sewage

M. Han
S. E. Vlaeminck
Center for Microbial Ecology and Technology (LabMET),
Ghent University,
CoupureLinks 653, 9000 Ghent,
Belgium

M. Han
H. De Clippeleir
A. Al-Omari
S. Murthy
DC WATER, 500 Overlook Ave SW,
Washington, DC,
USA

B. Wett
ARAconsult, Unterbergerstr. 1,
6020 Innsbruck,
Austria

S. E. Vlaeminck (corresponding author)
Research Group of Sustainable Energy, Air and Water Technology, Department of Bioscience Engineering,
University of Antwerp, Groenenborgerlaan 171,
2020 Antwerpen,
Belgium
E-mail: *siegfried.vlaeminck@uantwerpen.be*

C. Bott
Hampton Road Sanitation District,
1436 Air Rail Ave,
Virginia Beach, VA,
USA

INTRODUCTION

The conventional mechanism of nitrogen removal in municipal wastewater is nitrification/denitrification converting ammonium nitrogen to nitrate aerobically using aerobic ammonium-oxidizing bacteria (AerAOB) and nitrite oxidizing bacteria (NOB), and then anoxically reducing nitrate to nitrogen gas through denitrification with an endogenous or exogenous organic carbon source (Tchobanoglous *et al.* 2002). This process not only requires a lot of energy input for aeration, but also has a high cost for carbon source if endogenous carbon is lacking. Around 20 years ago, researchers found out that autotrophic nitrogen removal through partial nitritation/anammox could be achieved which led to considerable savings in energy, carbon and sludge treatment (Jetten *et al.* 1999; Strous *et al.* 1999; Wett *et al.* 2007; Vlaeminck *et al.* 2012). Depending on the research group, this process is also known as oxygen-limited autotrophic nitrification/denitrification (OLAND) (De Clippeleir *et al.* 2013), DEMON (Wett *et al.* 2007), SHARON-Anammox (Van Dongen *et al.* 2001), CANON (Nielsen *et al.* 2005), or deammonification (Al-Omari *et al.* 2012), as it will be referred to in this paper. Deammonification is actually a two-step microbial process where AerAOB aerobically oxidize approximately half of the ammonium to nitrite and the anoxic ammonium-oxidizing bacteria (AnAOB) oxidize the remaining half of the ammonium with the produced nitrite to mainly nitrogen gas and some nitrate, without the need for organic carbon. Besides a key focus on AerAOB and AnAOB activities, aerobic and denitrifying contribution by heterotrophic organisms along with NOB activity determine

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the overall process performance. In recent years, more stringent regulations have been driving many researchers and engineers to work on more resource-efficient processes for nitrogen removal in wastewater, not only for sidestream treatment but also for mainstream low strength municipal wastewater.

There are several factors that play a role in determining the most viable nitrogen removal technology for a wastewater treatment plant, including but not limited to existing infrastructure, level of nitrogen removal required, and influent wastewater characteristics, most notably the carbon to nitrogen ratio. Since carbon is the energy source, the capture of carbon is essential to achieve energy recovery. The carbon removal efficiency will directly influence the type of nitrogen removal process option feasible for final polishing. On the other hand, if a certain nitrogen removal process is preferable, carbon management should be adjusted for that process to minimize cost. It is clear that carbon and nitrogen management are closely interlinked.

Under conditions of high nitrogen concentrations and low biodegradable chemical oxygen demand/nitrogen (bCOD/N) ratios as, for instance, prevailing in supernatant from dewatered digested sludge and industrial digestates, deammonification has been implemented successfully in up to 100 full-scale plants by now (Lackner *et al.* 2014). For low strength municipal sewage, deammonification shows potential due to its huge cost savings on carbon and aeration as, instead of only 20% of the nitrogen load (sidestream), all nitrogen is sent to this process when applied in mainstream (Al-Omari *et al.* 2012). However, autotrophic nitrogen removal under these conditions has not been maturely developed so far, with maintaining AnAOB activity under relatively high COD/N ratio and minimizing NOB activity under low temperature and N levels as main challenges (Vlaeminck *et al.* 2012; De Clippeleir *et al.* 2013).

The mainstream nitrogen removal process normally follows pre-treatment processes, such as primary sedimentation, chemically enhanced primary treatment (CEPT), a high-rate activated sludge (A-stage) and/or a combination of those. The range of biodegradable COD/N ratio in the influent of deammonification process could range between 1:1 and 10:1, depending on the pre-treatment processes applied (Wett *et al.* 2007; Regmi *et al.* 2014; Meerburg *et al.* 2015). Although it is believed that the deammonification process has better performance when biodegradable COD/N ratio is lower than 0.5 (Joss *et al.* 2009), there was a successful study that showed when biodegradable COD/N ratio increased from 0.5 to 1.7, nitrogen removal efficiency increased from 85% to >95% under sidestream conditions (Jenni *et al.* 2014). In order to achieve full nitrogen removal, a system consisting of both AnAOB, and denitrifiers might be preferred.

It is generally accepted that the anammox reaction is less favorable than denitrification reaction due to longer doubling time of anammox bacteria, which ranges from 5 to 10 days (Jetten *et al.* 1999; Strous *et al.* 1999; Molinuevo *et al.* 2009; Lotti *et al.* 2014b). Moreover, the coexistence of denitrifying bacteria and AnAOB has been reported to increase the granulation of AnAOB due to the competition for nitrite, and a total N removal efficiency of 93.8% could be achieved under COD/NO_3-N of 0.94–1.69 (Gao *et al.* 2013). Other studies showed that AnAOB would lose the capability to compete with denitrifiers when the COD/N ratio was above 1 in the cultivating environment containing volatile fatty acids (Güven *et al.* 2005). Therefore, more research is needed, especially under mainstream conditions, to understand the role of COD in the co-existence and competition between AnAOB and denitrifiers.

Another important challenge for mainstream deammonification is efficient NOB out-selection, since nitrate produced by NOB is undesirable, as it cannot be removed by AnAOB and, therefore, NOB activity decreases total nitrogen removal rates. NOB could compete with AerAOB for oxygen under aerobic conditions and compete with AnAOB for nitrite under oxygen-limited conditions. According to the literature on Monod kinetics for AerAOB and NOB (Blackburne *et al.* 2007; Martens-Habbena *et al.* 2009) and, as applied in sidestream deammonification, operating the reactor at low dissolved oxygen (DO) conditions could get the largest gap between AerAOB and NOB growth rates, hence achieving NOB out-selection by controlling aerobic sludge retention time. Whether high DO or low DO operation could result in a better deammonification performance under low nitrogen is, however, unclear as both strategies have been proposed before (Al-Omari *et al.* 2012; Hu *et al.* 2013). It is also reported in the literature that AerAOB did not show impact after anoxic disturbance; instead, the NOB showed reduced growth rate (Kornaros *et al.* 2010) and possible enzymatic lag phase allowing AerAOB to recover faster from oxygen limited conditions, resulting in nitrite accumulation (Gilbert *et al.* 2013).

The goal of this research was to identify the impact of COD/N fed to the system on the competition between denitrifiers and AnAOB and to study different aeration regimes (aerobic and anoxic exposure) as competitive factor to optimize NOB out-selection. Two aeration regimes were tested out: one aerating at a high frequency (e.g. 2 min air on and 13 min air off), the other one aerating at a low frequency (e.g. 7 min air on and 38 min air off).

METHODOLOGY

Experimental set-up

Two laboratory-scale sequencing bath reactors (SBRs) were operated at 25 °C since the year round temperature of the plant is between 12 °C and 30 °C. Each reactor had a liquid volume of 10 L, and the operation cycle time was 6 h.

The reactor was fed during the first 7 min of the cycle using effluent from the high-rate activated sludge system after CEPT (indicated as L phases) or with CEPT effluent only (indicated as H phases) from DC Water Blue Plains wastewater treatment plant (WWTP) after storage of the feed at 20 °C under UV disinfection to eliminate the artifact of biomass growth in the feeding tank. Influent total nitrogen concentrations were corrected to 22.0 ± 5.9 mg N/L, by addition of ammonium bicarbonate. The influent alkalinity was maintained at about 180 mg $CaCO_3$/L. Influent soluble COD (sCOD) to total nitrogen ratio was 1.5 and 3.0, for L phases and H phases, and total COD (tCOD) to total nitrogen ratio was 1.5 and 6.2, respectively. The average inert COD concentration in the influent of the plant was around 17 mg/L, which resulted in an average bCOD/N ratio of 0.6 and 2.0 for L and H phases. The influent volatile suspended solids (VSS) were below 2 mg/L for the low carbon phases and below 5 mg/L for the high carbon phases.

Every cycle began with a 4 h reaction phase where DO concentration was controlled at a DO set-point of 1.5 mg O_2/L using a DO controller and luminescent dissolved oxygen (LDO) probe (Hach, Düsseldorf, Germany). To achieve intermittent aeration in the reactor, aeration was also controlled using a timer at a frequency of interest. A parameter of cycle/h was used to describe the air on/off frequency of aeration. An anoxic phase (30 min) followed the aeration period, and the settling phase (65 min) and decanting phase (15 min) finished the cycle. The volume exchange ratio was 55%. During the whole cycle, pH was controlled between 7.00 and 7.20 using a pH controller and probe (Eutech Instruments, Singapore) and 0.3 N H_2SO_4 and 0.3 N NaOH. The AnAOB bacteria were seeded to the SBRs, initially originating from the DEMON reactor in Strass WWTP (Wett *et al.* 2007) (phases L 1–2), and later from a sidestream bench-scale DEMON (phases H 1–3, L 3–5) to maintain the AnAOB activity in the mainstream system (Zhang *et al.* 2016).

The influent flow rate was 21 L/d, resulting in hydraulic retention time of 0.47 d. The total solids retention time (SRT) was maintained at about 30 days, and wasting was done once a week through a screen (212 μm) to retain the AnAOB to increase the AnAOB SRT compared to SRT of AerAOB and NOB (Zhang *et al.* 2016). Aerobic SRT was calculated in total SRT multiplied by aerobic fraction. The reactor was cleaned every week to make sure there was no biomass growth on the walls to avoid an impact on the SRT of the system.

Reactor influent and effluent samples were taken daily for ammonium, nitrite, nitrate, tCOD, and sCOD analyses. All the nitrogen species (NH_4^+, NO_3^-, NO_2^-), and COD measurements were done in duplicate using Hach method (Hach, Düsseldorf, Germany). All reported measurements are averages of duplicates. Moreover, total suspended solids (TSS)/VSS of the reactor was measured daily according to standard method (USEPA Method 160.2, 1999 revision).

Profiling and activity tests

In-situ profiling tests were performed once a week to better understand how the reactor was performing. Starting from the beginning of the cycle, ammonium, nitrate, and nitrite concentration, as well as DO profiles, were monitored during the cycle. sCOD and ortho phosphorus were also measured during the cycle.

In-situ AnAOB activity tests were performed weekly to measure the maximum anammox activity in the reactor. For this, aeration was stopped in the beginning of the cycle and nitrogen gas was bubbled through the reactor. Ammonium and nitrite were spiked to the concentration of 10 and 5 mg N/L in the reactor, respectively, using NH_4Cl and KNO_2 stock solutions. After a 5 min mixing time, samples were taken every 15 min from the reactor to monitor ammonium, nitrite, and nitrate levels. The sCOD concentration was also measured in the beginning and end of the test.

Oxygen half saturation coefficients (K_O) and maximum oxygen uptake rates were measured through kinetic analysis. To make sure there was no readily biodegradable COD in the sample, the mixed liquid sample was aerated for 20 min, centrifuged at 5,000 rpm for 5 min and the residual pellet was re-dissolved in de-chlorinated tap water. When the test was started, bicarbonate and ammonium/nitrite were spiked using $NaHCO_3$, NH_4HCO_3 and KNO_2 stock solutions yielding concentrations of 100 mg HCO_3^-/L, 10 mg NH_4^+-N/L and 5 mg NO_2^--N/L, respectively. Ammonium was used for AerAOB tests and nitrite was used for NOB tests. After spiking the chemicals, DO was brought up to 4 mg O_2/L, and then logged until DO reached 0 mg O_2/L using a JENCO DO controller (JENCO Instruments, San Diego, CA, USA). The half saturation coefficients were estimated by fitting a modeled curve of 95% confidence through the data points, and were eventually calculated according to the reaction equations.

Mass balance calculations

Anammox, denitritation and denitratation were the three anoxic reactions taking place (Jetten *et al.* 1999; Strous *et al.* 1999; Molinuevo *et al.* 2009). Based on the reaction equations, nitrogen mass balance of NH_4^+, NO_3^-, NO_2^- removal and COD consumption were calculated. The calculation was based on the observed COD removal rate during aerobic and anoxic phases of the reaction cycle in profiling tests. The different ratios (Table S1, available with the online version of this paper) for the phases were derived from the COD removal patterns in the anoxic and aerobic reaction. The calculation of the observed rates was based on the total ammonia removal of the reactor corrected for the ammonia removal due to AnAOB activity and for the aerobic fraction of the reaction cycle. The relative nitrate production was calculated based on the percentage of nitrate production to ammonium removal (corrected for nitrite accumulation). In case no nitrification or denitrification occurs, the relative nitrate production is 11%, according to stoichiometry.

RESULTS AND DISCUSSION

The reactors were operated for 514 days, and the whole operation was divided into seven phases according to different conditions, including DO set-point, aerobic SRT and influent characteristic (high or low carbon content, later referred to as H and L phases, see Table 1).

Overall performance

For reactor A with high aeration frequency (aeration cycle of 15 min), the DO set point was kept at 1.5 mg O_2/L throughout the operation. The total SRT of the reactor was maintained at around 32 days for all phases, but the aerobic SRT varied by changing the aerobic time fraction during the reaction phase of the cycle (Table 1). During the first three low carbon phases when aerobic fraction decreased from 17% to 7%, both ammonium removal rate and maximum AerAOB rate were maintained at the same level, which showed the potential of reactor to reach higher rates. When the influent sCOD to nitrogen ratio increased from 1.4 to 2.7, the total removal efficiency increased from 18%

Table 1 | Operational parameters and influent characteristics of reactor A (high frequency aeration) and B (low frequency aeration)

Phase	L 1	L 2	L 3	H 1	H 2	L 4	L 5
Time duration (d)	19	77	27	54	91	68	171
Influent loading rate (mgN/L.d)	47.5 ± 3.8	44.9 ± 6.0	47.2 ± 4.5	44.3 ± 5.2	46.6 ± 4.0	45.5 ± 6.1	46.8 ± 6.8
Influent NH_4-N concentration (mg N/L.d)	21.9 ± 1.8	20.0 ± 2.9	16.6 ± 2.9	20.6 ± 2.5	21.9 ± 1.9	20.2 ± 2.9	16.8 ± 4.2
Influent sCOD/N ratio (–)	1.5 ± 0.2	1.6 ± 0.4	1.4 ± 0.4	2.7 ± 0.4	2.9 ± 0.6	1.3 ± 0.3	1.1 ± 0.3
AnAOB seeding rate (mg Ntot/gVSS.d)	2.7	4.0 ± 1.0	5.4	5.2 ± 3.0	9.9 ± 10.2	10.0 ± 2.7	20.4 ± 9.9
Reactor A							
DO set point (mg/L)	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Frequency (cycle/hr)	6	2.5	2.4	2.4	4.2	4	4
Aerobic fraction (%)	16.7	7.6	7.41 ± 0.6	7.41	13.0 ± 2.7	5.7 ± 1.5	3.9 ± 0.5
Aerobic SRT (d)	5.7 ± 1.1	2.5 ± 0.9	2.4 ± 1.5	2.5 ± 1.1	5.2 ± 4.1	1.7 ± 0.7	1.2 ± 0.3
Overall SRT (d)	34.0 ± 6.7	32.8 ± 10.7	31.8 ± 19.8	34.4 ± 14.5	41.7 ± 33.5	30.4 ± 11.9	30.4 ± 6.5
Reactor B							
DO set point (mg/L)	0.3	1.5	1.5	1	1.5	1.5	1.5
Frequency (cycle/hr)	2	2	2	2	1.8	1.3	1.3
Aerobic fraction (%)	22.2	22.2	22.2	22.2	14.0 ± 3.3	13.5 ± 6.5	7.4 ± 2.8
Aerobic SRT (d)	6.8 ± 0.4	6.3 ± 2.6	6.9 ± 3.2	7.8 ± 4.1	5.4 ± 3.3	3.7 ± 2.0	2.2 ± 1.2
Overall SRT (d)	30.4 ± 1.7	28.2 ± 11.5	31.2 ± 14.2	35.2 ± 18.3	40.9 ± 28.2	27.4 ± 7.5	28.7 ± 7.3

to 27%, and a total removal efficiency of 66% was reached after adaptation (Table 2, phase H1). This result was within range of other study reported (Lackner *et al.* 2015). Throughout the operation, it could be seen that the specific AerAOB rates during high carbon phases were lower than the low carbon phases, as indicated from the ammonium removal rates (Table 2). This could be due to diffusion limitation of oxygen in the mixed liquor, since high carbon phases had more particulate COD in the influent than low carbon phases (Zhang *et al.* 2016). The COD probably also stimulated heterotrophic growth, thereby lowered the AerAOB abundance in the biomass and the AerAOB specific rates. The rate of AnAOB kept decreasing during the operation while seeding rate was increased (Table 1), which showed the challenge of keeping active AnAOB bacteria in the system.

On the other hand, for reactor B with low aeration frequency (aeration cycle of 45 min), the DO set point was varied from 0.3 to 1.5 mg O_2/L (air flow rate 0.5 L/min) during the operation (phase L1–L2). Reactor B showed similar trends in terms of the nitrogen removal rates and efficiencies compared to reactor A (Table 2). Operation at low DO resulted in a lower ammonium removal rate of 36 mg N/L/d compared to that of 41 mg N/L/d for high DO (Table 2, phase L1). Therefore, from phase L2 onwards high DO set-point was applied.

Impact of the COD/N ratio

It was shown that when switching from low carbon to high carbon for feeding (Phase H1), effluent ammonium concentration increased significantly for both reactors (Table 2). For reactor A, after increased aeration frequency from 2.4 to 4.2 cycles/h and increased aeration percentage from 7% to 13%, the effluent ammonium level gradually decreased and stabilized around 3 mg NH_4^+-N/L at the end of Phase H2 (Tables 1 and 2). For reactor B, although the effluent ammonium concentration also decreased in phase H2 compared to Phase H1, but only reached about 8 mg NH_4^+-N/L.

When switching from high carbon (H phases) to low carbon phases (L phases), nitrate production increased dramatically for both reactors, although ammonium removal rate remained the same (Table 2). In order to lower the nitrate production while keeping the ammonium removal, the approach of decreasing SRT was applied in Phase L4 and L5 for both reactors. After about 150 days of operation, the reactors reached a balance where a total removal rate of 13 mg N/L/d and 10 mg N/L/d for reactor A and B was

Table 2 | Performance summary of reactor A (high frequency aeration) and B (low frequency aeration)

Phase		L 1	L 2	L 3	H 1	H 2	L 4	L 5
A	MLTSS (mg/L)	1,613 ± 196	1,445 ± 276	968 ± 123	1,799 ± 616	2,378 ± 554	2,196 ± 458	1,429 ± 418
	Effluent NH_4^+-N (mg/L)	2.5 ± 4.0	5.0 ± 4.3	6.4 ± 4.6	15.9 ± 4.8	3.0 ± 2.5	1.6 ± 1.8	3.9 ± 2.9
	Effluent NO_3^--N (mg/L)	8.0 ± 2.1	6.8 ± 3.7	11.9 ± 3.4	0.5 ± 1.6	2.7 ± 2.4	11.1 ± 3.1	12.3 ± 3.9
	Effluent NO_2^--N (mg/L)	0.1 ± 0.04	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.02	0.1 ± 0.1	0.04 ± 0.1	0.2 ± 1.2
	Effluent Ntot (mg/L)	10.3 ± 2.5	12.0 ± 3.4	18.5 ± 4.6	16.5 ± 4.1	5.8 ± 2.2	12.8 ± 2.6	16.4 ± 3.0
	Relative NO_3 production (%)	40.0 ± 4.7	38.9 ± 22.8	75.6 ± 40.3	−9.0 ± 20.9	10.0 ± 12.0	53.9 ± 14.7	61.4 ± 25.5
	NH_4 removal rate (mgN/L.d)	44.7 ± 5.2	33.6 ± 9.1	24.6 ± 27.7	27.1 ± 14.3	35.1 ± 8.6	40.9 ± 8.5	36.7 ± 8.4
	Ntot removal rate (mgN/L.d)	26.4 ± 3.2	20.3 ± 7.8	12.6 ± 8.9	12.7 ± 13.0	31.0 ± 6.7	18.8 ± 7.1	13.3 ± 4.3
	Ntot removal efficiency (%)	57.5 ± 4.2	43.2 ± 17.2	18.0 ± 22.8	27.1 ± 26.4	66.2 ± 14.6	40.9 ± 11.9	28.6 ± 8.5
	sAOB rmax (mg N/gVSS.d)	235.8 ± 220.5	248.4 ± 71.9	314.6 ± 142.7	149.6 ± 108.5	136.8 ± 48.6	167.2 ± 50.5	303.4 ± 187.6
	sNOB rmax (mg N/gVSS.d)	63.1 ± 41.8	142.9 ± 80.9	219.5 ± 52.4	141.4 ± 68.5	168.6 ± 63.1	147.5 ± 51.7	236.3 ± 150.1
	sAnAOB rmax (mg N/gVSS.d)	295.5 ± 88.1	120.6 ± 32.6	170.0 ± 28.3	83.0 ± 91.6	57.8 ± 27.0	56.9 ± 24.6	71.7 ± 21.6
B	MLTSS (mg/L)	1,556 ± 232	1,388 ± 287	1,031 ± 99	1,873 ± 508	2,157 ± 631	2,208 ± 554	1,406 ± 635
	Effluent NH_4^+-N (mg/L)	6.0 ± 2.2	2.8 ± 2.5	2.2 ± 2.5	14.6 ± 5.3	8.3 ± 5.3	1.9 ± 1.7	2.6 ± 3.4
	Effluent NO_3^--N (mg/L)	3.9 ± 0.8	8.9 ± 3.3	12.9 ± 3.9	0.5 ± 1.6	0.9 ± 1.3	10.5 ± 3.2	13.6 ± 4.7
	Effluent NO_2^+-N (mg/L)	0.1 ± 0.03	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.03	0.2 ± 0.6	0.04 ± 0.03	0.1 ± 0.9
	Effluent Ntot (mg/L)	10.0 ± 1.7	11.9 ± 2.7	15.1 ± 2.9	15.2 ± 4.6	9.3 ± 5.4	12.5 ± 3.4	16.4 ± 4.1
	Relative NO_3 production (%)	23.5 ± 1.5	46.4 ± 17.2	55.9 ± 18.3	−11.6 ± 27.7	4.1 ± 14.8	51.5 ± 15.5	73.7 ± 25.9
	NH_4 removal rate (mgN/L.d)	35.9 ± 3.3	38.7 ± 5.7	40.6 ± 9.3	15.2 ± 14.3	29.4 ± 10.5	41.2 ± 6.6	40.1 ± 10.6
	Ntot removal rate (mgN/L.d)	26.6 ± 3.0	20.0 ± 6.6	16.0 ± 5.8	15.4 ± 12.8	28.6 ± 8.9	19.7 ± 7.8	10.4 ± 4.3
	Ntot removal efficiency (%)	58.4 ± 3.3	43.9 ± 13.4	33.5 ± 11.5	32.6 ± 20.3	58.5 ± 23.9	42.9 ± 14.9	22.3 ± 8.6
	sAOB rmax (mg N/gVSS.d)	109.3 ± 160.7	315.2 ± 155.8	242.3 ± 110.8	131.9 ± 48.5	134.5 ± 48.8	178.8 ± 99.5	292.6 ± 129.3
	sNOB rmax (mg N/gVSS.d)	33.2 ± 21.4	143.9 ± 98.3	240.9 ± 53.2	195.1 ± 77.3	146.9 ± 51.8	108.9 ± 34.8	318.6 ± 186.9
	sAnAOB rmax (mg N/gVSS.d)	256.8 ± 108.0	112.8 ± 45.6	305.9 ± 9.7	122.9 ± 56.0	60.0 ± 31.2	57.6 ± 24.0	67.2 ± 28.8

MLTSS: mixed liquor total suspended solids.

achieved without any external carbon source addition under low aerobic fractions (Table 2).

It was also shown that when switching from low to high carbon influent, specific AerAOB rate had a decreasing trend in the system (Figure 1). When switching back from high carbon to low carbon influent, AerAOB rates showed a reverse trend, which confirmed the impact of higher available COD on the AerAOB content of the sludge and growth of OHO during high COD phases. This could also be confirmed by the maximum rate for AerAOB that were only about 140 mg N/gVSS/d in H phases, but as high as about 250 mg N/gVSS/d in L phases for both reactors (Table 2). Although NOB rates were almost 30% lower during H phases than the L phases, the influence of extra COD was not as big as the AerAOB (Figure 1). This means that the ratio of the AerAOB to NOB rates was also lower so, in other words, out-selection of NOB did not significantly improve when influent COD increased (Table 2). With the apparent K_O of AerAOB being around 0.9 mg/L and K_O of NOB being around 0.4 mg/L (Figure 4), the increase of COD potentially caused diffusion limitation in flocs due to adsorption of particles on flocs, yielding a competitive advantage for the NOB compared to the AerAOB.

According to the literature, the optimum range of influent COD to nitrogen ratio should be about 1.1–1.6 to get a stable performance for the system in which denitrifiers and AnAOB coexist at the same time and avoid AnAOB out-competition (Lackner et al. 2014). It was also reported that under an influent COD/NH_4^+-N ratio of 1.4–5.0, although the contribution of AnAOB declined from 89 to 58% at high COD/N ratios, stable total nitrogen removal and co-existence of denitrifiers and AnAOB in a fixed-bed reactor could be maintained in a mainstream system (Gao et al. 2013). Even for a COD/N ratio as high as 10, the total inorganic nitrogen (TIN) removal efficiency was still able to reach 57% under a short SRT of 4–8 days (Regmi et al. 2014). In this research, the sCOD to nitrogen ratios were as high as 2.7 for the high carbon phases (Table 1), and a stable contribution of 50 to 60% in total

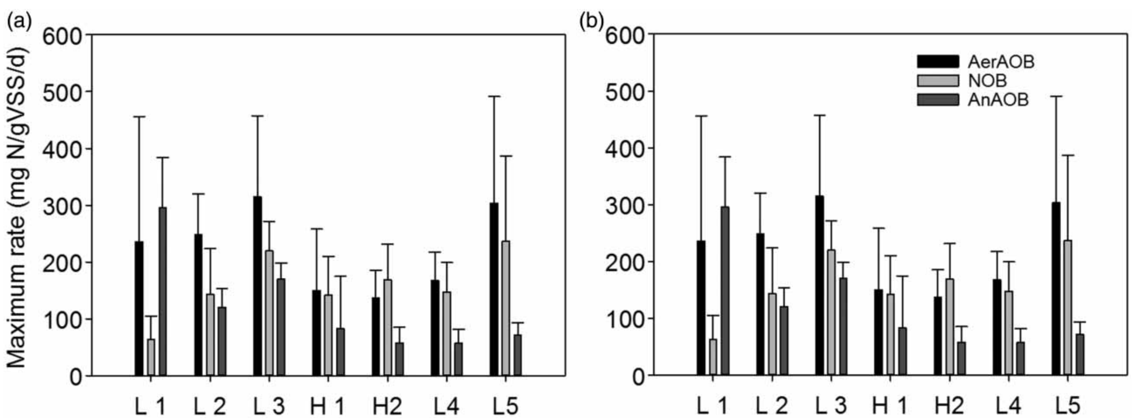

Figure 1 | Maximum specific conversion rates for AerAOB (NH_4^+), NOB (NO_2^-), and AnAOB (TN) from ex-situ activity tests during the whole operation phases for reactors A and B.

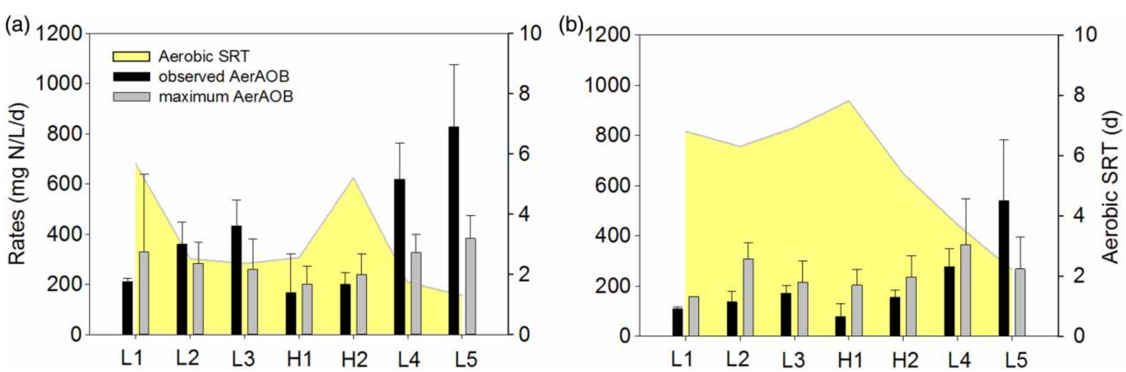

Figure 2 | Relationship of observed AerAOB rates in the reactor and maximum AerAOB rates from activity tests (measured) for both reactors A (left) and B (right).

nitrogen removal from AnAOB was still achieved (Figure 3). When sCOD to nitrogen ratio decreased to 1.1, the contribution from AnAOB increased to almost 70%.

Biomass activity

Throughout the whole operation, NOB rate had a trend of increasing and AnAOB a trend of decreasing (Table 2 and Figure 1), which showed that the main challenges of mainstream deammonification were NOB out-selection.

Due to the much lower growth rate of AnAOB compared to heterotrophs (Sözen et al. 1998; Strous et al. 1999; Molinuevo et al. 2009) and lower SRT compared to sidestream, the bioaugmentation of AnAOB in mainstream is supplemented through seeding procedure and retention techniques (Zhang et al. 2016). Although high activity of AnAOB was found under different conditions in mainstream systems (Hendrickx et al. 2014; Lotti et al. 2014a), it is not feasible to purely depend on the bioaugmentation of AnAOB. In this study, the maximum AnAOB rate was about six to seven times higher under ideal conditions than actual nitrogen removal rate through the anammox route measured in the reactor overall (Figures 1 and 3). This showed that the AnAOB rate was only limited by NOB out-selection in this case, in other words, by nitrite availability.

It was shown in Figure 2 that the maximum AerAOB rates stayed stable during the operation, but the observed AerAOB activity varied. One of the reasons that observed AerAOB rates were sometimes higher than maximum AerAOB activity could have been the presence of residual oxygen in the anoxic phases, in particular in low carbon phases, it took longer for the DO to reach zero after stopping the aeration. Although lower aerobic SRT resulted in an increase of AerAOB rates, it also increased the NOB rates (Figure 1 phases L4, L5). Previously, however, a high level of NOB out-selection in mainstream systems has been observed under similar conditions in a continuously stirred reactor system and at higher COD/N (Regmi et al. 2014). Compared to other types of reactors, SBR have limitations on settling time since a certain settling time has to be maintained to achieve good effluent quality, which does not allow total SRT to decrease significantly. Hence, it is difficult to wash NOB out by applying the SRT tool, as aerobic SRT decreases but total SRT remained long.

AnAOB contribution to N removal

Effluent nitrite concentrations were non-detected throughout the whole experiment, except for a short period of 4 weeks in the beginning of phase H2 for both reactors (up to 2 mg/L). The AnAOB bacteria showed lower maximum specific activity rates during H phases than L phases overall (Figure 1), which probably was the reason for the small nitrite accumulation.

The deammonification contribution to total nitrogen removal decreased about 20% during H phases compared to the L phases (Figure 3). However, a significant contribution of AnAOB (up to 57%) was still essential to obtain efficient total nitrogen removal efficiencies (Figure 3). An average of 70% of deammonification was achieved in total nitrogen removal overall in the L phases for both reactors (Figure 3). The total nitrogen removal rate increased from 13 to 31 mg N/L/d from low to high carbon, and this increase was mainly attributed to higher denitrification rate (Figure 3).

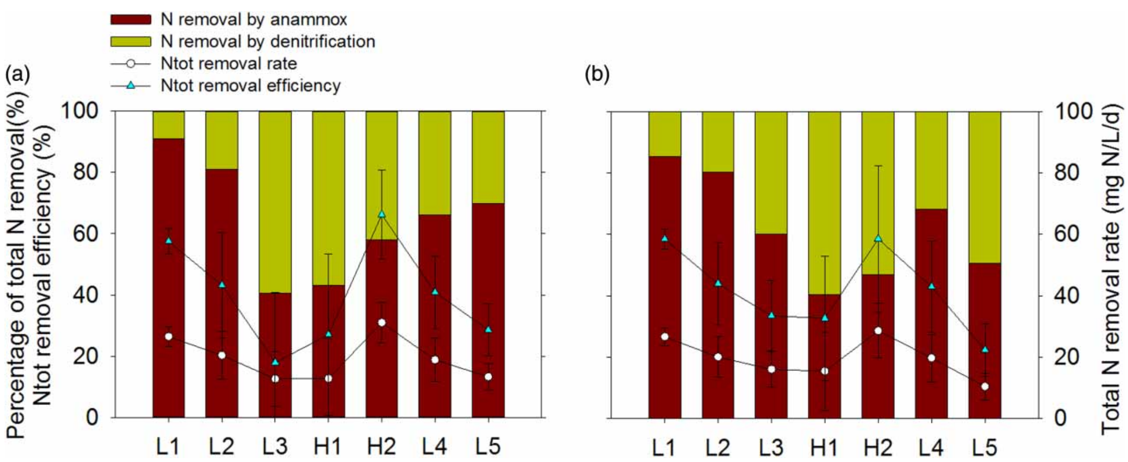

Figure 3 | Total nitrogen removal rate and ammonium removal efficiency of deammonification and denitrification comparison for all the operation phases in reactors A and B. Data were interpreted in percentage of total nitrogen removal. The usage of COD was based on observed COD removal during aerobic and anoxic phase of the reaction cycle (Table S1, available with the online version of this paper).

Effect of DO set-point and aeration regime

Generally, based on kinetics data collected, it is advised to operate the reactor under the DO set-point that favors AerAOB more than NOB, so that NOB out-selection could be achieved by applying aggressive SRT. In this study, operation at low DO set-point of 0.3 mg O_2/L resulted in a specific AerAOB rate of 109 mg N/gVSS/d (Table 2, phase L1). On the other hand, a DO set-point of 1.5 mg O2/L had a higher rate of 236 mg N/gVSS/d under similar aerobic SRT (Tables 1 and 2, phase L1), which showed the AerAOB was not performing at maximum rate for low DO reactor. The low DO set-point resulted in a higher apparent affinity for both AerAOB and NOB (Figure 4). From statistics, they were significantly different ($p_{AOB} = 0.02$ and $p_{NOB} = 0.03$). However, since the difference of AerAOB and NOB rates were larger under high DO conditions, it could be concluded that AerAOB has advantage when DO set-point was higher. When taking reactor operation into account, although maximum rates measured in ex-situ activity tests of AerAOB and NOB were similar for both DO level conditions, the high DO reactor had a lower aerobic fraction (16.7%) than the low DO reactor (22.2%). The two reactors were able to reach similar ammonium removal and total nitrogen removal rate (27 mg N/L/d), which showed higher oxygen utilization for high DO reactor.

According to previous reports, the K_O for AerAOB was lower than NOB and conventionally considered to be 0.24 mg O_2/L and 0.54 mg O_2/L, respectively (Blackburne *et al.* 2007). However, based on our observation, under high DO conditions, AerAOB had a higher nitrification rate and a higher K_O than NOB (Figure 4) and was on average 0.92 and 0.44 mg O_2/L for AerAOB and NOB, respectively. Based on kinetic tests results, it indicated that in the Monod curves for the two types of biomass, the crossing point was close to 2.0 mg O_2/L, where AerAOB and NOB have the same rate (Blackburne *et al.* 2008). The latter kinetic data confirm that operation under high DO conditions is preferred to increase NOB out-selection efficiency. This finding correlates well with other mainstream studies focused on NOB out-selection which achieved stable nitritation under high DO concentration (Ma *et al.* 2013; Regmi *et al.* 2014).

The aerobic SRT, together with K_O kinetics, were important control parameters in the system because NOB out-selection can only be achieved as the advantage of a rate differential between AerAOB and NOB is used to washout NOB by operating the reactor at a minimum SRT. It was shown that the maximum specific AerAOB rates did not change significantly ($p > 0.05$) over the operation phases (Figure 1), but NOB rates had an increasing trend. This might have been caused by the start inoculums which originated from a sidestream deammonification system (Wett *et al.* 2007) which contained mostly AnAOB and no NOB. After operating the reactor for some time, NOB out-selection in the reactor could not be maintained at the same level as the beginning. This could also be indicated from the reactor maximum AnAOB rates (Table 2), which kept decreasing while seeding was increased and total SRT was maintained the same. The latter indicated that not enough nitrite was available for AnAOB to grow. The total nitrogen removal rate decrease indicated that since AerAOB rates were the same, the level of NOB out-selection became the limiting factor of the reactor performance (Figure 3).

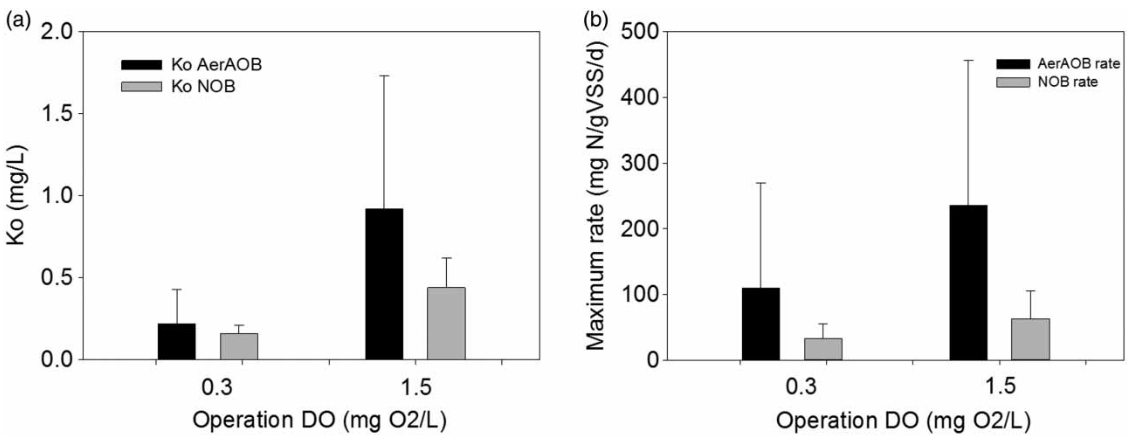

Figure 4 | The half saturation (K_O) coefficient (a) and maximum nitrification rate (b) for low DO (0.3 mg/L) and high DO (1.5 mg/L) scenarios (Table 1, phase L1 of reactors A and B).

In the aeration frequency study, during high carbon (H) phases, although the percentage of aeration, DO set-point and SRT were similar, reactor A (higher frequency) resulted in a better performance, since it had lower effluent ammonium concentration. Moreover, to achieve similar total N removal rates, reactor A had an aeration percentage of 4% compared to 11% for reactor B during the last phase (Table 1, L5), which also showed an advantage for higher aeration frequency regime. In general in bioaugmented systems, decay represents a more dominant process balancing both the seed and the growth rate (Ma *et al.* 2011). Others proposed that more frequent aerobic cycling could slightly enhance overall decay of NOB and thus increased the availability of organics for denitrification (Gilbert *et al.* 2013). During the last phase of this study, the relative nitrate production percentage for reactor A (frequency of 4 cycle/h) was 13% lower than reactor B (frequency of 1.3 cycle/h) (Tables 1 and 2, phase L5), which supported the literature.

Similar to other researchers' findings (Regmi *et al.* 2015) in this study, NOB out-selection was the limitation to obtain better performance, as nitrate formation determined the overall nitrogen removal performance and is therefore the most important factor in achieving efficient mainstream deammonification. It was reported that NOB could be inhibited by exposure of anoxic condition (Kornaros *et al.* 2010), although this is not observed in this experiment.

CONCLUSIONS

This research found that the affinity for oxygen is higher for NOB than for AerAOB, and the relationships between K_O and maximum activity rates revealed that higher DO set-point could decrease the affinity of both AerAOB and NOB communities. Moreover, this study revealed the impact of the organic carbon to nitrogen ratio on mainstream deammonification. Since the COD increase was mainly due to particulate COD in the influent, the diffusion limitation impacted AerAOB more than NOB, based on their respective K_O values. From the study of the aeration regimes, it was shown that a higher aeration frequency (more air on and off) performed better in terms of AerAOB activity and effluent quality. Overall, a total removal efficiency of 66% was reached under a low bCOD/N ratio of 2.0 after adaptation, a reasonably good result for one-stage suspended sludge mainstream deammonification (Lotti *et al.* 2014a; Lackner *et al.* 2015; Seuntjens *et al.* 2016).

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