Environmental Science & Technology Letters

Letter

pubs.acs.org/journal/estlcu

Biological Nitrogen Removal in a Photosequencing Batch Reactor with an Algal-Nitrifying Bacterial Consortium and Anammox Granules

Nathan D. Manser,[*,†] Meng Wang,[†] Sarina J. Ergas,[†] James R. Mihelcic,[†] Arnold Mulder,[‡] Jack van de Vossenberg,[§] Jules B. van Lier,[§,∥] and Peter van der Steen[§]

[†]Department of Civil & Environmental Engineering, University of South Florida, Tampa, Florida 33620, United States

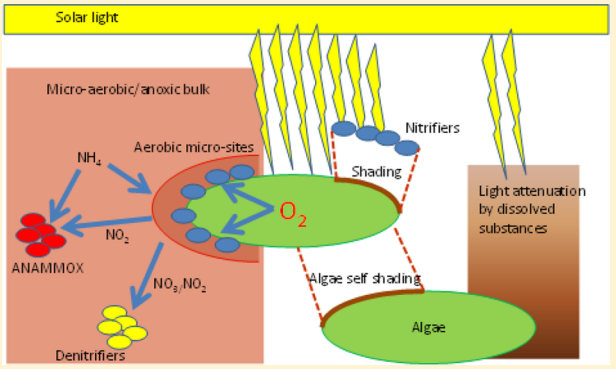
[‡]Amecon Environmental Consultancy, 2614 GK Delft, The Netherlands

[§]UNESCO-IHE Institute for Water Education, 2611 AX Delft, The Netherlands

[∥]Section Sanitary Engineering, Department of Water Management, Delft University of Technology, 2628 CD Delft, The Netherlands

Supporting Information

ABSTRACT: This study demonstrates the feasibility of combining microalgae, ammonia-oxidizing bacteria (AOB), and Anammox in a photosequencing batch reactor. Alternating light and dark periods were applied to achieve biological nitrogen removal without mechanical aeration or external electron donor addition. This process is termed ALGAMMOX (algal anaerobic ammonium oxidation) and differs from the SHARON−Anammox process in that oxygen is generated during light periods through microalgal photosynthesis, replacing mechanical aeration. Results from bench-scale ALGAMMOX experiments with high-ammonia strength wastewater (COD/TN from 1 to 3) showed that influent ammonia was converted to nitrite during light periods at a rate of 7.0 mg of NH_4^+-N L^{-1} h^{-1}. Nitrite was subsequently reduced by an average of 82% during the dark (anoxic) periods due to Anammox activity. Further studies are needed to optimize the system to maximize nitrogen removal rates and to assess long-term process stability.



1. INTRODUCTION

Biological nitrogen removal (BNR) processes are used for treatment of high-nitrogen (N) strength wastewaters, such as anaerobic digestion reject waters, livestock wastes, and landfill leachate. However, conventional BNR processes are resource intensive because of aeration and external electron donor requirements.[1−3] Algal-based BNR systems (i.e., paddle-wheel raceways and photobioreactors) can greatly reduce energy input requirements, as microalgae use photosynthesis to supply dissolved oxygen (DO) for nitrification.[4−7] Microalgae have also been shown to have a high N substrate affinity, improving BNR efficiency.[8,9] Furthermore, algal-based BNR processes can be designed to include anoxic zones or stages to promote denitrification,[10,11] which can simplify the treatment train. Despite these benefits, algal-based BNR of wastewaters with low readily biodegradable chemical oxygen demand to total N ratios (COD/TN < 8) often requires external electron donor addition for complete N removal.[10] In manure-free piggery reject waters, for instance, this ratio has been shown to be as low as 0.84[11] or as high as 14 when the manure is included,[12] while in effluent from an anaerobic digester processing swine manure, the ratio ranges from 1.7 to 8.2.[13−16]

Over the past two decades, BNR configurations, such as SHARON−Anammox, that combine nitritation with anaerobic ammonium oxidation (Anammox) for complete N removal from high-ammonia strength wastewaters have been developed.[17,18] In this process, stable nitrite formation is promoted by adjusting the temperature, pH, DO, and/or solids retention time (SRT) to select for ammonia-oxidizing bacteria (AOB) over nitrite-oxidizing bacteria (NOB).[19] The SHARON−Anammox process requires 25% less aeration than conventional BNR and does not require external carbon source addition;[20] however, mechanical aeration is still required. A novel algal-based BNR process has recently been developed at the lab scale, where high-ammonia strength wastewater is subjected to an anoxic zone for denitrification of the nitrate that is formed in a continuously illuminated aerobic zone.[21] This system couples cyanobacteria with AOB and NOB to achieve efficient removal of organic carbon (>95%) and TN (>90%). Drawbacks of this

Received: January 29, 2016
Revised: February 29, 2016
Accepted: March 2, 2016

DOI: 10.1021/acs.estlett.6b00034
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

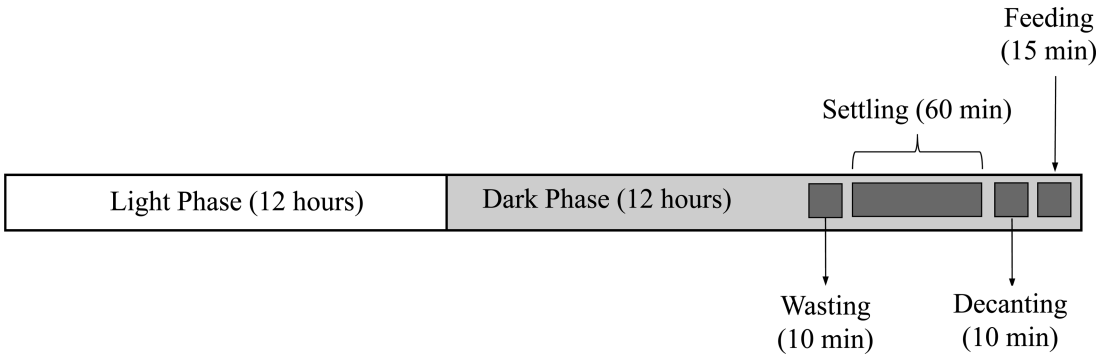


Figure 1. Sequencing batch reactor operation strategy used for the PSBR and ALGAMMOX systems.

system, however, are the additional carbon sources (glucose and HCO_3^-) that were needed to achieve TN removal.

Our study demonstrates the feasibility of a BNR process that integrates a unique consortium of microorganisms. This process is termed ALGAMMOX (algal anaerobic ammonium oxidation) and differs from the SHARON−Anammox because DO produced by microalgae replaces mechanical aeration. The relationships among microalgae, AOB, and Anammox are described in eqs 1−4. Equations 1 and 2 show oxygen production and ammonia uptake by algal biomass, respectively.[22]

$$CO_2 + H_2O + \lambda_{photon} \rightarrow CH_2O + O_2 \quad (1)$$

$$NH_4^+ + 7.6CO_2 + 17.7H_2O \rightarrow C_{7.6}H_{8.1}O_{2.5}N + 7.6O_2 + 15.2H_2O + H^+ \quad (2)$$

Equation 3 estimates the uptake and oxidation of ammonia to nitrite by autotrophic AOB using standard half-reactions, assuming that the amount of metabolic energy dedicated to cell synthesis (f_s) is 0.14.[23]

$$NH_4^+ + 1.24O_2 + 0.16CO_2 + 0.04HCO_3^- \rightarrow 0.04C_5H_7O_2N + 0.96NO_2^- + 0.92H_2O + 1.92H^+ \quad (3)$$

Finally, eq 4 describes how Anammox utilize the nitrite formed (eq 3) with available ammonia to produce N_2 gas, biomass, and nitrate.[24]

$$NH_4^+ + 1.3NO_2^- + 0.1HCO_3^- + 0.1H^+ \rightarrow N_2 + 0.3NO_3^- + 0.1CH_2O_{0.5}N_{0.15} + 2H_2O \quad (4)$$

In a prior study,[16] we showed that complete N removal could be achieved in anaerobic digestion reject waters without mechanical aeration using a photosequencing batch reactor (PSBR) with alternating light and dark stages, including an external organic carbon source. Alternatively, in this work, we couple an algal−bacterial biomass selected for nitritation with Anammox bacteria to promote complete N removal without external organic carbon for denitrification or the use of mechanical aeration.

2. MATERIALS AND METHODS

2.1. Bioreactor Description. Experiments were conducted in two phases, a PSBR phase with a mixed algal-nitrifying bacterial consortium and an ALGAMMOX phase with the same algal−bacterial biomass with added Anammox granules. Experiments were performed in a 2.0 L working volume water-jacketed cylindrical (50 mm diameter, 275 mm height) glass reactor (Applikon Biotechnology). This system was identical to the experimental setup used by Karya et al.[25] The experimental hydraulic retention time (HRT) was maintained at 4 days, resulting in a 500 mL daily exchange volume. The SRT of the algal−nitritation biomass was estimated to be 30 days based upon the wasting strategy of the mixed liquor (see Figure 1). The Anammox granule SRT for this experiment was not known; however, the granules demonstrated superior settling characteristics. Therefore, we assumed that the granules were almost fully retained in the system for the duration of the experiment. A Bio-Console (Applikon Biotechnology) control system maintained a Thermolyne (Dubuque, Iowa) Cimarec 2 magnetic stirrer at 50 rpm. Masterflex C/L and L/S (Cole-Palmer) peristaltic pumps were used to pump influent, mixed liquor, and effluent in and out of the reactor. The reactor was illuminated using four warm white lamps (Philips Standard 40 W E27 55 mm) equally spaced along the reactor circumference, at a distance of 2 cm from the outer wall. The average light irradiance was 109 ± 6.1 μmol m^{-2} s^{-1} measured inside an empty reactor (Photometer Li-COR model Li-250A). This intensity is above the inhibition threshold for AOB and NOB of 75 μmol m^{-2} s^{-1};[26] however, inhibition was not expected as light intensity decays exponentially as it passes deeper into the PSBR because of the self-shading by the microbial biomass,[27] and the intensity did not exceed the threshold for complete inhibition (300 μmol m^{-2} s^{-1}).[28] Therefore, AOB inhibition by light was not evaluated as part of this study.

Figure 1 shows the sequencing batch reactor operational strategy for each reactor's 24 h cycle. Mixing was provided during all stages except for settling and decanting. Note that influent was added to the reactor immediately after the supernatant had been decanted and before the aerobic light period to avoid the accumulation of DO in the reactor. This strategy was used in response to a prior study that showed high levels of DO (12 mg of O_2/L) can occur in an ammonia-limited PSBR,[25] and Anammox have been shown to be inhibited by DO above 1.5 mg of O_2/L at 25 °C.[29]

2.2. Influent Characteristics. Electron donors are likely to exist in anaerobic effluents that can support denitrification, such as sulfide and methane;[30] however, our study was an early attempt using simplified conditions that would exist if dissolved methane and sulfide concentrations were negligible or removed first by degassing. Therefore, anaerobic digester effluent (COD/TN ratio target range of 1−3) was simulated in this study by modifying primary wastewater from the Harnaschpolder wastewater treatment plant (WWTP) (Den Hoorn, The Netherlands). The COD was not directly measured in this

DOI: 10.1021/acs.estlett.6b00034
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

study as it was assumed to be no more than the value reported in the facility permits (125 mg of COD/L). Additionally, $NaHCO_3$ (1200 mg/L), NaH_2PO_4 (50 mg/L), and Na_2HPO_4 (300 mg/L) were added to the simulated effluent, resulting in an average influent alkalinity of 800 mg/L (as $CaCO_3$), and a phosphate pH buffer (0.2M) capable of maintaining the pH at 7.5. The primary effluent was stored at 4 °C within 1 h of collection until use, while the influent was prepared daily immediately before the fill stage. It was assumed that the primary effluent provided sufficient micronutrients to support uninhibited growth, although trace element concentrations were not measured. Influent nitrite and nitrate concentrations were below the method detection limits (MDLs) shown below.

2.3. Anammox Granules. Four liters of Anammox granules was harvested from the municipal WWTP Dokhaven-Sluisjesdijk (Rotterdam, The Netherlands) in March 2015. This side stream SHARON−Anammox system has achieved an N removal rate of 10 kg N m^{-3} day^{-1} after a startup period of 3 years.[31] Anammox granules were stored at 4 °C until they were used. The activity of the granules was demonstrated over 5 days using a reactor configuration identical to the one described in section 2.1 without illumination. The system DO and temperature were maintained at 1 mg/L and 25 °C, respectively, using a Bio-Console control system. The system pH was maintained at 7.5 using phosphate buffer (0.2M). The Anammox biomass was fed 500 mL of deionized (DI) water with $NaNO_2$ (3.6 g/L), NH_4Cl (2.0 g/L), and $NaHCO_3$ (6.0 g/L) each day. Influent and effluent concentrations of ammonia, nitrite, and nitrate were monitored during this phase to ensure granules were active. It should be noted that immediate activity was observed and documented (see Figure S1 of the Supporting Information showing consistent removal of ammonia and nitrite without nitrate accumulation). Using fresh granules from cold storage, 25 mL portions of granules were acclimated to 25 °C using a water bath (approximately 2 h) and then immediately transferred into the PSBR. This process was repeated for 3 days until a noticeable change in the nitrogen species profiles was observed. This seeding strategy was adopted during the experiment because only a small effect on the nitrogen removal rate was observed after the first and second additions.

2.4. Analytical Methods. Mixed liquor samples were collected immediately before the settling stage, and effluent samples were collected immediately after the decant stage. Influent, effluent, and mixed liquor samples were filtered through a 45 μm membrane filter and stored at 4 °C prior to analysis, with some filters retained for the algal−biomass analysis. Standard Methods were used for the following: alkalinity (ASTM 2320), ammonia (NEN 6472), nitrite (ASTM D3867), and nitrate (ASTM D3867). The DO and pH were measured and recorded using BioXpert (Applikon Biotechnology) software and AppliSens probes. Chlorophyll *a* was measured using an ethanol extraction method (NEN 6520, Dutch Standard). MDLs for all N species were 0.05 mg of N/L.

3. RESULTS AND DISCUSSION

3.1. PSBR Phase. The PSBR was operated for 90 days with a consortium of microalgae and AOB prior to the start of the ALGAMMOX phase. Concentrations of ammonia, nitrite, and nitrate were measured weekly during the final 38 days of this period, with a typical concentration profile shown in Figure 2. A decrease in ammonia concentration (average initial value of 124 mg of NH_4^+-N/L, average final value of 7 mg of NH_4^+-N/L)

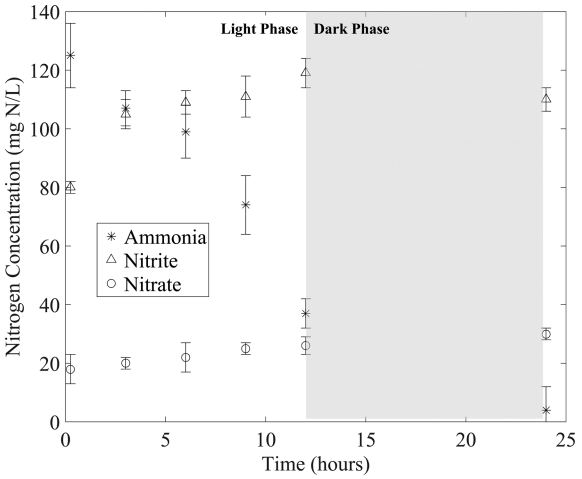


Figure 2. Average concentration profiles of ammonia, nitrite, and nitrate during a single 24 h cycle for the PSBR showing the conversion of ammonia to nitrite with little nitrate formation. Error bars represent the standard deviation of six sample days recorded via triplicate measurements.

during the illuminated phase was accompanied by increases in nitrite and nitrate concentrations. The lower average effluent nitrate (30 mg of NO_3^--N/L) relative to nitrite (115 mg of NO_2^--N/L) concentration indicated that, although some NOB activity was likely occurring, the high ammonium concentration, high nitrite concentration, and low DO concentration favored AOB over NOB.[16] During this time, the average bulk liquid DO concentration was observed to be 2.1% (0.17 mg/L) at 25 °C. From Figure 2, the ammonia removal rate during the illuminated phase of the PSBR operation was estimated to be 5 mg of N L^{-1} h^{-1}. This rate is within a range of ammonia removal rates observed (4.1−5.7 mg of N L^{-1} h^{-1}; DO = 0.5 mg of O_2/L) when treating high-ammonia strength effluent from an anaerobic digester processing swine manure. This variation likely resulted from a difference in the experimental light intensity, 109 μmol m^{-2} s^{-1} in our study compared to 75 μmol m^{-2} s^{-1} in ref 16. Our results were lower than those of a PSBR operated via illumination for 24 h and much higher DO concentrations, which achieved an ammonia removal rate of 7.7 mg of N L^{-1} h^{-1} with DO concentrations as high as 12 mg of O_2/L.[25]

After 90 days of the PSBR phase, stable conditions were demonstrated by comparing the observed ammonia removal rates, assuming that if they were within 10% of each other, then stable conditions existed. Rates were calculated for the illuminated stages only, by a linear fit to a minimum of four sample points for each data collection period. The five observations within the last 40 days of PSBR operation (shown in Figure 3) indicate that this requirement was satisfied, allowing the ALGAMMOX phase to begin.

3.2. ALGAMMOX Phase. As was observed during the PSBR phase, bulk DO concentrations did not change during the ALGAMMOX phase from an average value of 0.17 mg of O_2/L, suggesting that Anammox activity was not inhibited by DO during this experiment,[25] although this effect was not measured directly. A typical nitrogen species concentration profile for the ALGAMMOX phase is shown in Figure S2 of the Supporting Information. Improved nitrite removal was observed, 42 mg of NO_2^--N/L, in the effluent compared to a rate of 115 mg of NO_2^--N/L (Figure 2). The lower nitrite

DOI: 10.1021/acs.estlett.6b00034
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

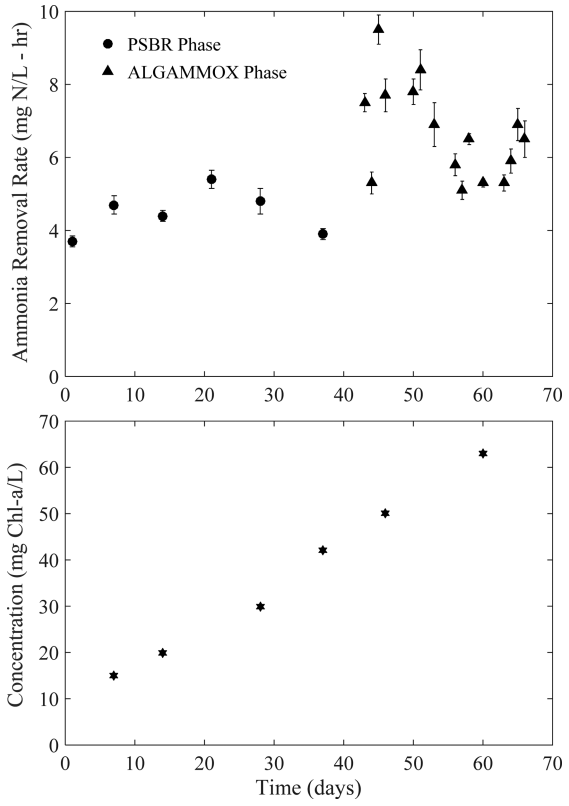


Figure 3. Chlorophyll *a* concentrations and ammonia removal rates (based on the illuminated period) during PSBR and ALGAMMOX phases. The average ammonium removal rate increased from 4.5 to 7.0 mg of NH_4^+-N L^{-1} h^{-1} after Anammox addition. Error bars represent standard deviations of triplicate measurements, except for chlorophyll results that are an average of one split sample.

concentrations favored process performance because Anammox bacteria are inhibited by nitrite accumulation.[32,33] Via comparison of the nitrate concentrations in the same manner, it can be inferred that no substantial accretion of nitrate occurred between the two phases, 30 mg of NO_3^--N/L compared to 32 mg of NO_3^--N/L (Figure 2). The error bars associated with the ammonia data in Figure S2 of the Supporting Information show cycles that achieved nearly complete removal of ammonia occurred during the experiment, indicating that there were periods of low ammonia concentrations allowing the uptake of nitrate by the microalgae to occur.[34,35] Freshwater microalgae have been shown to take up between 0.062 and 0.189 mg of NO_3^--N L^{-1} h^{-1} per 10^5 cells.[36] Furthermore, because the effect of denitrification was not studied in this experiment, it is possible that some denitrifying bacteria were present in the system and their uptake of organic carbon also controlled nitrate concentrations. These effects on nitrogen in the system were outside of the scope of this study and must be considered further.

Ammonia removal rates (milligrams per liter per hour) in the reactor during the PSBR (n = 5) and ALGAMMOX (n = 15) phases are shown in Figure 3. The average ammonia removal rate was 4.3 ± 0.6 mg of NH_4^+-N L^{-1} h^{-1} during the PSBR phase, which increased by 62% to 7.0 ± 2.1 mg of NH_4^+-N L^{-1} h^{-1} during the ALGAMMOX phase. An unpaired t test showed this difference was significant (α = 0.05; p = 0.011) despite the relatively small number of samples compared. This increase in ammonia removal rate is attributed to the addition of Anammox bacteria to the already active AOB biomass. It is possible that a fraction of the increase can be traced to the assimilation of ammonia by the microalgae; however, this is unlikely as Figure 3 shows that there was no substantial change in the ammonia removal rate during the first 40 days of the experiment (PSBR phase) even though the chlorophyll concentration doubled from 15 to 30 mg/L.

These results show that wastewaters that are low in COD relative to ammonia can be treated in the ALGAMMOX process without addition of an external carbon source for denitrification or energy intensive aeration. In this study, near complete ammonia removal was achieved without accumulation of nitrite, nitrate, or dissolved oxygen. Future research activities must include investigations into process stability and maximal conversion capacity (kilograms of N per cubic meter per day), so that HRT can be optimized. Reducing HRT is important for the ALGAMMOX process to lessen the areal requirements of the system. Equally essential will be development of a full-scale reactor design that offers optimal conditions for a good process performance. The challenges could be the effect of environmental conditions such as Anammox inhibition by oxygen peaks, AOB limitation by oxygen concentrations at or below the half-saturation constant, and finally temperature and light intensity on the performance of the reactor.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.6b00034.

Activity of the Anammox granules demonstrated by consistent removal of ammonia and nitrite without accumulation of nitrate (Figure S1) and average concentration profiles for ammonia, nitrite, and nitrate for a typical 24 h cycle of the ALGAMMOX reactor (Figure S2) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Address: 4202 E. Fowler Ave., ENB 118, Tampa, FL 33620. E-mail: ndmanser@mail.usf.edu. Phone: (906) 487-2197.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This material is based upon work supported by the National Science Foundation under Grant 1243510. This work was performed at the UNESCO-IHE Institute for Water Education in Delft, The Netherlands. Special thanks to Peter Heerings, Berend Lolkema, Angélica Rada Ariza, and Francisco Rubio Rincón. The Anammox granules were kindly provided by Paques BV and WaterBoard Hollandse Delta.

■ REFERENCES

(1) Plappally, A. K.; Lienhard V, J. H. Energy Requirements for Water Production, Treatment, End Use, Reclamation, and Disposal. *Renewable Sustainable Energy Rev.* **2012**, *16*, 4818−4848.

(2) McCarty, P. L.; Bae, J.; Kim, J. Domestic Wastewater Treatment as a Net Energy Producer − Can this be Achieved? *Environ. Sci. Technol.* **2011**, *45*, 7100−7106.

(3) Gao, H.; Scherson, Y. D.; Wells, G. F. Towards Energy Neutral Wastewater Treatment: Methodology and State of the Art, Environmental Science. *Processes Impacts* **2014**, *16*, 1223−1246.

DOI: 10.1021/acs.estlett.6b00034
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

(4) de Godos, I.; González, C.; Becares, E.; García-Encina, P.; Muñoz, R. Simultaneous Nutrients and Carbon Removal During Pretreated Swine Slurry Degradation in a Tubular Biofilm Photobioreactor. *Appl. Microbiol. Biotechnol.* **2009**, *82* (1), 187−194.
(5) González-Fernández, C.; Molinuevo-Salces, B.; García-González, M. C. Nitrogen Transformations under Different Conditions in Open Ponds by Means of Microalgae−bacteria Consortium Treating Pig Slurry. *Bioresour. Technol.* **2011**, *102* (2), 960−966.
(6) Jorquera, O.; Kiperstok, A.; Sales, E. A.; Embiruçu, M.; Ghirardi, M. L. Comparative Energy Life-cycle Analyses of Microalgal Biomass Production in Open Ponds and Photobioreactors. *Bioresour. Technol.* **2010**, *101*, 1406−1413.
(7) Mendoza, J. L.; Granados, M. R.; de Godos, I.; Acién, F. G.; Molina, E.; Banks, C.; Heaven, S. Fluid-dynamic Characterization of Real-scale Raceway Reactors for Microalgae Production. *Biomass Bioenergy* **2013**, *54*, 267−275.
(8) Di Termini, I.; Prassone, A.; Cattaneo, C.; Rovatti, M. On the Nitrogen and Phosphorus Removal in Algal Photobioreactors. *Ecol. Eng.* **2011**, *37*, 976−980.
(9) Boelee, N. C.; Temmink, H.; Janssen, M.; Buisman, C. J. N.; Wijffels, R. H. Nitrogen and Phosphorus Removal from Municipal Wastewater Effluent using Microalgal Biofilms. *Water Res.* **2011**, *45*, 5925−33.
(10) Janssen, M.; Tramper, J.; Mur, L. R.; Wijffels, R. H. Enclosed Outdoor Photobioreactros: Light regime, Photosynthetic Efficiency, Scale-up, and Future Prospects. *Biotechnol. Bioeng.* **2003**, *81*, 193−210.
(11) Meng, J.; Li, J.; Li, J.; Antwi, P.; Deng, K.; Wang, C.; Buelna, G. Nitrogen Removal from Low COD/TN Ratio Manure-free Piggery Wastewater within an Upflow Microaerobic Sludge Reactor. *Bioresour. Technol.* **2015**, *198*, 884−890.
(12) Wang, Y.; Guo, W.; Yen, H. W.; Ho, S. H.; Lo, Y. C.; Cheng, C. L.; Ren, N.; Chang, J. S. Cultivation of *Chlorella Vulgaris* JSC-6 with Swine Wastewater for Simultaneous Nutrient/COD Removal and Carbohydrate Production. *Bioresour. Technol.* **2015**, *198*, 619−625.
(13) Lei, Z.; Wu, T.; Zhang, Y.; Liu, X.; Wan, C.; Lee, D. J.; Tay, J. H. Two-stage Soil Infiltration Treatment System for Treating Ammonium Wastewaters of Low CPD/TN Ratios. *Bioresour. Technol.* **2013**, *128*, 774−778.
(14) Kinyua, M. N.; Cunningham, J. A.; Ergas, S. J. Effect of Solids Retention Time on the Bioavailability of Organic carbon in Anaerobically Digested Swine Waste. *Bioresour. Technol.* **2014**, *162*, 14−20.
(15) Manser, N. D.; Mihelcic, J. R.; Ergas, S. J. Semi-continuous Mesophilic Anaerobic digester Performance under Variations in Solids Retention Time and Feeding Frequency. *Bioresour. Technol.* **2015**, *190*, 359−366.
(16) Wang, M.; Yang, H.; Ergas, S. J.; van der Steen, P. A Novel Shortcut Nitrogen Removal Process using an Algal-bacterial Consortium in a Photo-sequencing Batch Reactor (PSBR). *Water Res.* **2015**, *87*, 38−48.
(17) van Hulle, S. W. H.; van den Broeck, S.; Maertens, J.; Villez, K.; Donckels, B. M. R.; Schelstraete, G.; Volcke, E. I. P.; Vanrolleghem, P. A. Construction, Start-up and Operation of a Continuously Aerated Laboratory-scale SHARON Reactor in View of Coupling with an Anammox Reactor. *Water SA* **2007**, *31*, 327−334.
(18) Guo, J. H.; Peng, Y. Z.; Wang, S. Y.; Zheng, Y. N.; Huang, H. J.; Ge, S. J. Effective and Robust Partial Nitrification to Nitrite by Real-time Aeration Duration Control in an SBR Treating Domestic Wastewater. *Process Biochem.* **2009**, *44*, 979−985.
(19) van Dongen, U.; Jetten, M. S.; van Loosdrecht, M. C. The SHARON-Anammox Process for Treatment of Ammonium Rich Wastewater. *Water Sci. Technol.* **2001**, *44*, 153−160.
(20) van Dongen, L. G. J. M.; Jetten, M. S. M.; van Loosdrecht, M. C. M. The Combined SHARON/Anammox Process: A Sustainable Method for N-removal from Sludge Water. *Water Intelligence Online*; International Water Association: London, 2007; Vol. 610.4028/www.scientific.net/amr.396-398.2044.
(21) de Godos, I.; Vargas, V. A.; Guzman, H. O.; Soto, R.; Garcia, B.; Garcia, P. A.; Munoz, R. Assessing Carbon and Nitrogen Removal in a Novel Anoxic-aerobic Cyanobacterial-bacterial Photobioreactor Configuration with Enhanced Biomass Sedimentation. *Water Res.* **2014**, *61*, 77−85.
(22) Rashid, N.; Ur Rehman, M. S.; Sadiq, M.; Mahmood, T.; Han, J. I. Current Status, Issues and Developments in Microalgae Derived Biodiesel Production. *Renewable Sustainable Energy Rev.* **2014**, *40*, 760−778.
(23) Rittman, B. E.; McCarty, P. L. *Environmental Biotechnology: Priniciples and Applications*; McGraw-Hill: Boston, 2001.
(24) van de Graaf, A. A.; de Bruijn, P.; Robertson, L. A.; Jetten, M. S. M.; Kuenen, J. G. Autotrophic Growth of Anaerobic Ammonium-oxidizing Micro-organisms in a Fluidized Bed Reactor. *Microbiology* **1996**, *142*, 2187−2196.
(25) Karya, N.; van der Steen, P.; Lens, P. N. L. Photo-oxygenation to Support Nitrification in an Algal-bacterial Consortium Treating Artificial Wastewater. *Bioresour. Technol.* **2013**, *134*, 244−250.
(26) Lipschultz, F.; Wofsy, S. C.; Fox, L. E. The Effects of Light and Nutirents on rates of Ammonia Tranformation in a Eutrophic River. *Mar. Chem.* **1985**, *16* (4), 329−341.
(27) Zhang, T. Dynamics of Fluid and Light Intensity in Mechanically Stirred Photobioreactor. *J. Biotechnol.* **2013**, *168*, 107−116.
(28) Yoshioka, T.; Saijo, Y. Photoinhibition and Recovery of NH_4^+ Oxidinzing Bacteria and NO_2^- Bacteria. *J. Gen. Appl. Microbiol.* **1984**, *30* (3), 151−166.
(29) Egli, K.; Fanger, U.; Alvarez, P. J. J.; Siegrist, H.; van der Meer, J. R.; Zehnder, A. J. B. Enrichment and Characterization of an Anammox Bacterium from a Rotating Biological Contactor Treating Ammonium-rich Leachate. *Arch. Microbiol.* **2001**, *175* (3), 198−207.
(30) Delgado Vela, J.; Stadler, L. B.; Martin, K. J.; Raskin, L.; Bott, C. B.; Love, N. G. Prospects for Biological Nitrogen Removal from Anaerobic Effluents During Mainstream Wastewater Treatment. *Environ. Sci. Technol. Lett.* **2015**, *2* (9), 234−244.
(31) van der Star, W. R. L.; Abma, W. R.; Blommers, D.; Mulder, J. W.; Tokutomi, T.; Strous, M.; Picioreanu, C.; Van Loosdrecht, M. C. M. Startup of Reactors for Anoxic Ammonium Oxidation: Experiences from the First Full-scale Anammox Reactor in Rotterdam. *Water Res.* **2007**, *41*, 4149−4163.
(32) Jin, R. C.; Yang, G. F.; Yu, J. J.; Zheng, P. The Inhibition of the Anammox Process: A Review. *Chem. Eng. J.* **2012**, *197*, 67−79.
(33) Hendrickx, T. L. G.; Kampman, C.; Zeeman, G.; Temmink, H.; Hu, Z.; Kartal, B.; Buisman, C. J. N. High Specific Activity for Anammox Bacteria Enriched from Activated Sludge at 10 °C. *Bioresour. Technol.* **2014**, *163*, 214−221.
(34) Ohmori, M.; Ohmori, K.; Strotmann, H. Inhibition of Nitrate Uptake by Ammonia in a Blue-Green Alga, Anabaena cylindrica. *Arch. Microbiol.* **1977**, *114*, 225−229.
(35) Haines, K. C.; Wheeler, P. A. Ammonium and Nitrate Uptake by the Marine Macrophytes *Hypnea Musvuformis* (Rhodophyta) and *Macrocystis Pyrifera* (Phaeophyta). *J. Phycol.* **1978**, *14* (3), 319−324.
(36) Halterman, S. G.; Toetz, D. W. Kinetics of Nitrate Uptake by Freshwater Algae. *Hydrobiologia* **1984**, *114* (3), 209−214.

DOI: 10.1021/acs.estlett.6b00034
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX