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Photo-oxygenation for nitritation and the effect of dissolved oxygen concentrations on anaerobic ammonium oxidation

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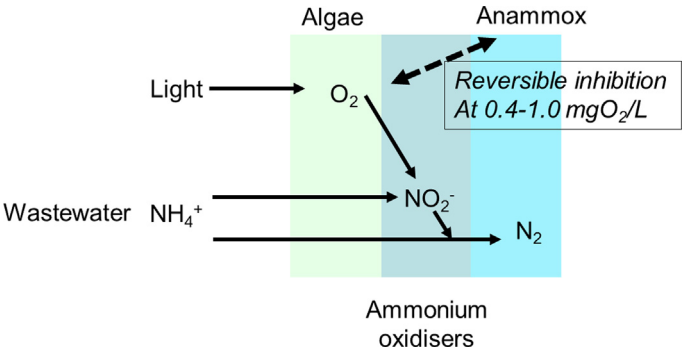
HIGHLIGHTS

- Algae, nitrifying and anammox bacteria were cultured in wastewater in one photo-bioreactor.
- Anammox bacteria survived photosynthetic oxygen production resulting in DO < 0.2 mg/L.
- Reversible inhibition of anammox was observed at bulk DO values > 0.4–1.0 mg/L.
- Recovery time for anammox bacteria increased with increasing DO concentrations.

GRAPHICAL ABSTRACT



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ABSTRACT

Removal of nitrogen from wastewater without using electricity consuming aerators was previously observed in photo-bioreactors with a mixed algal-bacterial biomass. Algammox is the particular process based on algae, ammonium oxidizing organisms and anammox bacteria. In this research the activity of anammox bacteria in such an oxygen-producing environment was tested, as well as the effect of short-duration increase in dissolved oxygen (DO) to values potentially inhibiting anammox activity. Sequencing batch photo-bioreactors were fed with settled domestic wastewater enriched with ammonium (200 mg NH_4^+-N/L) and exposed to light within the photosynthetic active range with intensity of about 500 μmol/m^2·s. Each cycle consisted of 12 h illumination and 12 h darkness. A well-settling biomass (10 days solids retention time) developed that carried out nitritation, nitrification and anammox. Ammonium removal rate during the light period was 4.5 mg N-NH_4^+/L·h, equal to 858 mg N-NH_4^+/m^2·h or 477 mg N-NH_4^+/(mol photons). When the reactors were aerated for 3 h to temporarily increase the DO, anammox was inhibited at bulk DO values larger than 0.4–1.0 mg/L. For almost oxygen saturated conditions, recovery time was about 9 days. Algammox photo-bioreactors are therefore able to overcome short periods of oxygen stress, provided they occur only occasionally.

1. Introduction

1.1. N removal in photo-bioreactors by nitritation and anammox

Nitrogen removal from wastewaters in photo-bioreactors by mixed cultures of algae, nitrifying bacteria and anammox bacteria is a way to

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reduce aeration and energy costs of wastewater treatment systems (Manser et al., 2016). The overall energy use in municipal treatment plants varies between 0.30 and 0.78 kWh m^{-3} (Nordlander et al., 2017), of which 45–75% may be due to the mechanised aeration process (Stenstrom and Rosso, 2008). In particular nitrogen removal in conventional activated sludge systems requires high inputs of energy for aeration. Therefore many researchers have investigated high rate algae ponds or photo-bioreactors for nitrogen uptake by rapidly growing algae, instead of using nitrification for ammonium removal. An alternative approach is using algae as producers of oxygen that subsequently can be used for full nitrification (Karya et al., 2013) or nitritation (Wang et al., 2015). In both cases was demonstrated that nitrogen removal through denitrification by heterotrophs in the mixed culture is also possible. However, the nitritation option seems more promising since it opens up the possibility for short-cut nitrogen removal by nitritation and denitritation. That short-cut significantly reduces the need for oxygen for nitrification and degradable organic matter for denitrification (Wang et al., 2015). However, the concentration of degradable organic matter was found to be insufficient in photo-bioreactors fed with wastewater that was anaerobically pre-treated. Moreover, degradable organic matter even further decreased during illuminated and aerobic phases in the photobioreactor (Wang et al., 2015). Therefore, Manser et al. (2016) tested the activity of anammox bacteria in a sequencing batch photo-bioreactor containing microalgae and ammonium oxidizing bacteria (Algammox system). Anammox bacteria do not require an organic substrate for nitrogen removal. The results showed that the inclusion of anammox increased nitrogen removal, most likely due to anammox activity (Manser et al., 2016), but the long-term performance and effects of operational or environmental conditions are not yet known. Optimal performance will require balancing the proportions of algae, nitrifiers and anammox to maintain a maximum flux for nitrogen conversion.

1.2. Effect of dissolved oxygen concentrations on anammox activity

If algae produce too much oxygen in relation to oxygen consumption by nitrifiers, then oxygen may reach inhibitory levels for anammox bacteria. Szatkowska et al. (2004) achieved the highest nitrogen removal rates by anammox bacteria at 0.2–0.4 mg O_2/L while treating reject water of anaerobically digested waste activated sludge. It was also shown that 0.8 mg O_2/L oxygen inhibited the efficiency of anammox bacteria in a reversible manner, but that at 1.4 mg O_2/L the inhibition became irreversible (Egli et al., 2001). However, Zekker et al. (2014) found that a high oxygen concentration in the bulk liquid of 3.2 ± 1.7 mg O_2/L did not inhibit anammox bacteria that were growing in aggregates (flocculent biomass). The ammonium oxidizing organisms (AOO) also present in the aggregates, probably caused steep oxygen gradients within the aggregates. Anammox bacteria inside granules or flocculent aggregates may therefore be protected from inhibitory levels of oxygen by oxygen scavenging AOO or heterotrophs. This is applied in full scale reactors (Remy et al., 2016) where so-called one-step anammox granules carry out nitritation in the outer layers of the granule, while anammox bacteria are active in the inner parts, not hindered by the oxygen present at low concentrations (<1 mg/L) in the bulk liquid. These observations predict the possibility of co-culturing algae, AOO and anammox bacteria.

1.3. Relation between light incidence, dark algae respiration and oxygen availability for nitrifiers

Oxygen concentrations are also affected by the ratio between algae in the reactor that photosynthesise and respire in the illuminated zone, versus the algae in the dark zone that only carry out respiration. Arashiro et al. (2017) tested the effect of solids retention time (SRT) in a photo-bioreactor (perfectly mixed sequencing batch reactor (SBR)) fed with reject water from an anaerobic digester treating swine manure. The longer SRT (11 days) resulted in a higher solids concentration than the shorter SRT (7 days) and therefore a larger dark zone. When nitrification was completed, the DO in the latter reactor increased to higher DO values than in the reactor with longer SRT. The higher DO production at lower SRT resulted in slightly higher nitrification rates. It shows that in a mixed culture of mainly algae, nitrifiers and anammox bacteria, the biomass will also cause shading of algae and therefore limit the oxygen production. Assuming that inorganic carbon or other substrates will not be limiting for either of the micro-organisms, then the incidence light intensity and the relative dark portion of the reactor are expectedly the main determinants for the flux of nitrogen through the nitritation/anammox pathway. By selecting the SRT and biomass concentration, one may determine the net oxygen production by algae and therefore the oxygen availability for nitritation and the oxygen concentration in the reactor.

1.4. Variations in DO as expected in full scale photo-bioreactors

In practice it will not always be possible to balance the nitrogen load and the associated oxygen consumption with the oxygen production by algae over the duration of a day (Subashchandrabose et al., 2011). This may lead to occasional high DO values, especially when using natural sunlight. For instance during periods of low loading rates around solar noon, the net oxygen production will be high. Therefore the aim of this study was to investigate the possible inhibition of activity of anammox bacteria by occasional high dissolved oxygen concentrations in an Algammox sequencing batch photo- bioreactor. A better understanding of how to balance oxygen production and oxygen consumption and the effect of such an unbalance on anammox activity is required to optimise the mixed culture proposed in this work.

2. Materials and methods

2.1. Bioreactor setup

The laboratory experiments were carried out during a period of two months in two identical Photo Sequencing Batch Reactors (PSBR) (Schott Duran 5000 mL beakers). The open reactors were placed about 40 cm beneath a metal-halide lamp (HQIBT 400 w/D proE40). Light intensity (as Photosynthetic Active Radiation - PAR) was measured at the open water surface of the reactors with a light meter (LI1400 data Logger LI-COR) and was found to vary between 450 and 600 μmol PAR/m^2·s. Alternating illuminated and dark periods (Light Period (LP) and Dark Period (DP)) were 12 and 12 h, respectively (Fig. 1).

The reactors were inoculated with a mixed biomass that consisted of pure cultures (5 mL each of the following microalgae: *Chlorella sp., Scenedesmus quadricauda, Anabaena variabilis, Chlorococcus* sp. and *Spirulina sp.* Furthermore, granules (150 mL) were added from a one-step anammox reactor that was treating a mixture of wastewater from potato starch industry and a concentrated side-stream from a municipal sludge treatment plant (Oldenburg, Germany, Paques BV). Such granules contain AOO as well as anammox bacteria. The reported nitrogen removal rate achievable by these granules in the above-mentioned reactor was 1.0–2.5 kg N/m^3/day (Remy et al., 2016).

The reactors were fed with primary effluent (Harnaschpolder municipal wastewater treatment plant, Den Hoorn, the Netherlands) enriched with ammonium chloride up to a concentration of 200 mg NH_4^+-N/L. Inorganic carbon concentration was increased to 1.00 g HCO_3^-/L using sodium hydrogen bicarbonate ($NaHCO_3$).

Each PSBR was operated at 4.0 L active volume and had an open surface area of 0.021 m^2. The daily cycle started 6 min before the beginning of the LP with the addition of 2.0 L of influent to the 2.0 L of mixed liquor that remained from the previous cycle. After 12 h of LP and 11.75 h of DP the mixing was stopped to allow biomass settling for 2 min, after which 2.0 L of supernatant was withdrawn (Fig. 1). Influent and effluent were added and withdrawn by using Masterflex L/S pumps (Cole-Parmer, USA). Reactor mixing was by magnetic stirring bars and stirring plates

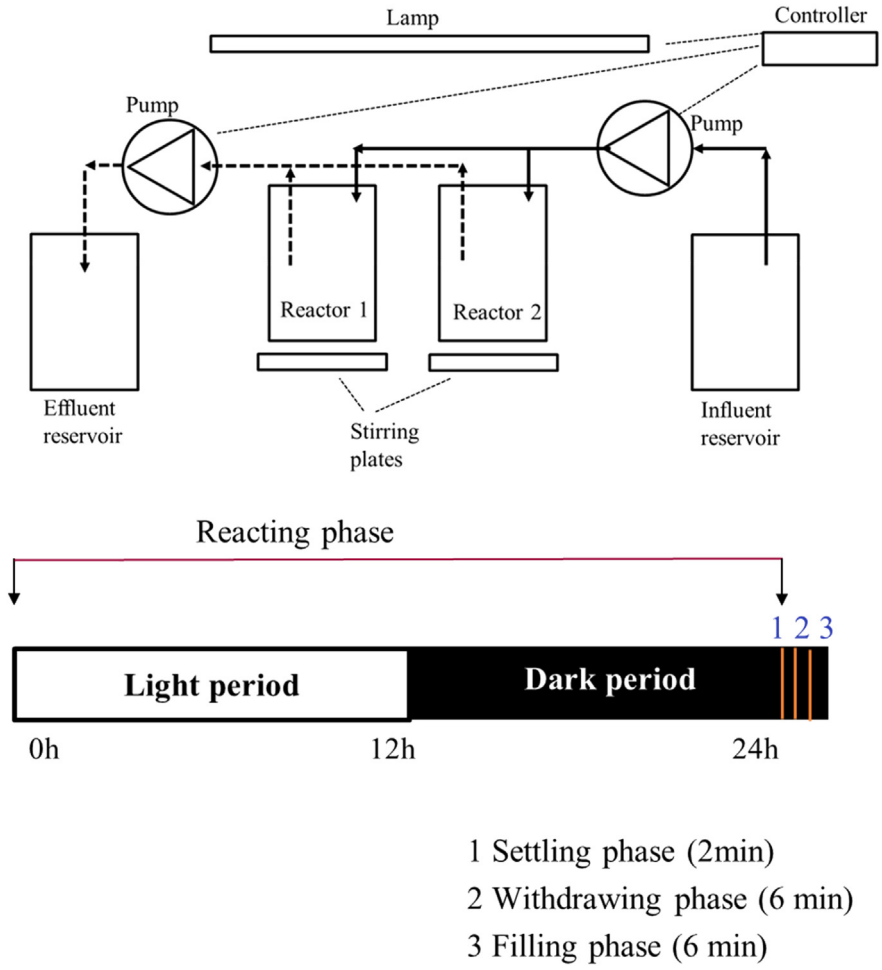

Fig. 1. The sequencing batch photo bioreactor set-up and the operational cycle of 24 h and its phases.

(Cimarec Komet Mono) at about 150 rpm. The lamp, pumps and stirrers were controlled by a GHL Profilux 3 Aquarium computer (GHL, Germany). The pH, DO, and temperature were read using WTW pH3310 and WTW Oxi 3310, respectively.

2.2. Bioreactor operation and in-situ activity tests

After inoculation with the mixture of algae and the 1-step anammox granules, the reactors were operated for 30 days to achieve stable operation. Subsequently, the performance of both reactors (operated as duplicates) was characterised by measuring profiles of temperature, DO, pH, NH_4^+, NO_2^- and NO_3^- along the LP and DP. The Total Suspended Solids (TSS) in the mixed reactor and the effluent were measured daily to determine the amount of mixed liquor to waste, in order to maintain an SRT of 10 days. Because the granules with anammox settled quickly, most of the wasted biomass were flocs of algae and suspended bacteria. Therefore the SRT of the anammox was most likely significantly longer than 10 days.

To test the activity of anammox bacteria two different experiments were carried out. In Activity test-1 extra nitrite was dosed to the reactor at the end of the light period until the concentration in the reactor was 30.3 ± 0.43 mg NO_2^--N/L, in order to study nitrite removal during the dark period.

2.3. Activity tests in side-experiments and control experiments

Activity test-2 was carried out with 200 mL of biomass harvested from one reactor (at the end of the dark period) in a covered (dark) and stirred beaker glass. Initial concentrations of NH_4^+, NO_2^- and NO_3^- were determined. After 2 h $NaNO_2$ was added to achieve about 30 mg NO_2^--N/L. The decrease in NO_2^- concentration was monitored and the nitrite spike was repeated if after 2 h the level was negligible. Concentrations of NH_4^+, NO_2^- and NO_3^- were determined for the 22 h duration of the experiment.

The objective of Activity test-3 was to determine activity of anammox bacteria in the effluent of the reactors, to estimate how much anammox activity was washing out. 200 mL of effluent was put in a mixed and covered (dark) beaker. Then, about 30 mg/L of NO_2^--N was added as $NaNO_2$. Every 2 h the concentrations of NH_4^+, NO_2^- and NO_3^- were determined.

A control-test-1 was done to exclude the possibility of nitrite removal by denitritation, instead of nitrite removal by anammox. Good conditions for denitritation were created by spiking 40 mg/L of sodium acetate to 200 mL of biomass from the reactor. Darkness ensured anoxic conditions. Initial concentrations of nitrite, nitrate and soluble Chemical Oxygen Demand (COD) were measured. Samples were collected every 2 h and analysed.

Control-test-2 was carried out to exclude other mechanisms than anammox for ammonium removal during the dark period in the reactor. For that purpose 200 mL biomass was harvested from the reactors just after the feeding and put in a covered (dark) beaker. Initial concentrations of NH_4^+, and NO_3^- were determined. NO_2^- was negligible in the sample from the reactor and the dark conditions ensured anoxic conditions and prevention of ammonium oxidation.

2.4. Temporarily increase in DO

Subsequently the operation of the test reactor was modified to test the effects of temporarily increase in DO, while the control reactor continued to be operated as before. The DO in the test reactor was increased by an aquarium aerator, from 5 h until 8 h into the LP. The aeration was regulated to achieve the following DO concentrations: 0.35–0.4, 0.8–1.0, 1.8–2.1, 3.8–4.0 and 6.8–7.1 mg O_2/L. The day of the aeration period was followed by a number of days without any aeration to test for recovery of anammox activity.

2.5. Analytical methods

Samples were taken manually using a 50 mL syringe and filtered using a glass fiber filter GF/C (pore size of 1.2 μm) and immediately analysed or stored at 4 °C for maximum 12 h. Ammonium nitrogen was analysed using Dutch standard method NEN 6472. All other analyses were according to APHA (2005). Both nitrite and nitrate were measured spectrophotometrically (UV/VIS Lambda 365 Perkin Elmer) in a 1 cm glass quarts cuvette, at 543 nm and 324 nm, respectively.

3. Results and discussion

3.1. Stabilization of reactors and tests for anammox activity

After 30 days, the patterns of DO, pH and nitrogen species during the daily cycles were very similar in both reactors. The DO was <0.2 mg/L, even during the light period (Fig. 2) and a further decrease during the dark period showed that the effect of oxygen diffusion from the air into the open reactors was negligible. The temperature slightly increased during the day due to heat from the lamp. The pH decreased during the light period, showing that the effect of nitritation on pH (i.e. a decrease) was more pronounced than that of photosynthesis (i.e. an increase). During the dark period the pH increased, possibly due to the stoichiometry of the anammoxor denitritation reactions (Manser et al., 2016). During the light period ammonium concentration decreased at an average rate of 4.5 mg N-NH_4^+/L·h and during the dark period the decrease continued at an average rate of 1.0 mg N-NH_4^+/L·h. Both nitrite and nitrate concentrations increased during the light period, indicating activity of both AOO and nitrite oxidizing organisms (NOO). The sum of the increase in nitrite and nitrate concentrations was 28 mg N/L, while the decrease in ammonium concentration was 54 mg N-NH_4^+/L. Part of this difference could be explained by ammonia volatilisation, simultaneous nitrification/denitrification, anammox activity and ammonium removal by algae uptake. For the latter process Karya et al. (2013) found 1.2 mg N-NH_4^+/L·h under similar conditions. During the dark period nitrite was removed down to negligible concentrations while the nitrate concentration did not decrease. This pattern could be explained by anammox activity during the dark period, but also by denitritation of nitrite. The latter explanation is less likely, as denitrification of nitrate did not occur.

3.2. Tests for anammox activity

Activity test-1 showed (Fig. 3) that an additional spike of nitrite at the start of the dark period, was completely removed. The most likely explanation for this is activity of anammox bacteria, because the nitrate concentration increased during the activity test, whereas the decrease in ammonium concentration was significantly higher than the ammonium removal shown in Fig. 2. Several spikes of nitrite to a batch test with biomass from the reactor (Activity test −2; Fig. 4) were removed at a rate of 8–15 mg NO_2^--N/L·h. Nitrification is not a possible explanation, as the DO was negligible and the batch was covered (no photosynthesis). The ammonium removal rate over the first 12 h was 9.7 mg N-NH_4^+/L·h, significantly larger than observed when no nitrite was added (Fig. 2; 4.5 mg N-NH_4^+/L·h). Meanwhile, the increase in nitrate,

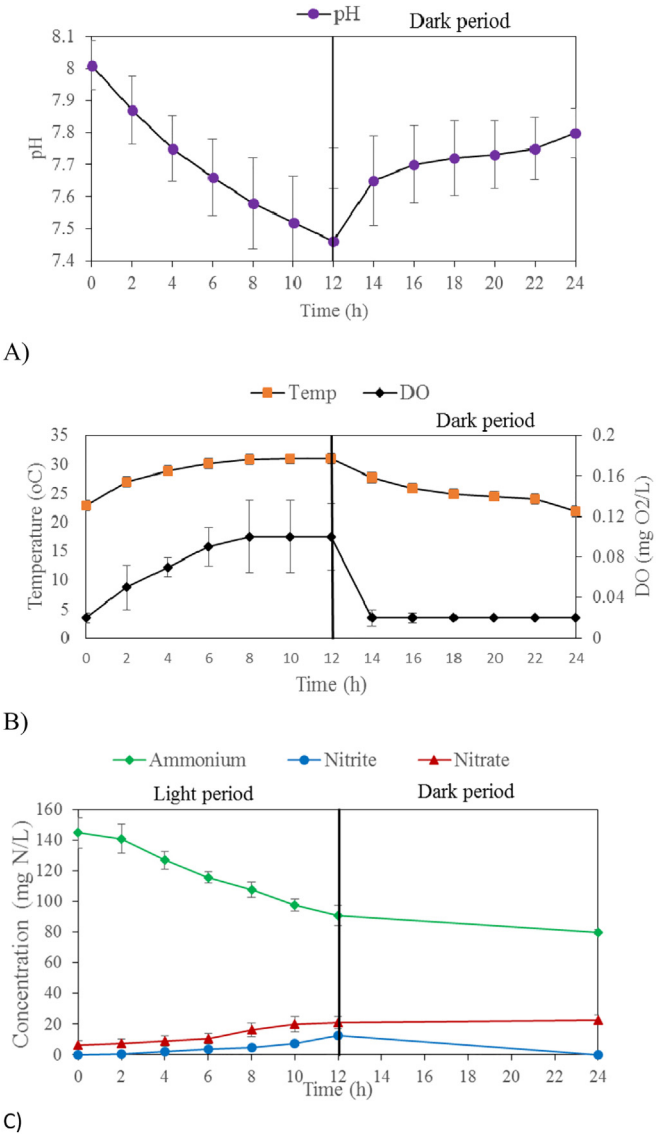

Fig. 2. Average profiles of pH (A), DO and temperature (B), and nitrogen species (C) after stabilization of the reactors. Samples were taken on three consecutive days, after 50 days of reactor operation.

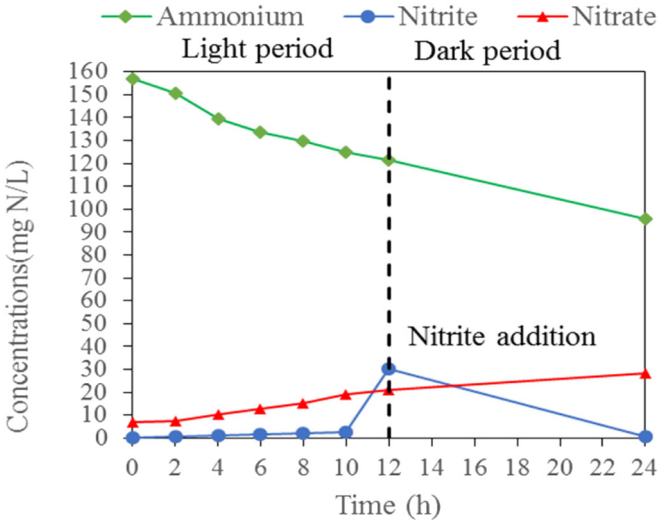

Fig. 3. Variation of N species in the photo-bioreactor when nitrites were added at the start of the dark period (Activity test-1) to test for anammox activity.

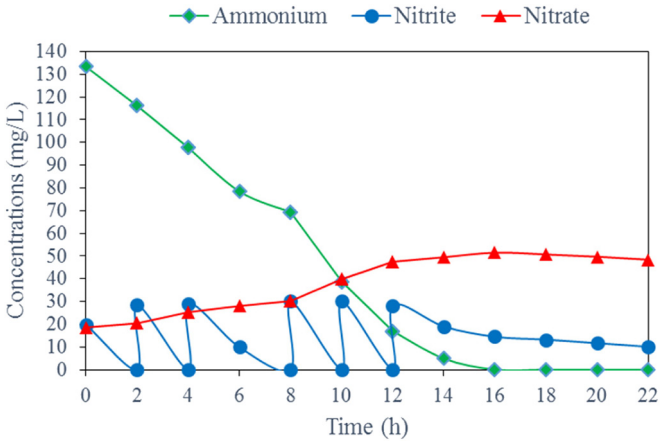

Fig. 4. Nitrite consumption by biomass that was harvested from the reactor and transferred to a dark and anoxic batch, with repeated additions of nitrite (Activity test-2) to test for anammox activity.

in the absence of nitrification, also pointed at anammox activity. The measured nitrite consumed over the first 14 h of the experiment (147 mg NO_2^--N/L), corresponds to a theoretical conversion of 113 mg/L of NH_4^+-N (stoichiometry according to van de Graaf et al., 1996), whereas the measured value was 129 mg/L of NH_4^+-N. This indicates that anammox could explain 88% of the observed ammonium removal. Further evidence of activity of anammox bacteria comes from the cessation of nitrite removal after the ammonium concentration had decreased to values below the detection limit (at 14–16 h) (Fig. 4).

The activity of anammox bacteria under dark conditions does not prove anammox activity for the light phase in the reactor. However, the low DO (<0.2) is unlikely to be inhibitory for anammox (Zekker et al. (2014), although locally higher oxygen concentrations could prevail in algae-aggregates. A mass balance for nitrogen species over the light phase (Fig. 2C), shows an ammonium decrease of 53.8 mg N, while nitrite increased by 8.0 mg N and nitrate by 17.4 mg N. Since volatilisation can be considered negligible (Karya et al., 2013), 28.4 mg NH_4^+-N/L is taken up by algae or converted by anammox. Ammonium uptake for light-limited growth of algae can be estimated assuming the maximum quantum yield of 0.125 mol O_2/mol photons, which gives for the applied light intensities 7.2 $gO_2/m^2/h$, or taking into account the reactor surface this gives 0.151 gO_2/h of maximum oxygen production through photosynthesis. The maximum N uptake by algae based on stoichiometry of photosynthesis (Karya et al., 2013) gives an N uptake of $0.151 * (1/32 * 16/118 * 14) = 8.95$ mg N/h, or 2.24 mg N/L·h or 27 mg N/L·12 h. So only if the maximum quantum yield is assumed, then algae growth could explain the ammonium mass balance. Therefore it is likely they also during the light phase there was some anammox activity.

3.3. Activity tests in side-experiments and control experiments

The activity-test-3 with effluent showed the same pattern (data not shown) as observed in the previous experiments; under anoxic conditions the ammonium and nitrite concentrations decreased, while the nitrate concentration increased. When all nitrogen species were supplied in excess, ammonium removal in the effluent was 1.5 mg NH_4^+-N/L·h, which is 15.4% of the rate observed in the mixed liquor, also under non-limiting conditions (Fig. 4; 9.7 mg N-NH_4^+/L·h). This shows that the effluent may remove about 8% of the anammox capacity per cycle, which means that anammox bacteria growth rate should be at least 0.0033 h^{-1} to maintain the anammox capacity in the reactor.

The control-test-1 showed that the biomass was not able to remove significant amounts of nitrite under anoxic conditions and in the presence of an easily biodegradable substrate (acetate). The measured NO_2^--N concentration decreased only from 12.5 to 11.3 mg NO_2^--N/L in 8 h (Fig. 5A). This shows that it is unlikely that canonical denitritation significantly contributed to nitrite removal observed in the reactors.

The control-test-2 was done to exclude other mechanisms than anammox for ammonium removal during dark and anoxic periods in the reactor. It showed that under such conditions and in the presence of ammonium and nitrate (but no nitrite) ammonium removal did not occur (Fig. 5B).

3.4. Photo-oxygenation as the limiting factor for ammonium removal by a mixed culture of algae, AOO and anammox bacteria

Removal of nitrogen from wastewater in photo-bioreactors through algae growth and combined nitritation-denitritation is limited by the efficient use of photo-synthetically generated oxygen. The oxygen production by algae growth in photo-bioreactors treating nutrient rich wastewater is in most cases light-limited, as anaerobic effluents contain high concentrations of inorganic carbon and macro- and micro-nutrients. Under such conditions, where the photosynthetic yield of algae per photon cannot be easily improved, it is important to benefit from the generated oxygen by using it for nitritation, with subsequent denitritation (Karya et al., 2013; Wang et al., 2015). Similarly, the use of oxygen for purposes that do not contribute to nitrogen removal, such as algae respiration in the dark zones of the reactor, should be prevented as much as possible by not applying too high biomass

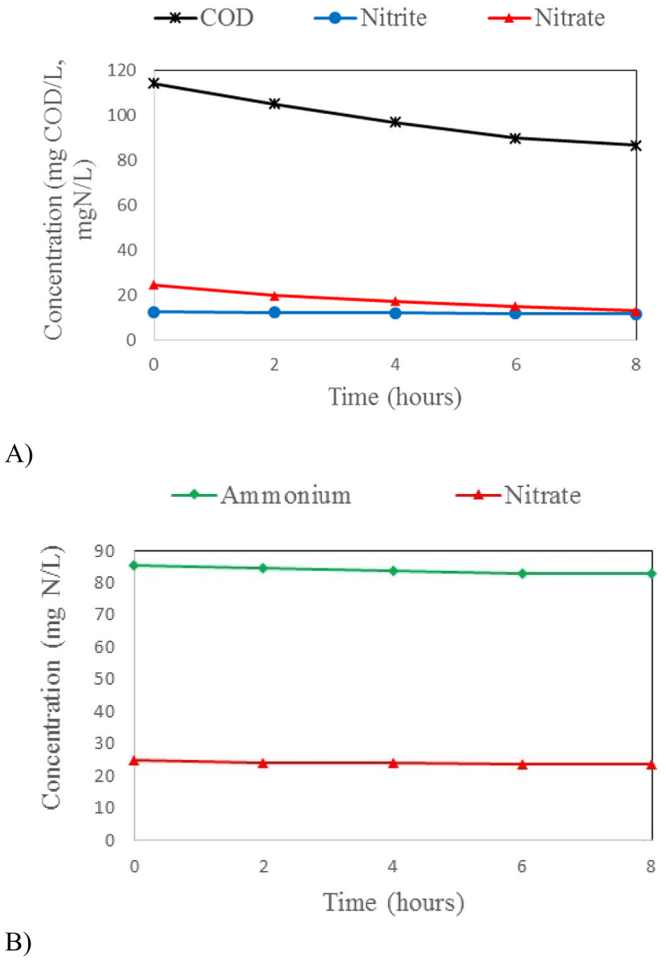

Fig. 5. Profiles of nitrogen species for Control-test-1 (A) and Control-test-2 (B). Control-test-1 was to exclude the possibility of nitrite removal by denitritation, instead of nitrite removal by anammox. Good conditions for denitritation were created by spiking 40 mg/L of sodium acetate to 200 mL of biomass from the reactor. Darkness ensured anoxic conditions. Control-test-2 determined the potential ammonium removal by other mechanisms than anammox by incubating biomass harvested from the photo-bioreactor under dark and anoxic conditions without nitrite.

concentrations or too high SRT (Arashiro et al., 2017). Nitrogen removal efficiency could be further improved by replacing nitritation-denitritation by nitritation-anammox (Manser et al., 2016). Also in a nitritation-anammox photo-bioreactor, the overall conversion of ammonium is limited by the oxygen production, though during the light period some nitrite may temporarily accumulate due to aerobic ammonium oxidation rates being higher than ammonium and nitrite conversion by anammox (Fig. 2C).

The average incidence light intensity applied at reactor surface in this research was 500 μmol photons/m^2·s, but as the reflection from the water surface was not measured, it is difficult to estimate the maximum achievable oxygen production based on theoretical quantum yields. Nevertheless, assuming the theoretical estimated quantum yield for algae growing on ammonium (instead of nitrate) at low light intensities as 0.125 mol O_2/mol photons (Iluz and Dubinsky, 2013), then maximum oxygen generation would be $6.25 * 10^{-5}$ mol O_2/m^2·s or 7.20 gO_2/m^2·h. This oxygen would be used for heterotrophic oxidation of organics, algae respiration, ammonium oxidation and nitrite oxidation, and the amounts can be calculated as follows. Oxygen consumption for heterotrophic oxidation of COD was not more than the observed maximum COD removal, which was 30 mg COD/L (data not shown), equivalent to 1.25 mg O_2/L·h, or 0.24 g O_2/m^2·h (3.3% of maximum oxygen production). Typical algae respiration is reported as 0.09 g O_2/g COD-biomass·day (Wolf et al., 2007). As the average TSS concentration in the reactors was 1.4 g TSS/L, the algae respiration would be 1.14 gO_2/m^2·h[1], or 16% of the maximum oxygen production. Furthermore, we will assume that 85% of the observed ammonium removal (85% * 4.5 mg N-NH_4^+/L·h) occurred via nitritation (Karya et al., 2013) and denitritation is assumed as negligible (see control-test-1). In that case the oxygen requirement for nitritation was 49 mg O_2/h or 2.33 g O_2/m^2·h (based on stoichiometry of 3.2 mgO_2/mg N-NH_4^+), which is 32% of maximum oxygen production. Finally, the maximum oxygen consumption by nitrite oxidation can be derived from the maximum increase in nitrate concentration during the LP (15 mg/L N-NO_3^-; Fig. 2C) as 1.4 mg O_2/L·h or 0.27 g O_2/m^2·h, which is 3.8% of maximum oxygen production. Making the assumption about denitritation, these processes together account for 55% of the maximum oxygen production, which would mean an effective quantum yield of 0.068 mol O_2/mol photon was achieved. This value is higher than observed by Boelee et al. (2011), who found 0.012–0.043 mol O_2/mol photons.

Reversing on the denitritation assumption, and assuming now that bacterial ammonium removal was completely performed by a balanced AOO-anammox biomass (no net nitrite production), then the ammonium removal is (85% * 4.5 mg N-NH_4^+/L·h * 4 L) 15.3 mg N-NH_4^+/h, which is according to the stoichiometry (1 mol ammonium converted by AOO into nitrite allows another 0.74 mol of ammonium to be converted by anammox bacteria; Manser et al., 2016) equal to 8.8 mg N-NH_4^+/h by nitritation and 6.5 mg N-NH_4^+/h by anammox. In this case the oxygen required for the nitritation is 8.8 mg N-NH_4^+/h * 3.2 mg O_2/-mg N-NH_4^+ = 28.2 mg O_2/h or 1.34 g O_2/m^2·h (or only 18.6% of theoretical maximum). The sum of the oxygen consuming processes is now only 42% of maximum oxygen production. Therefore, inclusion of anammox and an optimum balance between AOO and anammox bacteria could reduce overall oxygen requirements by 24% and therefore would result in a photobioreactor with significantly less surface area requirements. The effective quantum yield for this research would then reduce to 0.053 mol O_2/mol photons, which is close to the range as observed by Boulee et al. (2011).

From a different perspective and according to the stoichiometry, the maximum amount of ammonium removal by AOO-anammox per mol of oxygen is 74% higher than for nitritation-denitritation. Therefore one may conclude that including anammox as a nitrogen conversion mechanism in a mixed culture of AOO and algae results in up to 74% more ammonium removal per photon as compared to a system without anammox.

[1] Algae respiration is 0.09 g O_2/gCOD·day * 1.42 gCOD/gVSS * 0.8 gVSS/gTSS = 0.10 gO_2/gTSS·day = 4.26 mgO_2/gTSS.h
4.26 mg O_2/gTSS·h * 1.4 gTSS/L * 4 L * (1/0.021) m^{-2} = 1.14 gO_2/m^2/h

Table 1
The ammonium and total nitrogen removal, during the day that aeration took place, in activity-test-2 for different DO concentrations (AR = ammonium removal, LP = light period, DP = dark period).

DO (mgO_2/L)	0.1	0.1–0.25	0.35–0.4	0.8–1.0	1.8–2.05	4.8–5	6.8–7.1
AR LP(mg/L)	46.4	53.8	76.9	77.6	79.9	86.7	76.4
AR DP(mg/L)	15.0	15.3	23.0	15.4	5.2	5.3	3.5

3.5. Effect of temporary oxygen increase on anammox activity

The temporary increase in DO by aeration resulted in significant differences in nitrogen conversions in the test reactor as compared to the control (Table 1). On the same day as the aeration, the ammonium removal during the light period (AR LP) increased, while also the nitrite and nitrate production increased. Under normal operational conditions, apparently the AOO activity was limited by oxygen supply and therefore aeration had a positive effect.

Ammonium removal in the dark period (AR DP) did also increase with increasing DO applied during the light period, probably because more nitrite was available for anammox bacteria at the start of the dark period. However, when the temporary DO increase exceeded 0.4–1.0 mg/L, then the AR DP significantly decreased. This indicated that anammox bacteria were inhibited by DO concentrations above a value in the range 0.4–1.0 mg/L, and this resulted in a different profile of nitrogen species in Fig. 6 as compared to Fig. 2C. Apart from the changes in conversion rates, the lack of nitrite removal during the dark period is the strongest indication of anammox bacteria inhibition. Inhibition of anammox bacteria by oxygen may be reversible (Egli et al., 2001; Zekker et al., 2014) and therefore the nitrogen conversions were also monitored during the days following the cycle in which the DO was temporarily increased. System recovery could most clearly be seen from the nitrite concentrations at the end of the dark period (Table 2). For the highest DO increase (6.8–7.1 mg/L) the system had recovered after 7–9 days.

The nitrogen profiles observed in the reactor, as well as in the activity tests and control experiments pointed to significant anammox activity in the photo-bioreactor, which shows that anammox is not so sensitive to oxygen to that it excludes the possibility to maintain a combined algal-AOO-anammox biomass. However, the AOO do require higher oxygen concentrations for optimal growth than the anammox bacteria. The half-saturation constant K_s for AOO is reported as

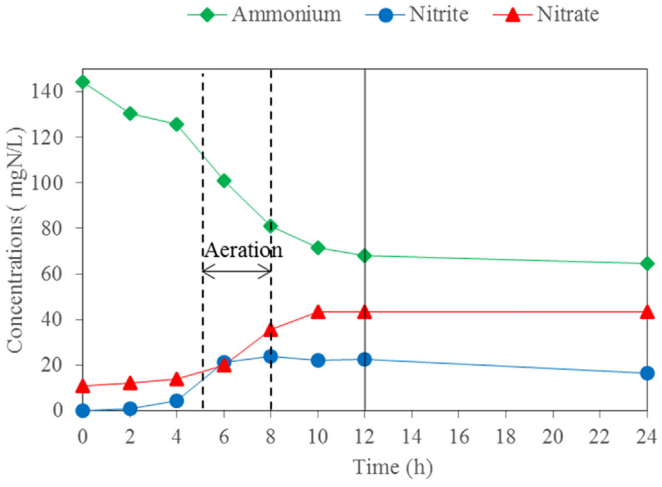

Fig. 6. Profiles of nitrogen species in the test reactor with temporary bubble aeration (for 3 h, from hour 5 to hour 8) to increase the DO concentration to 6.8–7.1 mg O_2/L.

Table 2
Variation of nitrogen species concentration after a DO concentration of 6.8–7.1 mg/L was applied to the system for 3 h during the LP. (Day 1 means one day after the cycle with aeration and LP = light period, DP = dark period.)

	Ammonium (mg N/L)			Nitrite (mg N/L)			Nitrate (mg N/L)		
	Initial concentration	End of LP	End of DP	Initial concentration	End of LP	End of DP	Initial concentration	End of LP	End of DP
Control reactor	139	95	86	0	11	0	18	25	27
Test reactor day 1	144	68	64	0	22	17	11	43	43
Test reactor day 3	146	82	76	2	7	4	7	22	23
Test reactor day 6	136	71	64	2	7	3	13	22	23
Test reactor day 9	134	69	57	1	13	0	13	23	27

0.79 mgO_2/L (Manser et al., 2005), which explains the increase in nitritation rates that were observed at higher DO values (Table 1). For anammox bacteria, the observed inhibition by oxygen was reversible, even when near saturation values were achieved (Table 2). This confirms literature reports on the protective layers that AOO are able to provide to anammox bacteria in aggregates (Morales et al., 2015; Zekker et al., 2014). The difference in this research is that oxygen is not provided from an external source and diffusing into the flocs, but that it is generated in-situ by algae growing in the same flocs. One may speculate that algal oxygen production takes place primarily at the outer edges, or outside, of the flocs, where light supply will be better than inside the flocs. Alternatively, AOO may also grow attached to algae and thus limit the oxygenated environment to a volume at close distance to the algal cells. Further research is required into the mechanism, but the observation that even at near oxygen saturation the inhibition was reversible shows the effectiveness of the oxygen scavenging by AOO.

The results showed that at least a 9 days recovery period is required when temporary oxygen saturation occurs, therefore in practical applications such an event should not occur frequently. The extent to which the DO fluctuates in a real world system for the treatment of domestic wastewater will depend on the fluctuations in load, the relative activity of photosynthesis and nitritation, and the variation in light incidence. Periods of low ammonium load and high sunlight incidence can easily lead to oxygen saturation (Karya et al., 2013). A proposed control strategy for a combined system of anaerobic pre-treatment and a photo-bioreactor is a dynamically controlled bypass of the raw wastewater directly into the photo-bioreactor. The bypass is expected to trigger higher autotrophic and heterotrophic oxygen consumption. Typical DO fluctuations (bulk DO > 0.4–1 mg/L) that are expected in a full-scale algammox system treating domestic wastewater will inhibit activity of anammox bacteria, but recovery is possible if the frequency is not too high allowing for several days of recovery.

4. Conclusions

- Stable biological nitrogen removal was achieved in an algal-bacterial photo-bioreactor, under alternating 12 h light and 12 h dark conditions. Ammonia oxidizing organisms produced nitrite, which was converted by anammox bacteria during the dark phase and possibly also during the light phase.
- Reversible inhibition of anammox bacteria was observed at bulk DO values larger than 0.4–1.0 mg/L. Recovery time increased with increasing DO concentrations. Temporary saturation with oxygen, as may occur under field conditions, will require at least 9 days for the anammox bacteria to recover from reversible inhibition.

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