Role of air scouring in anaerobic/anoxic tanks providing nitrogen removal by mainstream anammox conversion in a hybrid biofilm/suspended growth full-scale WWTP in China

Quan Yuan[1,2], Beiping He[3], Liang Qian[3], Helen Littleton[4], Glen T. Daigger[5], Mark van Loosdrecht[6],
George F. Wells[7], Kaijun Wang[1], Hulin Cai[8]

[1] School of Environment, Tsinghua University, Beijing, China

[2] School of Ecology and Environment, Beijing Technology and Business University, Beijing 100048, China

[3] Thunip Co., Ltd., Beijing 100084，P. R China

[4] LX environmental LLC. Richboro, PA, 18954, USA

[5] Department of Civil and Environmental Engineering, University of Michigan, 2350, Ann Arbor, USA

[6] Department of Biotechnology, Delft University of Technology, the Netherlands

[7] Department of Civil & Environmental Engineering, Northwestern University, Evanston, USA

[8] Xi'an Wastewater Treatment Co. Ltd., Xi'an, Shanxi province 710077, P. R China

Corresponding author: Quan Yuan, Email: yuanq15@126.com

WEF members: Quan Yuan, Helen Littleton, Glen T. Daigger, Mark van Loosdrecht, George F. Wells

Abstract:

A full-scale wastewater treatment plant in China experienced unintentional anammox bacteria enrichment on biofilm carriers placed in the anaerobic and anoxic zones of an anaerobic/anoxic/oxic process under ambient temperatures and without bioaugmentation. Here we show that microaerophilic conditions resulting from air scouring needed for biofilm carriers suspension in the anaerobic/anoxic zones can support a robust nitritation/anammox process. Results from an in-situ on/off air scouring test showed that air scouring strongly induced both ammonia and total inorganic nitrogen removal in the anaerobic/anoxic zones. Ammonium concentration in the anaerobic and anoxic tanks remained constant or even slightly increased when air scouring was off, indicating that air scouring made a noticeable difference in nitrogen profiles in the anaerobic/anoxic zones. Various batch tests further indicated that partial denitrification is not likely to generate nitrite for anammox bacteria. Robust nitritation, and anammox on the carriers, can occur at low dissolved oxygen conditions, as measured in the full-scale facility. The observations show that mainstream deammonification without sidestream bioaugmentation at moderate temperature is feasible and further optimization by a more dedicated design can result in improved nitrogen removal in cases when chemical oxygen demand is limited in mainstream wastewater treatment.

Keywords: Mainstream anammox, SND, partial nitritation/anammox, air scouring, IFAS

1 Introduction

Anaerobic ammonia oxidation (anammox) is the conversion of ammonia and nitrite to nitrogen gas under anoxic conditions. When combined with partial nitritation, anammox (PN/A) provides a resource-efficient pathway for nitrogen removal in wastewater treatment plants (WWTPs). This process requires less energy and organic carbon consumption (62.5% and 100% theoretical reduction, respectively), compared with the conventional nitrification and denitrification process (N/DN). Incorporating this nitrogen removal mechanism into mainstream biological treatment systems is considered to be key to achieve energy-neutral or even energy-positive wastewater treatment when combined with organic pre-concentration processes for energy recovery (Kartal *et al.* 2010, Lackner *et al.* 2014). Although PN/A has been successfully implemented in warm and ammonia-rich wastewater, such as anaerobic digester supernatant (known as “sidestream PN/A”, (Lackner *et al.* 2014)), its implementation under mainstream conditions is still challenged due to the dilute and cold characteristics of municipal wastewater (Cao *et al.* 2017).

Most recent knowledge concerning mainstream anammox has been obtained from bench-scale experiments (Liang *et al.* 2014, Laureni *et al.* 2016, Ma *et al.* 2017). Currently, only two full-scale

WWTPs achieving full-scale mainstream anammox have been reported: (1) the Strass WWTP in Austria (Wett *et al.* 2015) and (2) the Changi Water Reclamation Plant in Singapore (Cao *et al.* 2016). The Strass plant achieved net energy self-sufficiency using the adsorption/bio-oxidation process in the mainstream for carbon capture and deammonification (PN/A) in the sidestream for nitrogen removal. Anammox biomass granules produced in the sidestream deammonification process are constantly bioaugmented to the mainstream biological nitrogen removal process. Hydrocyclones and subsequently screens are used to selectively retain anammox granules in the mainstream deammonification system. The Changi plant achieved long-term operation by a mainstream anammox process, with the discovery of the fast-growing free suspended anammox bacteria *Candidatus* Brocadia sp. 40 benefitting from the local high temperature (28~32 ºC) throughout the year (Cao *et al.* 2016). The anammox pathway was found to contribute about 30% of the total nitrogen removal, providing confidence for the full-scale mainstream anammox application in tropical regions. The application of the approach from Changi has not yet been demonstrated in moderate temperate areas, however, and requires further investigation.

More recently, obvious anammox activity was reported on biofilm carriers placed in the anaerobic and anoxic zones in a full-scale anaerobic/anoxic/oxic WWTP in China (Yuan *et al.* 2020). The anammox contribution to total plant nitrogen removal was calculated to be around 15%. Different from the Strass plant with bioaugmentation from the sidestream anammox reactor and Changi with relatively high temperature, this WWTP spontaneously enriched anammox bacteria in the biofilms on suspended carriers in the anaerobic and anoxic zones. This case provides confidence in mainstream anammox implementation, especially in large-scale WWTPs. However, the underlying factors leading to generation of nitrite needed for anammox are yet poorly understood. Partial denitrification, which converts nitrate to nitrite, was hypothesized to provide the nitrite required for anammox growth (Li *et al.* 2019). This hypothesis contrasts with the observation that some air scouring is consciously introduced into the anaerobic and anoxic compartments of the system, suggesting that nitritation could also play an important role in providing nitrite for anammox growth (Yuan *et al.* 2020). The limited coarse bubble aeration was introduced as a means to keep biofilm carriers in suspension in the anaerobic and anoxic compartments.

To better understand the underlying factors allowing for this fortuitous enrichment of mainstream anammox activity, the effects of air scouring in the anaerobic and anoxic zones of this plant on nitrogen removal and anammox activity were investigated in this study. Nitrogen conversion was investigated by turning the air scouring on/off in the anaerobic and anoxic tanks in-situ, and batch tests were conducted for further validation. The role of air scouring in the anaerobic and anoxic tanks and the underlying causative factors are discussed.

2 Materials and methods

2.1 Brief introduction

Mainstream anammox activity was observed in train 1 of the Xi'an 4th WWTP after a process retrofit implemented November 2012 through April 2013, as illustrated in Figure 1 (for more detail see Yuan *et al.* (2020)). The existing train 1 was operated as an anaerobic/anoxic/oxic (AAO) process with 11 hours hydraulic retention time (HRT), including 2/2.6/6.4 hours for the anaerobic/anoxic/aerobic zones, respectively. The retrofit involved the addition of customized carriers (plastic hollow cylinder, each 10 mm high and 25 mm in diameter, made by Yulong Environment Co., Ltd.) to the anaerobic/anoxic tanks, converting it to a hybrid system. Located in a temperate region, the wastewater temperature routinely fluctuates between 12 ºC and 22 ºC. Horizontal propeller-type mixers are provided in each of the anaerobic and anoxic tanks to maintain solids in suspension and minimize the accumulation of carriers on the screens retaining them in each of the zones. Air agitation is also provided at each of the screen locations and was initially intended as a supplemental system to be used only occasionally to minimize carrier accumulation at the screens. Operational experience dictated, however, that aeration was needed on a nearly continuous basis to minimize blockage of the screens by the carriers. The impact of aeration in the anaerobic and anoxic zones on process performance is discussed below.

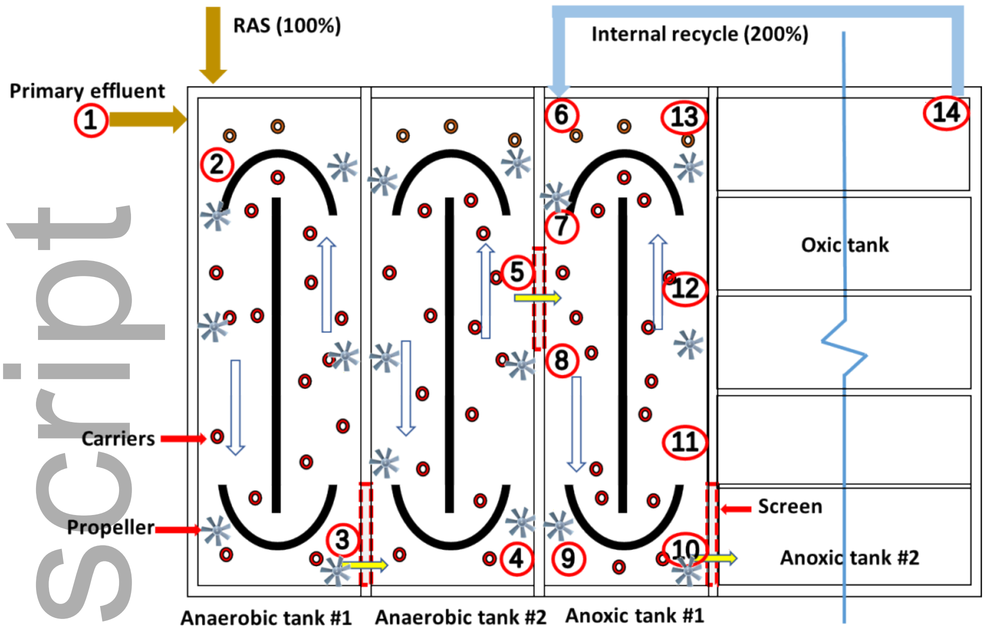

Figure 1 Detailed schematic diagram of train 1 in the full-scale WWTP with carriers in anaerobic and anoxic tanks. The red dash bar indicates the screens used to separate carriers from each tank; the aeration system is installed under each screen (Yuan *et al.* 2020)

2.2 Dissolved oxygen (DO) and Oxidation Reduction Potential (ORP) profiles in anaerobic/anoxic tank

DO and ORP profiles were conducted through the anaerobic and anoxic tanks to assess the impacts of air scouring. Portable probes (HQ40d, Hach Company, USA) were used, with measurements made at the fourteen locations indicated by numbered circles in Figure 1.

2.3 Oxygen transfer calculation caused by the air scouring in the anaerobic/anoxic tanks

Oxygen transfer in the anaerobic and anoxic tanks was calculated and compared to the nitrite production required for the amount of anammox activity occurring there. The oxygen transfer rate was calculated as follows (Tchobanoglous *et al.* 2014) :

$$R_{O2} = 0.28 G_s E_a \qquad (1)$$

where R_{O2} = Oxygen mass transfer rate under standard conditions (20 ^{o}C, standard atmosphere), m^3/h,

Gs = Aeration flowrate, 1300 m^3/h in this study

Ea = Oxygen utilization rate, 16%

$$C_{SW(T)} = C_{S(T)} \cdot \left[\frac{0.5 \times (1 - Ea)}{79 + 21 \times (1 - Ea)} + \frac{P + 9.8 \times 10^3 \times H}{2.06 \times 10^5}\right] \qquad (2)$$

$C_{sw(T)}$ = Average saturated dissolved oxygen in the tank, mg/L

T = Liquid temperature, 20 °C

$C_{S(T)}$ = Saturated dissolved oxygen under local atmospheric pressure, 9.17 mg/L

P = Atmospheric pressure, 1.013×10^5 Pa

H = Tank depth, 6 m

$$\mathrm{R} = \frac{\alpha(\beta \cdot \rho \cdot C_{SW(T)} - C) \times 1.024^{(T-20)} R_{O2}}{C_{s(20)}} \qquad (3)$$

R = Available oxygen, kgO_2/d, oxygen mass is 1331 g/m^3 under standard condition

Cs(20) = Saturated dissolved oxygen concentration at 20 °C, 9.17 mg/L

α= Correction factor for oxygen transfer, 0.82

β = Correction factor for oxygen solubility, 0.95

ρ = Correction factor for pressure, 1

C = liquid phase bulk oxygen concentration, 0.7 mg/L

2.4 In-situ air scouring on/off experiment design

A protocol (Table 1) was designed to better understand the effect of air scouring in the anaerobic and anoxic tanks on ammonium removal in these tanks. The actual hydraulic residence time for fluid and activated sludge in each tank was calculated based on all the flows entering a tank and the volume of each tanks, which we refer to as the "*effective Hydraulic retention Time (HRT)*" or "*instantaneous HRT*" to differentiate it from the nominal HRT generally based on the influent flow rate of a WWTP. The nominal HRT is independent of recirculation flows internal to the process in a continuous flow reactor, but the instantaneous HRT of one part of process is the actual HRT though this part of the full-scale bioreactor and must consider recirculation flows internal to the process that pass through it. To time sampling to reflect the actual flow-through time of the bioreactor, all the influent flows, including influent wastewater but also recirculation flows such as return activated sludge and mixed liquor recirculation must be considered to calculate the effective HRT. The effective HRT quantifies the length of time that a volume element resides within the reactor compartment. For example, return activated sludge (RAS) and/or internal recycle sludge (IRS) flow as appropriate, in addition to influent wastewater, are included when calculating the effective HRT. As a further example, consider that the volume of each anaerobic and anoxic tank is 2600 m^3, and the influent (primary effluent, PE) flow rate, Q, is 2600 m^3/h. The RAS ratio (R) is 100%, giving a flow to each anaerobic tank of (1+R)Q, i.e., 2Q, and the effective HRT is 0.5 hour (30 min) for each anaerobic tank. The IRS ratio (r) is 200%, so the effective HRT is 0.25 h (15 min) in the anoxic tank. We sampled the mixed liquor concentrations in the anaerobic and anoxic tanks on the same day with and without air scouring. Samples were taken at different spots with a time delay equal to the effective HRT (see table 1) of wastewater between entering the system and reaching the sample spot. Sampling was first conducted with air scouring on (normal condition). Air scouring was then turned off and, following a 2-hour period to allow tank contents from the previous operating mode to be displaced from the system, the sampling protocol was repeated. All the mixed liquor samples taken were filtered immediately on-site and preserved in an ice bath and analyzed for nitrogen species within 24 h. Profile sample collections were repeated three times.

Table 1 Sampling procedure with/without air scouring

Sampling time	Sampling spot
0 min	Primary effluent and RAS
30 min	Effluent of anaerobic tank #1
60 min	Effluent of anaerobic tank #2 and IRS
75 min	Effluent of anoxic tank

The N loss in the anaerobic and anoxic tanks was calculated.

The NH_4^+-N loss in the anaerobic tank #1 = $\frac{NH_4^+ - N_{PE} + NH_4^+ - N_{RAS} \times R}{1+R} - NH_4^+ - N_{Effluent\ of\ anaerobic\ \#1}$

The NH_4^+-N loss in the anaerobic tank #2 $= NH_4^+ - N_{Effluent\ of\ anaerobic\ 1\#} - NH_4^+ - N_{Effluent\ of\ anaerobic\ \#2}$

The NH_4^+-N loss in the anoxic tank = $\frac{NH_4^+ - N_{Effluent\ of\ anaerobic\ 2\#} \times (1+R) + NH_4^+ - N_{IRS} \times r}{1+R+r} - NH_4^+ - N_{Effluent\ of\ anoxic}$

Nitrate loss in each tank was calculated the same manner as ammonia. Total inorganic nitrogen (TIN) loss was the sum of ammonia and nitrate loss since nitrite was largely not detected (the limit of detection is 0.01 mg-N/L and the maximum nitrite concentration was 0.16 mg-N/L in the PE sample).

2.5 Batch tests for partial denitrification activity in the suspended solids

Partial denitrification (nitrate to nitrite) is related to sludge and carbon source characteristics (amount and type) (Ma *et al.* 2017). To determine whether the *in situ* sludge and carbon source resulted in partial denitrification process, sludge and wastewater taken from different locations in the treatment process were evaluated in batch tests designed to quantify partial denitrification. Batch denitrification tests (Table 2) were conducted in a 1.5 L beaker for 1 to 2 hours. Samples were collected every 15 min, filtered immediately, and N species were analyzed within 4 hours. Since a lower C/N ratio might make nitrite accumulation via partial denitrification more obvious, 4 mg-N/L nitrate was added in test groups 1 and 2.

Table 2 Partial denitrification processes under different scenarios

Group	Wastewater types	Aim
1	0.5 L PE + 0.5 L RAS	To mimic the starting conditions for the anaerobic tank
2	Anaerobic tank #2 effluent	To understand the effect of carbon on the partial denitrification process
3	0.5 L anaerobic tank effluent + 0.5 L IRS	To mimic the starting condition for the anoxic tank

2.6 Batch tests for denitrification activity with carriers

Since it is possible that partial denitrification could occur but that nitrite was consumed by heterotrophic denitrification, we conducted batch denitrification tests with carriers added. If the partial denitrification occurred, anammox on the carriers could compete with heterotrophic denitrifiers in the suspended growth, resulting in a noticeable decline in ammonia. In these batch tests (Table 3) anaerobic tank effluent and IRS were mixed and 50 carriers taken from the anoxic tank were added in a 1.5 L beaker. Ten mg-N/L of ammonia and nitrate were also added in batch test group 2 to lower the C/N ratio to further encourage partial denitrification. Samples were taken every 15 min for 1 hour and filtered immediately and refrigerated. Nitrogen species were analyzed within 4 hours.

Table 3 Partial denitrification processes under different scenarios

Group	Wastewater types	Dosing nitrate and ammonia	Aim
1	0.5 L anaerobic tank effluent + 0.5 L IRS+ 50 carriers	No	To mimic the anoxic tank situation without air
2	0.5 L anaerobic tank effluent + 0.5 L IRS+ 50 carriers	Yes, ~10 mg-N/L of each	To mimic the anoxic tank situation without air and enhance the possible partial denitrification process

2.7 Batch tests for low DO conditions

To understand the effect of limited oxygen on nitrogen transformations, 1 L of mixed liquor and 50 carriers taken from the anoxic tank were put in a 1.5 L beaker, and different small amounts of air were fed to the beaker through a fine air stone for 1 hour by adjusting a rotameter (as shown in Table 4). Samples were taken every 15 min, filtered immediately and refrigerated. Nitrogen species and concentration were analyzed within 4 hours. DO concentration was monitored throughout the tests.

Table 4 Nitrogen profiles under low DO conditions

Group	Airflow quality	Carriers
1	60 mL/min	No
2	20 mL/min	No
3	20 mL/min	Yes (50)

3 Results

3.1 Impact of air scouring on the DO and ORP in the anaerobic and anoxic tanks

Consistent operation of the screen air scouring system in the anaerobic and anoxic tanks resulted in continuous low level aeration of these tanks. DO and ORP profiles along the mixed liquor flow were measured at the spots indicated in Figure 1 to assess the conditions in these tanks. As indicated in

Table 5, DO was present in the anaerobic and anoxic tanks, and the ORP varied substantially. ORP variation in the anaerobic tanks showed an elevated redox condition near the screens (#3>#2 in anaerobic tank #1 and #5>#4 in anaerobic tank #2). The DO adjacent to the screens was as high as 0.3 mg-O_2/L and decreased to only around 0.2 mg-O_2/L as the mixed liquor flowed away from the screens. DO concentrations of this magnitude can be sufficient for ammonia oxidizing bacteria (AOB) to produce nitrite as a substrate for anammox via the nitritation pathway, as discussed further below. The DO concentration increased further where the IRS flowed into the anoxic tank, and subsequently decreased as the mixed liquid flowed through the anoxic tank.

Table 5 The DO and ORP profiles along with the water flow in train 1

Sites		DO	ORP
Number	Name	(mg-O_2/L)	(mV)
1	Primary effluent	-	-
2	Inlet of anaerobic tank (PE+RAS)	-	-300
3	Outlet of anaerobic tank #1	0.2	-202
4	A spot in anaerobic tank #2	-	-158
5	Outlet of anaerobic tank #2	0.27	-120
6	Inlet of mixed liquor recycle	0.52	-20
7	A spot before mixing with anaerobic effluent	0.31	-70
8	Inlet of anoxic tank #1 (Influent +IRS)	0.35	-80
9	A spot in anoxic tank #1	0.23	-120
10	Outlet of anoxic tank #1	0.33	-70
11	A spot in anoxic tank #1	0.31	-80
12	A spot in anoxic tank #1	0.24	-98
13	A spot in anoxic tank #1	0.21	-145
14	Outlet of aerobic tank	2.5	183

Oxygen transfer was calculated based on the total air introduced to the anaerobic and anoxic tanks of 1300 m^3/h, which would be sufficient to account for 14.3 mg/L of TIN removal by anammox. Previous work (Yuan *et al.* 2020) had indicated that total nitrogen removal by anammox activity in this system was about 5-8 mg/L. Thus, oxygen transferred by the air scour system in the anaerobic and anoxic tanks is sufficient to produce sufficient nitrite to account for nitrogen removal by anammox activity in these tanks.

3.2 Impact of Scour Air On and Off on Performance

Ammonia and nitrate profile data for the influent, RAS, outlets of anaerobic tank #1 and #2, IRS, outlet of anoxic tank #1, accounting for the effective HRT as described above, are presented in Figure 2 a. The data were the average of the three replicates. Nitrite concentrations ranged from 0.01 mg/L to 0.16 mg/L and were neglected. The inlet to anaerobic tank #1 was calculated as the mixture of the influent and RAS with the ratio of 1:1, and the inlet to anoxic tank #1 was calculated as the mixture of anaerobic tank #2 effluent and IRS with the ratio of 1:1. With air scour was on the nitrate concentration decreased to an undetectable level at the end of anaerobic tank #1 and the ammonia concentration decreased by 1.8 mg-N/L, resulting in a 4.8 mg-N/L of total inorganic nitrogen loss in anaerobic tank #1 (Figure 2 c). The ammonia concentration decreased by 2.9 mg-N/L in the anoxic tank while the nitrate concentration increased by 1.8 mg-N/L, resulting in a 1.1 mg-N/L of total inorganic nitrogen loss. An air scour in this tank apparently provided sufficient oxygen to AOB and nitrite-oxidizing bacteria (NOB) to allow some ammonia to be oxidized to nitrate and only part of the nitrate entering from the IRS and formed in the anoxic tank to be reduced to nitrogen gas, resulting in a less total inorganic nitrogen removal than ammonia removal. In contrast, when air scour was off nitrate decreases in both anaerobic tanks and the anoxic tanks #1 while the ammonia concentration remained relatively constant in anaerobic tank #1, or slightly higher at the end of the anaerobic tank #2 compared with the start of anaerobic tank #1. Nitrate was fully removed in the anoxic tanks, while the ammonia concentration increased by 1.9 mg-N/L probably due to the ammonification of organic nitrogen and perhaps also biomass decay (shown in Figure 2 b and d). The net result was net ammonia release when the air scour was off and much less total nitrogen removal compared to that occurring when air scour was on (Figure 2 c and d). The removal of ammonia simultaneously with total inorganic nitrogen removal in anaerobic /anoxic tanks with air scour, as shown in Figure 2 c, indicates that PN/A or simultaneous nitrification and denitrification (SND) is occurring. Considering the confirmed anammox activity on the carriers in anaerobic/anoxic tanks (Yuan *et al.* 2020), it is reasonable to conclude that PN/A occurred. Ammonia was not removed in anaerobic/anoxic tanks with air scour off, which is consistent with the hypothesis that anammox activity was absent and that partial denitrification process was not the main pathway for nitrite generation to support anammox.

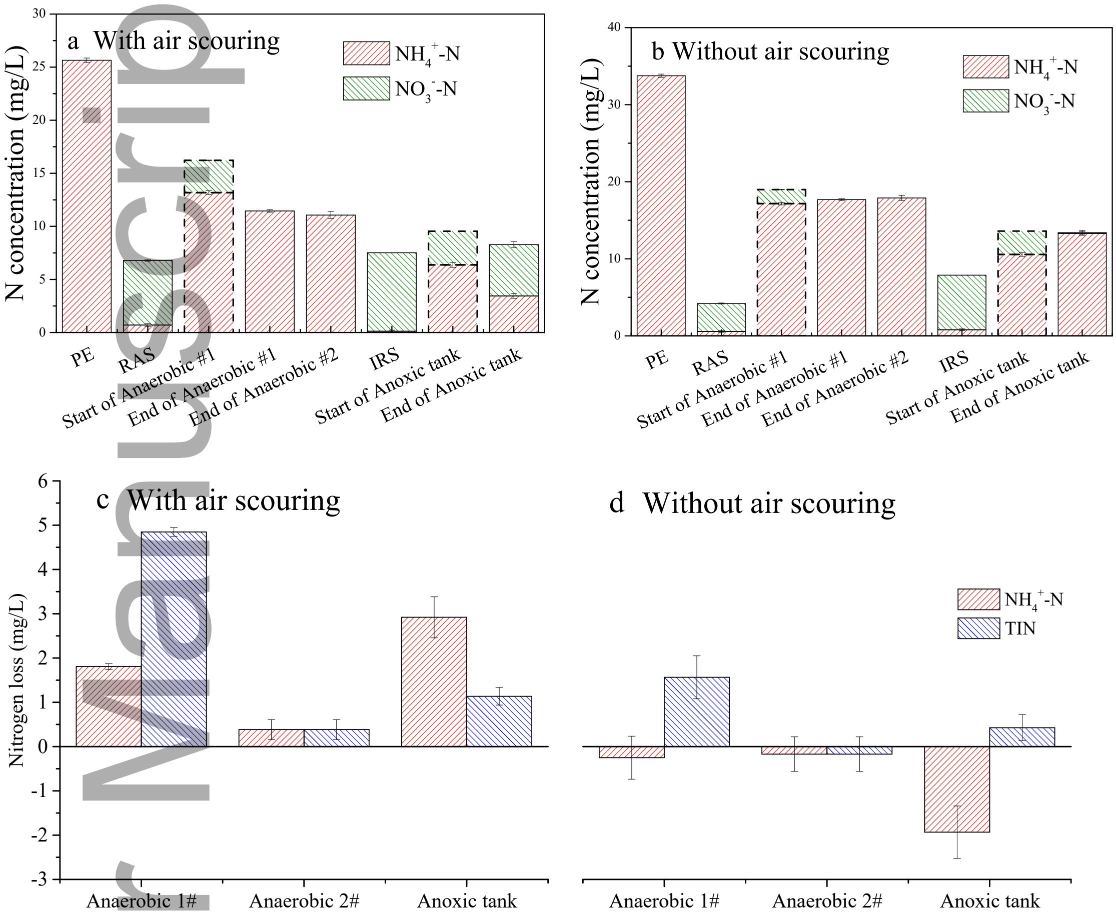

Figure 2 In-situ ammonia and nitrate profiles with (a) /without air scouring (b) (dash line bar in starting of anaerobic #1/anoxic tanks indicates calculation based on the mixing ratio of PE and RAS or IRS and anaerobic effluent); ammonia and TIN losses in the anaerobic/anoxic tanks with (c)/without (d) air scouring

3.3 Batch test results

3.3.1 Partial denitrification results

Results for the partial denitrification batch tests are presented in Figure 3. The results for the three conditions investigated indicated that nitrate was rapidly consumed with very little nitrite formed. For

PE and RAS, which simulates the condition in the anaerobic tank, only approximately 3% of the nitrate present was converted to nitrite (Figure 3 a. Similar results were obtained with the effluent from anaerobic tank and the mixture of anaerobic effluent and IRS (Figures 3 b and c), with about 6% of the influent nitrate converted to nitrite. The maximum nitrite concentration was 0.4 mg-N/L in all cases, which is insufficient to account for the anammox activity in these tanks. Ammonia was also detected but remained essentially constant throughout the tests (not shown in Figure 3). These results suggest that partial denitrification was not a significant source of nitrite for anammox under these conditions.

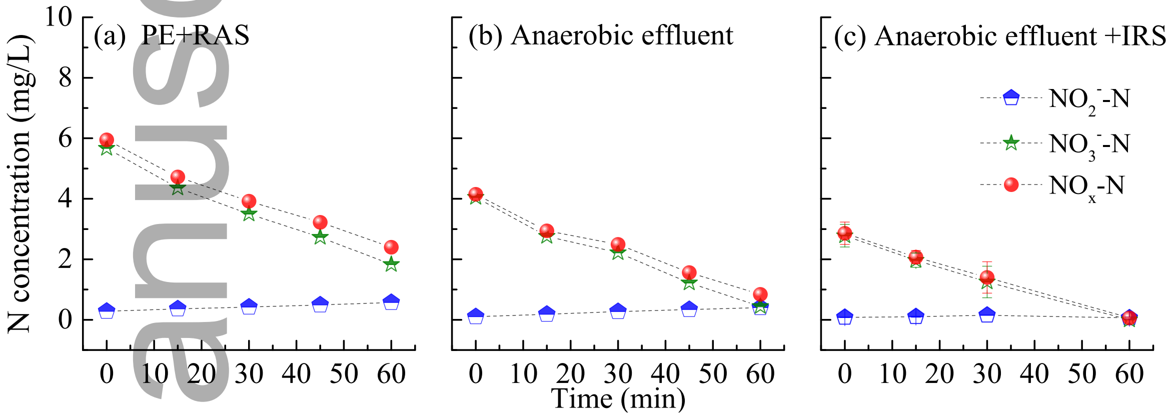

Figure 3 Partial denitrification activities under different scenarios (About 4 mg-N/L nitrate was dosed in batch tests (a) and (b) to make the denitrification trend more distinctive)

3.3.2 Partial denitrification coupled with anammox batch test results

In contrast to the partial denitrification batch tests, results reported immediately above, carriers containing anammox were added in these batch tests to introduce competitors with heterotrophic denitrifiers for nitrite that could be produced by partial denitrification. Nitrate was consumed rapidly in these batch tests, but ammonia concentration remained constant with carriers present (Figure 4 a), even when we elevated the nitrate and ammonia concentrations at the beginning of the test (Figure 4 b). No anammox activity was evident in these batch tests, in spite of the fact that anammox and ammonia were present, suggesting that the anammox reaction was limited by the lack of nitrite. Since nitrite could only be produced by partial denitrification, these results further indicate that partial denitrification is not occurring in this system.

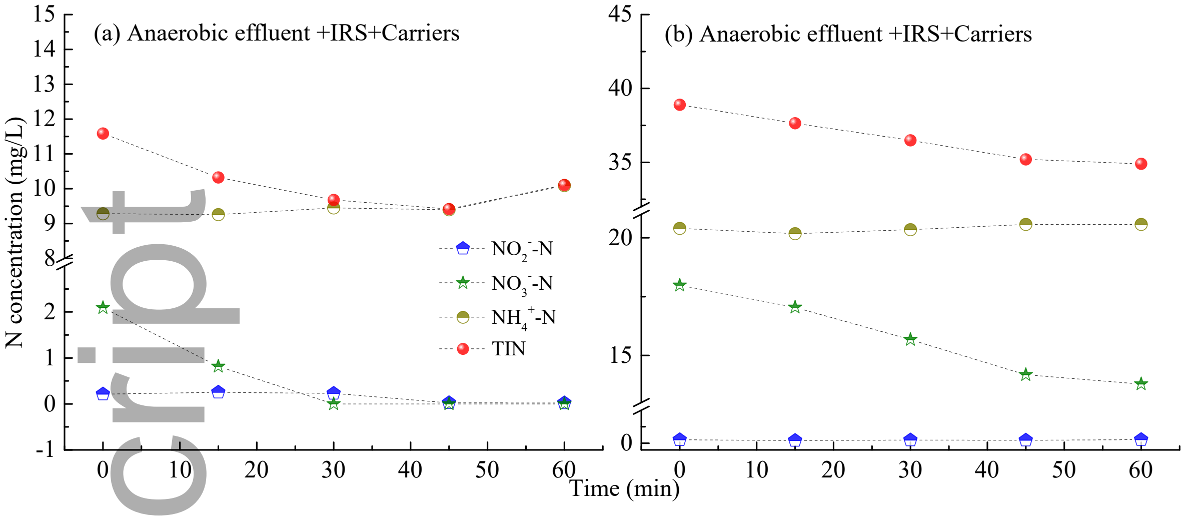

Figure 4 Batch tests to mimic the anoxic tank without air introduction. Nitrate and ammonia concentrations elevated for Figure (b).

3.3.3 Batch test results with aeration

These batch tests were conducted to investigate whether the presence of carriers and a modest amount of air can lead to apparent PN/A. The suspended solids and carriers were taken from the anoxic tank to mimic the situation there. When 60 mL/min air was introduced (batch test 1), the resulting DO ranged between 0.1 and 0.15 mg-O_2/L, the ammonia removal rate was 105.1 mg-N/(L·d), and nitrate was produced resulting in a nitrate generation rate of 74.5 mg-N/(L·d) (Figure 5). Total inorganic nitrogen removal rate was 30.5 mg-N/(L·d), indicating that PN/A and/or SND was occurring. When the air flow was reduced from 60 mL/min to 20 mL/min in batch test 2, DO drop to 0.05 mg-O_2/L and the ammonia removal rate decreased to 41.3 mg-N/(L·d), apparently due to the insufficient oxygen. Nitrate decreased (rather than was produced) at a rate of 20.6 mg-N/(L·d), while a higher nitrogen removal rate of 75.1 mg-N/(L·d) was observed. When the carriers were removed in batch test 3, the ammonia removal rate decreased to 21.8 mg-N/(L·d) and the nitrate concentration increased with a production rate of 22.2 mg-N/(L·d), resulting in almost no total inorganic nitrogen removal. Compared with batch test 2 with carriers, the reduced ammonia and total inorganic nitrogen removal rate without carriers (batch test 3) can be attributed to two possible factors: (1) anammox activity and (2) denitrification activity created by the biofilm due to the limited oxygen transfer from bulk to the deeper side of biofilm and some carbon source available for denitrification absorbed on the biofilm.

Comparing the batch tests with 60 mL/min and with 20 mL/min, the amount of air obviously affects total inorganic nitrogen removal. Increased bulk DO concentration will accelerate AOB activity, and possibly NOB activity but can hinder heterotrophic and autotrophic (anammox) denitrification. Ammonia decreased in all batch tests, with the rate of decline higher at the higher aeration rate, even though the DO was as low as 0.05 mg-O_2/L. The suspended solids and carriers were taken from the full-scale reactor directly, so the batch test results represent the microbial community and organic carbon situation present there. Although the air in the batch tests does not directly simulate air scouring in the full-scale reactor, the batch reactor results demonstrate that robust nitrification/nitration can occur even when DO concentrations are quite low and organic carbon is present (Third *et al.* 2001). When the air flow increased from 20 mL/min to 60 mL/min with carriers, the nitrate concentration increased over time. This is in line with the conversion at the full scale test, when air scouring was applied there was production of nitrate/nitrite and decrease in ammonium.

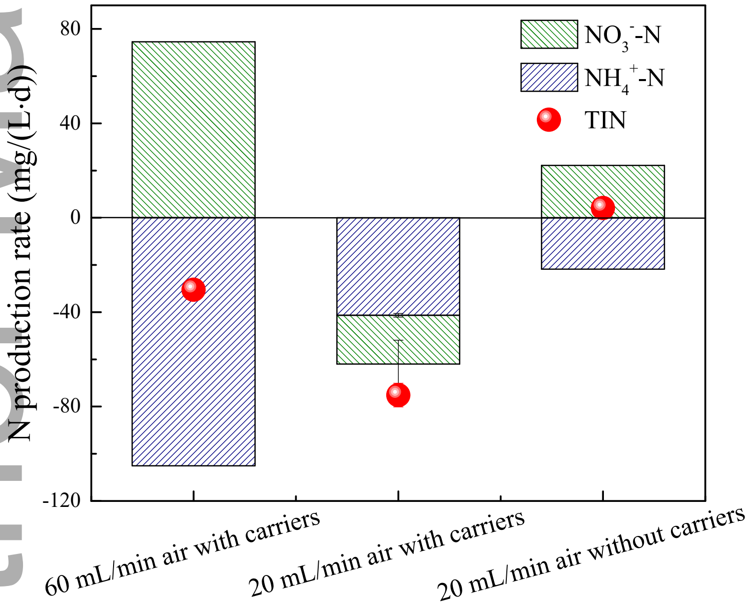

Figure 5 Nitrogen production rate with a limited amount of air with/without carriers

4 Discussion

The results presented here consistently demonstrate that the presence of continuous air scouring required to prevent carriers blockage of the screens in the anaerobic/anoxic tanks (Yuan *et al.*, 2020) resulted in nitritation that largely accounts for nitrogen removal by annamox activity in the process.

The results supporting this conclusion include DO and ORP profiles in the anaerobic/anoxic tanks, calculation of the likely amount of oxygen transferred due to the air scour, in-situ air on/off tests, and various batch tests. This result conflicts with that of Li *et al.* (2019) who apparently were either unaware of the air scouring occurring or judged without supporting data that nitritation resulting from the air scour was insufficient to account for nitrite production required by the anammox present on the carriers in the anoxic and anaerobic tanks. As demonstrated by the results presented here, AOB activity can be present even when the DO concentration is quite low and in the present of organic carbon.

Firstly, it is well established that nitritation, nitrification, and anammox activity can occur effectively under low DO aerated anoxic conditions, even if DO is not detectable (Third *et al.* 2001, Littleton *et al.* 2003, Park and Noguera 2004, Fitzgerald *et al.* 2015, Laureni *et al.* 2016, Keene *et al.* 2017). This can make nitrite available for anammox. Third et al. (2001) operated a 2-L CANON reactor with very limited airflow (7.9 mL/min air) under a non-limiting ammonia supply, and the results showed stable ammonia and TIN removal even though only 0~0.24 mg-O_2/L DO was detected. Littleton et al. (2003) also observed ammonia and TIN loss in a batch test when accidental air leakage happened, indicating that even an air amount, like air leakage, can cause nitrification/nitritation, and that the produced nitrate or nitrite was consumed immediately. Roots *et al.* (2020) operated a single-stage anammox granular sludge reactor at DO of 0.2 mg-O_2/L to treat municipal wastewater with low ammonia concentration, and robust nitration was achieved. As one of the well-known NOB genus, *Nitrospira* has been widely reported as a complete aerobic ammonia-oxidizing bacteria (comammox) (Gonzalez-Martinez *et al.* 2016, Lawson and Lücker 2018), and some *Nitrospira*, such as *Nitrospira inopinata,* have a relatively low affinity for nitrite, making the symbiotic association with anammox bacteria possible. Sustained nitrogen loss has been observed in a combination of Comammox *Nitrospira* and anammox bacteria, especially in low ammonia condition and sometimes in low DO condition (Roots *et al.* 2019, Gottshall *et al.* 2020, Shao and Wu 2021). The dominant NOB genus in this case, as shown in the reference (Yuan *et al.* 2020), was *Nitrospira*, and its relative abundance in the suspended solids was 2 times higher in summer and 7 times higher in winter than the biofilm. It is possible that *Nitrospira* in the suspended solids in this case acts as Comammox and cooperated with anammox bacteria attached on the carriers. Cooperation between Comammox and anammox bacteria were found both in a sequencing batch reactor for sludge digester liquor treatment (Wu *et al.* 2019) and a continuous reactor for low-strength ammonia-containing wastewater treatment (Shao and Wu 2021). van Kessel *et al.* (2015) reported that Comammox bacteria is capable to produce nitrite and cooperate with anammox for nitrogen removal. The consortium relationship between Comammox and anammox bacteria in this study needs further investigation. Along with the known AOB, such as *Nitrospira* and *Nitrosomonas* which can perform nitrification in low DO conditions, some unknown AOB have been found capable of autotrophic and heterotrophic ammonia utilization, such as *Pseudomonas*, *Xanthomonadaceae*, *Rhodococcus*, and *Sphingomonas* (Fitzgerald *et al.* 2015). Current knowledge about aerobic nitrification is incomplete and more work is needed to unravel the microbiological puzzles of nitrification at low DO conditions. Abundance evidence shows, however that nitrification/nitration can occur at very low DO conditions, indicating that it cannot be ignored in this instance.

Secondly, carbon oxidation happens with ammonia oxidation simultaneously, as has been well demonstrated in many full-scale facilities. In fact, this is the basic operating principle for SND. Daigger and Littleton (2000) studied several full-scale municipal oxidation ditch wastewater treatment plants, significant nitrification occurs in the first channel, as indicated by decreased soluble total Kjeldahl nitrogen and ammonia concentrations, the nitrite or nitrate concentration do not increase appreciably. This suggests that nitrification and denitrification are occurring simultaneously in the first channel. In the first channel, influent containing sufficient organic carbon mixed with mixed liquor in a closed-loop section of the bioreactor, air was input in the first channel. Ammonia depletion was observed in the first channel although the DO was non-detected, while nitrate or nitrite did not increase correspondingly, resulting in total inorganic nitrogen loss in the first channel. Three mechanisms can be responsible for SND (Daigger and Littleton 2014): (1) the macro-anoxic environment, (2) the micro-anoxic environment and (3) aerobic denitrification processes. Keene *et al.* (2017) operated a pilot-scale anaerobic/anoxic/aerobic reactor treating the primary effluent from local WWTP, and stepwise reduced the DO in the aerobic tank to 0.23 mg-O_2/L over a 16-month period, the reactor achieved excellent nutrient removal efficiency and SND in the low-DO tanks contributed more than 40% to total nitrogen removal. In this study, the macro-anoxic environment created by local oxygen transfer by operation of the air scour system contributed to nitrogen loss in the anaerobic and anoxic tanks. The biofilm attached on the suspended carriers also contributes to the micro-anoxic environment. The aerobic denitrification pathway was not tested, and can be a future research focus. The macro- and micro-anoxic environments make SND possible in the anaerobic/anoxic tanks, indicating that ammonia and carbon oxidation can occur simultaneously in this case.

Anammox activity is known to be present on the carriers in the anaerobic and anoxic zones, and ammonia decrease was observed in the full-scale in-situ profile testing when air scour was on. Ammonia did not decrease after air scour was turned off, and showed a slight increase possibly due to organic nitrogen conversion or microbial decay. This suggests that oxygen provided by aeration resulted in nitritation to provide the nitrite needed for anammox activity, rather than partial denitrification. Turning off air scour would stop nitritation but not partial denitrification. The batch denitrification results also indicated very little nitrite production, now was ammonia reduction observed even in the presence of carriers where the anammox activity is known to reside. The results of in-situ and batch tests in this study showed that air scour in the anaerobic/anoxic tanks is critical to ammonia and TIN removal and provided nitrite by the nitritation process for anammox activity. Partial denitrification may occur, but it does not play an important role in this case.

The air scouring in the anaerobic and anoxic tanks did not impede the denitrification process and, in fact, improved nitrogen removal instead, as demonstrated by the in-situ results. Without air scour, only the nitrate recycled back to the anaerobic tank by RAS and anoxic tank by IRS were denitrified to N_2. Air scour provided oxygen needed to allow conversion of some ammonia to nitrite or nitrate, allowing increased denitrification, denitritation and anammox in the anaerobic and anoxic tanks, resulting in increased total inorganic nitrogen removal. The air amount in the anaerobic/anoxic could be further optimized to intensify the SND and PN/A processes, resulting in improved system nitrogen removal efficiency. In fact, if nitrogen removal by SND and PN/A in the anaerobic/anoxic tanks is increased by introducing additional air, the ratio of IRS could be reduced accordingly since less ammonia would enter the aerobic tank and less nitrate would be present in the effluent of the aerobic tank. Operation cost could be reduced accordingly by lowering air in the aerobic tank and the IRS ratio.

In addition to nitrite provided by the nitritation pathway, the carriers for the retention of slow-growing anammox bacteria are also essential for achieving mainstream PN/A. This hybrid system combined suspended growth AOB with attached growth anammox bacteria retained in a zone with modest aeration to achieve PN/A and supplement conventional heterotrophic denitrification. Understanding the relative contributions of these factors is important, not only for this case but also for its wider application. Only when we have a better understanding of the relative importance of these factors can this knowledge be extended to other reactor designs and operation. Note, of course, that, compared with the partial denitrification/anammox process, PN/A is more energy/carbon efficient.

Anammox bacteria have been widely reported to be synergetic with heterotrophic bacteria, especially with the denitrifiers, making it possible for anammox growth when some carbon sources are present (Mulder *et al.* 1995, Winkler *et al.* 2011, Ge *et al.* 2018). Evidence showed that anammox bacteria are robust and act as "nitrite-sink" when competing for nitrite with denitrifiers at relatively low C/N condition (Winkler *et al.* 2012, Winkler *et al.* 2012, Laureni *et al.* 2019, Roots *et al.* 2020). Although the boundary conditions for nitrite competition between anammox bacteria and denitrifiers are still unclear, the cooperation of anammox bacteria and denitrifiers results in stable and excellent nitrogen removal efficiency in spite of influent variations, as evidence by performance of Xi'an 4th Wastewater Plant. After all, stable operation and good effluent quality meeting discharge standard are the ultimate goals for WWTP.

Overall, the effect of minor air scouring in the anaerobic and anoxic tanks should not be neglected, and it can be simply evaluated by calculating the oxygen transfer rate and measuring the variation of DO and ORP, as shown in Section 3.1. If the calculated oxygen transfer by air addition are higher or in the same order as the possible nitrogen removal caused by anammox, and the variations of DO and ORP also indicate the possibility of nitritation or nitrification, then the possibility of PN/A can be validated further by batch tests and in-situ air on/off tests. The in-situ air on/off test results provide the most direct evidence of whether air scouring in the anaerobic and anoxic tanks affects nitrogen removal. The lesson we can learn from this case is that any details should not be neglected when making judgments.

5 Conclusions

The effect of the minimal aeration introduced by air scouring for biofilm carrier suspension in the anaerobic and anoxic tanks cannot be neglected and was enough to induce simultaneous nitritation and anammox in a full-scale WWTP at moderate temperature. This biofilm carriers-suspended solids hybrid system shows that mainstream PN/A without sidestream bioaugmentation under normal northern climate temperatures and low ammonia substrate concentration is possible. This is mainly enabled by allowing for biofilm growth in a low DO environment. Furthermore, nitritation and anammox under mainstream conditions are robust and can function well with conventional nitrification and denitrification process. Aerated anoxic condition stabilized the mainstream anammox process. Simultaneous nitrification/denitrification, nitritation/denitritation and anammox reaction can occur in the IFAS system depending on the substrates of dissolved oxygen, ammonium and organic carbon concentration. Batch denitrification tests in the anaerobic tank and full scale text in the anoxic test indicated that partial denitrification did not play a significant role in anammox nitrogen removal. Optimizing the aeration and amount of biofilm carrier can be used to further improve the autotrophic nitrogen removal process allowing COD to be diverted to anaerobic digestion enabling an energy neutral or energy positive treatment plant operation.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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