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

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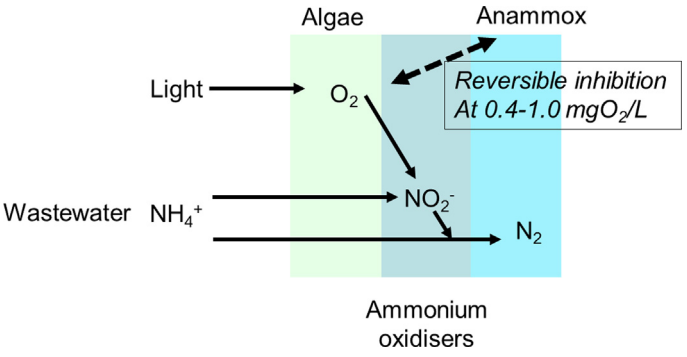
## HIGHLIGHTS

- 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