Laboratory study on factors influencing nitrogen removal in marble chip biofilters incorporating nitritation and anammox

Wendong Tao, Jianfeng Wen and Christopher Norton

ABSTRACT

It remains challenging to integrate nitritation and anammox in ecologically engineered treatment systems such as passive biofilters that are packed with natural materials and have low energy inputs. This study explored the factors influencing nitritation-anammox through parallel operation of two laboratory-scale biofilters packed with large and small marble chips respectively. Clean marble chips (mainly $CaCO_3$) had an alkalinity dissolution rate of 130 mg $CaCO_3$/kg marble d when water pH approached 6.5. Marble chips effectively increased water pH and provided sufficient alkalinity to support nitritation-anammox in the biofilters. Ammonium and total nitrogen removal decreased by 47 and 26%, respectively, when nutrients were not amended to influent. An influent nitrite concentration above 8.9 mg N/L could inhibit anammox in thin biofilms of biofilters. Nitritation-anammox was enhanced with a hydraulic retention time of 2 d relative to 7 d, likely due to enhanced air entrainment. Size of marble chips rarely made a significant difference in nitrogen removal, possibly due to sufficient surface area available for bacterial attachment and alkalinity dissolution.

Key words | alkalinity, biofilter, biological nitrogen removal, ecologically engineered system

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INTRODUCTION

As a sustainable alternative to the conventional nitrification-denitrification process, partial nitrification (nitritation) and anaerobic ammonium oxidation (anammox) have been integrated to remove nitrogen from ammonium-rich wastewaters. Anammox bacteria use nitrite (NO_2^-) as an electron acceptor to oxidize ammonium (NH_4^+) under strictly anaerobic conditions as Equation (1) (Strous *et al.* 1998) shows. When nitritation and anammox are coupled, only about ½ of NH_4^+ needs to be oxidized aerobically to NO_2^- first.

$$\begin{aligned}\text{Anammox}: NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \\ = 1.02N_2 + 0.066CH_2O_{0.5}N_{0.15}(\text{anammox biomass}) \\ + 0.26NO_3^- + 2.03H_2O \qquad (1)\end{aligned}$$

Integration of nitritation and anammox has been accomplished in several types of bioreactors through high energy and material inputs such as heating, artificial aeration, alkalinity addition, and bubbling dinitrogen (Gaul *et al.* 2005; van der Star *et al.* 2007; Liao *et al.* 2008; Yang *et al.* 2010). Unlike conventional bioreactors, ecologically engineered systems such as biofilters and subsurface flow constructed wetlands are simple and less costly to operate due to utilization of natural materials and self-maintenance. However, design considerations for integration of nitritation and anammox in ecologically engineered treatment systems have only been investigated in a few recent studies (Dong & Sun 2007; Paredes *et al.* 2007; Sun & Austin 2007; Tao & Wang 2009).

The first objective of this study was to examine the effects of nutrient and alkalinity amendment, influent NO_2^- concentration, and hydraulic retention time (HRT) on nitrogen removal through the novel nitritation-anammox process in biofilters that were packed with marble chips. Alkalinity and nutrients are usually supplemented to influent of anammox reactors (van de Graaf *et al.* 1996; Tsushima *et al.* 2007; Liao *et al.* 2008; Yang *et al.* 2010), which increases operational costs. Liao *et al.* (2008) and Yang *et al.* (2010) confirmed the positive impact of bicarbonate or alkalinity addition on stimulating anammox activity. However, no studies have examined the effects of nutrient amendment on anammox. A low NO_2^- concentration will

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result in substrate limitation to anammox, while a higher concentration can lead to inhibition. The inhibitory level of NO_2^- has been reported in a wide range (Dapena-Mora *et al.* 2007; van der Star *et al.* 2007; Wett *et al.* 2007; Bettazzi *et al.* 2010). No study on NO_2^- inhibition to anammox has been conducted in ecologically engineered systems, in which microorganisms are attached to packing materials as biofilms and thin biofilms are subject to less NO_2^- transport limitation. HRT also affects integration of nitritation and anammox (Gaul *et al.* 2005; van der Star *et al.* 2007), which is one of the major design parameters for biofilters.

To enhance anammox for total nitrogen removal from ammonia-rich wastewaters, more NO_2^- should be produced by nitritation and NO_2^- oxidation be inhibited. Preference of nitritation to NO_2^- oxidation has been achieved at basic pH values (Ciudad *et al.* 2007; Park *et al.* 2007). Dissolution of marble chips (mainly $CaCO_3$) in the biofilters may supplement alkalinity to water and buffer pH. The second objective of this study was to investigate dissolution rate of alkalinity from marble chips.

MATERIALS AND METHODS

This study constructed two biofilters and operated in a laboratory under various conditions over 345 days. Two 189-L rectangular polypropylene tanks ($45.7W \times 45.7L \times 91.4H$ cm) were used to build the biofilters (Figure 1). One biofilter was packed with large marble chips (effective chip size $d_{10} = 1.35$ cm) that had a porosity of 0.49 and specific surface area of 2,400 cm^2/L (bulk). The other biofilter was packed with small marble chips ($d_{10} = 0.49$ cm) that had a porosity of 0.47 and specific surface area of 3,897 cm^2/L (bulk). The biofilter with small marble chips was expected to provide more surface area for biofilm adhesion and dissolution of alkalinity, while the biofilter with large marble chips was of less concern for clogging over long-term operation. A 71.4 cm long baffle was mounted vertically in the middle of each biofilter to extend the flow path. Effluent overflowed at the height of 84.4 cm, 5 cm below the marble chip surface at the outlet. A 5-cm sand layer overlying the marble chips in the inlet aided even distribution of influent.

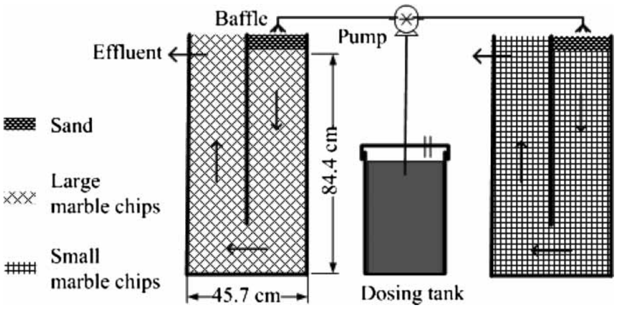

Figure 1 | Schematic of baffled marble chip biofilters for enhancement of nitritation-anammox.

The biofilters were initially inoculated with nitritation-anammox biofilms (5 mg/L of biomass) scraped from pebbles of constructed wetlands (Tao & Wang 2009). Influent was synthesized with NH_4Cl and $NaNO_2$ in dechlorinated tap water, and fed to the biofilters continuously with a peristaltic pump. The synthetic influent represents organic-carbon-poor, ammonium-rich wastewaters, such as anaerobic sludge digestate, pretreated landfill leachate, and secondary anaerobic effluent (van Dongen *et al.* 2001; Wett *et al.* 2007; Kartal *et al.* 2010). Alkalinity and nutrients were added to the influent in $NaHCO_3$ (360 mg/L), KH_2PO_4 (26.3 mg/L), $MgSO_4$ (58.6 mg/L), $CaCl_2$ (133.2 mg/L), and trace element solutions I (1 mL/L) and II (1 mL/L) in the amounts used by van de Graaf *et al.* (1996) for anammox enrichment. Influent NO_2^- concentration, alkalinity and nutrient amendment to the influent, and HRT varied stepwise as summarized in Table 1.

Influent and effluent samples were analyzed for NH_4^+, NO_2^-, and nitrate with a QuickChem 8500 series flow injection autoanalyzer (Lachat Instruments, Loverland, CO, USA). Total nitrogen (TN) was calculated as the sum of NH_4^+-N, NO_2^--N, and nitrate-N. Dissolved oxygen, pH, and oxidation-reduction potential were measured with portable meters while sampling. Dissolved metals in one effluent sample and two tap water samples were determined with an ELAN DRC-E ICP-MS (Perkin Elmer, Waltham, MA, USA). One-way analysis of variance was performed to assess differences of nitrogen removal rates between the biofilters and between operational periods.

Batch tests were conducted in two 1-L beakers to determine the rate of alkalinity dissolution from clean marble chips at room temperature. Each beaker had 50 g of large marble chips, 50 g of small marble chips, and 500 mL of influent. Initially, 0.1 N H_2SO_4 was added to deplete alkalinity in water. Recovery of water pH and alkalinity was tracked over 24 h. Alkalinity was determined with Standard Method 2320-M titration method (APHA, AWWA and WEF. 1998). Alkalinity dissolution rate, r_d (mg $CaCO_3$/kg marble d) was calculated as:

$$r_d = \Delta A \times V / (m \times \Delta t) \qquad (2)$$

where Δt is the time interval (d); ΔA the change of water

Table 1 | Variation of influent loading to marble chip biofilters over operational periods

	Start-up	Period 1	Period 2	Period 3	Period 4
Elapsing time, d	0–40	40–134	134–241	241–282	282–345
Amendment of alkalinity and nutrients to influent	Yes	Yes	Yes	No	No
Hydraulic retention time, d	7	7	7	7	2
Number of samples	4	8	10	5	8
Influent to the biofilter packed with large marble chips (mean ± S.D.)					
NH_4^+, mg N/L	69.2 ± 3.5	65.3 ± 3.9	71.1 ± 7.9	69.9 ± 1.0	96.6 ± 2.7
NO_2^-, mg N/L	8.4 ± 1.7	12.9 ± 0.7	8.9 ± 1.8	6.6 ± 0.2	1.4 ± 0.6
Influent to the biofilter packed with small marble chips (mean ± S.D.)					
NH_4^+, mg N/L	69.2 ± 3.5	65.3 ± 3.9	71.1 ± 7.9	69.9 ± 1.0	94.3 ± 4.1
NO_2^-, mg N/L	8.4 ± 1.7	12.9 ± 0.7	8.9 ± 1.8	6.6 ± 0.2	5.4 ± 0.5

alkalinity over Δt; V the water volume (L); and m the mass of marble chips in each beaker (kg).

RESULTS AND DISCUSSION

Operational conditions and nitrogen removal processes

Effluent pH was often higher than influent pH and maintained at 7.3–7.9 (Table 2), suggesting that release of calcium carbonate from marble chips buffered pH over 345 d of operation. Batch dissolution tests with marble chips demonstrated a pH increase even when water pH was approaching 6.5 (Figure 2(a)). The optimum pH for anammox is around 7.5 (van de Graaf *et al.* 1996; Tsushima *et al.* 2007). pH at 7.5–8.5 is usually considered to be favourable to nitritation over NO_2^- oxidation (Ciudad *et al.* 2007; Park *et al.* 2007). Therefore, the generally basic effluent pH (Table 2) suggested favourable conditions of marble chip biofilters for nitritation-anammox.

Dissolved oxygen concentrations below 1.5 mg/L are favourable to nitritation over NO_2^- oxidation (Park &

Table 2 | Operational conditions of marble chip biofilters over four periods

	Period 1	Period 2	Period 3	Period 4
Influent of biofilter packed with large marble chips (mean ± S.D.)				
pH	7.1 ± 0.1	7.4 ± 0.1	6.9 ± 0.2	6.6 ± 0.1
Dissolved oxygen, mg/L	1.1 ± 1.3	1.3 ± 1.2	1.9 ± 0.4	1.8 ± 0.6
Oxidation-reduction potential, mV	−26 ± 6	−52 ± 7	20 ± 4	23 ± 6
Effluent of biofilter packed with large marble chips (mean ± S.D.)				
pH	7.4 ± 0.2	7.4 ± 0.2	7.7 ± 0.2	7.4 ± 0.0
Dissolved oxygen, mg/L	0.0 ± 0.0	0.3 ± 0.2	0.0 ± 0.0	0.2 ± 0.1
Oxidation-reduction potential, mV	−37 ± 14	−37 ± 10	−28 ± 4	−20 ± 3
Influent of biofilter packed with small marble chips (mean ± S.D.)				
pH	7.1 ± 0.1	7.4 ± 0.1	6.9 ± 0.2	6.8 ± 0.1
Dissolved oxygen, mg/L	1.1 ± 1.3	1.3 ± 1.2	1.9 ± 0.4	2.2 ± 0.8
Oxidation-reduction potential, mV	−26 ± 6	−52 ± 7	20 ± 4	14 ± 4
Effluent of biofilter packed with small marble chips (mean ± S.D.)				
pH	7.3 ± 0.2	7.4 ± 0.2	7.9 ± 0.3	7.6 ± 0.0
Dissolved oxygen, mg/L	0.0 ± 0.0	0.3 ± 0.2	0.0 ± 0.0	0.2 ± 0.1
Oxidation-reduction potential, mV	−38 ± 13	−38 ± 10	−37 ± 3	−33 ± 2

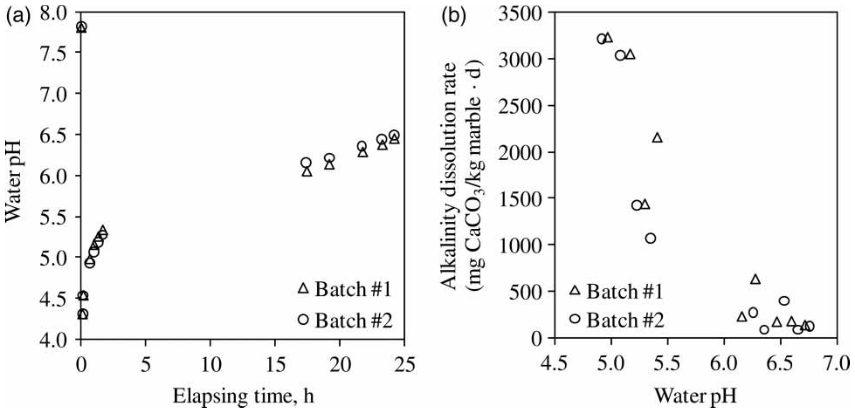

Figure 2 | (a) Recovery of water pH with dissolution of marble chips over time, and (b) variation of alkalinity dissolution rate with water pH in two batches of water (0.5 L) and marble chips (100 g).

Noguera 2004; Ciudad *et al.* 2007). The influent to the marble chip biofilters had dissolved oxygen concentrations between 0.8 and 2.2 mg/L (Table 2), suggesting that NO_2^- could be produced by nitritation near the inlet under slightly aerobic conditions in addition to NO_2^- provided with the influent. The biofilter effluent had dissolved oxygen concentrations $\leq$0.3 mg/L and low oxidation-reduction potentials (Table 2), suggesting presence of anoxic niches for anammox in the biofilters.

There was a lack of organic substrates in the influent. Heterotrophic denitrification in the marble chip biofilters was hence negligible. Batch adsorption tests with clean marble chips demonstrated negligible sorption of NH_4^+ to marble chips (data not given here). As Figure 3 shows, nitrate was accumulated in the biofilters, possibly due to anammox (Equation (1)) and complete nitrification. Therefore, TN removal in the marble chip biofilters could be attributed to autotrophic anammox and NH_4^+ removal could be attributed to nitrification and anammox. Nitrogen gas production due to anammox (Equation (1)) could be represented by the TN removal rate of the biofilters. Subsequently, stoichiometric estimation indicated that anammox in the biofilters accounted for 26, 21, 30, and 15% of NH_4^+ removal during the 4 periods respectively.

Specific surface area and associated biomass of anammox bacteria make a significant difference in nitrogen removal rate in anammox reactors (Tsushima *et al.* 2007; van der Star *et al.* 2007). The two biofilters had different sizes of marble chips and similar operational conditions during periods 1–3. However, mostly there were no significant differences in nitrogen removal ($P = 0.01$–0.65) between them (Figure 3). Larger marble chips can hence be packed in biofilters to avoid clogging over long-term operation. Considering the low growth yield of anammox bacteria (0.056 g biomass/g TN removed based on Equation (1)), thin biofilms are anticipated in biofilters treating organic-carbon-poor wastewaters. Scanning electron microscopy showed scattered biofilms on the marble chips (not shown here), which confirmed no limitation of surface area for bacterial attachment to marble chips.

Effects of influent nitrite concentration

NO_2^- concentration in the biofilter influent was lower during period 2 than period 1 (Table 1). Consequently, ammonium removal rate (Figure 3) was significantly higher during period 2 than period 1 ($P \leq 0.002$). TN removal rate appeared to be higher during period 2 than period 1 ($P = 0.12$ and 0.18). During period 4, there were insignificant differences in NH_4^+ and TN removal rates between the two biofilters ($P = 0.51$ and 0.40) that had different influent NO_2^- concentrations (Table 1). Therefore, an influent NO_2^- concentration of >8.9 mg N/L could be inhibitory to anammox in marble chip biofilters. Similarly, Wett *et al.* (2007) found that anammox could be inhibited at a NO_2^- concentration $\geq$4.8 mg N/L. Nevertheless, the inhibitory level of NO_2^- to anammox has also been reported to be much higher by Dapena-Mora *et al.* (2007), van der Star *et al.* (2007), and Bettazzi *et al.* (2010). The low threshold in this study could be due to the thin biofilms, into which NO_2^- diffusion was less restricted.

Effects of alkalinity and nutrient amendment

Periods 2 and 3 had similar influent except that there was no amendment of alkalinity and nutrients to the influent during period 3 (Table 1). Ammonium removal rate in the biofilters was significantly lower ($P \leq 0.02$) and TN removal rate appeared to be lower ($P = 0.06$ and 0.21) during period 3

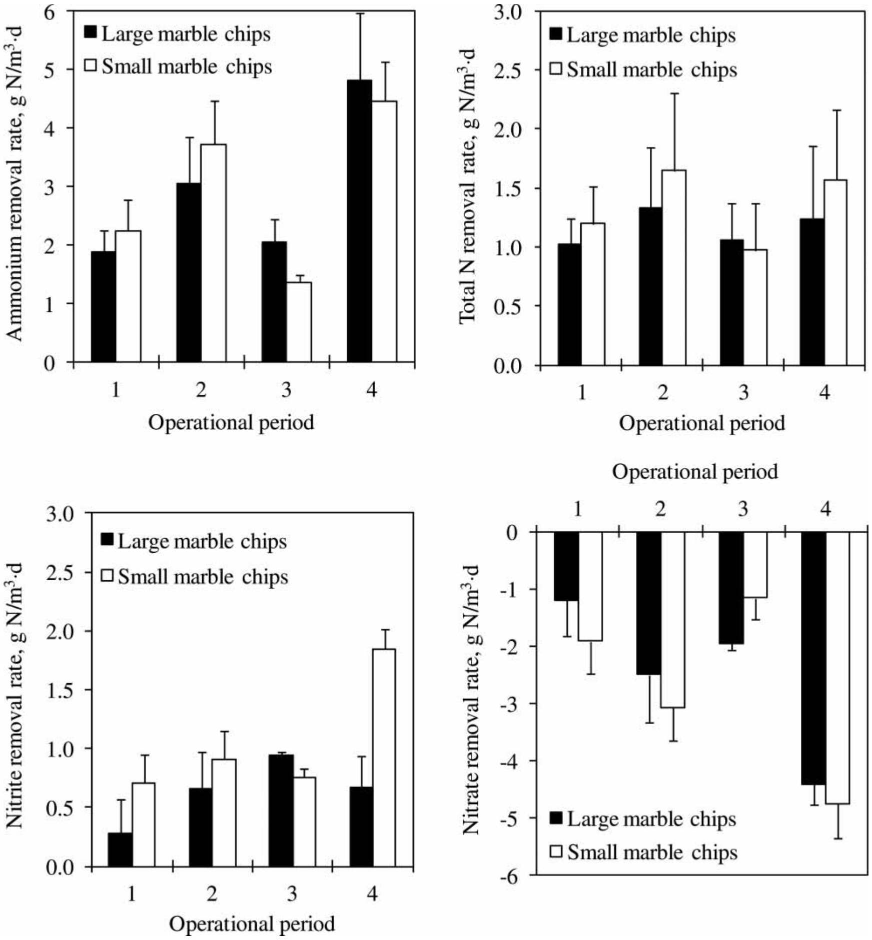

Figure 3 | Nitrogen removal rates in two biofilters packed with large and small marble chips respectively.

than period 2 (Figure 3), suggesting limitation of alkalinity or nutrients to nitritation-anammox. At water pH close to the biofilter effluent pH (Table 2), marble chips may release alkalinity about 130 mg $CaCO_3$/kg d (Figure 2(b)) or 420 mg $CaCO_3$/L d in the marble chip biofilters, which could support 59 g N/m^3 d of NH_4^+ removal via nitritation-anammox. In addition, the tap water that was used to make influent contained 100 mg/L alkalinity (City of Syracuse 2010). Therefore, nitritation-anammox was unlikely to be limited by alkalinity in the marble chip biofilters.

Nutrient limitation to nitrification can occur when the concentrations of nitrogen and phosphorus are in the range of 0.1 and 0.3 mg/L. Trace element concentrations that have been found to stimulate the growth of nitrifying bacteria in pure culture are above 0.50 mg/L Ca, 0.01 mg/L Cu, 0.03 mg/L Mg, 0.001 mg/L Mo, 0.10 mg/L Ni, and 1.0 mg/L Zn (Metcalf & Eddy 2003). Tap water that was used to make influent contained 1.75 mg/L orthophosphate (City of Syracuse 2010). Tap water had 0.09–0.16 mg/L Cu, 0.07–0.09 mg/L Zn, 0.02–0.03 mg/L Ba, 0.08–0.10 μg/L Se, 3.8–10.7 μg/L Fe, 0.5–1.2 μg/L Mo, and 1.6–7.7 μg/L Ni. Concentrations of some metals were increased in biofilter water by marble dissolution, having 21.6 mg/L Mg, 78.9 mg/L Ca, and 0.13 mg/L Mn. Therefore, nitritation in the marble chip biofilters could be limited by availability of micronutrients such as Ni and Zn. Comparing the recipe for anammox enrichment (van de Graaf *et al*. 1996) with nutrient concentrations in the tap water indicated that anammox in the marble chip biofilters could be limited by inadequacy of such nutrients as orthophosphate, Fe, Se, and Mo. Nevertheless, nutrient limitation during period 3 just resulted in a decrease in NH_4^+ and TN removal rates by 47 and 26% respectively.

Effects of hydraulic retention time

The biofilter with small marble chips during period 4 had similar operational conditions to both of the biofilters during period 3 except for a shorter HRT and higher influent NH_4^+ concentration during period 4 (Table 1). Ammonium removal rate was significantly increased ($P < 0.001$) and TN removal rate appeared to be increased ($P = 0.08$ and 0.11) during period 4 relative to period 3, suggesting that nitritation-anammox could be enhanced at a shorter HRT and higher NH_4^+ loading. Similarly, Sliekers *et al.* (2003) and Tsushima *et al.* (2007) reported enhanced anammox at shorter HRTs and subsequently higher NH_4^+ loading rates. The loading rates of nutrients were also higher at a shorter HRT during period 4, which supported higher nitrogen removal rates.

HRT could also affect autotrophic nitrifying and anammox bacteria by changing oxygen and CO_2 supply to the saturated marble chip biofilters. The increased flow velocity at the short HRT during period 4 was likely able to entrain more air or oxygen and CO_2 into the deeper zones, resulting in higher NH_4^+ and TN removal by autotrophic nitritation and anammox bacteria.

CONCLUSIONS

- Marble chips can be packed in biofilters to increase pH and supplement alkalinity for enhancement of the novel nitritation-anammox process.
- Alkalinity dissolution rate of clean marble chips decreases as pH increases, being 130 mg $CaCO_3$/kg marble d at water pH 6.5.
- An influent NO_2^- concentration above 8.9 mg N/L is likely to inhibit anammox in the thin biofilms of biofilters.
- Nitrogen removal in marble chip biofilters can be improved with a shorter HRT such as 2 d versus 7 d due to increased NH_4^+ loading and entrainment of oxygen and CO_2 from air.
- Nitritation and anammox can be integrated in marble chip biofilters to remove nitrogen from both nutrient-poor and nutrient-rich wastewaters.
- Marble chip size had little effect on nitritation-anammox in the biofilters. Large marble chips rather than small marble chips can hence be packed in biofilters to minimize clogging issues over long-term operation.

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