Anammox moving bed biofilm reactor pilot at the 26th Ward wastewater treatment plants in Brooklyn, New York: start-up, biofilm population diversity and performance optimization

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ABSTRACT

New York City Environmental Protection in conjunction with City College of New York assessed the application of the anammox process in the reject water treatment using a moving bed biofilm reactor (MBBR) located at the 26th Ward wastewater treatment plant, in Brooklyn, NY. The single-stage nitritation/anammox MBBR was seeded with activated sludge and consequently was enriched with its own 'homegrown' anammox bacteria (AMX). Objectives of this study included collection of additional process kinetic and operating data and assessment of the effect of nitrogen loading rates on process performance. The initial target total inorganic nitrogen removal of 70% was limited by the low alkalinity concentration available in the influent reject water. Higher removals were achieved after supplementing the alkalinity by adding sodium hydroxide. Throughout startup and process optimization, quantitative real-time polymerase chain reaction (qPCR) analyses were used for monitoring the relevant species enriched in the biofilm and in the suspension. Maximum nitrogen removal rate was achieved by stimulating the growth of a thick biofilm on the carriers, and controlling the concentration of dissolved oxygen in the bulk flow and the nitrogen loading rates per surface area; all three appear to have contributed in suppressing nitrite-oxidizing bacteria activity while enriching AMX density within the biofilm.

Key words | anaerobic ammonia oxidation, MBBR, qPCR, reject water treatment

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INTRODUCTION

New York City Environmental Protection (NYCEP) owns and operates 14 wastewater treatment plants (WWTPs), with a combined design capacity of approximately 1.8 billion gallons per day, (6.8×10^6 m^3/day). All WWTPs were originally designed to achieve secondary treatment quality effluent using the stepfeed activated sludge process and to stabilize the combined primary and secondary sludge in mesophilic anaerobic digesters. The stabilized sludge is dewatered using centrifuges located in eight centralized locations, one of which is the 26th Ward WWTP. The dewatered sludge solids are then trucked offsite for land application or disposal in landfills, while the reject water is returned to the head of the plant for treatment. Consequently some of the plants are loaded with an excessive amount of nitrogen that in some cases reaches as much as 40% of the plant's total nitrogen load.

To address recently imposed nitrogen discharge limits, NYCEP developed a nitrogen control plan that requires the affected plants to be upgraded for nitrogen removal. This is being achieved by converting the existing plants to step-feed biological nitrogen removal, (BNR), while providing separate treatment for the reject water. In four of the plants that are subject to nitrogen discharge limits, full-scale separate reject water treatment is being provided. The 26th Ward, Bowery Bay and the Hunts Point WWTPs use the typical nitrification/denitrification process while at the Wards Island WWTP the SHARON® process was installed. All four reject water treatment facilities require an external carbon source, such as methanol or glycerol,

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and sodium hydroxide to supplement the available alkalinity, thus imposing high operating costs.

To reduce the operating costs, NYCEP engaged the City College of New York to initiate a bench-scale study of the anammox (anaerobic ammonium oxidation) process using two different configurations: the first was a two-stage nitritation followed by an anammox granular sequencing batch reactor and the second was a single-stage nitritation/anammox moving bed biofilm reactor (MBBR). The granular reactor was seeded with biomass from a previously operated pilot-scale anammox process and achieved a nitrogen removal efficiency of 88% after 200 days of operation. The single-stage MBBR was inoculated with activated sludge from the Red Hook WWTP which was operating as a step-feed BNR plant. Anammox bacteria (AMX) was successfully enriched in the biofilm after 180 days of operation, and after 375 days of operation the reactor achieved 66% nitrogen removal without chemical addition. The doubling time of anammox in the two-stage granular reactor and in the single-stage MBBR were 5.3 and 8.9 days, respectively (Park *et al*. 2010b).

Based on the favourable results from the bench-scale studies, a single-stage nitritation/anammox MBBR process was selected for further evaluation in a pilot plant built at the 26th Ward WWTP, which is the subject of this paper. The single-stage MBBR configuration was selected by the NYCEP because of its smaller footprint, apparent simplicity and yet robustness in operation, and ability to withstand variations in the influent characteristics typically found in reject water.

Thus the objectives of the pilot study included collection of additional process kinetic and operating data, and to assess the effect of nitrogen loading rates on process performance, provide guidance for design and startup of a full scale facility, and refine the potential savings of a single-stage MBBR compared to alternatives currently in use at the NYC plants.

Startup of the pilot began in March 2011 and at the direction of NYCEP no seeding of the pilot MBBR with AMX was allowed. Instead only activated sludge solids were added to the MBBR as seed, and thus the AMX bacteria grown on the carriers were termed 'homegrown' and reflected the actual reject water with all the variability in strength and composition that would be experienced in a full-scale facility. The focus of this paper is on the following aspects of the pilot study:

- Startup challenges and the modes of operation attempted that enabled enrichment of the biofilm with ammonia-oxidizing bacteria (AOB) and AMX bacteria by August 2011.
- The process optimization phase where the target was to remove 60 to 70% nitrogen, limited by the available alkalinity in the reject water, at an operating temperature of 32–34 °C.
- Quantification of the prevalent bacteria present in the biofilm and in suspension throughout startup and process optimization phases.

MATERIALS AND METHODS

The MBBR pilot facility was located at the 26th Ward WWTP in Brooklyn, NY. Reject water was heated up to 35 °C in a feed tank and was transferred to the reactor within which Kaldnes K1® carriers occupied half of the volume. More information on the reactor configuration, process monitoring and analysis is provided in the supplementary material (available online at http://www.iwaponline.com/wst/070/362.pdf).

RESULTS AND DISCUSSION

Startup of the reactor

The startup period began in March 2011 with an initial seeding of return activated sludge from the 26th Ward WWTP. The reject water fed to the MBBR initially was diluted inadvertently with wash water by plant operations, reducing the ammonia concentration to within the range of 200 mg/L, much lower than the average of approximately 400 mg/L. The low influent ammonia concentrations proved to be advantageous towards the initial objective of startup, which was to grow AOB in conjunction with biofilm thickness on the carriers while limiting the concentration of nitrite in the bulk liquid. During this period, the reactor was operated at 33 °C to promote higher growth and consequently enrichment of AOB in preference to nitrite-oxidizing bacteria (NOB) (Hellinga *et al*. 1998). Concurrently, aeration was cycled between continuous and intermittent phases in order to sustain the nitrite concentration within the range of 50 to 70 mg/L. To encourage growth of a thicker biofilm, the reactor was operated at low turbulence and at a hydraulic retention time (HRT) of 1 day, which provided both a high ammonia surface loading rate and washout of suspended growth. As the biofilm thickened, anaerobic conditions were established in the inner biofilm layers, conditions conducive for enrichment of AMX. Operations continued adjusting dissolved oxygen (DO) concentration and length of anoxic periods in an

attempt to maximize nitritation while sustaining the select range of nitrite concentration. Figure S2 in the supplementary material (available online at http://www.iwaponline.com/wst/070/362.pdf) shows an increase in total inorganic nitrogen (TIN) removal rate that is concurrent with an increase in nitrate concentration by August 2011 as shown in Figure 1. This combined effect of nitrate production and TIN removal marked the first sign of AMX activity. However, as stated in section 'Identified species of bacteria using qPCR analysis', qPCR (quantitative real-time polymerase chain reaction) data confirmed the presence of AMX in the biofilm in June, 3 months earlier, when the concentration of nitrite in the bulk phase was in the range of 100 mg/L. The observed lag between apparent initiation of growth and observance of activity as measured by reaction byproducts questions previously reported lower levels of nitrite inhibiting the growth of AMX (Fux *et al.* 2002; Wett *et al.* 2007; Fernández *et al.* 2012). Additionally, several studies report inhibition of anammox activity at short-term exposures, such as an immediate 50% loss of activity at a nitrite concentration of 350 mg NO_2^--N/L (Dapena-Mora *et al.* 2007), or an immediate 50% reversible loss of activity at 400 mg NO_2^--N/L (Lotti *et al.* 2012).

MBBR pilot operation

The nitrogen removal performance of a single-stage nitritation/anammox MBBR process depends on balancing the activities of AOB and AMX bacteria while restricting the growth of NOB. Ideally, AOB should grow on the surface of the biofilm to supply nitrite to the AMX residing deep within the biofilm.

Once anammox activity became evident during startup as inferred from the removal of TIN, ratio of ammonia to nitrite conversion, and the stoichiometry of nitrate production, aeration was switched to a continuous mode in order to increase the nitritation rate to support higher AMX bacteria activity. However, although the enhanced nitritation increased ammonia conversion to 70–75%, average removal rate for TIN ranged between 50 and 60%, thus eluding the target objective of 70%. Figure 1 shows that during the initial continuous aeration period when DO was maintained at 0.8–1.0 mg/L, the nitrate production increased beyond the expected stoichiometric level of 11%, indicating NOB activity. It reinforced the rudimentary concept of this process that it is essentially a balancing act wherein the dual populations of AOB and AMX have to be nurtured and the NOB have to be suppressed. This was one of the vexing operating challenges faced through the beginning of March 2012.

The recommended methods to suppress NOB activity typically involve lowering the DO concentration or introducing anoxic periods in the operation since NOB appear to have a higher oxygen affinity constant compared to AOB ($K_{O_2}^{NOB} = 1.1$, $K_{O_2}^{AOB} = 0.3$ mg/L) (Volcke *et al.* 2010; Yang & Yang 2011; Jardin & Hennerkes 2012). Therefore the operation was switched to the aerobic/anoxic mode in March 2012. The total aerobic/anoxic cycle was in the range of 40–50 minutes and the aeration time was varied between 10–20 minutes of the total cycle time. The expectation was that the percent

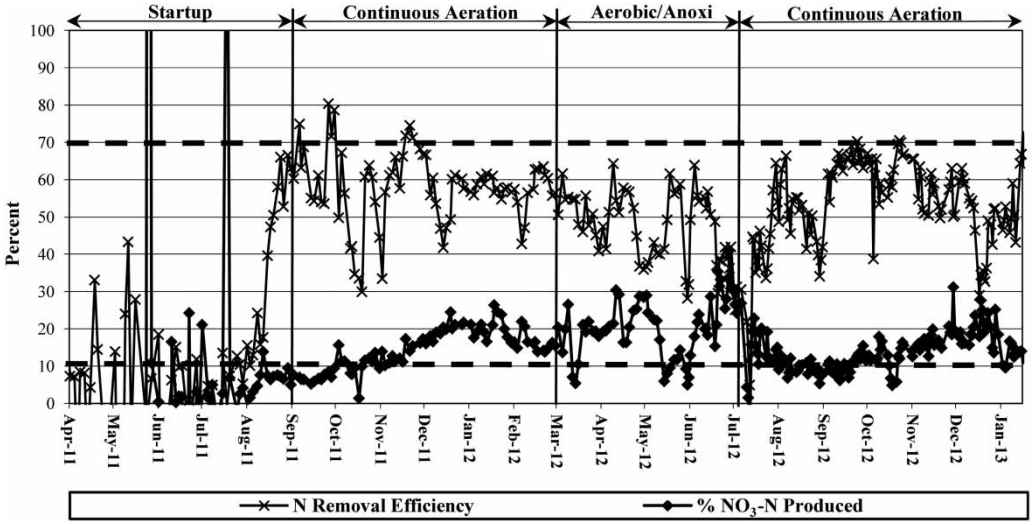

Figure 1 | Total nitrogen removal (TIN) efficiency and percent nitrate produced in the MBBR pilot. The dashed lines show the 70% objective TIN removal and 11% theoretical value of nitrate produced.

nitrate would decrease with diminished NOB activity and consequently increase the total nitrogen removal. The target removal of 70% still proved to be elusive as AOB activity was impaired due to the long anoxic periods although the DO concentration was 2–3 mg/L during the oxic period, as shown in Figure S2. In addition NOB activity persisted, resulting in deterioration of process performance. Furthermore, *ex situ* batch tests under anoxic conditions (data not shown here) conducted during this period with non-limiting nitrite concentration indicated that the anammox activity in the main reactor was primarily limited due to the scavenging of the nitrite by the NOB, as evidenced by the increase in NO_3-N concentration in the reactor as shown in Figure 1 during this period. At this juncture, the configuration and operation of the reactor were reassessed to address this inability to achieve the target TIN removal rate and the observed thin biofilm on the carriers. It was thus decided to increase the volume of the reactor from 1,100 to 1700 gallons (4.2 to 6.4 m^3) which resulted in an increase in water depth from 6 ft. (1.8 m) to 10 ft. (3 m) but with the same volume of carriers, now at a reduced 30% of the total volume. This modification led to reduced turbulence due to aeration and increased nitrogen load per unit area on the carriers, which resulted in a thicker biofilm. The aeration regime during this next phase was continuous with the DO concentration set at approximately 2 mg/L. The nitritation rate increased but the overall nitrogen removal still stayed lower than expected due to the persistence of NOB activity. At this stage, in July 2012, it was decided to switch to an aggressive mode of operation and the HRT was reduced from 1.5 to 0.5 days. The higher loading rate resulted in an exponential growth of the biomass on the carriers as shown in Figure 2 and coincided with a step up in nitrogen removal and reduction in reactor nitrate concentration. Once this level of operation was achieved, the HRT was re-adjusted to 1 day to optimize removal. The operating data starting September 2012 show that the average removal was between 60 and 70% as shown in Figure 1. The performance had significant variability because of incoming reject water quality, periodic excessive polymer dosages used during dewatering and process disruption due to super storm Sandy in October 2012. However, the process recovered and removal of nitrogen returned to the range of 65–70%, limited by the available alkalinity in the reject water based on stoichiometry. Removal of nitrogen by AMX requires a ratio of nitrite to ammonia of 1.32:1, (Strous *et al.* 1998). Consequently the removal of 1 mg/L ammonia requires a nitritation of 0.57 mg/L ammonia destroying 4.1 mg/L of alkalinity. Table S1 (available online at http://www.iwaponline.com/wst/070/362.pdf) shows an alkalinity of 1,300 mg $CaCO_3$/L and, assuming a residual alkalinity of 200 mg/L for stable pH, the ammonia removed will be approximately 268 mg/L. For an average ammonia concentration in the reject water of 400 mg/L the calculated removal is 67%. Higher nitrogen removal is possible when supplemental alkalinity was provided as shown in Figure S3 (available online at http://www.iwaponline.com/wst/070/362.pdf).

Identified species of bacteria using qPCR analysis

The startup period began in March 2011 with the initial inoculum of activated sludge solids, and, soon after beginning in April 2011, sampling for qPCR was initiated to monitor the growth of bacteria speciation in the biofilm and the suspension. Figure 3 shows the progression of the different bacteria species through the various phases of the

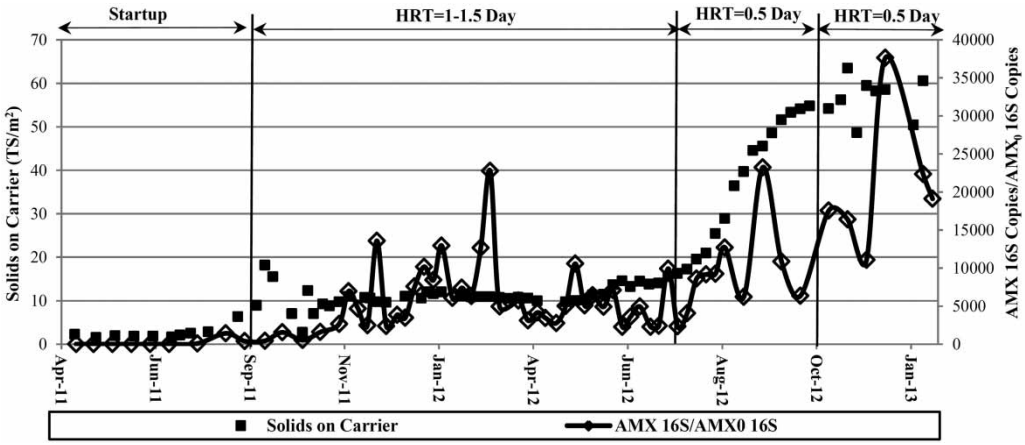

Figure 2 | The ratio of AMX 16S copies for each sample to initial AMX 16S copies and total solids on the carriers.

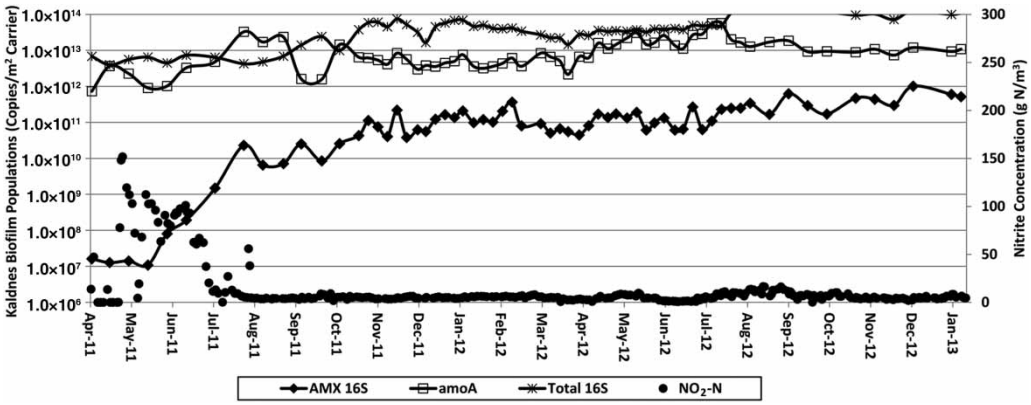

Figure 3 | Kaldnes biofilm *amoA*, AMX, total 16S population (copies/m^2 of carrier), and reactor nitrite concentration (g N/m^3) (AOB, anammox and total bacterial abundance were quantified targeting *amoA*, AMX, and total 16S genes).

study. Anammox, AOB and NOB were determined by quantifying of targeting AMX 16S rRNA genes, *amoA*, *Nitrobacter* 16S rRNA (Nb 16S) genes and *Nitrospira* 16S rRNA (Ns 16S) genes, respectively. Total bacterial abundance was quantified using eubacterial 16S rRNA gene targeted primers. The first group of samples analyzed show a high concentration of *amoA* on the Kaldnes K1® carriers, a consequence of the inoculum being from the 26th Ward WWTP which was operating in the BNR mode. Figure 3 also confirms that attachment and growth of AOB on the carriers is rapid, within 30 days, as documented by the high population of AOB on the Kaldnes K1® carriers equivalent to 7.3×10^{11} copies/m^2 of carrier. By November 2011, the abundance of AOB on the Kaldnes K1® media reached 6.4×10^{12} copies/m^2 of carrier and remained stable during the long-term operation of the reactor in both suspension and on the carriers. The concentration of AOB in suspension was slightly lower and exhibited larger variability than the concentration in the biofilm (data not shown). It appears that, as the HRT was modified during the optimization phases, loss of AOB coincided with the loss of suspended solids at the higher flow rates.

Since the population of AOB in the reactor remained relatively constant, the low nitritation rates occasionally observed during the different phases of operation may be primarily attributable to reduced activity of AOB. Reduced activity of AOB could have been caused by the low DO concentrations or prolonged anoxic periods attempted, low ammonia loading rates experienced, or, by inhibition during abrupt changes in the characteristics of the reject water experienced throughout the study.

In contrast, the activated sludge inoculum, as expected, was characterized with low concentration of AMX bacteria at 2×10^7 copies/m^2 in the biofilm as shown in Figure 3. The population of AMX remained relatively constant on the Kaldnes K1 carriers from March through May 2011. Beginning in June, the AMX population in the biofilm appeared to have grown exponentially to 2.3×10^{10} by August 2011 over a period of 70 days. The growth of AMX bacteria in suspension was less pronounced.

There are several reports on different levels of anammox activity in nitrite concentrations from 5 to 60 mg/L (Fux *et al.* 2002; Wett *et al.* 2007; Fernández *et al.* 2012). However, in this study the apparent exponential growth of anammox within the biofilm occurred when the average nitrite concentration in the bulk liquid was approximately 90 mg/L as shown in Figure 3.

Assuming that the AMX resides in the deeper layers of the biofilm (Nielsen *et al.* 2005, Volcke *et al.* 2010), a negligible amount is sheared off due to turbulence. On the basis of the concentrations shown in Figure 3 for the period June 8 through August 17, 2011, the doubling time of AMX bacteria was calculated at 6.3 days in accordance to Equation (S1) (available online at http://www.iwaponline.com/wst/070/362.pdf). This value is lower than AMX bacteria doubling times reported for both laboratory and full-scale reactors, typically 10–11 days (Strous *et al.* 1998, van der Star *et al.* 2007), although for suspended growth processes Tsushima *et al.* (2007) reported a lower value of 5.4 days for AMX doubling time, characterized by qPCR in semi-batch experiments inoculated with anammox seed from a rotating disc reactor. In addition, Park *et al.* reported that the average doubling time for AMX bacteria in granular and single-stage MBBRs was 5.3 and 8.9 days, respectively (Park *et al.* 2010b). The low values reported in this study may be due in part to the analytical method used, but also to the fact that growth of

anammox was taking place within the biofilm, a more secure and stable environment shielded from external forces of granule breakup and shearing caused by high turbulence.

The exponential growth of AMX bacteria in August 2011 resulted in a sharp increase in TIN removal rate as shown in Figure S2. When growth rate was compared to TIN removal rate, a linear relationship between the AMX bacteria and the TIN removal rate was evident with an $R^2 = 0.95$.

Comparing AOB and anammox population, AOB was the dominant species during the operation. The initial specific fractions of AOB and NOB were $23\% \pm 2\%$ and $3\% \pm 0.2\%$, respectively, while the fraction of AMX bacteria was negligible on the carriers. Cell concentrations were calculated by assuming 2 *amoA* gene copies per AOB, 1 rRNA operon per AMX and NOB, and 4.13 rRNA operons per Eub cell (Park *et al*. 2010a). By January 2013, the fractions were $21\% \pm 1.5\%$, $8\% \pm 1\%$ and 2% for AOB, NOB and AMX, respectively. The initial high fraction of AOB is related to the characteristics of the seed which was obtained from a BNR process, where the AOB population was already enriched in the sludge solids.

Beginning in July 2012, when the HRT was reduced from 1 d to 0.5 d, the growth of solids on the carriers increased exponentially. This increase coincided with an eightfold increase in the concentration of the AMX bacteria and an increase of 1.5–2 times for the *Nitrobacter* spp. population while the population of the AOB and N*itrospira* spp. remained relatively constant. The AMX 16S/AMX_0 16S ratio is also shown in Figure 2. The variability in the AMX ratio as seen in Figure 2 is probably a consequence of the small number of carriers per sample collected and hence being not representative of the total in the reactor and of the qPCR method of analysis.

The population of NOB (as measured by 16S rRNA gene abundance) on the carriers along with the percentage of nitrate produced is shown in Figure 4. Initially, the dominant species contributing to the NOB population was *Nitrobacter*, again reflecting the source of the inoculums being a wastewater BNR plant. However, starting August through November 2011, the *Nitrospira* population increased by a count of 10,000 and became the dominant NOB species in the reactor. The specific growth rate of *Nitrospira* for the apparent exponential period of growth, August through October 2011, was 0.11 day^{-1} ($R^2 = 0.97$). Further there is a linear correlation between the number of Ns 16S gene and nitrate produced ($R^2 = 0.87$) during the exponential growth (data not shown).

The reactor operations during the startup used the oxic/anoxic mode of operation to promote nitritation and anammox growth. Once anammox activity was established by August 2011, the operation was switched to a continuous aeration mode to promote additional nitritation. With increasing anammox activity, the residual nitrite concentration in the reactor decreased to below 10 mg/L. It has been hypothesized that NOB related to the genus *Nitrospira* are K-strategists and can exploit low amounts of nitrite more efficiently than NOB related to genus *Nitrobacter* (Kim & Kim 2006). The conditions in the MBBR were hence conducive to *Nitrospira* spp. growth as evidenced by their significant increase over *Nitrobacter* spp., as shown in Figure 4.

Additionally, DO concentrations in the reactor were below 1 mg/L, which can also promote *Nitrospira* growth over *Nitrobacter* (Schramm *et al*. 2000; Huang *et al*. 2010) since *Nitrospira* supposedly have a competitive advantage over *Nitrobacter* for the available oxygen in oxygen-limited environments.

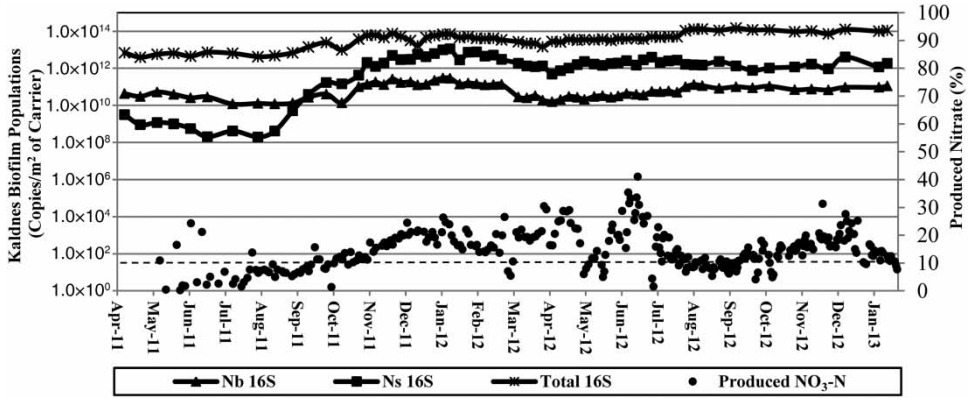

Figure 4 | Kaldnes biofilm Nb 16S, Ns16S, total 16S population (copies/m^2 of carrier) and percentage of produced nitrate. The dashed lines shows 11% theoretical percent nitrate produced (*Nitrobacter* spp., *Nitrospira* and total bacterial abundance were quantified targeting *amoA*, AMX, and total 16S genes).

One pertinent observation of the data is that excess nitrate production and removal rate increased exponentially along with the *Nitrospira* and anammox growth. Therefore, a sharp increase was observed in a short period of time although the growth initiated several weeks earlier. It appears that qPCR detects initiation of growth much earlier than evidenced by the gross measurement of the byproducts of the reactions undertaken by the stated species.

In high solids retention time systems (such as biofilm processes), reactor performance might take a while to manifest itself (Park *et al*. 2010a). The concentration and activities of the biocatalyst itself typically precede performance measures. Hence, this demonstrates the benefit of directly inspecting the bacterial speciation as well as activities, especially during startup, or process upsets, or intentional modifications in process operations.

The NOB population remained at high levels over this operating period of continuous aeration from November 2011 to March 2012. By the end of March 2012, as the reactor operation mode was switched to aerobic/anoxic mode, the NOB population slightly decreased. However, the long anoxic period did not inhibit the NOB activity completely and the produced nitrate percentage remained above 11%, indicating that the NOB activity persisted. Nevertheless, even at this level of nitrate production, the TIN removal of the pilot MBBR averaged between 65 and 70%.

CONCLUSIONS

A single-stage nitritation/anammox MBBR process seeded with only activated sludge solids from a step-feed BNR plant and fed reject water without any chemical conditioning developed its own 'homegrown' AMX in approximately 150 days. Process optimization efforts that involved control of DO concentration, nitrogen loading rates, and biofilm thickness achieved a nitrogen removal within the range of 60 to 70%. Nitrogen removal was limited to a large extent due to the limited alkalinity available in the reject water as shown in Table S1 and the inability to restrict NOB activity within the pilot MBBR. The startup and optimization phases of the study were also monitored with intermittent sampling for qPCR analyses, which when compared to the process performance data showed the following:

- Rapid attachment of AOB on the carriers within the first 30 days when the MBBR was seeded with solids from a step-feed BNR plant.
- Three months after initiation of startup, apparent exponential growth of AMX was evident although the average concentration of nitrite was 90 mg/L in the bulk flow.
- The doubling time of AMX calculated was 6.3 days.
- The species of NOB bacteria shifted from predominantly *Nitrobacter* in the seed to *Nitrospira* after approximately 8 months. The doubling time of *Nitrospira* was calculated to be 5.8 days.
- Monitoring microbial speciation during startup using qPCR would be a beneficial tool to the plant operators since it would provide a level of confidence that startup is proceeding successfully. In addition, it could be used also as a diagnostic tool during process upsets to identify the species affected and thus take the appropriate corrective actions.

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