Anaerobic digestion effluent treatment using microalgae and nitrifiers in an outdoor raceway pond with fluidized carriers

Shinichi Akizuki, Germán Cuevas-Rodríguez and Tatsuki Toda

ABSTRACT

Combining microalgae and nitrifiers in a single photobioreactor has attracted attention as an alternative approach for conventional nitrogen removal from wastewater. However, nitrifiers are known to be sensitive to light exposure. This study demonstrated the effectiveness of using fluidized carriers to mitigate light stress in nitrifiers. An outdoor raceway pond containing microalgae and nitrifiers with fluidized carriers was used to treat two-fold diluted anaerobic digestion effluent (785 mg-N L^{-1} as a form of dissolved total Kjeldahl nitrogen: TKN) over 50 days. The average daily sunlight intensity reached the inhibition level of nitrifiers (423 μmol photons m^{-2} s^{-1}); however, stable nitrification with a specific ammonium oxidation rate of 55 mg-N g-total suspended $solid^{-1}$ day^{-1} was observed. TKN was mostly removed via nitrifier metabolism (ammonium oxidation and uptake: 40.1%) and partially via microalgae uptake (5.7%). Different microalgae-based processes including that of this study were compared in terms of tolerances to a high dissolved TKN concentration and strong light. Our system showed a relatively higher resistance to not only light exposure but also TKN because the nitrification process decreased the free ammonia level to less than 0.25 mg L^{-1}, which allowed microalgae to grow despite the high ammonium concentration.

Key words | high-ammonia-containing wastewater, immobilization, light stress, microalgal-bacterial consortia, nitrification, photo-oxygenation

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HIGHLIGHTS

- Tested total Kjeldahl nitrogen removal from digestate in microalgae-nitrifiers consortia.
- Fluidized carriers were added as a strategy to mitigate light stress in nitrifiers.
- Stable nitrification with a specific activity of 55 mg-N g-TSS^{-1} day^{-1} was achieved.
- Co-occurrence of nitrification and microalgae growth was observed.
- Fluidized carriers protected nitrifiers against natural light in summer.

INTRODUCTION

Anaerobic digestion is widely considered to be a sustainable technology to decompose and stabilize different types of organic wastes and wastewaters because of its cost-effectiveness and energy recovery via recovering biogas (Chynoweth *et al.* 2000; Rajeshwari *et al.* 2000). As the commercial technology for biogas production through anaerobic digestion is mature, the world biogas production has been increasing in different regions including Europe, North America, the Asian Pacific region, Latin America, the Middle East, and Africa, with an estimated production of 17 Mtoe per year in 2012 expected to increase to 33 Mtoe per year in 2022 (Pike Research 2012). During the anaerobic digestion

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process, dissolved nutrients such as ammonium (NH_4^+) are not completely reduced because the microorganisms employed generally lack sufficient autotrophic metabolism of inorganic nitrogen. The anaerobic digestion effluent (ADE), can be used as a fertilizer applied to agricultural land given its high nutrient content (Chen *et al.* 2014). However, a biogas plant may lead to an ADE oversupply (excess demand) for local agricultural land because the surrounding agricultural land area is often too small for adequate use of the ADE (Li *et al.* 2018). Thus, ADE is frequently subjected to further treatment before discharge to streams, rivers, or sewers to meet discharge standards. Sequential nitrification and denitrification are widely accepted as the main processes for nitrogen removal, in which NH_4^+ is oxidized to a nitrogen oxide, i.e. nitrite: (NO_2^-) or nitrate (NO_3^-), via nitrification, and then, a nitrogen oxide is reduced to nitrogen gas (N_2) via denitrification. These processes require a huge amount of energy for nitrification as well as chemical additives for denitrification (Ruiz *et al.* 2006). A more energy-efficient and cost-effective post-treatment technology needs to be developed.

As an alternative to conventional NH_4^+ removal processes, anaerobic ammonia oxidation (anammox) has been developed during the last two decades (Strous *et al.* 1997; Gustavsson *et al.* 2020). In this process, NH_4^+ and NO_2^- are directly reacted to form N_2 by anoxic anammox bacteria, without requirement for aeration and chemical additives (Strous *et al.* 1997). Nevertheless, partial nitrification before anammox is required to produce sufficient available NO_2^- for the anammox bacteria, which constitutes an economical burden for treatment plants. Recently, co-culture of microalgae and nitrifiers in a single reactor has been expected to be another attractive alternative to classical NH_4^+ removal as it has the following advantages: (1) microalgal photosynthesis supplies oxygen to the nitrifiers, which results in an effective reduction of the energy consumption for aeration (Karya *et al.* 2013); and (2) nitrogen ions (NH_4^+, NO_2^-, and NO_3^-) uptake by microalgae lowers the nitrogen loading on the subsequent denitrification, mitigating the required chemical dosage. It was found that excess free ammonia (FA; NH_3), which is determined by the pH and NH_4^+ when the value of temperature is constant (Anthonisen *et al.* 1976), inhibits microalgal growth (Azov & Goldman 1982). As ADE commonly has a high NH_4^+ concentration, previous literature has suggested that ADE should be diluted to decrease the FA concentration to avoid growth inhibition in microalgae-only systems (Cho *et al.* 2013). Instead of considerable water consumption for dilution, nitrification in a microalgal system can play a role in effectively reducing the FA level in a more sustainable manner due to the NH_4^+ oxidation accompanied by the pH decrease in their reaction. Thus, co-culture of nitrifiers and microalgae can be expected to be a robust system in situations that treat high NH_4^+-containing wastewater such as ADE.

Even so, it is known that light irradiation inhibits nitrifier activity because of the inactivation of ammonia monooxygenase in nitrifiers (Hyman & Arp 1992). For instance, Merbt *et al.* (2012) reported that ammonia oxidizers were inhibited by continuous illumination at a light intensity of 60 μmol photons m^{-2} s^{-1}. Vergara *et al.* (2016) also evaluated the effect of light intensity on nitrifiers and showed that an irradiance of 500 and 1,250 μmol photons m^{-2} s^{-1} resulted in a significant decrease of nitrifier activity. Sunlight often exceeds 1,000–2,000 μmol photons m^{-2} s^{-1} and may severely inhibit nitrification, resulting in instability or even failure of the co-culture of nitrifiers and microalgae, particularly in the treatment of high-NH_4^+-containing wastewater. However, in practical plants for nitrogen removal from wastewater, such as advanced oxidation ditches, the nitrifiers function well despite exposure to natural light, probably because of the considerable depth of the reactor (>3–5 m from the surface of water), which decreases the light transmittance into the reactor. Meanwhile, microalgal-based processes operate in flat reactors that are typically less than 0.2–0.3 m in depth, possibly leading to inhibition of nitrifiers. Based on the aforementioned, development of some strategies to attain light-stress tolerance in nitrifiers is important to use co-culture of nitrifiers and microalgae in practical applications. Another aspect should be noted is that the requirement of large areas for microalgae cultivation may be a barrier to the commercialization of the proposed co-culture system, especially in land-limited and populated areas. On the other hand, this concern can be eliminated if the systems are installed in rural areas where there is abundant available land.

Our previous work found that light inhibition of nitrifiers can be mitigated by immobilizing them to fluidized carriers, possibly due to attached biofilms in the carriers shielding the inner bacteria from light penetration (Akizuki *et al.* 2020). The aim of this study was to evaluate the effectiveness of the use of fluidized carriers immobilized in a co-culture of microalgae and nitrifiers for ammonia removal from ADE. A raceway pond, which is commonly employed as a large-scale microalgal reactor, was operated under outdoor conditions during the summer in Tokyo, Japan, and analyzed.

METHODS

Substrate and inoculums preparation

Digester supernatant after dewatering was collected from a sewage sludge treatment plant of the Hokubu Sludge Treatment Centre (Kanagawa Prefecture, Japan). The supernatant was rudimentarily filtrated through a commercial filter and used as a substrate (ADE). The ADE was stored in a refrigerator at 4 °C until its use.

Aerobic sludge that contains abundance nitrifiers was collected from the full-scale anaerobic-anoxic-oxic (A_2O) treatment plant of the Hokubu Sludge Treatment Centre. The sludge was immediately transported to a laboratory of Soka University and allowed to settle for a few hours, after which the supernatant was discarded. The concentrated sludge was used for biofilm formation into fluidized carriers. The microalgae *Chlorella sorokiniana* NIES 2173 was obtained from the Microbial Culture Collection, National Institute for Environmental Studies (NIES), Japan.

Biofilm formation and microalgae cultivation

Before beginning the raceway pond operation, the seed sludge was immobilized into fluidized carriers. The used carriers were a cubic-shaped polyurethane foam $10 \times 10 \times 10$ mm in size after water absorption (APG, Nisshinbo Chemical, Inc., Japan). The ADE was diluted three-fold and then used as a substrate. A 20-L cylindrical reactor was used as a reactor. The fluidized carriers were added to the reactor to establish the carrier–filling ratio, which is defined as the volume occupied by the carrier in a reactor, at approximately 30%. Then, the seed sludge and distilled water were added to the reactor to achieve a total suspended solids (TSS) concentration of 2.0 g L^{-1}. The reactor was operated using sequential batch operation under the following conditions: a filling time of 10 min, a reaction time of 23.5 h, and a settling and decanting time of 20 min. Aeration was conducted during the reaction time. The reactor's hydraulic retention time (HRT) and temperature were 10 days and 25 °C ± 1 °C, respectively. The reactor was operated for approximately 1 month. At the end of the immobilization, the average value of the dry weight of attached solids into carrier was 0.80 mg-TSS per each carrier. Both the immobilized and suspended sludges in the reactor were used as nitrifier inoculum for the main experiment.

Prior to the experiment, the microalgae were cultivated in identical conical flasks filled with an ADE-based medium, which was prepared as follows: (1) the ADE was filtrated through a 0.45 μm glass-fiber filter and diluted five-fold with distilled water; and (2) 3 mL of PIV metals, consisting of $Na_2EDTA{\cdot}2H_2O$ (1,000 mg L^{-1}), $FeCl_3{\cdot}6H_2O$ (196 mg L^{-1}), $MnCl_2{\cdot}4H_2O$ (36 mg L^{-1}), $ZnSO_4{\cdot}7H_2O$ (22 mg L^{-1}), $CoCl_2{\cdot}6H_2O$ (4 mg L^{-1}), and $Na_2MoO_4{\cdot}2H_2O$ (2.5 mg L^{-1}) in distilled water, was dosed into the diluted ADE. The microalgae were cultivated in an incubator illuminated by a light intensity of around 200 μmol photons m^{-2} s^{-1} at 25 °C ± 1 °C for 5 days. The cultivated microalgae were concentrated via centrifugation (5,000 rpm for 10 min) and the concentrated biomass was used as the microalgal inoculum.

Experimental set-up of the outdoor raceway pond

An open raceway pond with a working volume of 35 L (length: 1,000 mm, width: 340 mm, depth: 100 mm) was used. The fluidized carriers were added to the raceway pond at a carrier–filling ratio of approximately 10%, which led to an attached solid concentration of 200 mg L^{-1}. Then, the raceway was inoculated with suspended microalgal and nitrifier inoculums, resulting in a TSS concentration of 60 and 600 mg L^{-1}, respectively. The two-fold diluted ADE, whose characteristics on average were a pH of 7.71 ± 0.12, TSS equal to 26.3 ± 25.5 mg L^{-1}, a dissolved total Kjeldahl nitrogen (TKN) content of 785 ± 49 mg-N L^{-1}, a NH_4^+ content of 440 ± 66 mg-N L^{-1}, and a phosphate (PO_4^{3-}) content of 26.2 ± 2.1 mg-P L^{-1}, was used as substrate. The substrate was continuously fed at an HRT of 10 days, resulting in a dissolved TKN loading rate of 78.5 ± 4.9 mg-N L^{-1} day^{-1}. The paddlewheel rotational speed was 10 rpm. The raceway was covered with a plastic translucent plate to prevent rainwater infiltration. The experiment was run for 50 days under outdoor conditions from July to August 2018, at Soka University in Hachioji City (Tokyo, Japan). The pH in the raceway pond was not adjusted. Mixtures from the raceway pond and effluent tank were sampled between 6:00 and 8:00 pm. Dissolved oxygen (DO) measurement in the raceway pond was conducted between 6:00 and 8:00 pm, immediately after sampling. A schematic diagram of a series of experimental procedure is shown in Figure 1.

Analytical methods

Ambient temperature and light intensity (μmol photons m^{-2} s^{-1}) were monitored by pyranometers (MS-402, Eko

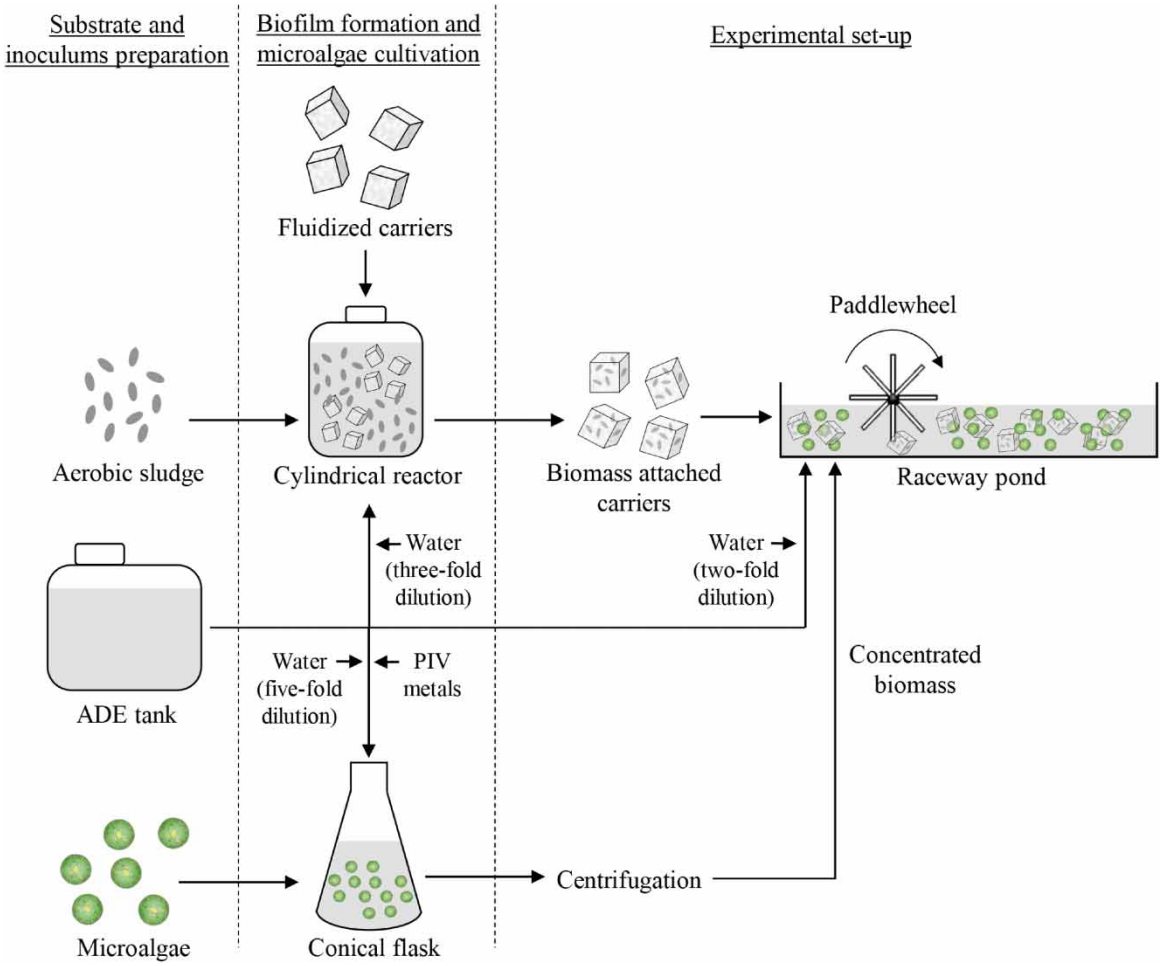

Figure 1 | A schematic diagram of a series of experimental procedures. Bacterial inoculum for the raceway pond also includes suspended form.

Instruments, Japan). For the mixture samples collected from the raceway pond, pH, DO, TSS, and Chlorophyll *a* (Chl. *a*) were analyzed. The mixture samples collected from the effluent tank were immediately filtrated through a 0.45 μm glass-fiber filter (GC-50, Advantec, Toyo Kaisha, Ltd, Japan) and then total dissolved nitrogen (TDN), and nitrogen ions of the filtrated samples were analyzed. The pH and DO were measured using a pH probe (Multiparameter Orion 4-star plus, Thermo Scientific, USA) and a DO probe (SensION 5, Hach, USA), respectively. The TSS concentration was measured according to the sewage analysis method of the Japan Sewage Works Association (1997). The Chl. *a* was extracted with *N, N*-dimethylformamide and its concentration was determined fluorometrically (Model 10-AU, Turner Design, Inc., USA) according to Welschmeyer (1994). The TDN concentration was measured using a Hach DR2000 portable instrument (Hach, USA). The nitrogenous ion was determined using a high-performance liquid chromatography system with electrochemical detector (CD-5, Showa Denko, Japan) and two types of columns (IC YS-50 for cation analysis and IC I-524A for anion analysis, Showa Denko, Japan). The dry weight of attached solids into carriers were determined by washing them with distilled water several times to remove almost all solids from the carriers, after which suspended solids in the water were analyzed.

Calculations

The attached solids concentration was determined using the following equation:

$$\text{Attached solid (mg L}^{-1}) = \frac{\text{AS}_C \times \text{N}_C}{\text{V}_\text{L}} \quad (1)$$

where AS_C and N_C refer to the dry weight of attached solids in each carrier (unit: mg) and the number of carriers in the

raceway pond, respectively. V_L refers to the volume of the raceway (i.e. 35 L).

The nitrogen balance during the stable nitrification period was determined based on measured nitrogenous ions values and stoichiometry of the nitrification reactions (Haug & McCarty 1972):

$$55NH_4^+ + 5CO_2 + 76O_2 \rightarrow C_5H_7O_2N + 54NO_2^- + 52H_2O + 109H^+ \quad (2)$$

$$400NO_2^- + 5CO_2 + NH_4^+ + 195O_2 + 2H_2O \rightarrow C_5H_7O_2N + 400NO_3^- + H^+ \quad (3)$$

The amount of NH_4^+ uptake by microalgae was determined from the following equation (modified from Karya *et al.* 2013):

$$NH_4^+ \text{ uptake by microalgae (mg)} = \text{Dissolved TKN } \textit{in} - (\text{Dissolved TKN } \textit{eff} + NO_x^- \textit{eff} + \text{FA } \textit{eff} + \text{Uptake by nitrifiers}) \quad (4)$$

where dissolved TKN *in (eff)*, NO_x^- *eff*, FA *eff*, and uptake by nitrifier refer to the daily average amounts of dissolved TKN in the influent (effluent), NO_x^- and FA in the effluent, and nitrogen uptake by nitrifiers calculated by Equations (2)–(4) (unit: mg).

The specific TKN removal rate and the specific NH_4^+ oxidation rate during the stable nitrification period were calculated using the following equations:

$$\text{Specific TKN removal rate (mg-N g-TSS}^{-1}\text{ day}^{-1}) = \frac{\text{Dissolved TKN removal rate}}{\text{TSS concentration}} \quad (5)$$

$$\text{Specific } H_4^+ \text{ oxidation rate (mg-N g-TSS}^{-1}\text{ day}^{-1}) = \frac{NO_x^- \text{ production rate}}{\text{TSS concentration}} \quad (6)$$

where dissolved TKN removal and NO_x^- production rates refer to daily dissolved removal rate and daily NO_x^- production rate, respectively (unit: mg-N L^{-1} day^{-1}). TSS concentration includes the attached solids concentration.

RESULTS AND DISCUSSION

Ambient conditions and process stability in the raceway pond

The ambient temperature fluctuated from 16.3 to 38.2 °C, with an average value of 28.0 °C ± 3.9 °C (Figure 2(a)). Previous research reported that *C. sorokiniana* effectively grew within a temperature range of 28–32 °C (Cordero *et al.* 2011), but can accommodate a wider range of temperature between 14 and 38 °C (Patterson 1970). The optimal temperature for stable nitrification was also found to be within a similar temperature range around 30 °C (Jones & Hood 1980). It was found that extreme high (45 °C) and low (5 °C) temperatures severely inhibited nitrification, whereas a moderate temperature range of 20–35 °C allowed maintenance of their activity (Neufeld *et al.* 1986; Zhang *et al.* 2014). The temperature exceeded or did not reach transiently allowable values for *C. sorokiniana* and the nitrifiers, but the temperature was within an appropriate range during most of the experimental period. The light intensity fluctuated from 0 to 2,020 μmol photons m^{-2} s^{-1} with an average value of 423 μmol photons m^{-2} s^{-1} (Figure 2(b)). Previously, a light

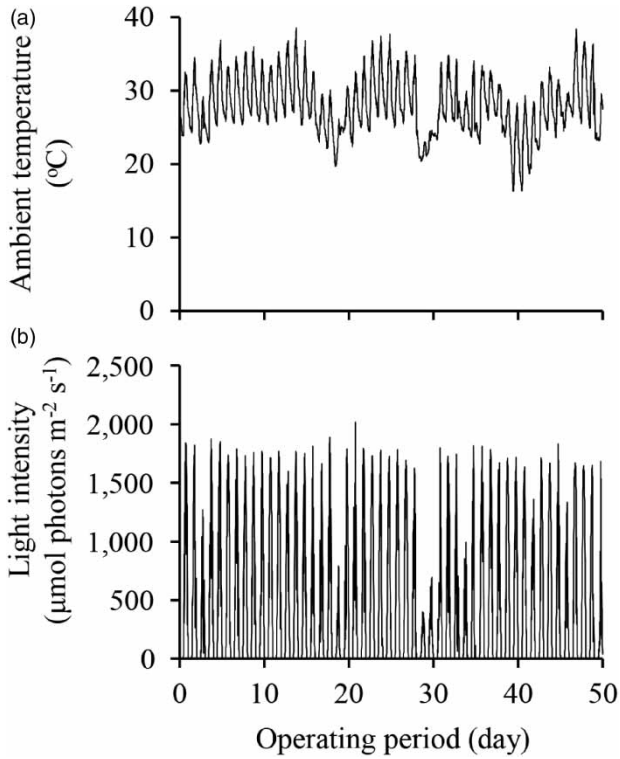

Figure 2 | Variations in ambient temperature (a) and light intensity (b) during the experiment.

intensity greater than 60–250 μmol photons m^{-2} s^{-1} was found to inhibit nitrifier activity (Merbt *et al.* 2012; Vergara *et al.* 2016), which means that light intensities frequently exceeded the critical level for stable nitrification.

In the co-culture of the microalgae and nitrifier, the pH of the culture medium changed via photosynthesis and/or nitrification; the value increased due to inorganic carbon consumption and the release of basic bioreaction metabolites by the microalgae, while it decreased due to NH_4^+ oxidation through nitrification. At the beginning of the experiment, the pH in the raceway pond increased up to 7.42 within 1 day and then slightly decreased during the first month (Figure 3(a)). Thereafter, the pH stabilized and a mildly acidic condition, with an average value of 5.63 ± 0.32, occurred which indicated the nitrification process. The DO concentration also varied over the experimental period (Figure 3(b)). During the first one-half of the experiment (0–22 days), the DO concentrations were relatively high, with an average value of 5.12 ± 0.77 mg L^{-1}, whereas the concentrations decreased thereafter to an average value of 2.67 ± 0.63 mg L^{-1}. It has been reported that preferable DO concentrations for complete nitrification to NO_3^- are greater than 1.7 mg L^{-1} (Ruiz *et al.* 2003); thus, the DO values were probably sufficient for nitrification during this experiment.

The TSS concentration in a suspended form quickly increased within the first 1 day and then gradually decreased (Figure 4(c)). During the last 20 days (30–50 days), the average TSS and attached solids concentrations, calculated using Equation (1), were 509 and 66 mg L^{-1}, respectively. In contrast to the TSS, Chl. *a* concentration tended to increase with experimental time from approximately 600 to 1,450 μg L^{-1} (Figure 4(d)). These results indicate that the microalgal biomass increased with the experimental time whereas the nitrifiers biomass decreased. This opposing tendency may be interpreted to be because of the difference in the specific growth rates, in that *C. sorokiniana* generally grows faster than the nitrifiers, both the NH_4^+-oxidizing bacteria (AOB) and NO_2^--oxidizing bacteria (NOB) (Wu *et al.* 2017; Huesemann *et al.* 2018).

Profiles of nitrogen compounds

The main nitrification products (i.e. NO_2^- and NO_3^-) varied with the experimental time (Figure 4). During the first 10 days, NO_2^- was the main product, but NO_3^- proportion

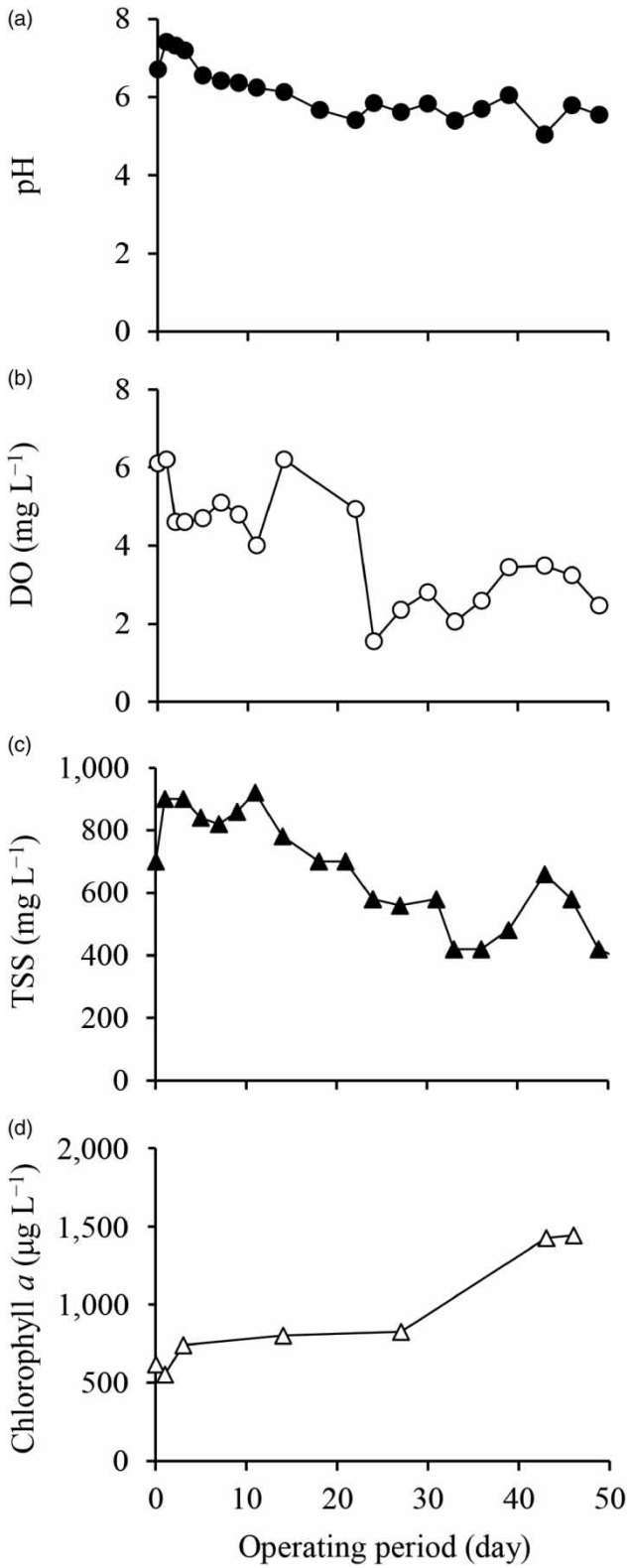

Figure 3 | Variations in pH (a), DO (b), TSS (c), and (d) Chl. *a* during the experiment.

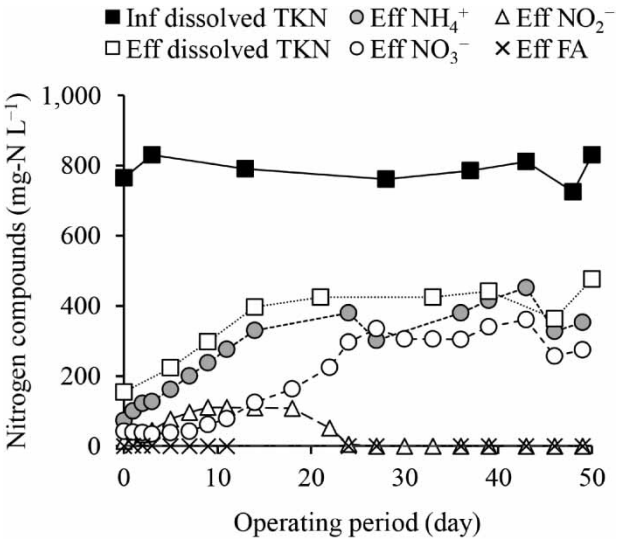

Figure 4 | Profiles of nitrogen compounds during the experiment.

gradually increased during the first month and NO_2^- disappeared thereafter. It was reported that AOB is recovered more rapidly from photoinhibition than NOB (Guerrero & Jones 1996). The other study reported that intensive light (blue range) was found to accumulate NO_2^- by selective photoinhibition of NOB, of which c-type cytochrome is known to be photo-bleachable at 408 nm (Kang *et al.* 2018). In the same way, most researchers have accepted that NOB are more sensitive to light exposure than AOB (Diab & Shilo 1988; Guerrero & Jones 1996; Vergara *et al.* 2016), although there is no determinate reason for this difference in photoinhibition. In this study, NOB might be partially inhibited by light exposure but their activity recovered with experimental time. There are several possible reasons for this recovery phenomenon. For example, the presence of microalgae, which has a high microbial extinction coefficient (Vergara *et al.* 2016), at a high density in the raceway pond can mitigate nitrification photoinhibition. However, this speculation may not be the case because the Chl. *a* concentration did not appreciably increase during the first one-half of the experiment (Figure 3(d)). Another possible reason is due to the acclimation of the nitrifiers to light. Previous literature implies that ammonia oxidizers such as thaumarchaeota can develop light-stress tolerance when inhabiting a shallow marine ecosystem because DNA photolyase was exclusively detected in the epipelagic clade (Luo *et al.* 2014). However, short-term acclimation of nitrifiers to light has not yet been proved and further investigations is needed.

During the stable nitrification period (i.e. from approximately 30 to 50 days), the average NO_3^- concentration was 310 ± 34 mg-N L^{-1}, which corresponds to a specific NH_4^+ oxidation rate of 54 ± 6 mg-N g-TSS^{-1} day^{-1}. A certain NH_4^+ concentration within a range of 330–452 mg-N L^{-1} remained during this stable period. Such NH_4^+ concentrations are expected to severely inhibit microalgal growth in microalgae-only systems due to the high FA level (Uggetti *et al.* 2014) unless the pH is maintained within a neutral range. In this study, the pH values were mildly acidic during the stable period as a result of the nitrification process. This resulted in maintaining FA concentrations less than 0.25 mg-N L^{-1}, which are markedly less than the reported inhibition levels for microalgae (>20–30 mg-N L^{-1}, Azov & Goldman 1982; Uggetti *et al.* 2014) as well as nitrifiers (>1–10 mg-N L^{-1}, Anthonisen *et al.* 1976). These results confirmed that co-existence of microalgae and nitrifiers shows robustness against high nitrogen-containing wastewater.

Nitrogen balances

The nitrogen mass balance during the stable nitrification period is shown in Table 1. The average dissolved TKN removal reached 46.6%, which corresponds to a specific TKN removal rate of 65 mg-N g-TSS^{-1} day^{-1}. The TKN removal was mainly performed via nitrification (i.e., NH_4^+ oxidation plus nitrifier uptake: 40.9%) followed by microalgal uptake (5.7%). Nearly one-half of the added TKN remained in the form of NH_4^+. The remaining NH_4^+ could be removed by means of an increase in the biomass concentration in the raceway. For instance, an appropriate biomass concentration to handle a two-fold diluted ADE can be estimated as 1,220 mg L^{-1} by the results of the specific TKN removal rate (64 mg-N g-TSS^{-1} day^{-1}) and average TSS and attached solids concentrations (total 575 mg L^{-1}). To maintain such a biomass level, equipping a settling tank and returning the settled biomass to the raceway pond would be effective.

Specific NH_4^+ oxidation rates in different studies on the co-culture of microalgae and nitrifiers, including here, are shown in Table 2. In the table, different types of reactors (e.g. sequencing batch reactor: SBR, continuous stir tank reactor: CSTR, and raceway), substrates (e.g. synthetic wastewater and ADE), inoculums, light intensities, and

Table 1 | Nitrogen mass balance (unit: % of the influent soluble TKN)

NH_4^+ oxidation				Uptake	
via NO_3^-	via NO_2^-	NH_4^+	Soluble organic nitrogen[a]	Nitrifiers	Microalgae
40.1	0	48.1	5.3	0.8	5.7

[a]Soluble organic nitrogen was calculated by the difference between soluble TKN and NH_4^+.

Table 2 | Specific NH_4^+ oxidation rates for different studies on the co-culture of microalgae and nitrifiers

Reactor type	Substrate	Inoculums		Average daily light intensity (μmol m^{-2} s^{-1})	NLR (mg-N L^{-1} day^{-1})	Specific NH_4^+ oxidation rate (mg-N g-SS^{-1} day^{-1})	References
		Nitrifiers	Microalgae				
SBR	Synthetic wastewater	Activated sludge	*Scenedesmus quadricauda*	63	25	77	Karya *et al.* (2013)
CSTR	Synthetic wastewater	n.d.	Mixed culture	67.5	140	50	Vargas *et al.* (2016)
SBR	Municipal wastewater	Bacteria from PBR in lab.	Mainly *Chlorella*, diatoms	100	37	58	Foladori *et al.* (2018)
Raceway	ADE	Aerobic sludge (A_2O)	*Chlorella sorokiniana*	423	78.5	55	This study

NLR: nitrogen loading rate; SBR: sequential batch reactor; CSTR: continuous stirred tank reactor.

dissolved TKN loading rates are also summarized (Karya *et al.* 2013; Vargas *et al.* 2016; Foladori *et al.* 2018; this study). The specific NH_4^+ oxidation rate obtained in this study (55 mg-N g-TSS^{-1} day^{-1}) was comparable to that of previously reported values (50–77 mg-N g-TSS^{-1} day^{-1}). Notably, the applied light intensity in this study (423 μmol photons m^{-2} s^{-1}) was markedly stronger than that of previous studies (63–100 μmol photons m^{-2} s^{-1}), indicating that the proposed system with fluidized carriers can offer adequate light-stress tolerance to nitrifiers under outdoor conditions.

Apart from the use of fluidized carriers, the use of concentrated microalgae biomass in the raceway can be another possible approach to mitigate the influence of the light on nitrifiers. Vergara *et al.* (2016) reported that the light extinction coefficient for *C. sorokiniana* (0.1045 m^2 g^{-1}) is considerably higher than that of nitrifiers (0.0003 m^2 g^{-1}). In their developed model, an algal–bacterial culture containing 95% *C. sorokiniana* and 5% of nitrifiers at a concentration of 500 mg-SS L^{-1} may have reduced nitrifier activity by only 11%. Nevertheless, sustaining such a high biomass ratio of microalgae may be an aspect worth considering in practical applications.

TKN and light-stress tolerances during different microalgal-based processes

Figure 5 shows the light intensities and influent dissolved TKN concentrations that facilitated stable reactor operation during the reported microalgae-based process. The term 'stable reactor operation' refers to the fact that the reactor did not face any process instability and/or failure due to reductions in the activities of nitrifiers and/or microalgae, which are frequently caused by strong light irradiation, high FA concentrations, high pH values, depletion of DO, and other reasons. Notably, these influent concentrations are not the ones to which microalgae are directly exposed as a result of ammonia removal in the continuous experiments. Instead, these concentrations are considered the ones to which microalgae are directly exposed in the batch experiments (at least the state of the experiments). In systems of a consortium of microalgae and nitrifiers, a wide

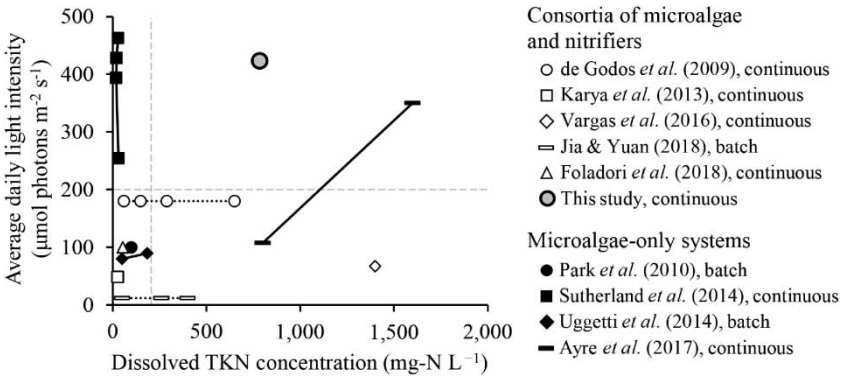

Figure 5 | Light intensities and influent dissolved TKN concentrations that facilitated stable reactor operation during the reported microalgae-based processes.

range of dissolved TKN concentrations from 27 to 1,400 mg-N L^{-1} have been reported as acceptable (de Godos *et al.* 2009; Karya *et al.* 2013; Vargas *et al.* 2016; Jia & Yuan 2018; Foladori *et al.* 2018). This tolerance to a high influent dissolved TKN concentration was probably due to the system's capability of decreasing FA levels through the nitrification process, which was also confirmed in this study. The previous studies operated systems under low and moderate daily light intensities ranging from 12 to 180 μmol photons m^{-2} s^{-1}, whereas the proposed system in this study could tolerate a high intensity of 423 μmol photons m^{-2} s^{-1}. Microalgae-only systems generally do not show adequate tolerance to a high influent dissolved TKN (<200 mg-N L^{-1}) (Park *et al.* 2010; Sutherland *et al.* 2014; Uggetti *et al.* 2014). Ayre *et al.* (2017) reported successful growth of a microalgal culture on 1,600 mg-N L^{-1} in the form of NH_4^+ in outdoor raceway ponds. This was possible by maintaining the pH at 8 via the addition of CO_2. Except for Ayre *et al.* (2017), only this study has shown adequate tolerances to both high influent dissolved TKN and high light intensity. In contrast to Ayre *et al.* (2017), this study did not apply any additional pH adjustment because a self-pH-buffering process occurred. Therefore, the proposed system can possibly serve as an energy-efficient and cost-effective approach to treat wastewaters containing high levels of nitrogen, such as ADE. Further techno-economic analyses, such as life cycle cost assessments, are needed to make decisions pertaining to the implementation of the proposed approach in practical applications. In this study, we used two-fold diluted ADE. This level of dilution can considerably increase the consumption of freshwater and the level of land occupation, which might hinder full-scale applications of the proposed system. In practical operation, the use of effluent instead of freshwater for dilution could help reduce water consumption, while improvement of the system performance would be necessary to shorten HRT (i.e. reduce land use). In addition, the operating period of this experiment was set to 50 days in summer. However, given that temperature and light levels fluctuate throughout year, microorganism performance in the system will vary depending on the seasons. Therefore, evaluation of the seasonal variability of the system throughout the year will be important for improving it further and using it in practical applications.

CONCLUSIONS

In this study, TKN (mainly NH_4^+) removal from two-fold ADE was evaluated using a co-culture of microalgae and nitrifiers in an outdoor raceway pond with fluidized carriers. Several conclusions can be made as follows:

- Stable nitrification with a specific NH_4^+ oxidation of 55 mg-N g-TSS^{-1} day^{-1} was achieved even at a high daily average light intensity of 423 μmol photons m^{-2} s^{-1}.
- During the stable nitrification period (30–50 days), the total TKN removal efficiency was 46.6%. Nitrifier metabolism (NH_4^+ oxidation and uptake) was the principal removal pathway (40.9%), followed by microalgal uptake (5.7%).
- Microalgae can grow even at a high NH_4^+ concentration (>300 mg-N L^{-1}) because the nitrification process helped to decrease the FA concentration to an acceptable level (<0.25 mg-N L^{-1}).
- By comparing this study's results to those of previous studies, we found that our proposed system has a higher tolerance to both a high dissolved TKN concentration and strong light intensity without any additional pH control.

FUTURE OUTLOOK

To enhance the TKN removal efficiency, biomass settling and recirculation will be incorporated into the proposed system and its effectiveness will be evaluated. In addition, investigations of undiluted ADE treatment, scaling-up, and seasonal variation in process performance are needed to further develop this approach.

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