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

SUPPLEMENTARY MATERIAL

SINGLE-STAGE NITRITATION/ANAMMOX MBBR PILOT FACILITY

The MBBR pilot facility was located at the 26th WWTP in Brooklyn, NY. The WWTP has a design capacity of 85 million gallons per day (3.2×10^5 m^3/d) and is a centralized dewatering facility which accepts additional anaerobically digested sludge from the Coney Island and Jamaica WWTPs and occasionally from other plants as required. The digested sludge is dewatered by centrifuges, producing a sludge 'cake' and reject water. The reject water is then directed to a wet well where ferric chloride and dilution water are added to prevent the formation of struvite in downstream reactors and equipment. From the wet well the reject water is pumped to Aeration Tank 3, now converted for reject water treatment using conventional nitrification/denitrification with glycerol addition. A small portion of this same reject water was diverted to the anammox MBBR pilot facility. The reject water pumped was characterized by high concentrations of suspended solids (SS), ammonia nitrogen and alkalinity, and a relatively low concentration of biodegradable chemical oxygen demand (sCOD) as shown in Table S1. Operating experiences have shown periodic spikes in the concentration of all parameters cited, in response to the quality and source of the digested sludge being dewatered and the solids capture efficiency of the centrifuges.

Figure S1 depicts the process flow schematic of the MBBR pilot facility. The facility included a lamella clarifier, three storage tanks with volumes of 850 gal (3.2 m^3), 2,000 gal (7.6 m^3), and 1,000 gal (3.8 m^3), respectively, a 1,000 gal (3.8 m^3) feed tank, and the MBBR, which was operated initially at a capacity of 1,100 gal and at a later phase of the study adjusted to 1,700 gal, (6.4 m^3). Several centrifugal and submersible pumps were used to convey reject water from the lamella clarifier through the series of storage tanks and from the feed tank to the MBBR. The pumps were operated either manually or with pre-set timers. The lamella clarifier captured the suspended solids in the feed reject water, especially during excursions from the average, at an average capture efficiency of 85%.

MONITORING PROCESS PERFORMANCE

On-line instrumentation monitored the following process parameters: total suspended solids in the Lamella influent using the Insite Model 15 total suspended solids (TSS) probe, MBBR DO concentration using the Insite Model 10 DO probe (Slidell, LA, USA), MBBR temperature and pH both by the Hach sc pH probe (Loveland, CO,

Table S1 | Characteristics of reject water pumped from the wet well

	Alkalinity (mg/L as $CaCO_3$)	NH_3-N (mg/L)	NO_2-N (mg/L)	NO_3-N (mg/L)	sCOD (mg/L)	TSS (mg/L)	VSS (mg/L)	pH
Average	1,289	378	0.0	0.9	584	1,427	1,364	7.59
St. Dev.	355	171	0.1	0.3	517	1,357	1,335	0.29
Maximum	2,140	774	0.3	1.6	2,576	9,732	7,632	8.19

St. Dev: standard deviation, VSS: volatile suspended solids.

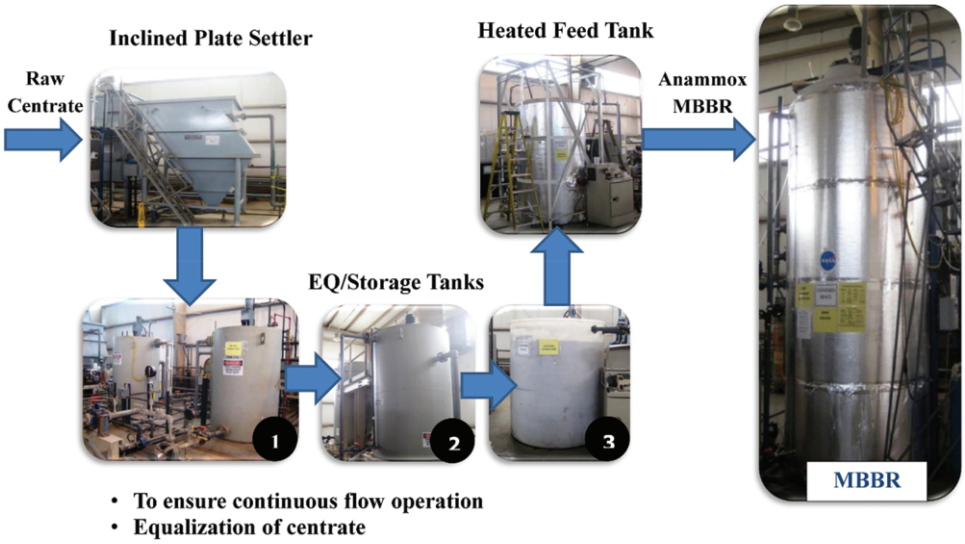

Figure S1 | Reject water process flow schematic of the MBBR pilot facility.

USA), ammonia–nitrogen by the S::can Ammo::lyser E-531-4, nitrite/nitrate nitrogen using the S::can Spectro::lyser SP-1-500-p0-s-NO-075 probe (Vienna, Austria), and airflow rate (2–7 ft^3/min) to the MBBR. A programmable logic controller supported the selected modes of operation in the MBBR. For example, aeration could be programmed on the basis of a desired level of DO concentration, or on a sequence of oxic/anoxic intervals, or a combination of both modes.

As part of the routine analysis, grab samples with a specific time stamp were collected either every day or at least three times a week. All samples were analyzed in duplicate for the specified parameters according to *Standard Methods* (American Public Health Association *et al.* 2012). The on-line data were compared to the time stamped grab samples or with readings from portable units that were calibrated against laboratory standards.

Flow from the storage tanks was first pumped into the feed tank, where electric heaters raised the temperature of the reject water to maintain the optimum range of 32 to 34 °C in the MBBR. From the feed tank a peristaltic pump delivered flow to the 1,700 gal (6.4 m^3) MBBR, within which the Kaldnes K1® carriers occupied half of the volume. The carriers provided an effective surface area of 500 m^2/m^3 where the biofilm would grow. The reactor was equipped with a variable-speed mixer specifically sized to move the media continuously within the reactor, especially during anoxic periods. An air compressor supplied air to the MBBR through a set of four fine-bubble membrane diffusers. Effluent from the MBBR overflowed into a drain and was returned to the head of the main treatment plant.

MBBR OPERATIONAL PROTOCOL

The guidelines used to achieve the balance of the bacterial population and thus achieve the highest possible nitrogen removal were as follows:

- Maintain continuous operation of the pilot MBBR, emphasizing conditions that promote biofilm thickness, i.e., high nitrogen loading rates and low turbulence within the reactor.
- Maintain the optimum temperature for growth of AMX bacteria, in this study selected as being within the range of 32° to 34 °C.
- Promote nitritation while restricting NOB activity by varying the DO concentration and/or the ratio of oxic/anoxic periods.

QUANTITATIVE PCR ANALYSIS

Biofilm samples from 30–50 carriers and SS from a 100 mL bulk flow were collected every week and stored at −80 °C for molecular analysis.

Fifty carriers collected from the MBBR were initially weighed after drying at 105 °C for 24 hours. Afterwards, the solids on the carriers were removed by shaking

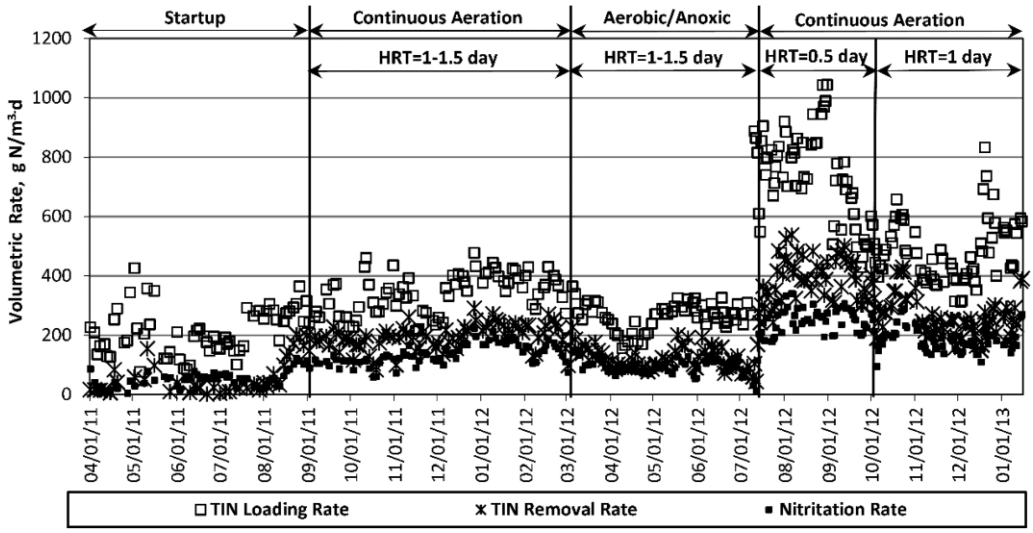

Figure S2 | TIN loading rate, TIN removal rate and nitritation rate in MBBR pilot.

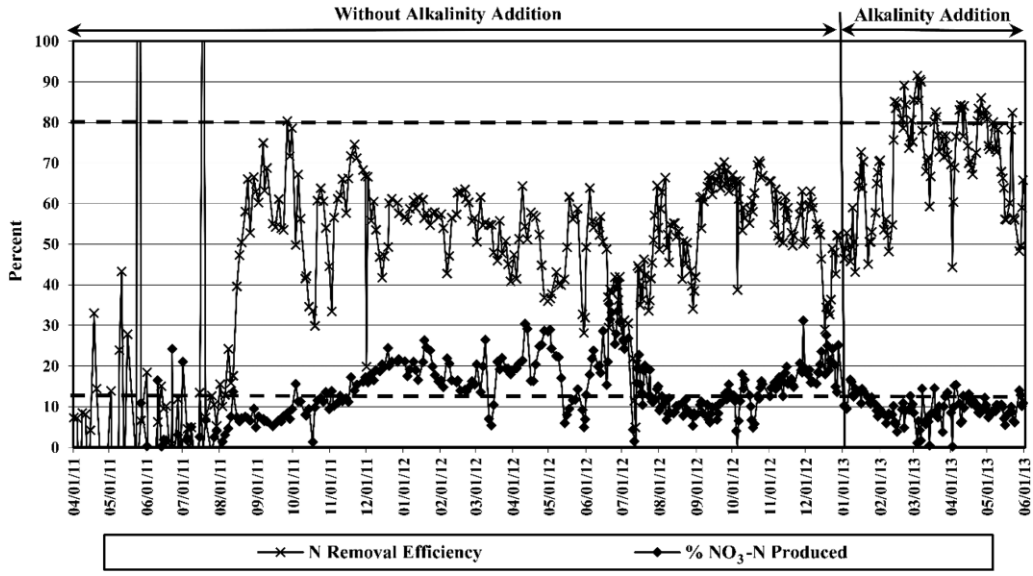

Figure S3 | Total nitrogen removal (TIN) efficiency and percent nitrate produced in the MBBR pilot before and after alkalinity addition. The dashed lines show the 70% objective TIN removal and 11% theoretical value of nitrate produced.

the carriers vigorously in a small container followed by inserting the carriers in a 5 M NaOH solution at 70 °C for 30–45 minutes. Finally, the carriers were reweighed after 24 hours drying at 105 °C. The difference between initial and final weight of the carriers indicated the mass of biofilm solids that had accumulated on the carriers.

DNA extraction was conducted using the DNeasy mini kit. The population of anammox, AOB and NOB were determined via SYBR Green chemistry qPCR assays 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 (Park *et al.* 2010). All qPCR assays were conducted on an iQ5 real-time PCR thermal cycler (BioRad Laboratories, Hercules, CA, USA). Standard curves for qPCR were generated via serial decimal dilutions of plasmid DNA containing specific target gene inserts. Primer specificity and the absence of primer-dimers were

confirmed via melt curve analysis (Park *et al.* 2010). qPCR was performed in triplicate for each sample.

CALCULATION OF GENERATION (DOUBLING) TIME

The doubling time (t_d) was estimated from the qPCR data with the least-squares method from the logarithm of the number of gene copies/volume of reactor with respect to time during an apparent log growth phase (van der Star *et al.* 2007).

$$t_d = \frac{\ln(2)(t - t_0)}{\ln\left(\frac{C}{C_0}\right)} \quad \text{(S1)}$$

where C_0 and C are AMX concentrations (copy/L reactor) at initial time t_0 and at time t days, respectively.

TIN loading rate, TIN removal rate and nitritation rate in the MBBR pilot are depicted in Figure S2.

TIN removal efficiency and percent nitrate produced before and after alkalinity addition are shown in Figure S3.

REFERENCES

American Public Health Association, American Water Works Association and Water Environment Federation 2012 *Standard Methods for the Examination of Water and Wastewater*, 22nd edn. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.

Park, H., Rosenthal, A., Jezek, R., Ramalingam, K., Fillos, J. & Chandran, K. 2010 Impact of inocula and growth mode on the molecular microbial ecology of anaerobic ammonia oxidation (anammox) bioreactor communities. *Water Research* **44** (17), 5005–5013.

Van der Star, W.R.L., Abma, W.R., Blommers, D., Mulder, J.-W., Tokutomi, T., Strous, M., Picioreanu, C. & van Loosdrecht, M. C. M. 2007 Startup of reactors for anoxic ammonium oxidation: Experiences from the first full-scale anammox reactor in Rotterdam. *Water Research* **41** (18), 4149–4163.