A novel shortcut nitrogen removal process using an algal-bacterial consortium in a photo-sequencing batch reactor (PSBR)

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Abstract

Removal of nitrogen from anaerobically digested swine manure centrate was investigated in a photo-sequencing batch reactor (PSBR) with alternating light and dark periods. Microalgal photosynthesis was shown to provide enough oxygen for complete nitrification during the light period. With addition of an organic carbon source during the dark period, the reactor removed over 90% total nitrogen (TN) without aeration other than by mixing. Overall, 80% of the TN removal was through nitrification/denitrification and the rest was due to biomass uptake. The high concentrations of ammonia and nitrite and low dissolved oxygen concentration in the PSBR effectively inhibited nitrite oxidizing bacteria, resulting in stable nitrification. The hybrid microalgal photosynthesis and shortcut nitrogen removal process has the potential to substantially reduce aeration requirements for treatment of anaerobic digestion side streams. The PSBR also produced well settling biomass with sludge volume index of $62 \pm 16 \text{ mL mg}^{-1}$.

Key words

Algae, bacteria, photo-sequencing batch reactor, nitrification/denitrification, nitrogen removal, high ammonia strength wastewater.

Graphical abstract

1. Introduction

Treatment and discharge of high ammonium (NH_4^+) strength wastewater, such as industrial wastewater (e.g. from food, fertilizer, plastics industries), centrate from anaerobic digestion of domestic, industrial and agricultural wastes and landfill leachate, results in serious environmental problems due to the toxicity of free ammonia (FA) to aquatic life (Carrera et al., 2003; Effler et al., 1990). Although anaerobic digestion is an attractive and effective method to recover energy and stabilize organic waste resources, centrate from anaerobic digestion systems is of particular concern due to its high NH_4^+ concentration and low bioavailability of chemical oxygen demand (COD) (Appels et al., 2011). This results in high oxygen and external carbon source requirements for effective nitrification and denitrification in conventional biological nitrogen removal (BNR) processes.

The shortcut N removal through nitrite (NO_2^-) process has been developed to treat high NH_4^+ strength wastewater with lower oxygen and organic carbon requirements than conventional BNR systems (Fux et al., 2006). In this process, NH_4^+ is oxidized to NO_2^- , which is then reduced to gaseous N_2 . Shortcut N removal requires inhibition of NO_2^- -oxidation to nitrate (NO_3^-) by selecting environmental and operating conditions that favor the growth of NH_4^+ -oxidizing bacteria (AOB) over NO_2^- -oxidizing bacteria (NOB). These conditions include: (1) high FA and/or free nitrous acid (FNA) concentrations (Anthonisen et al., 1976); (2) high temperatures and low solids residence time (SRT) (Hellings et al., 1998); and/or (3) low dissolved oxygen (DO) concentrations (Pollice et al., 2002; Ruiz et al., 2003). Major advantages of shortcut N removal are

reduced aeration, alkalinity and electron donor requirements (Bae et al., 2001). However, the cost of aeration and carbon source provision has the potential to be further reduced through the novel process investigated in this study, which combines microalgal photosynthesis with shortcut N removal via nitrification/denitrification.

The synergetic activity of algal-bacterial consortia has the potential to improve organic carbon, N and P removal and reduce energy costs in wastewater treatment (Subashchandrabose et al., 2011). Several prior studies have coupled algal photosynthesis with conventional BNR for wastewater treatment (Boelee et al. 2014; de Godos et al., 2014; González-Fernández et al., 2011; Karya et al., 2013). In these systems, algal photosynthesis produces oxygen for nitrification, resulting in reduced aeration demands. N removal in these systems can be achieved through assimilation by biomass and nitrification/denitrification, with the pathway dependent on the wastewater characteristics and reactor operating regime. He et al. (2013) treated municipal wastewaters in batch reactors with varying N concentrations using an algal-bacterial consortium. At NH_4^+ -N concentrations between 29 and 174 mg L⁻¹, 47-63% of the N supplied was assimilated by algal and bacterial biomass. Su et al. (2011) found that assimilation of N into biomass accounted for 61-93% of N removal in batch reactors with mixed algal-bacterial cultures treating wastewater. Karya et al. (2013) achieved full NH_4^+ removal while using synthetic wastewater (50 mg NH_4^+ -NL⁻¹) without mechanical aeration using an algal bacterial consortium. In this study, 81-85% of the NH_4^+ supplied was converted to NO_3^- by nitrification. Biological nitrification has also been shown to mitigate problems with FA stress during algal treatment of high NH_4^+ strength wastewaters (de Godos et al., 2009; Gonzalez et al., 2008). However, there is no prior

research aimed at treating high NH_4^+ -N strength wastewater via the shortcut N removal pathways using an algal-bacterial consortium.

The overall goal of this study was to evaluate shortcut N removal from high NH_4^+ strength wastewater using an algal-bacterial photo-sequencing batch reactor (PSBR) with alternating light and dark periods. During the light period, photosynthetic oxygen production stimulates AOB. During the dark period, DO is quickly consumed by microbial activity and algal respiration, promoting denitrification. The high FA, FNA and low DO concentration favors the growth of AOB over NOB. The specific objectives of this study were to investigate the effect of light intensity, operating cycle and carbon source addition on TN removal from anaerobically digested swine waste centrate. To our knowledge, this is the first time shortcut N removal has been combined with algal oxygenation for treatment of high NH_4^+ strength wastewater in a single reactor.

2. Materials and methods

2.1 Anaerobically digested swine waste centrate

A pilot-scale anaerobic digester, with a working volume of 26-L, was used as the centrate source for these experiments. The digester was initially seeded with sludge from an anaerobic digester at a local wastewater treatment plant (Clearwater, Florida). Swine manure was collected from Twenty Four Rivers Farm (Plant City, FL) on a weekly basis, mixed with urea and local groundwater and fed to the digester three times per week. This operation provided the centrate characteristics typical of anaerobic digestion at swine concentrated animal feed operations (CAFO). The SRT was maintained at 21 days, based on a prior study showing that high biogas production and volatile solids (VS) removal could be achieved at this SRT (Kinyua et al., 2014). The digester was operated under

mesophilic conditions ($35\pm 2^{\circ}\text{C}$) for more than one year prior to this study. Effluent was collected from the digester and was centrifuged at 3500 g for 10 minutes using a Thermoscientific Sorvall Legend RT Plus (Waltham, MA) centrifuge. After centrifugation, the supernatant (centrate) was filtered through a Sefar nylon mesh filter (Buffalo, New York) with a pore size of 35 μm to remove large particles. The centrate was diluted three times with DI water based on a preliminary study that showed the NH_4^+ did not present a toxicity problem to the algal consortium at this concentration (Nguyen, 2013). Note that no sterilization steps were performed. Characteristics of the diluted centrate, which was used as the PSBR influent, are shown in Table 1. Note that concentrations of heavy metals, pharmaceuticals or other potential toxicants that might affect the growth of the microbial community were not measured.

2.2 Photo-sequencing batch reactor (PSBR)

A bench-scale (2-L) cylindrical glass PSBR (diameter 15 cm, height 12 cm) was set up in a 21°C constant temperature room (Figure 1). Although the wastewater treated was from a mesophilic anaerobic digester, a typical temperature for wastewater treatment was applied because it was assumed that centrate treatment would be carried out under ambient outdoor conditions. Masterflex C/L (Cole-Palmer, US) peristaltic pumps were used to pump the influent and carbon source to the reactor and a Masterflex L/S pump (Cole-Palmer, US) was used to decant the effluent from the reactor. The reactor was illuminated using sixteen cool white fluorescent tubes (Philips Cool White-20W, 24 inches). A Thermolyne Cimarec 2 magnetic stirrer (Dubuque, Iowa) set at 200 rpm was used to maintain the biomass in suspension during the dark and light periods. The pumps, mixer and lights were controlled by a ChronTrol timer (San Diego, CA). A batch oxygen

mass transfer test was performed to determine the mass transfer coefficient (k_1a) under the experimental conditions following the method described in Tchobanoglous et al. (2003). Briefly, the reactor was initially purged with N_2 , after which the DO concentration was measured over time. The determination of k_1a is described in the supplementary information (S1).

The reactor was inoculated with a consortium of wild strain algae, consisting primarily of *Chlorella* spp. (95.2% of the total cell population), *Chlamydomonas* (3.1%), and *Stichococcus* (1.1%). The genera of algae inoculum was identified at the University of Florida based on algal cell morphology, as described in Halfhide et al. (2014). During the startup period, the PSBR was continuously illuminated at an intensity of $74 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$. After 25 days of operation, consistent nitrification was observed, after which light conditions were shifted to 12 hours light/12 hours dark for the rest of the experiment. Note that the reported days of operation were counted after the light condition was shifted to alternating light/dark periods.

Each cycle of the PSBR (feed, react, settle, decant) was 24 hours. The sequence of steps during each experimental phase was varied, and is shown in Figure 2. The PSBR was operated in two different phases: Phase 1 (Day 0-80) without an additional carbon source and Phase 2 (Day 90-163) with sodium acetate addition. During Phase 1, the light intensity was initially set at $74.2 \pm 5.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ for the first 50 days (Phase 1a), which was increased to $105 \pm 7.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Phase 1b) for the rest of the experiment. During Phase 2, sodium acetate was provided at the beginning of the dark period, based on the stoichiometry of $2.2 \text{ gCOD g}^{-1} \text{ NO}_2^- \text{-N}$ removed. Centrate feed was fed either concurrently with acetate addition at the beginning of the dark period (Phase 2a) or separate from

acetate addition at the beginning of the light period (Phase 2b). Settling and decanting steps took place at the end of the dark period.

The hydraulic retention time (HRT) in the PSBR was maintained at 4 days. A portion (220 mL) of the mixed liquor was wasted each day just before the settling period to maintain the SRT at approximately 8 days based on the following calculation:

$$SRT = \frac{TSS_R V_R}{TSS_R \cdot V_w + TSS_E \cdot V_E} \quad (1)$$

Where TSS_R is the biomass concentration of the mixed liquor; TSS_E is the biomass concentration of the effluent; V_R is the reactor volume; V_w is the daily volume of wasted mixed liquor and V_E is the daily volume of effluent.

2.3 *Ex situ* denitrification tests

Ex situ batch experiments (Table 2) were carried out during Phase 1 to investigate the effect of biomass concentration, carbon source addition and NO_2^- concentration on the rate of denitrification. Effluent from the PSBR was used as the feed source for these experiments. Batch reactors were set up in 170 mL glass serum bottles sealed with aluminum crimp caps with rubber septa. For external carbon source addition experiments, sodium acetate (NaAc) was added stoichiometrically to allow for complete denitrification/denitrification. The reactors were placed on a shaking table at 100 rpm for 31 hours. Samples were collected from each reactor over time using syringes and filtered through 0.45 μ m membrane filters for measurement of N species as described below.

2.4 Analytical methods

Mixed liquor samples were collected just before the settling period and effluent samples were collected just after the decanting period. The influent and effluent were kept in a refrigerator at 4°C to minimize the biodegradation. DO concentrations, pH and

conductivity were measured using an Orion GS9156 pH and DO meter and calibrated electrodes (Thermo Fisher Scientific Inc., Waltham, MA). Light intensity was measured at the surface of the reactor using an ExTech Easyview 30 light meter (ExTech Inc., Waltham, MA). Alkalinity, biomass dry weight (total suspended solids [TSS]), volatile suspended solids (VSS), COD, five-day biochemical oxygen demand (BOD₅) and sludge volume index (SVI) were measured according to *Standard Methods* (APHA et al., 2012). Chlorophyll *a* was measured using the ethanol extraction method according to the NEN 6520 – Dutch Standard. NH₄⁺, NO₂⁻ and NO₃⁻ concentrations were measured using a Metrohm Peak 850 Professional AnCat ion chromatography (IC) system (Metrohm Inc., Switzerland). Total N (TN) concentrations were measured using Hach TN Reagent kits TNT 828 (Hach Inc., US). The microscopic observation of algal and bacterial biomass was conducted using a Nikon Eclipse E200 Microscope (Tokyo, Japan).

3. Results

3.1 Nitrification and photosynthesis under varying light conditions

The algal-bacterial consortium grew well in the anaerobically digested swine waste centrate, which was in agreement with prior studies using anaerobically digested municipal sludge and swine waste centrate (Godos et al., 2009; Halfhide et al., 2014). Concentrations of TSS and Chlorophyll *a* during Phase 1 are shown in Figure 3a. The average TSS during Phase 1a was $774 \pm 126 \text{ mg L}^{-1}$ and increased to $1,229 \pm 28 \text{ mg L}^{-1}$ during Phase 1b after the light intensity was increased. Chlorophyll *a* showed the same trend as TSS (Figure 3a). The average Chlorophyll *a* concentration during Phase 1a was $17.4 \pm 3.6 \text{ mg L}^{-1}$ and increased to $28.5 \pm 2.5 \text{ mg L}^{-1}$ during Phase 1b. The ratio of Chlorophyll *a* to TSS was $2.4 \pm 0.3\%$ in Phase 1a and $2.3 \pm 0.2\%$ in Phase 1b. Park and

Craggs (2010) reported that Chlorophyll *a* accounted for about 1.5% of algae biomass in high rate algal ponds (HRAP). The measured Chlorophyll *a* fraction indicated that the dominant microorganisms in the biomass were algae, which was in agreement with the microscopic observations and calculations based on the stoichiometry (Arashiro, 2015; 6% AOB, 19% heterotrophs, 73% algae on a COD basis). The microbial growth rates would most likely be higher if centrate from the mesophilic anaerobic digester (~35°C) was directly fed to the algae cultivation system due to the increased temperature.

Light intensity is one of the most important factors affecting algal biomass growth. The selection of light intensity was based on our prior research on algae treating anaerobically digested sludge centrate (Yuan et al. 2012, Wang et al. 2014a). According to Ogbonna et al. (1999), algal activity increases with light intensity up to 200 – 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, where the photosynthetic apparatus becomes saturated. In Phase 1a, the light intensity was only $74.2 \pm 5.3 \mu\text{mol m}^{-2} \text{s}^{-1}$. The increase in light intensity in Phase 1b to $105 \pm 7.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ resulted in an immediate increase in biomass production. However, the potential for photoinhibition of AOB and NOB should also be considered when selecting the light intensity for algae-bacterial systems. Illumination at $75 \mu\text{mol m}^{-2} \text{s}^{-1}$ was shown to inhibit the growth of AOB and NOB under 12h light/12h dark conditions (Yoshioka and Saijo, 1984). Nearly completely inhibition of nitrification was observed when the illumination was $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Lipschultz et al. 1985). Moderate light intensities of $74 \sim 105 \mu\text{mol m}^{-2} \text{s}^{-1}$ were used in this study.

Profiles of N species during Phase 1 (no external carbon addition) are shown in Figure 3b. The average influent $\text{NH}_4^+\text{-N}$ concentration was $301 \pm 16 \text{mg L}^{-1}$. The average $\text{NH}_4^+\text{-N}$ removal efficiency was $65 \pm 6\%$ during Phase 1a and increased to $93 \pm 2\%$

208 during Phase 1b. The average concentration of NO_2^- -N in the effluent increased
209 significantly with increased light intensity, from $97 \pm 19 \text{ mg L}^{-1}$ to $159 \pm 20 \text{ mg L}^{-1}$. Less
210 than 5 mg L^{-1} of NO_3^- -N was detected in the effluent during Phase 1, indicating that AOB
211 activity was favored over NOB. The method used to estimate the fate of NH_4^+ -N during
212 each Phase is described in the supplementary information (S2). The overall NH_4^+
213 removal efficiency was relatively low during Phase 1a, with approximately 9.3%
214 assimilated into biomass and 52.9% removed by nitrification. Increasing the light intensity
215 on Day 50 improved the nitrification efficiency to 76.3% and the total NH_4^+ -N removal
216 efficiency increased to $93 \pm 2\%$, with TN removal efficiency at $46 \pm 4\%$.

217 To gain a greater understanding of N removal processes in the PSBR, samples were
218 collected on an hourly basis during an entire cycle (24 hours) on Day 54. Profiles of
219 different N species, DO, pH, alkalinity and BOD_5 are shown in Figure 4. During the
220 feeding stage, increases in NH_4^+ -N, alkalinity and DO were observed due to the higher
221 concentrations of these constituents in the influent. NO_2^- -N concentration decreased due
222 to the effect of the dilution. NH_4^+ -N and NO_2^- -N concentrations remained relatively
223 constant during the dark period during Phase 1b, which will be discussed later. NO_3^- -N
224 remained at a very low level ($< 5 \text{ mg L}^{-1}$) during the whole cycle, indicating that AOB
225 activity was selected over NOB activity. The DO concentration remained at 0.19 ± 0.03
226 mg L^{-1} during the dark period and increased to $0.31 \pm 0.04 \text{ mg L}^{-1}$ during the light period.
227 The relatively constant DO concentration in bulk solution was consistent with a balanced
228 oxygen supply and consumption by the algal-bacterial consortium in the biomass flocs.
229 Nitrification during the light phase led to an increase in NO_2^- -N and a decrease in pH and
230 alkalinity. The pH decreased continuously to 7.1 at the end of the light period. No

significant denitrification occurred during the dark period, therefore the alkalinity remained constant during this stage. Approximately 50% of the alkalinity that was present in the feed remained in the effluent. The consumption of BOD_5 took place constantly throughout the cycle, most likely due to the metabolism of heterotrophic bacteria. However, *Chlorella* have also been shown to have heterotrophic metabolic pathways (Perez-Garcia et al. 2011), so may also consume organic carbon, especially during the dark period.

3.2 *Ex situ* denitrification test

Batch *ex situ* denitrification experiments were conducted using biomass and effluent from the Phase 1b PSBR to identify the factors limiting denitrification/denitrification processes during the dark period. N profiles over time in the batch reactors are shown in Figure 5. During the first 5 to 8 hours, a decrease in NH_4^+ and an increase in NO_2^- and NO_3^- was observed in all reactors due to AOB and NOB utilization of DO in the feed at the beginning of the test, after which NH_4^+ concentration remained constant. NO_3^- -N and NO_2^- -N concentrations in reactor A1 (no carbon source addition as a control) did not change significantly over the time, indicating that the low rate of denitrification/denitrification could not be improved by increasing the biomass density if no external carbon source was provided. Reactors with acetate addition (A3 and A4) showed significant decreases in NO_2^- and NO_3^- , indicating that addition of a bioavailable external carbon source was needed to promote denitrification/denitrification. Even though the anaerobically digested centrate had a COD of 800 mg L^{-1} , the BOD_5 concentration was only 209 mg L^{-1} , which was insufficient for denitrification. NO_x ($NO_2^- + NO_3^-$) removals from A3 and A4 were 41.6 and 95.5 mg N L^{-1} , respectively, indicating

that if an external carbon source was provided a higher biomass concentration could improve the NO_2^- removal performance.

3.3 Hybrid photosynthesis-nitrification-denitrification

Based on the results of the batch tests, sodium acetate was added at the beginning of the dark phase during Phases 2a and 2b (Figure 2). The reactor was operated under completely dark conditions during Days 80-90 to decrease NO_2^- concentrations, which had accumulated during Phase 1. The extended dark period also resulted in a decrease in the TSS concentration from $1,195 \text{ mg L}^{-1}$ (D80) to 710 mg L^{-1} (D92). However, TSS concentrations in the PSBR increased rapidly after the light/dark cycle was restored and reached steady state on Day 99 (Figure 6a). The average TSS during Phase 2 was $1,235 \pm 125 \text{ mg L}^{-1}$, which was similar to Phase 1b. Chlorophyll *a* concentrations were highly correlated with the biomass concentration. The average Chlorophyll *a* concentration after steady state was $28.0 \pm 3.1 \text{ mg L}^{-1}$. The average Chlorophyll *a*/VSS ratio was $2.4\% \pm 0.2\%$, showing no significant change from Phase 1.

N species profiles during Phase 2 are shown in Figure 6b. The NH_4^+ concentration in the influent fluctuated during Phase 2 due to the rainy season in Southwest Florida, which affected the manure properties (Days 90-165). The average NH_4^+ -N concentration in the influent was $321 \pm 49 \text{ mg L}^{-1}$. The PSBR operating cycle during Phase 2a (Figure 2) was similar to a Modified Lutzak Ettinger (MLE) process, where the carbon source (acetate) was provided along with the centrate feed at the beginning of the anoxic (dark) period and the reactor is partially decanted at the end of the aerobic (light) period. During this period, $59 \pm 22 \text{ mg NO}_2^- \text{ NL}^{-1}$ remained in the effluent (Figure 6b). The NH_4^+ removal efficiency ($94 \pm 6\%$) in Phase 2a was similar to Phase 1b. The NH_4^+ -N

concentration in the effluent increased from day 110 in Phase 2a, most likely due to the carryover of unused sodium acetate into the light period and the competition between aerobic heterotrophs and AOB for the limited DO. The high NH_4^+ concentration from day 149 to 152 during Phase 2b resulted in incomplete nitrification, indicating that the biomass present in the PSBR at that moment did not have sufficient capacity for full nitrification.

During Phase 2b the PSBR operating cycle (Figure 2) was similar to a separate stage BNR process, where centrate was fed at the beginning of the aerobic (light) period and the carbon source (acetate) was added at the beginning of the anoxic (dark) period. As shown in Figure 5b, after changing the operating cycle only 3 mg L^{-1} of NO_2^- -N and almost no NO_3^- remained in the effluent. Accordingly, the TN removal efficiency increased to $93 \pm 3\%$. The NH_4^+ removal efficiency was $95 \pm 6\%$, which is similar to Phase 2a.

Profiles of different N species over time for Phases 2a and 2b are shown in Figure 7. After sodium acetate was added to the reactor, NO_2^- concentrations decreased rapidly. Almost all NO_2^- was consumed within 2 hours, leaving the rest of the dark phase idle. In Phase 2a, effluent decanting was performed during the light phase when there was a high concentration of NO_x in the PSBR. While in Phase 2b, effluent decanting was performed at the end of the dark period when both NH_4^+ and NO_x concentrations were at the lowest values.

3.4 Algal-bacterial floc properties

The algal-bacterial floc in the PSBR had an average size of $0.6 \pm 0.3 \text{ mm}$, with no observable differences between phases (Figure 8). No pure bacterial biomass aggregates were observed in the PSBR during the study, possibly because bacteria attached to algae

biomass or because small aggregates were washed out during decanting steps in the startup phase of the operation. The majority of biomass settled within 20 mins, leaving a relatively clear supernatant and a low TSS, although there was still some color and turbidity from colloids. The average TSS in the effluent was $71 \pm 26 \text{ mg L}^{-1}$. SVI values ($62 \pm 16 \text{ mL mg}^{-1}$) indicated good settling ability of the algal-bacterial biomass.

4. Discussion

4.1 Nitrogen metabolism in the PSBR

The fate of N as a percentage of the influent $\text{NH}_4^+\text{-N}$ load during each experimental Phase was calculated using the method presented in S2 and is summarized in Table 3. NH_4^+ was removed from the system mainly through biomass uptake and nitrification, with a small amount of nitrification observed during Phase 1. Volatilization of FA was estimated (Arogo et al., 1999) and found to be negligible. Little denitrification was observed during Phase 1 due to the limited bioavailable carbon in the centrate, as was shown by the *ex situ* denitrification experiments. N removal by biomass uptake increased from 9.3% to 16.2% between Phase 1a and 1b due to more algal growth at higher light intensity. Nitrification rates during Phases 1b, 2a and 2b were 5.6, 4.1 and $4.7 \text{ mg L}^{-1} \text{ h}^{-1}$, respectively. These values are lower than those observed during treatment of centrate in an activated sludge process ($\sim 83 \text{ mg L}^{-1} \text{ h}^{-1}$; Fux et al., 2006); however, the system was not optimized to maximize nitrification rates. When sodium acetate was added at the beginning of dark period, the ratio of $\text{NH}_4^+\text{-N}$ removed by denitrification was significantly increased. Over 80% of $\text{NH}_4^+\text{-N}$ was removed by denitrification in Phase 2b when the operational strategy was optimized.

A key factor for successful shortcut N removal is inhibition of NOB. NOB are more sensitive than AOB to FA and FNA concentrations. The NH_4^+ rich influent resulted in initial NH_4^+ concentrations greater than 50 mg NL^{-1} (2.4 mg L^{-1} FA under reactor conditions), which is higher than the FA concentration shown to inhibit NOB (1.5 mg L^{-1}) (Villaverde et al., 1997). As NH_4^+ was oxidized during the light period, NO_2^- accumulated in the reactor, resulting in approximately 0.013 mg L^{-1} FNA under reactor conditions, which is higher than the FNA concentration that has been shown to inhibit NOB (0.011 mg L^{-1}) (Vadivelu et al., 2006). Alternating FA and FNA inhibitory conditions has been shown to select for AOB over NOB. The low bulk DO concentration ($<0.5 \text{ mg L}^{-1}$) in the PSBR also favored nitrification, as NOB have been shown to be more inhibited than AOB by low DO (Tchobanoglous et al., 2003). The combination of alternating high NH_4^+ and high NO_2^- concentrations and low DO provided ideal conditions for shortcut N removal.

NH_4^+ oxidation through nitrification is a process that consumes bicarbonate and releases protons, thus decreasing alkalinity. The photosynthesis of algae consumes both bicarbonate and CO_2 , which may result in a reduction in alkalinity. In this PSBR, the pH decrease during the light period (Figure 4c) indicated that production of protons by nitrification/nitrification was faster than the release of OH^- by algal photosynthesis. A slight increase in pH was observed during the dark (anoxic) period, due to OH^- and alkalinity production by denitrification.

4.2 Effect of DO and carbon source on denitrification

Based on the calculated fate of NH_4^+ in the PSBR, denitrification/denitrification was found to take place during both light and dark periods (Table 3). The observed

simultaneous nitrification and denitrification and the low DO in the bulk liquid indicates that there were both aerobic and anoxic zones in the reactor. Although the DO concentration in the bulk liquid was less than 0.5 mg L^{-1} during both dark and light periods, DO gradients may have existed within the algal-bacterial floc.

A readily bioavailable COD (rbCOD) is required as electron donor for heterotrophic denitrification. Based on the average $\text{NH}_4^+\text{-N}$ loading rate in the PSBR, the rbCOD required for complete denitrification was $178 \text{ mg L}^{-1} \text{ d}^{-1}$. However, the average BOD_5 loading in the influent was only $45 \text{ mg L}^{-1} \text{ d}^{-1}$. In addition, *Chlorella* sp. have been shown to exhibit heterotrophic metabolism under dark conditions (Perez-Garcia et al. 2011). Therefore, *Chlorella* sp. may have competed with denitrifiers for the available rbCOD.

The type of rbCOD is a key factor affecting denitrification rates (Onnis-Hayden & Gu, 2008; Tam et al., 1992). Sodium acetate has the highest denitrification rate because it is more readily metabolized than other organic carbon substrates present in wastewater (Tam et al., 1992). Acetic acid can form Acetyl Co-A, which is the key component in the tricarboxylic acid (TCA) cycle and glycolytic metabolic pathway (Tam et al., 1992). The denitrification rates in our system were $24.0 \text{ mg L}^{-1} \text{ h}^{-1}$ during Phase 2a and $31.4 \text{ mg L}^{-1} \text{ h}^{-1}$ during Phase 2b. A potential supplementary organic carbon source for denitrification in this application, could be VFAs from the raw swine manure, which has been shown to support denitrification at similar rates as acetate (Obaja and Mata-Alvarez, 2005).

4.3 Oxygen mass balance

A mass balance approach was used to estimate the percent of the total oxygen requirements supplied by algal photosynthesis during Phase 2b (Table 4). It was assumed

that oxygen was supplied by photosynthesis and mixing and consumed by nitrification and aerobic sCOD oxidation. The net algal oxygen production accounts for the combined production via photosynthesis and removal via respiration of algae. Sodium acetate was added at the beginning of the dark period based on the stoichiometric requirements for denitrification. Therefore it was not considered part of the influent sCOD in this calculation. An oxygen mass balance for one cycle (24 h) can be expressed as:

$$f_l k_1 a (C_s - C_l) + f_d k_1 a (C_s - C_d) + R_{\text{photo}} = \frac{(sCOD_i - sCOD_e)}{\theta} + 3.4 f_l R_N \quad (2)$$

Where, C_l and C_d are the bulk DO concentrations (mg L^{-1}) in the PSBR during the light and dark periods, respectively; $k_1 a$ is the oxygen mass transfer coefficient (8.4 d^{-1} based on the oxygen transfer tests); C_s is the saturation DO concentration (8.92 mg L^{-1} ; assumed to be the same as the saturation concentration in pure water at 21°C); f_l and f_d are the light and dark period fractions of one cycle (dimensionless), respectively; R_{photo} is the net oxygen production of algae ($\text{mg L}^{-1} \text{d}^{-1}$); θ is the HRT (d); $sCOD_i$ (890 mg L^{-1}) and $sCOD_e$ (578 mg L^{-1}) are sCOD concentrations in the influent and effluent, respectively; R_N is the nitrification rate ($\text{mg NL}^{-1} \text{d}^{-1}$) and 3.4 is an DO consumption coefficient based on the stoichiometry of nitrification ($\text{mg O}_2 \text{ mg N}^{-1}$). Based on the calculation, 74% of the total oxygen demand was supplied through algal photosynthesis and 26% was supplied through mechanical aeration (mixing). This results in significant energy and cost savings over conventional aeration processes. The estimated R_{photo} provides a comparison with the conventional shortcut N removal aeration requirements.

4.4 Settling ability of the biomass

Chlorella sp. are small spherical green algae with a diameter $<10 \mu\text{m}$, which have been shown to have poor settling properties (Wang et al. 2014a). In this study, the

391 use of an algal-bacterial consortium in a PSBR produced biomass with good settling
392 properties. This may have been due to bacterial enhanced bioaggregation and washout of
393 microorganisms with poor settling properties during decanting stage. Both algae and
394 bacteria produce extracellular polymeric substances (EPS; Wang et al. 2013) and EPS has
395 been shown to play an important role in bioflocculation(Lee et al. 2009). Biomass
396 harvesting is a major limitation for full-scale algae wastewater treatment applications
397 (Muñoz & Guieysse, 2006; Su et al., 2011). Flocculation followed by gravity settling is
398 the most common harvesting method for large pond systems (Nurdogan & Oswald,
399 1996). However, this approach is not always effective for single celled microalgae
400 (Muñoz & Guieysse, 2006; Udom et al., 2013). Other commercial technologies, such as
401 filtration and centrifugation, are not economical for large scale microalgae harvesting
402 (Muñoz & Guieysse, 2006). Poor biomass settling also causes poor effluent quality in
403 HRAPs (Muñoz & Guieysse, 2006). In this study, decanting the PSBR after settling
404 helped in the selection of rapidly-settling biomass and led to an effluent with low TSS
405 concentrations (average $71 \pm 26 \text{ mgL}^{-1}$). The high density of biomass at the bottom of the
406 PSBR may have induced environmental stress in the algal-bacterial biomass, such as
407 limited access to substrates or competition among microorganisms, triggering the
408 production of EPS and promoting the formation of large flocs. The high
409 NH_4^+ concentration in the wastewater may also have introduced another environmental
410 stress, which can produce large amounts of EPS (Wang et al., 2014a). The harvested
411 biomass PSBR was used for bioenergy production by anaerobic co-digestion with swine
412 manure. The results of that study are reported elsewhere (Wang et al. 2014b).

4.5 PSBR Operating Regime and Land Area Estimate

The uncoupling of HRT and SRT, which is commonly used in the activated sludge process resulted in a higher biomass concentration compared to conventional HRAPs (50-450 mg L⁻¹) (Park et al., 2011a). The SBR operational mode is easy to adapt to varying wastewater loading rates, allowing for flexible operation. The settling and reaction times are also easy to adjust to achieve different functions (Bungay et al., 2007). Uncoupling HRT and SRT avoids washout of slow growing AOB, while reducing overall reactor volume requirements. This operational strategy should be relatively easy to adapt to algal pond systems at CAFOs.

A potential disadvantage of the proposed algal-bacterial shortcut N removal process is the large land area required due to limited light penetration in ponds containing dense algal-bacterial biomass. A large size CAFO using anaerobic digestion to treat waste from 7,000 pigs with an average weight of 63.5 kg pig⁻¹ (US EPA, 2004), would generate approximately 56 m³ d⁻¹ of anaerobic digester centrate, assuming the total solids production rate was 0.36 kg pig⁻¹ d⁻¹ (Hamilton et al., 1997). The depth of algal pond system was assumed to range from 0.2~1.0 m (Park et al. 2011b; Sutherland et al. 2014). Although HRAPs are normally operated at depths ≤0.4 m a deeper pond may be warranted for this process to provide a dark region where simultaneous nitrification denitrification can occur (Arashiro, 2015). Based on an N loading rate in our preliminary results, between 672~3,360 m² of land will be required for a pond employing this process. The land area required to treat 1 m³ d⁻¹ of digested centrate would be between 12 ~ 60 m².

5. Conclusions

A photo-sequencing batch reactor (PSBR) was used to treat centrate from anaerobically digested swine manure at a HRT of 4 days, an SRT of 8 days and an NH_4^+ -N loading rate of $76 \pm 14 \text{ mg L}^{-1} \text{ d}^{-1}$. Alternating high FA and high FNA concentrations and low DO concentrations successfully favored AOB over NOB activity. An increase in incident light intensity from 74.2 ± 5.3 to $105 \pm 7.2 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ resulted in increased biomass production and nitrification efficiency. The maximum observed nitrification rate was $5.6 \text{ mg L}^{-1} \text{ h}^{-1}$. The bioavailability of the COD in the centrate was found to limit denitrification. Addition of sodium acetate at the beginning of the dark period resulted in $95 \pm 6 \%$ NH_4^+ removal and $93 \pm 3 \%$ total nitrogen removal, of which over 80% of N was removed through nitrification/denitrification. Photosynthesis was found to supply 74% of the required DO, with mechanical aeration (mixing) supplying the remainder. The uncoupling of HRT and SRT in PSBR produced biomass with average SVI of $62 \pm 16 \text{ mL mg}^{-1}$. The hybrid photosynthesis-nitrification-denitrification process using an algal-bacterial consortium has the potential to be developed into a sustainable and low cost technology for high NH_4^+ strength wastewater treatment.

Acknowledgements

This material is based upon work supported by the National Science Foundation under Grant No. 1243510. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation. The authors also acknowledge the “Erasmus Mundus International Master of Science in Environmental Technology and Engineering” program.

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Table 1. Characteristics of the PSBR influent (diluted centrate).

NH₄⁺-N (mg L⁻¹)	297±29
TN (mg L⁻¹)	313±137
TP (mg L⁻¹)	27.5±4.2
Soluble TP (mg L⁻¹)	22.9±4.7
Total COD (mg L⁻¹)	1,045±158
Soluble COD (mg L⁻¹)	800±135
pH	8.08±0.19
Alkalinity (mg CaCO₃ L⁻¹)	1,574±46

Table 2. *Ex situ* denitrification experimental design.

Reactor	External C Source	Biomass (VSS) g L⁻¹	Purpose
A1	None	0.9	Control
A2	None	1.8	Effect of biomass density
A3	NaAc	0.9	Effect of C source addition
A4	NaAc	1.8	Effect of biomass and C source

Table 3. Fate of NH_4^+ -N in the PSBR.

Phase	Effluent- NH_4^+-N	Effluent- NO_2^--N	Effluent- NO_3^--N	Biomass uptake	Denitrification
Phase 1a	37.8%	29.2%	1.8%	9.3%	21.9%
Phase 1b	7.8%	54.2%	2.0%	16.2%	19.8%
Phase 2a	14.1%	14.3%	BDL	18.8%	52.8%
Phase 2b	BDL	BDL	BDL	18.3%	81.7%

BDL: below detection limit.

Table 4. Oxygen mass balance and DO concentrations during the light and dark periods of Phase 2b (percentage of the total are shown in parentheses).

Volumetric oxygen production (mg L ⁻¹ d ⁻¹)		Volumetric oxygen consumption (mg L ⁻¹ d ⁻¹)		Dissolved oxygen (mg L ⁻¹)	
Gas transfer	Algal photosynthesis	Nitritation & nitrification	Organic matter oxidation	Light period (C _l)	Dark period (C _d)
68 (26%)	193 (74%)	183 (70%)	78 (30%)	0.19±0.02	0.09±0.01

Figure 1

Figure 1. Bench-scale PSBR system schematic.

Figure 2

Figure 2. PSBR operating cycles during different experimental phases.

Figure 3

Figure 3. Concentrations of: a) biomass TSS and chlorophyll *a* and b) N species during Phase 1.

Figure 4

Figure 4. Profiles of a) N species, b) DO, c) pH, d) alkalinity and e) BOD₅ in the PSBR during one cycle operation during Phase 1b (Day 54).

Figure 5

Figure 5. Profiles of a) $\text{NH}_4^+\text{-N}$, b) $\text{NO}_2^-\text{-N}$ and c) $\text{NO}_3^-\text{-N}$ during batch denitrification/denitrification experiments (A2 showed the same trend as A1 and is not shown).

Figure 6

Figure 6. Concentrations of: a) biomass TSS and chlorophyll *a* and b) N species during Phase 2.

Figure 7

Figure 7. Profiles of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ during one cycle operation during a) Phase 2a (Day 125), and b) Phase 2b (Day 135).

Figure 8

a.

b.

Figure 8. Photomicrograph of the algal-bacterial biomass: a) 1x10 and b) 1x100.

Graphical abstract

Shortcut nitrogen removal using an algal-bacterial consortium