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INVESTIGATING AMMONIUM TOXICITY IN THE PERFORMANCE OF RECIRCULATING PERIPHYTIC ALGAL WASTEWATER TREATMENT SYSTEMS

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APPROVAL PAGE

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ABSTRACT

Freshwater nutrient enrichment from wastewater facilities and other sources can lead to freshwater eutrophication, a threat to global aquatic ecosystems. Mechanical and chemical ways to curb this threat are either too expensive or not sustainable, and thus, not feasible. Compared to mechanical and other methods, sustainable, inexpensive biological methods (for example, algal treatment systems) have therefore been developed for the removal of excess nutrients from wastewater. By design, secondary waste treatment facilities (WWTF) remove organics and solids and lower oxygen demanding substances; however, they may not remove enough nutrients to protect freshwater ecosystems in all cases. While algae-based biological methods have proven successful in treating primary and secondary wastewater, less is known about the use of algae in treating biodigester filtrate. Since biodigester filtrate is characterized by elevated concentration of ammonia and high pH, it may inhibit algae in algal treatment systems. An experiment was conducted to test the hypothesis that diluting biodigester filtrate concentration improves algal biomass production and nutrient removal in recirculating algal treatment systems. Three concentrations of biodigester filtrate were created with different volumes of secondary wastewater (1:7, 1:14, and 1:28 for high, medium, and low concentrations respectively). The results showed algae were inhibited by high concentrations of biodigester filtrate. One possible explanation for the result is ammonia/ammonium toxicity. To test this hypothesis, a second experiment that added ammonium chloride to diluted biodigester filtrate (target concentrations of 20mg/L, 40mg/L, and 80mg/L NH_3-N for control, low and high treatments respectively) was conducted. Eight replicates recirculating floways of each treatment were operated for 21days. Total algal biomass production and nutrient removal were higher in control than in the elevated ammonium chloride treatments. Results from this study clearly demonstrate that ammonium toxicity is an inhibitory factor in algal productivity. This study shows the feasibility of using algal wastewater treatment systems to treat highly concentrated biodigester filtrate if the filtrate is diluted prior to treatment.

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Contents

INTRODUCTION

Global human population is predicted to exceed 9 billion by 2050 (Rahimifard et al., 2013). As population continues to increase, humans continue to degrade fresh water by discharging pollutants into aquatic ecosystems from wastewater effluent and non-point runoff. These releases include nutrients, such as nitrogen and phosphorus, that cause ecosystem-wide changes referred to as eutrophication (Hansen et al., 2000).

Eutrophication fundamentally changes the biotic structure and function of aquatic ecosystems. An example of a change in function is the increase in biomass of primary producers. Eutrophication is characterized by excessive algal growth. Anoxia, the loss of available oxygen in aquatic ecosystems, is another common side-effect of eutrophication (Ladwig et al., 2021). The death of fishes and some aquatic invertebrates caused by anoxia is one of the most dramatic manifestations of eutrophic and hypereutrophic aquatic ecosystems (Camargo and Alonso 2006). Eutrophic ecosystems also typically experience high turbidity and limited underwater light availability (Gordon et al., 2006). In lentic ecosystems, these conditions can result in the loss of benthic macrophytes and cause the lakes to shift to a phytoplankton dominated water column (Smith and Schindler, 2009). Eutrophication stresses the planet's finite global freshwater resources (Holden, 2013) like rivers.

To protect the biotic integrity of freshwater aquatic ecosystems, it is therefore important to regulate the release of nitrogen (N) and phosphorus (P), the primary causes of eutrophication (Kaiser, 2001). An important step to solving the problem of freshwater eutrophication is the identification of the source of nutrient pollution. Reducing non-point source pollution is typically challenging because nutrient pollutants are generally diffuse across the landscape and their location within a watershed, for example, agricultural fields, parking lots, impervious surfaces can be difficult to identify and mitigate (Carpenter et al., 1998). Conversely, a practical approach to ecological remediation is to remove nutrients from point sources. Point sources emit from a single known location (examples include pipes discharging from a factory or wastewater treatment plant). Point sources of nutrients are easier to identify and less complicated to treat. One potentially controllable source of nutrients is municipal wastewater (Boesch 2002) typically processed by wastewater

treatment facilities. Wastewater treatment facilities are the largest point source contributor of nutrient loads to streams, contributing over 94 percent of the total nitrogen load for the conterminous United States (Skinner & Maupin, 2019).

While primary and secondary treatment techniques at wastewater treatment plants are effective at removing oxygen-demanding substances, nutrient removal is mainly accomplished using tertiary techniques. In the United States of America, only about 32% of wastewater treatment facilities use tertiary treatment to remove nutrients due to its prohibitive cost (EPA 2013). The Clean Water Act (CWA 1972), administered by the EPA, made it unlawful to discharge any pollutant from a point source into the nation's waters without a permit. Strict enforcement of the Clean Water Act may require wastewater treatment facilities to reduce the nutrients in their discharge effluent.

The South Columbus Water Resource Facility (SCWRF) is a regional facility serving the city of Columbus and the community of Fort Benning. It has a treatment capacity of $3.8x10^7$ liters of wastewater per day. The facility uses the primary and secondary treatment processes to remove dissolved carbon and suspended solids but fails to completely remove the nutrients in wastewater. Consequently, it is an ideal place to investigate novel nutrient removal technologies. One of the most concentrated sources of nutrients is the filtrate from the biodigester treatment process. The solids collected during primary and secondary treatments are often degraded into sludge using anaerobic digestion. The supernatant from the sludge thickening process is the biodigester filtrate or side stream, a highly corrosive and nutrient-rich liquid. Biodigester filtrate has high oxygen-demanding ammonia content and does not meet the standards of the Environmental Protection Agency for discharge into aquatic ecosystems. Thus, biodigester filtrate requires additional treatment.

To further treat filtrate and achieve nutrient reductions, the South Columbus Water Resource Facility (SCWRF) would have to incorporate a new treatment technique. Currently, the SCWRF sends the filtrate back into the treatment process at the head of the plant. This recirculation of biodigester filtrate complicates the nutrient removal of the facility by constantly enriching and reintroducing large quantities of phosphorus and nitrogen into the treatment process. It also has the potential to corrode the assets of the

treatment facility (for example, pipes, pumps and motors). By comparison, the average ammonia concentrations for biodigester filtrate and secondarily treated wastewater collected and analyzed by Columbus Water Works between July and September 2018, were 844.9 mg/L for biodigester filtrate and 1.06 mg/L for secondarily treated wastewater respectively while the phosphorus concentrations were 129.5 mg/L for biodigester filtrate and 2.37 mg/l secondarily treated wastewater respectively (Table 1).

Although membrane techniques have the capacity to solve the problem of nutrient enrichment in biodigester filtrate, the high cost associated with its installation and operation (Judd 2017) limits the adoption of this technology. An alternative way of mitigating the problem is to bioremediate the biodigester filtrate. Bioremediation is the use of biological organisms (e.g., plants, algae, and bacteria) to help remove or detoxify pollutants in the environment. The use of algae to bioremediate wastewater has a long history beginning in the early 20th century (Sukačová & Červený, 2017), but it has gained considerable scientific attention (Zhao et al., 2009, Christenson, & Sims, 2011; Salama et al., 2017).

Using algae in (ATS) system is a type of water treatment technology that utilizes various species of algae to remove excess nutrients, pollutants, and organic matter from water sources. Algal turf scrubber systems are designed to mimic natural processes where algae grow and thrive, absorbing nutrients like nitrogen and phosphorus while producing oxygen through photosynthesis (Yun, et al., 2015). Algal turf scrubber systems have been primarily used for wastewater treatment, nutrient removal from agricultural runoff, and improving water quality in ponds and lakes (Craggs, 2001). My thesis studied one approach of bioremediation that involves the use of filamentous algae (i.e., algal turf scrubbers™, Adey, 2013) for the remediation of highly concentrated wastewater such as biodigester filtrate.

In this study, I investigated factors affecting the performance of recirculating periphytic algal treatment systems for treating biodigester filtrate. My primary question was would chemical characteristics of biodigester effluent, such as high ammonia concentrations, be toxic to algae and impair treatment system performance. This question was tested in two separate experiments. The first experiment tested the hypothesis that

algal system performance improves when highly concentrated biodigester filtrate is diluted. The second experiment was designed to test the hypothesis that algal biomass accumulation will be lower in treatments with higher ammonia concentrations than in treatments with lower ammonia concentrations. The results of these experiments can be used to increase effectiveness of algal treatment systems used to treat highly concentrated biodigester filtrate and to reduce nutrients discharged to receiving water bodies.

METHODS

General Experimental Conditions

To successfully accomplish this study, biodigester filtrate was obtained from the South Columbus Water Resource Facility (SCWRF). The SCWRF (32° 23' 52" N 84° 57' 15" W) houses three biodigesters each with the capacity to hold and digest $4.5x10^6$ liters of biosolids, fats, oils, and grease turning them into sludge. Sludge was separated from the supernatant using a belt-press. Samples of SCWRF biodigester filtrate and secondarily treated wastewater were filtered using Drain-Sleeve® silk sock to reduce flocculants and were collected twice (October 18th, 2018 and November 10th, 2019) to supply material for two different experiments. Samples were transported to Columbus State University where experiments were conducted in the laboratory under controlled environmental conditions (Columbus, Georgia, USA).

Experimental Recirculating Floways (i.e., miniature algal treatment systems)

Rectangular-shaped floways (61cm x 121cm, height x length) made of polyvinyl chloride pipes (PVC: 5.1cm, inside pipe diameter) were used to conduct experiments (Fig. 1). Each floway had a 7.6cm wide opening on top to allow the penetration of light to the algae. To facilitate algal biofilm attachment, floways were lined with unglazed ceramic tiles. Circulation of wastewater in the floways was induced using bubbles created by compressed air and an aeration stone (Fig. 1). Flow rates were standardized across treatments using either a clear plastic strip (5cm long x 0.5mm wide x 0.1mm thick, experiment 1) or a plastic V-shaped weir inserted at the downstream end of the floways (experiment 2). Floway volumes were maintained by adding Milli-Q® Ultrapure filtered water as needed to account for evaporation. To stimulate photosynthesis, full-spectrum fluorescent lights [GE™ F40 T12, 1.2 m x 38.1mm (length x diameter)] were used to illuminate the floways (Fig. 2). The lights were controlled by a timer. Because algae colonized and grew naturally in the biodigester filtrate-wastewater mix, it was unnecessary to add additional algal propagules.

Experiment 1: The influence of biodigester filtrate concentration on algal treatment effectiveness

Experimental Design and Setup

To examine the influence of biodigester filtrate concentration on algal treatment effectiveness, this experiment tested algal growth in three ratios of biodigester filtrate to wastewater: high concentration (1:7), medium concentration (1:14) and low concentration (1:28). Each treatment was evaluated using four replicate, recirculating floways (Fig. 1) for a total of 12 floways. The experiment was conducted for three weeks (October 3rd through 24th 2018).

To conduct this experiment, biodigester filtrate samples were diluted with secondary treated wastewater before placing 7L of the appropriate concentration (low, medium, high) into each floway. Floways were lined using 40 salmon-colored unglazed (5cm x 5cm square), ceramic tiles to serve as substrates for algal attachment. The 12 floways were bound together and covered with six pairs of fluorescent grow lamps to evenly distribute light to stimulate photosynthesis. A timer was connected to the grow lamps and set for 15 hours light and 9 hours dark to simulate Georgia's summer light conditions.

To quantify algal treatment system performance, the floways were sampled for algal biomass and nutrient concentrations. Water samples for orthophosphate, ammonia, and nitrate were collected in small vials (60mL) from each floway once each week for a total of four times. Samples were stored at 4ºC until analyzed (within 48 hr). Temperature and pH were measured using a calibrated Hach EC-10 pH meter three times a week. Algal biomass was measured from samples collected on days 7, 14, and 21. Two rows of algae-covered tiles were randomly selected, placed in Whirl-Paks®, and stored frozen at -4°C until analysis. One tile was used for quantifying volatile solids and dry mass; and the second tile served as a backup.

Experiment 2: The influence of ammonium in biodigester filtrate on algal treatment effectiveness

Experimental Design and Setup

To examine the influence of ammonium on algal treatment system effectiveness, an experiment was conducted that varied the concentration of ammonium in diluted biodigester filtrate. Biodigester filtrate concentration was diluted with secondary wastewater to create the equivalent of the lowest ammonia concentration used in experiment 1 (~20mg/L-N). The target ammonium concentrations for this second experiment were 20mg/L-N (control, n=8), 40mg/L-N (low, n=8), and 80mg/L-N (high, n=8). To make the needed concentrations, eight floways were spiked with 0, 477mg, or 1,401mg of ammonium chloride. To replicate the conditions from experiment 1, the ammonia/ammonium concentrations in the control were allowed to vary naturally. In contrast, ammonia/ammonium concentrations in the low and high treatments were supplemented when depleted by more than 10 mg/L (Table 2) as measured using an ion-selective ammonia probe. The experiment was conducted for three weeks (November 11th through 28th 2019).

The setup of the experiment was similar to that used in experiment 1. However, in this experiment, floways were lined using 17 2.5cm by 5cm peach-colored unglazed, ceramic tiles that served as substrates for algal attachment. Tiles were covered with a piece of silver-gray PVC fiberglass screen (~1 mm mesh). The tile and mesh were held together using black, silica bands. The mesh functioned as a substrate for algal biofilm attachment. Treatments were assigned randomly to prevent location effects. Floways were bound together into two sets of 12. Each set was covered with seven pairs of grow lights to distribute light and stimulate photosynthesis. Grow lamps were placed on a timer set for 14 hours light and 10 hours dark to simulate Georgia's summer light conditions.

The floways were sampled for algal biomass and nutrient concentrations to quantify the effects of ammonium on algal treatment system performance. Water samples were collected from each floway weekly starting the first day of the experiment and were preserved using concentrated sulphuric acid (pH<2) and stored at 4ºC before analysis.

Algal biomass was determined from tile samples collected on days 7, 14, and 21. Three rows of algae-covered tiles were picked at random, placed in Whirl-Paks®, labeled and stored frozen at -4°C until analysis. One tile was used for quantifying volatile solids and dry mass, the second for identification of algae, and the third for backup. All algal samples were stored frozen at -4°C in Whirl-Paks® before being analyzed within 30 days of collection. The dominant algal taxa were identified from digital photos collected from wet mounted slides viewed using a Motic® Panthera Brightfield Microscope (100-400x magnification).

Measuring Algal Biomass

To quantify dry mass and volatile solids, pre-rinsed Wyvern Scientific filters (0.7 µm porosity, GF/F 90 mm circular diameter) were placed in aluminum pans and ignited at 550ºC in a muffle furnace for 15 minutes to remove organics. Ashed aluminum pans with filters were cooled to room temperature in Drierite™ filled desiccators and weighed to the nearest 0.1mg using an Ohaus Explorer® Analytical Balance**.** Moisture interference during weighing was minimized using recharged Drierite™ placed inside the corners of the scale's weighing chamber.

To prepare the algae for weighing, tiles-covered with algae were scraped using a nylon-bristled brush. The tile contents and brush were then rinsed onto filters using Milli-Q® water. Filtered algae were oven dried at 105ºC for 24 hours and weighed to the nearest 0.1mg using an Ohaus Explorer® Analytical Balance for algal dry mass. Dry mass filters were heated to 550ºC for 30 minutes in a muffle furnace to determine volatile solids. Ashed filters were cooled in desiccators and then weighed to the nearest 0.1mg (Furnish & Keller, 2020).

Water Chemistry Analyses

Treatment effects on water chemistry were quantified by assessing orthophosphate and nitrate concentrations in floways. Orthophosphate samples were analyzed using a molybdate ascorbic acid colorimetric reaction following Hach® Method 8048 within 48 hours to minimize sample degradation. Other preserved samples were neutralized using sodium hydroxide (5 N) before analysis (within 30 days of collection). Nitrate (NO_3-N)

concentrations were measured using a cadmium reduction method following Hach® Method 8039. In accordance with EPA Method 350.3, ammonia concentrations were analyzed using an Oakton Ion 6+ ion selective electrode (Thomas, and Booth, 1973). The pH of each floway was recorded every three days using a Hach® EC10 pH probe. The pH probe was recalibrated before use on each occasion using pH standard solutions 4.01, 7.00, and 10.01.

Statistical Analysis

The results of both experiments were compared among treatments using repeated measures analysis of variance models (RM ANOVA). Employing this statistical model was necessary because the experiment involved making multiple measurements from each floway throughout each experiment (Keselman et al., 2001). Pairwise, post-hoc comparisons were made using Bonferroni and Tukey corrections to minimize the likelihood of the inflation of statistical significance. Alpha was set to a probability of 0.05. For RM ANOVA results, Greenhouse-Geiser (GG) corrections were used when Mauchly's Test was statistically significant—an indication that the assumption of sphericity was not valid. All statistical analyses were performed using IBM SPSS® version 26 (Cai et al., 2019).

RESULTS

Experiment 1: The influence of biodigester filtrate concentration on algal treatment effectiveness

General Experimental Conditions

Testing conditions were compared to assess the validity of the experimental setup. Average water temperature throughout the experiment was 24.4ºC (24.3-24.5ºC, 95% confidence interval, Fig. 3A). Average water temperature differed across time (RM ANOVA, $F_{2,18}$=632.47, p<0.001) and over time by treatment (RM ANOVA, $F_{4,18}$=3.41, p=0.03). However, it did not differ across treatments (RM ANOVA, $F_{2,9}$=1.45, p=0.28). Water temperatures dropped by 1ºC in week 2 and by 1.5ºC in week 3 (RM ANOVA, Bonferroni p<0.001 for all). Even though there was a significant interaction between time and treatment, average temperature among the treatments did not differ on any day of measurement (RM ANOVA, Bonferroni p≥0.16).

The pattern of water pH variability differed from that of temperature. The pH differed statistically across time (RM ANOVA-GG, $F_{1.3,12.3}$ =475.13, p<0.001), treatment (RM ANOVA, $F_{2,9}$=28.31, p<0.001) and time by treatment interaction (RM ANOVA-GG, $F_{2.7,\ 12.3}$ =8.93, p=0.002). Average pH on the first day of the experiment was 8.36 but pH declined to 5.40 by the final day of the experiment (Fig. 3B). Water pH differed statistically across the dates of sampling (Bonferroni p≤0.03 for all). Similarly, all treatments differed statistically (Bonferroni p≤0.024 for all). The low concentration had a pH of 6.39 while the high concentration had a pH of 7.41. The pattern of decline in pH was not consistent in all concentrations. In the low concentration treatments, water pH declined progressively till day 14 and then stabilized by day 21. In the high concentration, pH increased slightly on day 7 and then declined steadily while in the medium concentration, pH consistently decreased (Fig 3B).

Initial ammonia concentrations differed across biodigester filtrate treatments (ANOVA, p<0.001 for all). On day one, the medium and low concentration treatments had an average ammonia concentration 61% and 83% lower (respectively) than the high concentration treatment (Bonferroni, p<0.001 for both). Although medium concentration

had a higher ammonia concentration than the high concentration treatment, it was not statistically significant (Bonferroni, p=0.103). The high concentration treatment filtrate had a mean ammonia concentration of 115.6 mg/L (Fig. 4A). Initial phosphate concentrations in the medium and low concentration treatments averaged 41% and 62% respectively lower than the high concentration biodigester filtrate (Bonferroni, $p<0.001$ for both). The low concentration treatment had 36% lower ammonia concentration than the medium concentration treatment (Bonferroni, $p<0.001$). The least diluted biodigester filtrate had a mean phosphate concentration of 86.6 mg/L (Fig. 4B). Nitrate concentration, on the other hand, in the low concentration was 16% and 20% lower than the high (Bonferroni, p=0.14) and medium (Bonferroni, p=0.019) concentrations respectively. The medium concentration had an average nitrate concentration of 9.925mg/L (Fig. 5A). Across time and treatment, nitrate was statistically significant (RM ANOVA-GG $F_{1.73,15.58}=130$, $p<0.001$ and RM ANOVA $F_{2,9} = 6.4$, $p = 0.19$ respectively). Furthermore, the treatment and time interaction showed statistical significance for nitrate concentration (RM ANOVA-GG $F_{3.46,15.58}=13.704$, $p<0.001$).

To test the influence of biodigester filtrate concentrations on algal treatment effectiveness, algal biomass production rate was measured (Fig. 5B). There were three levels of concentration treatments of biodigester filtrate to wastewater: low (1:7), medium (1:14) and high concentrations (1:28). Across all concentrations, algal biomass growth rate differed (RM ANOVA $F_{2,9}=9.72$, $p=0.006$). The high concentration treatment had an average 62% and 60% (respectively) lower algal dry biomass than the medium and low concentration treatments (Bonferroni, $p<0.017$ for both). There was no statistically significant difference in algal biomass growth rate over time (RM ANOVA $F_{2,8}=2.763$, $p=0.09$). Furthermore, the concentration treatment and time interaction showed no statistical significance for algal dry mass growth rate (RM ANOVA $F_{4,18}=1.543$, $p=0.232$).

Unlike the algal biomass growth rate data, phosphate uptake i.e., mass of phosphorus removed over the course of the experiment showed no statistically significant differences among all concentrations (ANOVA $F_{2,9}=3.489$, $p=0.076$).

Experiment 2: The influence of ammonia in biodigester filtrate on algal treatment effectiveness (Main Study*)*

General Experimental Conditions

Treatments were compared to ensure consistency of testing conditions. Average water temperature throughout the experiment was 25.9ºC (25.8-25.9ºC, 95% confidence interval, Fig. 6A). Average water temperature did not differ statistically across treatments (RM ANOVA, $F_{2,21}$=0.858, p=0.439), time (RM ANOVA, $F_{2,42}$=0.684, p=0.510), and their interaction (RM ANOVA, $F_{4,42}$=0.762, p=0.556). The average pH differed across treatments (RM ANOVA-GG, $F_{2,21}$=437.412, p<0.001) and time (RM ANOVA-GG, $F_{1.4,42}$=14.861 p<0.001). There was no difference in pH between weeks 1 and 2 (RM ANOVA, Bonferroni p=0.297); however, pH in week 3 dropped by 5.6% (RM ANOVA, Bonferroni p=0.006), and 6.9% (RM ANOVA, Bonferroni p<0.001) from weeks 1 and 2 respectively. The interaction between time and treatment did not differ statistically (RM ANOVA-GG, $F_{2.8,42}$=0.546 p=0.644). The range of average pH values were 7.9-8.5, 5.3-5.8 and 5.4-5.7 for the control, low and high treatments respectively (Fig. 6B).

After the addition of ammonium chloride, ammonia concentrations differed significantly across treatments (RM ANOVA, $F_{2,21}$=1307.5, p<0.001). Ammonia concentrations continued to vary over time (RM ANOVA, $F_{3,63}$=11.73, p<0.001). There also existed a significant interaction between treatment and time (RM ANOVA, $F_{6,63}$=35.99, p<0.001). The control treatment had 85% and 94% lower ammonia concentration than the low and high ammonia treatments respectively (Bonferroni, p<0.001 for both). The low ammonia treatment had 60% lower ammonia concentration than the high ammonia treatment (Bonferroni, p<0.001). On average, ammonia concentrations were generally consistent across time, only day 1 differed statistically from day 7 (14% lower than day 1) and day 14 (13% lower than day 1, Bonferroni, $p \leq 0.001$ for both). Time by treatment interaction showed that whereas ammonia concentrations declined progressively in the control across time, the low and high treatments were relatively constant (Fig. 7A).

Average orthophosphate concentrations differed significantly across treatments (RM ANOVA, $F_{2,21}$=6.64, p=0.006), time (RM ANOVA-GG, $F_{2.27,47.71}$=197.23, p<0.001), and their interaction (RM ANOVA-GG, $F_{4.54,47.71}$=4.27, p<0.001). The control treatment had 18% and 20% lower orthophosphate concentration than the low and high ammonia treatments respectively (Bonferroni, p<0.001 for both). The low ammonia treatment had 3% lower orthophosphate concentration than the high ammonia treatment, but this result was not statistically significant (Fig. 4C, Bonferroni, p=1). There was a general decline in phosphate concentrations over time (Fig. 8A). Orthophosphate concentrations were not significant on day 1 across all treatments, however, relative to day 1, the control dropped 9% by day 7, 72% by day 14 and 78% by day 21 (Bonferroni, p≤0.001 for all). There was no detectable statistical difference in phosphate concentrations between the low and high ammonia treatments on any of the days measured (Bonferroni, p=1 for all).

Average nitrate concentrations differed across treatments (RM ANOVA, $F_{2,21}$=19.71, p<0.001), time (RM ANOVA-GG, $F_{2.155,45.254}$=94.29, p<0.001), and their interaction (RM ANOVA-GG, $F_{4.31,45.25}$=8.55, p<0.001). The control had 61% and 60% lower nitrate concentrations than the low and high ammonia treatments respectively (Bonferroni, p<0.001 for both). There was no statistical difference in average nitrate concentrations between the low and high treatments (Bonferroni, p=1). Nitrate concentrations differed statistically on all days except for days 7 and 14 (Bonferroni, p = 1). Relative to day 1, nitrate concentration dropped 35% by day 7, 39% by day 14 and 52% by day 21 (Bonferroni, p≤0.001 for all). The control showed a steady decline in nitrate concentration that was not apparent in the low and high ammonia treatments (Fig 7B.) Over the 21-day experiment, nitrate concentration in the control dropped 78%; alternatively, the low and high ammonia treatments declined 39% (Bonferroni, p<0.001 for all).

An important test of the effect of spiking wastewater with ammonium chloride on algal treatment system performance is to examine treatment effects on algal biomass production. Algal dry mass differed statistically across treatments (RM ANOVA $F_{2,21}$=5.42, p=0.013) and across time (RM ANOVA-GG $F_{1.49,31.29}$=9.073, p<0.001). The treatment by time interaction was not statistically significant (RM ANOVA-GG

$F_{2.98,31.29}$=0.965, p=0.421). The low and high treatments had 49% and 43% lower algal dry mass (respectively) than the control treatment (Bonferroni, p≤0.048 for both). There was no statistically significant difference in algal dry mass between the low and high treatments (Bonferroni, p=1). There was a general increase in algal dry mass across time (Fig 8B). Algal dry mass on day 7 was 75% less than day 14 and 79% less than day 21 (Bonferroni, p≤0.021 for both). Algal dry mass was statistically indistinguishable in the low and high ammonia treatments (Bonferroni, p=1), except in week 3 between the control and the low ammonia treatments (Bonferroni, p=0.036).

Unlike the algal biomass growth rate data, phosphate uptake i.e., mass of phosphorus removed over the course of the experiment showed no statistically significant differences among all concentrations (ANOVA $F_{2,9}$=3.489, p=0.076).

The experimental floways were dominated by two genera of filamentous green algae: *Ulothrix* and *Microspora* (Fig. 9A & B). *Ulothrix* is a genus of non-branching, broad and long celled low temperature thriving algae generally found in fresh and marine water during the spring and winter seasons. *Microspora* are unbranched cylindrical algae with cells having lamellate walls which sometimes are contracted at the cross wall. The control floways also had noticeable colonies of *Scenedesmus* (Fig. 9C), a colonial planktonic algae. Diatoms were also present, especially *Nitzschia palea* (Fig. 9D). *Nitzschia palea* are species of diatoms associated with freshwater habitat. Although they were present, *Nitzschia* was not abundant.

Discussion

The goal of this study was to investigate ammonia toxicity in the performance of recirculating periphytic algal treatment systems. There is a need to better understand the value of using algae to treat ammonia-rich, highly concentrated filtrate extracted from biodigesters. There were two experiments that were implemented in which algal biomass responses to ammonium (NH_4) were observed. In the first experiment, biodigester filtrate (characterized by concentrated ammonia) was diluted with secondary treated wastewater (1:7 – 1:28, biodigester filtrate:wastewater) to determine how filtrate concentration influences treatment performance. Consistent with the initial hypothesis, ammonium inhibits algal biomass production, algal biomass accumulation was greater in the medium and low concentrations, where more wastewater was used to dilute biodigester filtrate. Whereas it can be inferred that ammonium inhibited algae production in the minimally diluted treatments, this effect could not be confirmed in the first experiment. This result influenced the design of experiment 2 which tested the hypothesis that ammonium in biodigester filtrate inhibits algal biomass production. To test this hypothesis, diluted biodigester filtrate was spiked with no, low, and high amounts of ammonium chloride salt. The control, without ammonium chloride added, had the highest algal biomass production, whereas the low and high ammonium treatments had 49% and 43% lower algal dry mass than the control respectively.

To confirm the patterns seen in the algal biomass results, the nutrient uptake within the treatment systems was also investigated. In experiment 1, there was no clear difference in the nutrient uptake among the high, medium, and low biodigester filtrate concentrations, even though there existed differences in algal biomass among treatments. In contrast, nutrient uptake in experiment 2 did correspond to patterns in algal biomass growth. The ammonium in the ammonium chloride-treated floways inhibited the growth of algae and reduced nutrient uptake. Parker et al., (2012) studied elevated ammonium concentrations from wastewater discharge in the Sacramento River and reported that ammonium can suppress the primary production of diatom spring blooms. Similar to Parker et al. (2012), Dugdale et al., (2007) showed that ammonium inhibited nitrate uptake and delayed diatom blooms in San Francisco Bay.

Ammonium toxicity in water can be caused by the combined effect of both the ionized ammonium (NH_4^+) and the un-ionized ammonia (NH_3, Collos & Harrison, 2014). Both forms exist in solution and their relative proportions are dependent on pH. In freshwater systems, when the pH is greater than 9.0, ammonia is the dominant species. Alternatively, when the pH is less than 8, the system is predominantly ammonium (Collos & Harrison, 2014). In experiment 2, average pH of all treatments ranged from 8.3 (control) to 6.3 (NH_4Cl added), suggesting that both ammonium and ammonia were present in our study and could have caused toxicity.

Other studies have documented effects of ammonia/ammonium on algae. For example, ammonia toxicity on the microalga, *Nephroselmis pyriformis*, a marine green alga was studied by Källqvist et al., (2003). It was found that industrial process effluent, mainly characterized by high concentrations of ammonia (432 mg/L), inhibited *Nephroselmis* growth (Källqvist et al., 2003). In a study of *Euglena* (a freshwater autotroph/heterotroph) and *Chlorella* (a freshwater green alga), Konig et al., (1987) found that un-ionized ammonia was toxic to both species at pH 9 and above. There was also a reduction in growth of both *Euglena* and *Chlorella* at pH 8.3 (Konig et al., 1987). In experiment 2, the control (pH 8.3) had an estimated 10.2% un-ionized ammonia and 89.8% ionized ammonium. Therefore, the estimated concentration of un-ionized ammonia and ionized ammonium in the control was 0.41 mg/L and 3.6 mg/L respectively. In contrast, the low and high treatments (assuming a pH 6.3) had low un-ionized ammonia (NH_3-N) concentrations 0.030 mg/L and 0.074 mg/L respectively. The low and high treatments also had 26.2 mg/L and 65.4 mg/L ionized ammonium (NH_4^+-N) respectively. Thus, in this experiment, the more abundant ammonium ions in the low and high treatments likely inhibited algae in these recirculating treatment systems.

Ammonia, pH Control and Buffer

Municipal wastewater has ammonia as a common constituent (Wang, 1991). Raw municipal wastewater may be unsafe because it contains ammonia concentrations in the range 9 to 30 mg/L, sometimes more than 50 mg/L (Ruffier, et al., 1981). Ammonia concentration increases in wastewater by the microbial hydrolysis of urea and often, by the degradation of amino acids and other nitrogenous organic matter (Shen et al., 2017).

The maximum permissible ammonia (NH_3-N) concentration of 20 mg/L in wastewater is deemed safe, however, higher concentrations of ammonia may decrease the performance of waste stabilization ponds (Konig et al., 1987). In waste stabilization ponds, ammonium uptake leads to the release of hydrogen ions decreasing pH shifting the balance toward a higher concentration of ammonium. Such a decrease in pH and an increase in ammonium may be toxic to the microalgal population (Konig et al., 1987). Therefore, it may be necessary to regulate pH using an amendment such as calcium carbonate.

Because ammonia in wastewater can volatilize into the air and create particulate pollution (Camargo & Alonso 2006), it is important to manage wastewater sources to minimize ammonia loss. In this study, the tendency for ammonia volatilization was likely rather low, because biodigester filtrate was maintained at a low pH (e.g., pH 6.3 for low and high treatments) which minimizes the availability of ammonia and limits its volatilization (Camargo & Alonso 2006). Also, the biodigester filtrate was diluted using secondarily treated wastewater, which has been shown to have nitrogen primarily in the form of nitrate (2.0 – 4.4 NO_3 – N mg/L) rather than ammonia (Furnish & Keller, 2020).

Conceptually, algae grow to the degree of available nutrients in freshwater systems thereby depleting the amount of nutrients in fresh water (Smith 2006). Hence, algal nutrient uptake could be a tool for treating wastewater (Krustok et al., 2016; Kube et al., 2020; Solimeno et al., 2017). In analyzing the nutrient uptake by algae in the experiment's treatments, there was greater nutrient uptake in the controls which had more algal biomass compared with the low and high ammonia treatments. This concurrence of nutrient uptake and algal biomass production was also documented by Cole et al., (2015), where freshwater microalgal production was associated with increases in nutrient removal. Similarly, Craggs (2001) described the gradual decline in the productivity of algae along a turf scrubber. This pattern was thought to be caused by a reduction in the availability of nutrients as the wastewater traveled along the floways.

Despite having available nutrients like nitrogen and phosphorus in this experiment, algal growth was limited in the low and high treatments. This finding provides evidence of possible ammonium toxicity. Throughout this study, light, temperature, and other potential confounding variables (e.g., grazers, substrate) were unaltered and variation in

experimental treatments came exclusively from ammonium chloride augmentation. Ammonium chloride when added to water dissociates into ammonium and chloride ions. Chloride ions are not known to affect algae, except when found as a chlorine gas (Gao, et al., 2010). Thus, a reasonable explanation for the results of experiment 2 is that ammonium, not chloride, reduced algal productivity.

Wastewater is different from freshwater lakes and streams because nutrients in freshwater, especially phosphorus, may be limited (Correll, 1999). Wastewater, due to its high nutrient concentrations, can be considered a valuable nutrient resource for growing algae (Van Der Hoek et al., 2016). Secondarily treated wastewater would be expected to be a nutrient reservoir that can support algal photosynthesis and biomass accumulation. While studies have focused on algae grown in wastewater (Mennaa et al., 2015; Krustok et al., 2016; Kube et al., 2020), less is known about using algae to treat biodigester filtrate, a more nutrient-rich wastewater source. The success of nitrogen and phosphorus management in North America may depend on the effective treatment of biodigester filtrate. Therefore, more attention should be paid to nutrient recovery from biodigester filtrate using filamentous algal treatment systems, such as those used in the present study.

SUMMARY

Wastewater is a rich source of nitrogen and phosphorus; nutrients which can support algal biomass growth and accumulation (Withers et al., 2014; Lawton et al., 2017; Xia et al., 2020). In contrast, biodigester filtrate is an even richer source of nutrients for growing algae than wastewater. Both effluents are produced by many wastewater treatment facilities. When released into the environment without treatment, both wastewater and biodigester filtrate causes ecological harm because the amount of nitrogen and other nutrients being dumped into rivers and lakes can facilitate eutrophication.

There are over 1,200 biodigesters that produce biodigester filtrate (EPA, 2022) at wastewater treatment facilities in the United States, yet few studies have examined biodigester filtrate as a pollutant or a nutrient source for algal biomass production (e.g., Tua et al., 2021). Given the high cost of employing mechanical methods, e.g., membrane filtration technologies, to treat biodigester filtrate to remove nutrients, alternative nutrient removal methods such as algal treatment systems could be beneficial to wastewater treatment facilities. Algal treatment systems could serve as a low-cost, sustainable, and feasible alternative nutrient removal technology. In light of their benefits, it is appropriate to study the limitations of algal treatment systems for use in treating biodigester filtrate.

This study of biodigester filtrate showed that ammonium toxicity inhibits algal productivity in periphytic algae wastewater treatment systems. This problem can be minimized by diluting biodigester filtrate with freshwater or secondarily treated wastewater before treatment. This study showed that treated wastewater from the secondary clarifier could serve as a source of water for dilution of biodigester filtrate. Recycling secondary wastewater is more feasible than getting EPA permits to withdraw water from natural water sources (e.g., river or lake) for the dilution process. Using water from the clarifiers for dilution will also further reduce the cost of pumping water from outside a wastewater treatment facility. This study showed that dilution is required if an algal treatment system is used for nutrient recovery, because it reduces the concentration of potentially toxic ammonia in the biodigester filtrate. It also confirmed that it is the high

levels of ammonia and ammonium that inhibit algal growth and thus algal treatment system performance.

Consistent with other research, this study showed that algal treatment systems can effectively treat wastewater by removing nutrients like nitrogen and phosphorus, which are often found in sewage. Algae can be used to reduce pollution and help prevent eutrophication in water bodies (Pizarro et al., 2006). Additionally, algae grow rapidly and produce substantial biomass. This biomass can be harvested and used for various applications, such as biofuels, soil amendments, and animal feed (Yun et al., 2015). When converted to biofuels, algae can serve as a renewable source of energy (Adey et al., 2011).

List of figures

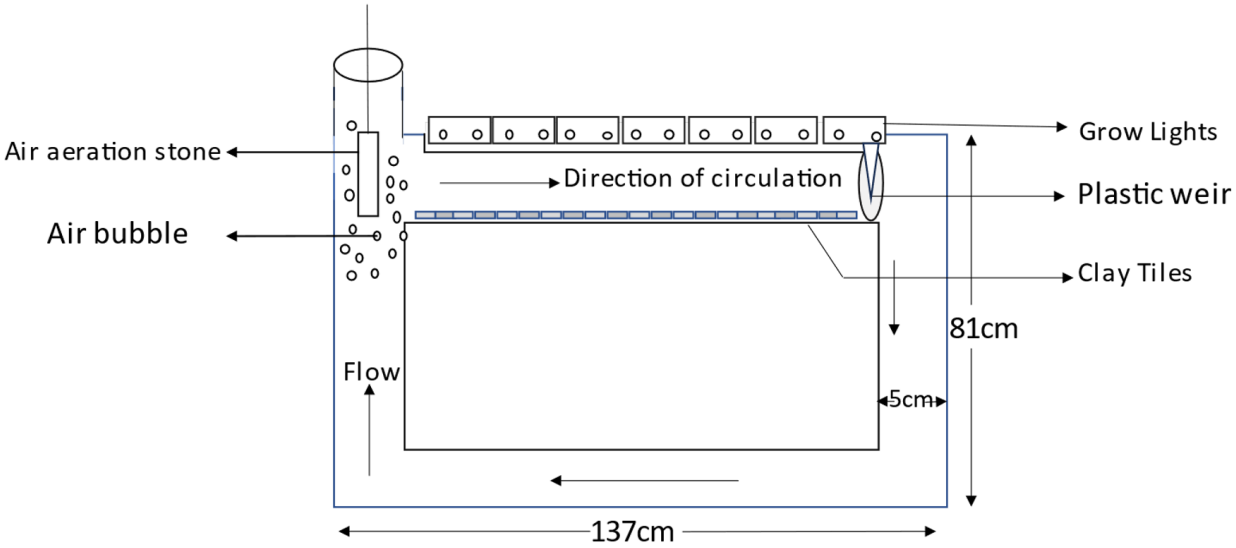

Figure1: Sideview of a recirculating PVC floway used in experiment one and two

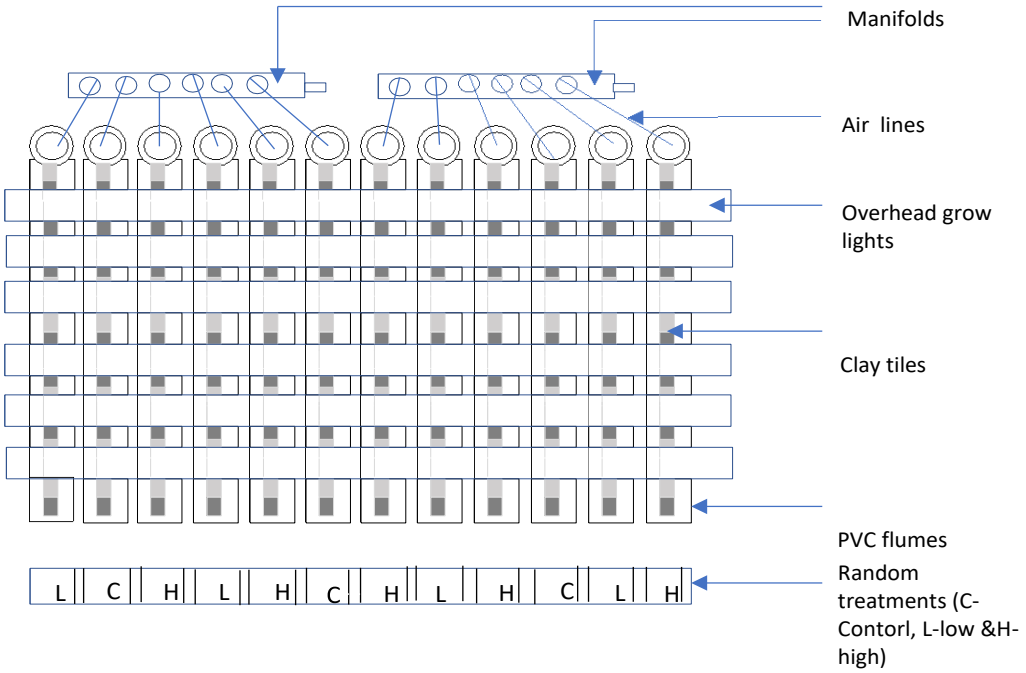

Figure 2. Aerial view of a bank of recirculating floways with the overhead grow lights as used in experiment 2

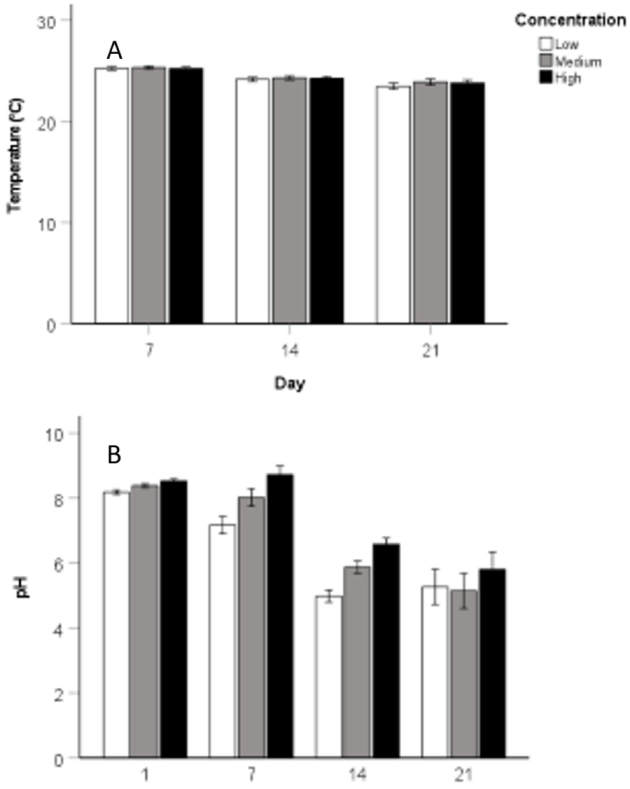

Figure 3. A - showing the temperature variation of treatments in experiment 1 and B - showing the pH variation of treatments in experiment 1. Error bars represent 95% confidence limit

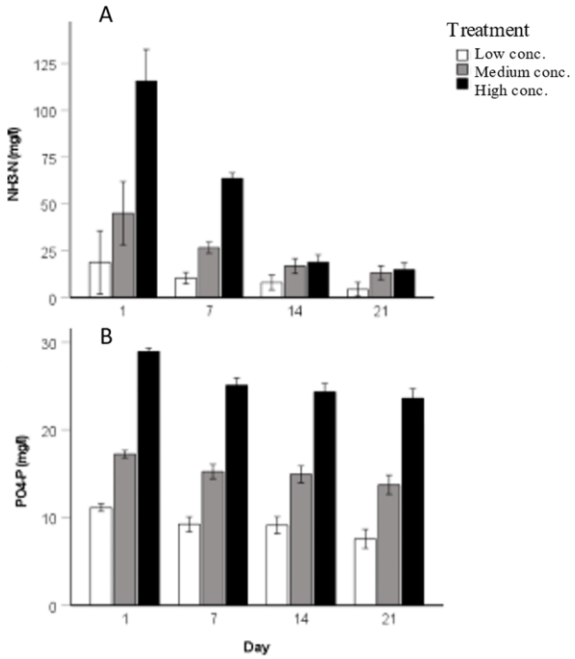

Figure 4. A - showing ammonia concentration of treatments in experiment 1 and B- showing orthophosphate concentration of treatments in experiment 1 . Error bars represent 95% confidence limit

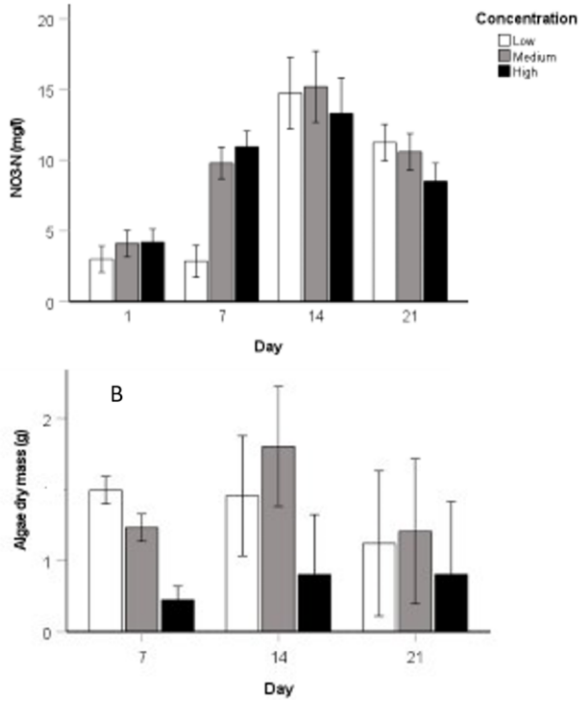

Figure 5. A - showing nitrate concentration of treatments in experiment 1 and B showing algal dry mass variation in treatments in experiment 1 . Error bars represent 95% confidence limit

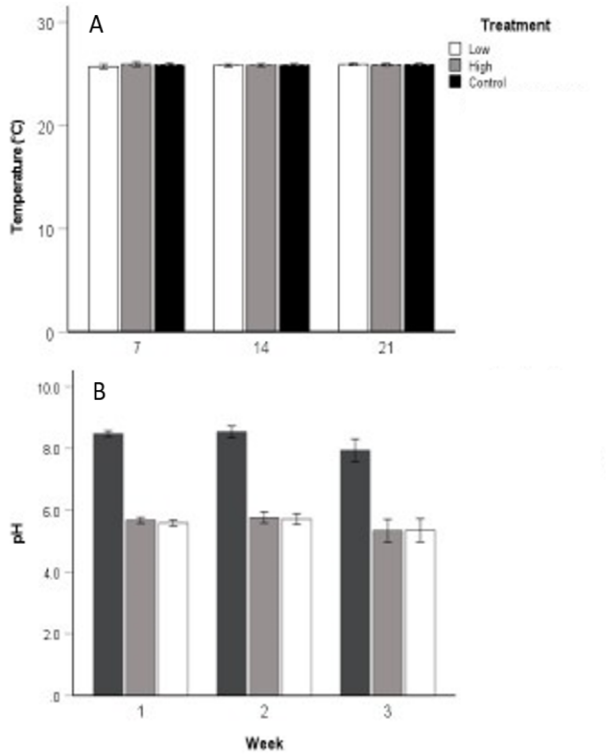

Figure 6. A - showing the temperature variation of treatments in experiment 2 and B showing the pH variation of treatments in experiment 2. Error bars represent 95% confidence limit

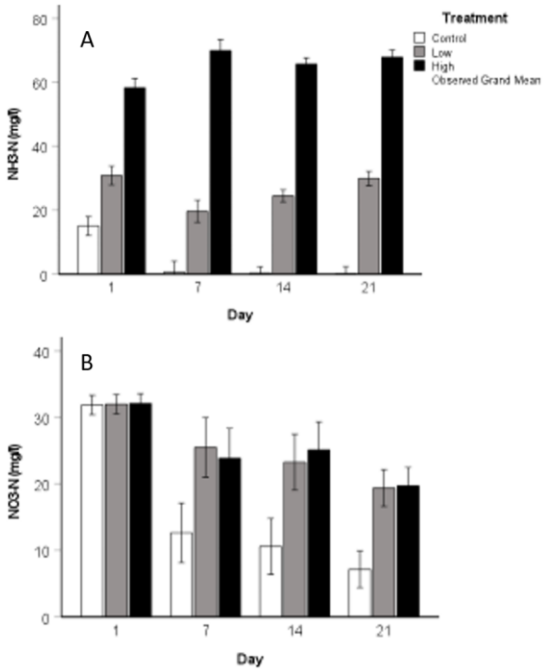

Figure 7. A - showing ammonia concentration of treatments in experiment 2 and B- showing nitrate concentration of treatments in experiment 2 . Error bars represent 95% confidence limit

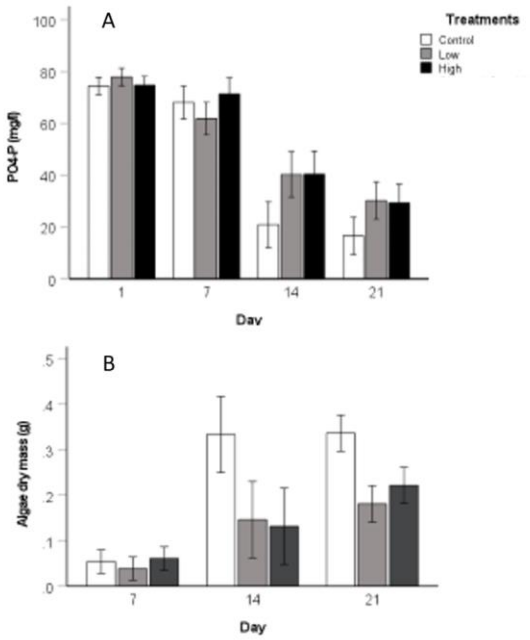

Figure 8. A - showing orthophosphate concentration of treatments in experiment 2 and B showing algal dry mass rate in treatments in experiment 2 . Error bars represent 95% confidence limit

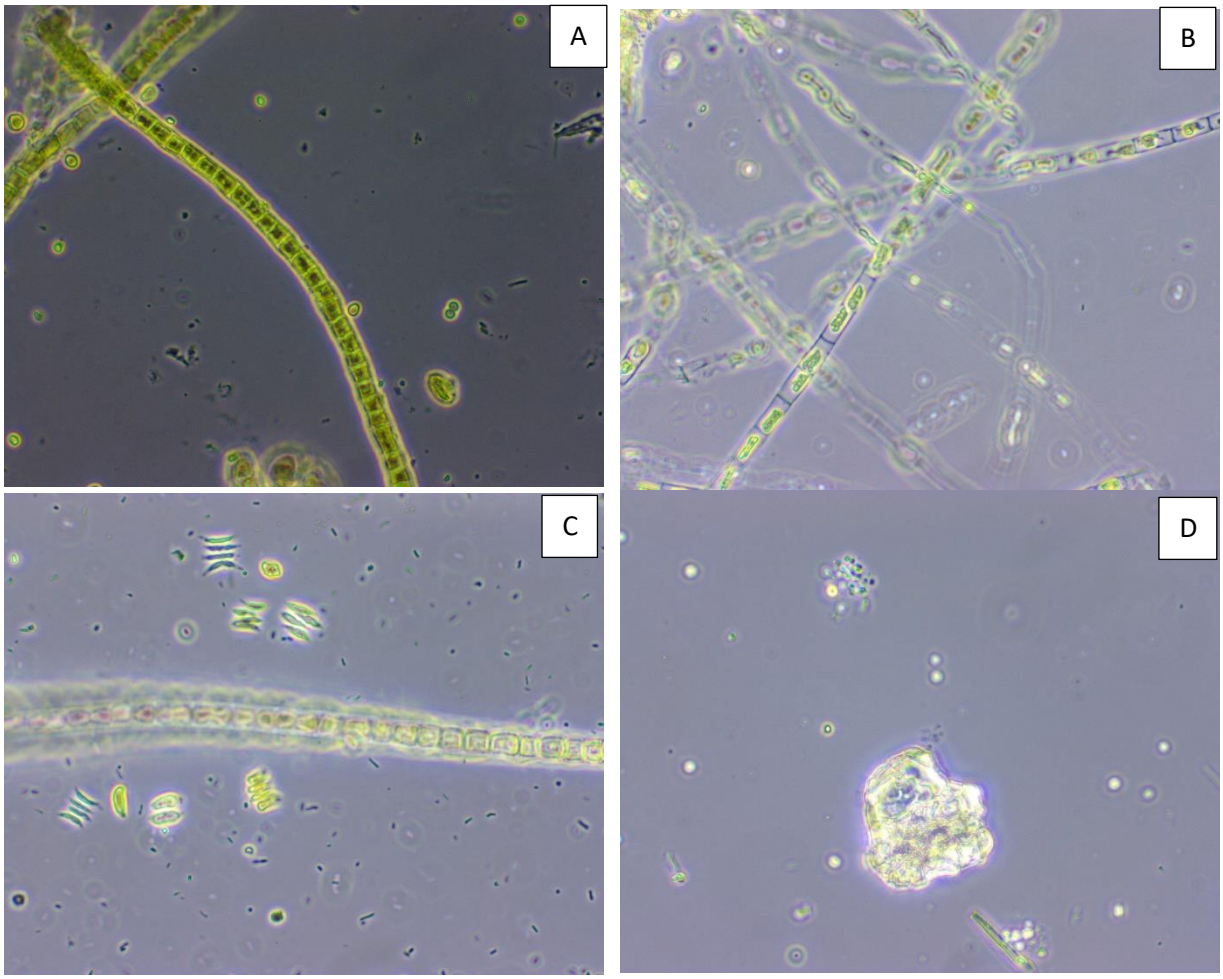

Figure 9. A – *Ulothrix*, B – *Microspora*, C – *Scenedesmus* and D – *Nitzschia palea* (Diatom)

Tables

Table 1: Characterization of secondarily treated wastewater and biodigester filtrate collected from Columbus Water Works from July to September 2018

	Secondarily treated Wastewater	**Biodigester filtrate**
Ammonia-Nitrogen (mg/L)	1.06 ± 0.49	844.88 ± 3.41
Total Phosphorus (mg/L)	2.37 ± 2.4	129.47 ± 2.67
Nitrate-nitrogen (mg/L)	6.93 ± 1.8	N/A

Table 2: Summary of ammonium chloride replenishment in treatments and replicates in experiment 2 (Low = Low treatment and High = High treatment)

Experimental Days	**Replicates and mass of replenished NH_4Cl (g)**							
	1	2	3	4	5	6	7	8
9	Low - 0.25 High - 0.23	Low - 0.50 H (0.23)	Low - 0.50 H (0.23)	Low - 0.50 H (0.23)	Low - 0.30 H (0.23)	Low - 0.50 H (0.23)	Low - 0.25 H (0.23)	L - 0.50 H (0.23)
17	Low - 0.25 High - 0.24	Low - 0.50 High - 0.24	Low - 0.50 High - 0.24	Low - 0.50 High - 0.24	Low - 0.30 High - 0.24	Low - 0.50 High - 0.24	Low - 0.25 High - 0.24	Low - 0.50 High - 0.24

REFERENCES

Adey, W. H., Kangas, P. C., & Mulbry, W. (2011). Algal turf scrubbing: cleaning surface waters with solar energy while producing biofuel. Bioscience, 61(6), 434-441

Adey, W. H., Laughinghouse IV, H. D., Miller, J. B., Hayek, L. A. C., Thompson, J. G., Bertman, S., ... & Puvanendran, S. (2013). Algal turf scrubber (ATS) floways on the Great Wicomico River, Chesapeake Bay: productivity, algal community structure, substrate and chemistry1. *Journal of phycology*, *49*(3), 489-501

Boesch, D. F. (2002). Challenges and opportunities for science in reducing nutrient over-enrichment of coastal ecosystems. *Estuaries*, *25*(4), 886–900. https://doi.org/10.1007/bf02804914

Cai, C., Liu, H., Dai, X., & Whalen, J. K. (2019). Multiple selection of resistance genes in arable soil amended with cephalosporin fermentation residue. *Soil Biology and Biochemistry*, *136*. https://doi.org/10.1016/j.soilbio.2019.107538

Camargo, J. A., & Alonso, Á. (2006). Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International*, *32*(6), 831–849. https://doi.org/10.1016/j.envint.2006.05.002

Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., & Smith, V. H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. In *Ecological Applications* (Vol. 8, Issue 3). https://doi.org/10.1890/1051-0761(1998)008[0559: NPOSWW]2.0.CO;2

Christenson, L., & Sims, R. (2011). Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnology advances, 29(6), 686-702.

Cole, A. J., de Nys, R., & Paul, N. A. (2015). Biorecovery of nutrient waste as protein in freshwater macroalgae. *Algal Research*, *7*, 58–65. https://doi.org/10.1016/j.algal.2014.12.005

Collos, Y., & Harrison, P. J. (2014). Acclimation and toxicity of high ammonium concentrations to unicellular algae. *Marine Pollution Bulletin*, *80*(1–2), 8–23. https://doi.org/10.1016/J.MARPOLBUL.2014.01.006

Correll, D. L. (1999). Phosphorus: a rate limiting nutrient in surface waters. *Poultry Science*, *78*(5), 674–682. https://doi.org/10.1093/PS/78.5.674

Craggs, R. J. (2001). Wastewater treatment by algal turf scrubbing. *Water Science and Technology*, *44*(11–12), 427–433. https://doi.org/10.2166/wst.2001.0862

Dugdale, R. C., Wilkerson, F. P., Hogue, V. E., & Marchi, A. (2007). The role of ammonium and nitrate in spring bloom development in San Francisco Bay. *Estuarine, Coastal and Shelf Science*, *73*(1–2), 17–29. https://doi.org/10.1016/J.ECSS.2006.12.008

Furnish, B. J., & Keller, T. A. (2020). Carbon limitation in hypereutrophic, periphytic algal wastewater treatment systems. *PLoS ONE*, *15*(10 October 2020). https://doi.org/10.1371/JOURNAL.PONE.0240525

Gordon, D. A., Toth, G. P., Graham, D. W., Lazorchak, J. M., Reddy, T. V., Knapp, C. W., & Lattier, D. L. (2006). Effects of eutrophication on vitellogenin gene expression in male fathead minnows (Pimephales promelas) exposed to 17α-ethynylestradiol in field mesocosms. *Environmental pollution*, 142(3), 559-566.

Hansen, N. C., Gupta, S. C., & Moncrief, J. F. (2000). Snowmelt runoff, sediment, and phosphorus losses under three different tillage systems. *Soil and Tillage Research*, *57*(1–2), 93–100. https://doi.org/10.1016/S0167-1987(00)00152-5

Holden, J. (Ed.). (2013). Water resources: an integrated approach. Routledge.

Judd, S. J. (2017). Membrane technology costs and me. *Water research*, *122*, 1-9.

Kaiser, K. (2001). Dissolved organic phosphorus and sulphur as influenced by sorptive interactions with mineral subsoil horizons. *European Journal of Soil Science*, *52*(3), 489–493. https://doi.org/10.1046/j.1365-2389.2001.00396.x

Källqvist, T., research, A. S.-W., & 2003, undefined. (n.d.). Assessment of ammonia toxicity in tests with the microalga, Nephroselmis pyriformis, Chlorophyta. *Elsevier*. Retrieved November 11, 2022, from https://www.sciencedirect.com/science/article/pii/S0043135402003615.

Keselman, H. J., Algina, J., & Kowalchuk, R. K. (2001). Analysis of repeated measures designs: A review. *British Journal of Mathematical and Statistical Psychology*, *54*(1), 1–20. https://doi.org/10.1348/000711001159357

Konig, A., Pearson, H. W., & Silva, S. A. (1987). Ammonia Toxicity to Algal Growth in Waste Stabilization Ponds. *Water Science and Technology*, *19*(12), 115–122. https://doi.org/10.2166/WST.1987.0135

Kriens, M. (1994). Grenzen der Belastbarkeit von aquatischen Oekosystemen mit Stickstoff. Literaturarbeit im Rahmen des Forschungsschwerpunktes" N achhaltige Ressourcenbewirtschaftung", EA WAG Dubendorf.

Krustok, I., Odlare, M., Truu, J., & Nehrenheim, E. (2016). Inhibition of nitrification in municipal wastewater-treating photobioreactors: Effect on algal growth and nutrient uptake. *Bioresource Technology*, *202*, 238–243. https://doi.org/10.1016/J.BIORTECH.2015.12.020

Kube, M., Spedding, B., Gao, L., Fan, L., & Roddick, F. (2020). Nutrient removal by alginate-immobilized Chlorella vulgaris: response to different wastewater matrices. *Journal of Chemical Technology & Biotechnology*, *95*(6), 1790–1799. https://doi.org/10.1002/JCTB.6377

Ladwig, R., Hanson, P. C., Dugan, H. A., Carey, C. C., Zhang, Y., Shu, L., & Cobourn, K. M. (2021). Lake thermal structure drives interannual variability in summer anoxia dynamics in a eutrophic lake over 37 years. Hydrology and Earth System Sciences, 5(2), 1009-1032.

Lawton, R. J., Cole, A. J., Roberts, D. A., Paul, N. A., & de Nys, R. (2017). The industrial ecology of freshwater macroalgae for biomass applications. *Algal Research*, *24*, 486–491. https://doi.org/10.1016/j.algal.2016.08.019

Mennaa, F. Z., Arbib, Z., & Perales, J. A. (2015). Urban wastewater treatment by seven species of microalgae and an algal bloom: Biomass production, N and P removal kinetics and harvestability. *Water Research*, *83*, 42–51. https://doi.org/10.1016/j.watres.2015.06.007

Parker, A. E., Dugdale, R. C., & Wilkerson, F. P. (2012). Elevated ammonium concentrations from wastewater discharge depress primary productivity in the Sacramento River and the Northern San Francisco Estuary. *Marine Pollution Bulletin*, *64*(3), 574–586. https://doi.org/10.1016/J.MARPOLBUL.2011.12.016

Prouty, N. G., Yates, K. K., Smiley, N., Gallagher, C., Cheriton, O., & Storlazzi, C. D. (2018). Carbonate system parameters of an algal-dominated reef along West Maui. *Biogeosciences*, *15*(8), 2467–2480. https://doi.org/10.5194/bg-15-2467-2018

Rahimifard, S., Sheldrick, L., Woolley, E., Colwill, J., & Sachidananda, M. (2013). How to manufacture a sustainable future for 9 billion people in 2050. *Re-Engineering Manufacturing for Sustainability - Proceedings of the 20th CIRP International Conference on Life Cycle Engineering*, 1–8. https://doi.org/10.1007/978-981-4451-48-2_1

Ruffier, P. J., Boyle, W. C., & Kleinschmidt, J. (1981). Short-term acute bioassays to evaluate ammonia toxicity and effluent standards. Journal (Water Pollution Control Federation), 367-377.

Salama, E. S., Kurade, M. B., Abou-Shanab, R. A., El-Dalatony, M. M., Yang, I. S., Min, B., & Jeon, B. H. (2017). Recent progress in microalgal biomass production coupled with wastewater treatment for biofuel generation. *Renewable and Sustainable Energy Reviews, 79*, 1189-1211.

Shen, Y., Yang, T., Zhu, W., & Zhao, Y. (2017). Wastewater treatment and biofuel production through attached culture of Chlorella vulgaris in a porous substratum biofilm reactor. Journal of applied phycology, 29, 833-841.

Skinner, K. D., & Maupin, M. A. (2019). Point-source nutrient loads to streams of the conterminous United States, 2012. *Data Series*. https://doi.org/10.3133/DS1101

Smith, V. H., & Schindler, D. W. (2009). Eutrophication science: where do we go from here? *Trends in Ecology and Evolution*, *24*(4), 201–207. https://doi.org/10.1016/j.tree.2008.11.009

Smith, V. H. (2006). Responses of estuarine and coastal marine phytoplankton to nitrogen and phosphorus enrichment. *Limnology and oceanography*, *51*(1 part 2), 377-384.

Solimeno, A., Parker, L., Lundquist, T., & García, J. (2017). Integral microalgae-bacteria model (BIO_ALGAE): Application to wastewater high-rate algal ponds. *Science of The Total Environment*, *601–602*, 646–657. https://doi.org/10.1016/J.SCITOTENV.2017.05.215

Sukačová, K., & Červený, J. (2017). Can algal biotechnology bring effective solution for closing the phosphorus cycle? Use of algae for nutrient removal - Review of past trends and future perspectives in the context of nutrient recovery. *European Journal of Environmental Sciences*, *7*(1), 63–72. https://doi.org/10.14712/23361964.2017.6

Thomas, R. F., & Booth, R. L. (1973). Selective electrode measurement of ammonia in water and wastes. *Environmental science & technology*, *7*(6), 523-526.

Tua, C., Ficara, E., Mezzanotte, V., & Rigamonti, L. (2021). Integration of a side-stream microalgae process into a municipal wastewater treatment plant: A life cycle analysis. *Journal of Environmental Management*, *279*, 111605. https://doi.org/10.1016/j.jenvman.2020.111605

Van Der Hoek, J. P., De Fooij, H., & Struker, A. (2016). Wastewater as a resource: Strategies to recover resources from Amsterdam's wastewater. *Resources,*

Conservation and Recycling, *113*, 53–64. https://doi.org/10.1016/j.resconrec.2016.05.012

Wang, W. (1991). Ammonia toxicity to macrophytes (common duckweed and rice) using static and renewal methods. *Environmental Toxicology and Chemistry*, *10*(9), 1173–1177. https://doi.org/10.1002/etc.5620100909

Withers, P. J. A., Neal, C., Jarvie, H. P., & Doody, D. G. (2014). Agriculture and eutrophication: Where do we go from here? *Sustainability (Switzerland)*, *6*(9), 5853–5875. https://doi.org/10.3390/su6095853

Xia, Y., Zhang, M., Tsang, D. C. W., Geng, N., Lu, D., Zhu, L., Igalavithana, A. D., Dissanayake, P. D., Rinklebe, J., Yang, X., & Ok, Y. S. (2020). Recent advances in control technologies for non-point source pollution with nitrogen and phosphorous from agricultural runoff: current practices and prospects. *Applied Biological Chemistry*, *63*(1). https://doi.org/10.1186/s13765-020-0493-6

Zhao, S., Hu, N., Chen, Z., Zhao, B., & Liang, Y. (2009). Bioremediation of reclaimed wastewater used as landscape water by using the denitrifying bacterium Bacillus cereus. *Bulletin of environmental contamination and toxicology*, *83*(3), 337-340.

APPENDIX A

Table 1: Dissolved nutrient levels in floways from experiment 1

Date	*Treatment*	*Flume*	*Nitrate (NO_3-N mg/l)*	*Phosphate (PO_4^{3-} mg/l)*	*Dilution*	*Total Phosphorus (PO_4^{3-} mg/l)*
10/3/2018	A	1	3.00	2.15	Low dilution	28.93
10/3/2018	A	5	2.40	2.39	Low dilution	29.20
10/3/2018	A	8	2.80	2.11	Low dilution	28.80
10/3/2018	A	11	3.80	2.49	Low dilution	28.80
10/10/2018	A	1	3.20	2.55	Low dilution	24.80
10/10/2018	A	5	3.00	1.89	Low dilution	24.00
10/10/2018	A	8	2.40	1.79	Low dilution	26.00

10/10/2018	A	11	2.80	1.72	Low dilution	25.60
10/17/2018	A	1	14.40	2.10	Low dilution	23.86
10/17/2018	A	5	15.6	2.63	Low dilution	23.06
10/17/2018	A	8	11.6	2.30	Low dilution	25.73
10/17/2018	A	11	17.4	2.53	Low dilution	24.66
10/24/2018	A	1	11.2	2.58	Low dilution	22.93
10/24/2018	A	5	11.2	2.67	Low dilution	22.93
10/24/2018	A	8	11.4	2.52	Low dilution	24.53
10/24/2018	A	11	11.2	2.30	Low dilution	24.00
10/3/2018	B	3	4	2.40	Medium dilution	18.13
10/3/2018	B	4	5.4	2.24	Medium dilution	16.93
10/3/2018	B	7	4	2.50	Medium dilution	16.93
10/3/2018	B	12	3.40	2.27	Medium dilution	16.93
10/10/2018	B	3	10.4	2.90	Medium dilution	15.87
10/10/2018	B	4	10.6	2.51	Medium dilution	14.80
10/10/2018	B	7	8.6	2.15	Medium dilution	14.53

10/10/2018	B	12	9.6	2.35	Medium dilution	15.73
10/17/2018	B	3	17.4	2.53	Medium dilution	15.60
10/17/2018	B	4	14.8	1.65	Medium dilution	14.13
10/17/2018	B	7	12.2	2.48	Medium dilution	14.40
10/17/2018	B	12	16.4	2.15	Medium dilution	15.60
10/24/2018	B	3	9.8	2.37	Medium dilution	13.33
10/24/2018	B	4	12	2.40	Medium dilution	13.47
10/24/2018	B	7	10.2	1.67	Medium dilution	12.54
10/24/2018	B	12	10.4	2.45	Medium dilution	15.60
10/3/2018	C	2	4.2	1.90	High dilution	11.07
10/3/2018	C	6	3.6	2.45	High dilution	11.33
10/3/2018	C	9	3.6	2.67	High dilution	11.07
10/3/2018	C	10	5.4	1.67	High dilution	11.06
10/10/2018	C	2	12.2	2.54	High dilution	9.33
10/10/2018	C	6	9.2	1.59	High dilution	8.53

10/10/2018	C	9	10.4	2.41	High dilution	9.07
10/10/2018	C	10	12	2.42	High dilution	10.00
10/17/2018	C	2	10.8	2.42	High dilution	9.20
10/17/2018	C	6	13.2	2.30	High dilution	8.53
10/17/2018	C	9	15.6	2.04	High dilution	9.07
10/17/2018	C	10	13.6	1.76	High dilution	9.73
10/24/2018	C	2	6.4	1.59	High dilution	7.20
10/24/2018	C	6	9.8	1.58	High dilution	7.73
10/24/2018	C	9	7.8	2.25	High dilution	6.93
10/24/2018	C	10	10	2.44	High dilution	8.40

Table 2: Algae mass in grams per square meter collected from one randomly selected tile per flume from experiment 1

Flume	Treatment	Dilution	Dry mass (g/m2) Day 7	Dry mass (g/m2) Day 14	Dry mass (g/m2) Day 21
1	A	Low dilution	1.87	3.24	4.92
5	A	Low dilution	1.12	5.98	8.60

8	A	Low dilution	1.12	8.84	10.41
11	A	Low dilution	2.12	4.42	9.970
3	B	Medium dilution	6.11	16.39	15.27
4	B	Medium dilution	5.36	8.97	6.48
7	B	Medium dilution	4.96	18.44	31.03
12	B	Medium dilution	4.05	29.16	6.48
2	C	High dilution	6.72	9.60	1.99
6	C	High dilution	7.35	13.96	22.18
9	C	High dilution	6.85	15.20	23.49
10	C	High dilution	6.92	14.77	4.49

APPENDIX B

Table 1: Dissolved nutrient levels in floways from experiment 2

Date	***Day***	***Flume***	***Treatment***	***Nitrate (NO_3-N mg/l)***	***Phosphate (PO_4^{3-} mg/l)***	***Total Phosphorus (PO_4^{3-} mg/l)***
11/7/2019	1	1	High ammonia conc	3.00	2.15	3.52
11/7/2019	1	2	Control	3.20	2.39	3.01
11/7/2019	1	3	Low ammonia conc	3.30	2.11	3.2
11/7/2019	1	4	Control	3.30	2.49	3.0
11/7/2019	1	5	Low ammonia conc	2.90	2.55	3.61
11/7/2019	1	6	High ammonia conc	3.40	1.89	3.4
11/7/2019	1	7	Control	3.20	1.79	3.27
11/7/2019	1	8	Low ammonia conc	3.30	1.72	3.51
11/7/2019	1	9	Control	3.40	2.10	3.35
11/7/2019	1	10	High ammonia conc	3.00	2.63	2.90
11/7/2019	1	11	Low ammonia conc	3.00	2.30	3.14
11/7/2019	1	12	Control	3.00	2.53	3.15
11/7/2019	1	13	Low ammonia conc	3.30	2.58	3.70

11/7/2019	1	14	Control	3.00	2.67	3.20
11/7/2019	1	15	High ammonia conc	3.1	2.52	3.00
11/7/2019	1	16	High ammonia conc	3.2	2.30	3.30
11/7/2019	1	17	Low ammonia conc	3.0	2.40	3.20
11/7/2019	1	18	High ammonia conc	3.2	2.24	3.30
11/7/2019	1	19	Control	2.9	2.50	3.20
11/7/2019	1	20	High ammonia conc	3.40	2.27	3.10
11/7/2019	1	21	Low ammonia conc	3.50	2.90	3.10
11/7/2019	1	22	Control	3.50	2.51	3.35
11/7/2019	1	23	Low ammonia conc	3.30	2.15	3.27
11/7/2019	1	24	High ammonia conc	3.50	2.35	3.13
11/14/2019	8	1	High ammonia conc	3.20	2.53	3.26
11/14/2019	8	2	Control	0.90	1.65	2.70
11/14/2019	8	3	Low ammonia conc	2.30	2.48	3.13
11/14/2019	8	4	Control	1.25	2.15	2.87
11/14/2019	8	5	Low ammonia conc	2.90	2.37	3.38

11/14/2019	8	6	High ammonia conc	1.70	2.40	2.93
11/14/2019	8	7	Control	1.19	1.67	3.28
11/14/2019	8	8	Low ammonia conc	2.20	2.45	2.71
11/14/2019	8	9	Control	1.32	1.90	3.11
11/14/2019	8	10	High ammonia conc	1.40	2.45	3.10
11/14/2019	8	11	Low ammonia conc	2.46	2.67	2.49
11/14/2019	8	12	Control	1.30	1.67	2.62
11/14/2019	8	13	Low ammonia conc	1.70	2.54	2.20
11/14/2019	8	14	Control	0.80	1.59	3.18
11/14/2019	8	15	High ammonia conc	3.30	2.41	2.69
11/14/2019	8	16	High ammonia conc	1.70	2.42	2.72
11/14/2019	8	17	Low ammonia conc	2.36	2.42	2.29
11/14/2019	8	18	High ammonia conc	2.70	2.30	3.68
11/14/2019	8	19	Control	0.80	2.04	3.09
11/14/2019	8	20	High ammonia conc	3.90	1.76	3.84
11/14/2019	8	21	Low ammonia conc	2.20	1.59	2.77
11/14/2019	8	22	Control	0.92	1.58	2.51

11/14/2019	8	23	Low ammonia conc	2.50	2.25	2.25
11/14/2019	8	24	High ammonia conc	2.20	2.44	2.70
11/21/2019	14	1	High ammonia conc	3.00	2.15	3.52
11/21/2019	14	2	Control	3.20	2.39	3.01
11/21/2019	14	3	Low ammonia conc	3.30	2.11	3.20
11/21/2019	14	4	Control	3.30	2.49	3.00
11/21/2019	14	5	Low ammonia conc	2.90	2.55	3.61
11/21/2019	14	6	High ammonia conc	3.40	1.89	3.40
11/21/2019	14	7	Control	3.20	1.79	3.27
11/21/2019	14	8	Low ammonia conc	3.30	1.72	3.51
11/21/2019	14	9	Control	3.40	2.10	3.35
11/21/2019	14	10	High ammonia conc	3.00	2.63	2.90
11/21/2019	14	11	Low ammonia conc	3.00	2.30	3.14
11/21/2019	14	12	Control	3.00	2.53	3.15
11/21/2019	14	13	Low ammonia conc	3.30	2.58	3.70
11/21/2019	14	14	Control	3.00	2.67	3.20
11/21/2019	14	15	High ammonia conc	3.10	2.52	3.00

11/21/2019	14	16	High ammonia conc	3.20	2.30	3.30
11/21/2019	14	17	Low ammonia conc	3.00	2.40	3.20
11/21/2019	14	18	High ammonia conc	3.20	2.24	3.30
11/21/2019	14	19	Control	2.90	2.50	3.20
11/21/2019	14	20	High ammonia conc	3.40	2.27	3.40
11/21/2019	14	21	Low ammonia conc	3.50	2.51	3.35
11/21/2019	14	22	Control	3.50	2.51	3.35
11/21/2019	14	23	Low ammonia conc	3.30	2.15	3.27
11/21/2019	14	24	High ammonia conc	3.40	2.35	3.13
11/28/2019	21	1	High ammonia conc	2.60	2.15	1.88
11/28/2019	21	2	Control	0.70	0.67	1.11
11/28/2019	21	3	Low ammonia conc	1.93	2.11	0.79
11/28/2019	21	4	Control	0.80	0.67	0.54
11/28/2019	21	5	Low ammonia conc	0.24	2.55	2.16
11/28/2019	21	6	High ammonia conc	1.20	1.89	1.06
11/28/2019	21	7	Control	0.90	0.70	0.72
11/28/2019	21	8	Low ammonia conc	1.85	1.72	1.63

11/28/2019	21	9	Control	0.60	0.72	0.48
11/28/2019	21	10	High ammonia conc	1.20	2.63	1.01
11/28/2019	21	11	Low ammonia conc	2.10	2.30	1.70
11/28/2019	21	12	Control	0.80	0.68	0.67
11/28/2019	21	13	Low ammonia conc	1.52	2.58	1.66
11/28/2019	21	14	Control	0.62	0.54	0.68
11/28/2019	21	15	High ammonia conc	2.30	2.52	1.74
11/28/2019	21	16	High ammonia conc	1.50	2.30	1.02
11/28/2019	21	17	Low ammonia conc	1.90	2.40	1.72
11/28/2019	21	18	High ammonia conc	2.50	2.24	0.89
11/28/2019	21	19	Control	0.60	0.48	0.70
11/28/2019	21	20	High ammonia conc	2.50	2.27	1.41
11/28/2019	21	21	Low ammonia conc	1.90	2.39	0.81
11/28/2019	21	22	Control	0.70	1.11	0.67
11/28/2019	21	23	Low ammonia conc	1.90	2.15	0.79
11/28/2019	21	24	High ammonia conc	1.96	2.35	2.05

Table 2: Algae dry mass in milligrams per centimeter square, collected from one randomly selected tile per flume from experiment 2

Flume	***Treatment***	***Dry Mass (mg/cm2) Day7- 11/14/2019***	***Dry Mass (mg/cm2) Day15- 11/21/2019***	***Dry mass (mg/cm2) Day21- 11/28/2019***
1	High ammonia	3.56	143.91	434.62
2	Control	130.20	171.19	509.60
3	Low ammonia conc	32.16	264.62	120.58
4	Control	160.38	397.98	447.53
5	Low ammonia conc	105.56	175.67	238.79
6	High ammonia conc	65.89	80.52	134.16
7	Control	21.74	235.89	462.69
8	Low ammonia conc	12.65	151.55	522.12
9	Control	57.98	277.93	1737.16
10	High ammonia conc	5.27	157.22	381.91
11	Low ammonia conc	4.35	262.78	427.37
12	Control	150.76	373.60	528.45
13	Low ammonia conc	3.03	152.60	353.04
14	Control	30.57	165.65	378.74
15	High ammonia conc	81.97	83.29	142.33
16	High ammonia conc	8.43	152.60	402.33
17	Low ammonia conc	45.86	237.34	110.43
18	High ammonia conc	1.58	152.87	387.84
19	Control	9.49	252.63	389.16

20	High ammonia conc	122.95	132.44	430.80
21	Low ammonia conc	97.78	141.53	81.44
22	Control	5.80	177.12	549.14
23	Low ammonia conc	51.26	103.58	153.92
24	High ammonia conc	44.54	370.57	440.02