OPTIMIZATION OF ALGAL TURF SCRUBBERS FOR LARGE-SCALE SUPER-INTENSIVE AQUACULTURE

A Thesis

by

JACQUELINE M. MITCHELL

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This thesis meets the standards for scope and quality of
Texas A&M University-Corpus Christi and is hereby approved.

Anthony Siccardi III, PhD
Co-Chair

John Scarpa, PhD
Co-Chair

Joe Fox, PhD
Committee Member

Ronald Pate
Committee Member

May 2018

ABSTRACT

Since their invention in the 1980s, algal turf scrubbers (ATS) have been successfully used to remove nitrogen and phosphorus from eutrophic waterways. Along with the nutrient mitigation properties of ATS, the biomass generated is also of interest as a potential source of biofuels. Much of the research has focused on nutrient removal by ATS systems rather than increasing biomass production. Therefore, two experiments were conducted sequentially to test the effect of substrate material and harvest interval on biomass productivity. For the substrate experiment, three substrate types (2-D, 3-D, and advanced 3-D) were tested against a control (no mesh substrate). Mean (n=3, ±s.d.) productivity of the advanced 3-D substrate (6.1±2.5 g AFDW/m^2/day) was significantly higher than both the control (3.5±2.4, $P < 0.001$) and the 2-D substrate (4.5±1.9, $P= 0.033$), but not the 3-D substrate (4.9±1.8, $P > 0.05$). The advanced 3-D material was then used to test the effect of harvest interval (4, 7, 10, and 14 days) on biomass production. The 4 day harvest schedule produced significantly ($P < 0.004$) more biomass (21.9 ± 7.0 g AFDW/m^2/day) than the other treatments (7, 10, and 14 day harvest intervals were 17.7 ± 7.1, 13.0 ± 5.0, and 10.3 ± 4.6, respectively). Compositional analysis was performed for the ATS biomass in each experiment to determine its potential use as a biofuel. For both experiments there was no difference for protein (% of dry biomass), fat (%), and fiber (%) content among treatments ($P > 0.05$). Protein, fat, and fiber averaged 8.1% (± 2.1), 0.09% (± 0.1), and 1.66% (±0.5), respectively, for the substrate experiment and 7.03% (± 0.6), 0.06% (±0.1), and 1.34% (±0.4), respectively, for the harvest interval experiment. During each of the experiments water samples were taken from the head tank and at the end of each ATS lane to determine the nitrogen removal rate. The low ammonia-nitrogen levels in the incoming water combined with the short residence time on the flow ways and measurement variation between

samples prevented the nitrogen removal rate from being determined for either experiment. With these parameters somewhat optimized, future experiments should determine the nitrogen removal rate for the ATS system so that an appropriately sized ATS for super intensive shrimp aquaculture can be modeled.

DEDICATION

I dedicate this work to my Ma and Dad (I'm sure writing "Joanne and Thomas" would have been more professional but it just felt strange and wrong when I tried so here we are), who love and support me through everything (including bouts of downward spirals and existential crises). I don't know how I would have gotten through it all without you—I love you both so much. Sam and Terry – I look up to you guys more than you probably realize, so thanks for being such great siblings and friends. For Jenna – as a friend I'm sorry I'm not cooler but how were either of us supposed to know at 10 years old that I'd grow up to like seaweed so much? Such is life, I suppose. And Alex, thank you for loving me. Having you with me has made all the difference and I'm so grateful for that. For the rest of my family and friends who have listened to me talk about algae for much longer than they probably ever wanted; don't get your hopes up that my graduating means that will change. Oh, and to Josue who, if I don't mention his name here, will most definitely make sure to remind me of the slight 50 years from now.

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INTRODUCTION

Aquaculture, the practice of farming aquatic organisms under controlled conditions, has been employed for centuries with some of the earliest accounts dating from the 5th century B.C. in Asia (Bardach et al. 1972, Parker, 2002). In many early systems, fish were often grown at relatively low densities and in conjunction with other organisms. This kind of polyculture practiced in Asia relied on taking advantage of, and integrating, ecological niches (Parker, 2002). Over time, aquaculture techniques evolved and adapted to increased commercialization. Generally speaking, aquaculture can be divided into extensive, semi-intensive, and intensive culture. These classifications are based on the densities at which organisms are grown and, as an extension of that, the amount of inputs and management needed (Baluyut, 1989).

Extensive culture utilizes low stocking densities and no supplementary feeding (Baluyut, 1989). Feeding is determined by natural productivity in the pond and fertilizers may be added to stimulate productivity. Water exchange occurs through tidal changes (Baluyut, 1989). This type of culture is relatively low maintenance compared to other types of culture methods. Semi-intensive culture uses higher densities than extensive methods and, as such, organisms require supplementary feeding (Baluyut, 1989; Edwards, 2015). Intensive culture employs the highest densities of culture organisms and is dependent on feeding and supplemental aeration or oxygenation. Water exchange still takes place in semi-intensive and intensive culture systems but they are not dependent on the tidal cycles. Rather, pumps are used to bring in new water and discharge effluent (Baluyut, 1989). Additionally, in semi-intensive and intensive systems, some if not all of the water is destined for re-use in the system (Cripps and Bergheim, 2000).

As global capture fisheries plateau, aquaculture becomes increasingly more important, especially as the world per capita consumption of fish continues to increase (FAO, 2016). In 2014, aquaculture, for the first time, contributed more fish for human consumption than capture fisheries (FAO, 2016). According to projections made by the U.N. Food and Agricultural Organization (FAO), capture fisheries is expected to remain stagnant through 2025 while aquaculture is expected to continue growing (FAO, 2016). To keep up with the demand for seafood, intensive systems are being more frequently employed. Because the amount of organisms grown in intensive systems is so large, as is the number of inputs (e.g., feed), water quality is the biggest challenge facing aquaculturists. In modern intensive systems water is re-used. In order for the water quality to be suitable for re-use in the system it must undergo several types of filtration (mechanical and biological) and clarification processes. Different methods are usually used in conjunction with one another rather than alone and the types utilized are dependent on the waste needing to be removed and what is most cost-effective (Sutherland and Craggs, 2017).

Mechanical filtration deals with the removal of suspended solids from the system. Mechanical filtration includes settling basins, bead filters, and rotating drum filters. Settling basins rely on flow velocity to separate out large particles from the water column (Cripps and Bergheim, 2000). They can be various shapes and sizes depending on the type and size of the system to which they are integrated. For pond culture, spare ponds can be used for sedimentation. For intensive recirculating aquaculture systems (RAS) that utilize tanks they can be conical in shape. High flow rates can be a problem because the water does not move slow enough to allow particles to settle out (Cripps and Bergheim, 2000). In some settling basins, baffles are added to decrease

flow rate and allow particles time to settle. Floating bead filters can be both a means of mechanical and biological filtration although most culturists use it as mechanical filtration (Cripps and Bergheim, 2000). In a floating bead filter, polyethylene beads are housed within the filter. Water is forced upward through the beads, trapping sediment, before exiting at the top of the filter. To remove the solids, water flow to the filter is stopped and the system is backwashed (Cripps and Bergheim, 2000). Rotating drum filters utilize screens with different micropore sizes to separate particles from the water column. As the water passes through the drum, particles are retained on the screen. As the drum rotates solids are scraped or sprayed off and discharged (Cripps and Bergheim, 2000). Because rotating drum filters have a structural axis that allows rotation, over time in marine systems salt water can cause damage.

In contrast to mechanical filters, biological filters provide a substrate for growth of bacteria so that toxic ammonia (NH_3) can be converted to nitrite (NO_2) and then, finally, to relatively non-toxic nitrate (NO_3). There are a number of important factors to consider with respect to biofilters to insure optimal performance. These include maximizing surface area for bacterial growth, uniform water flow through the media, dissolved oxygen for the bacteria to utilize, and shearing action. Types of biofilters include trickling bed, fluidized bed, and rotating biological contactors, among others. Trickling bed filters consist of a fixed bed of media over which water is sprayed evenly. Water trickles downward through the media and nitrification occurs. If the media is packed too densely oxygen will become limiting. Fluidized bed filters, utilize water to suspend the media within the filter housing. The constant movement of the media promotes shearing and gas exchange and can increase nitrification efficiency. Rotating biological contactors utilize media discs lined-up on a shaft. A motor turns the shaft, rotating the discs so

only a part of the disc is submerged in water at a given time. Similar to fluidized bed filters, this promotes gas exchange and shearing.

Other methods of water quality control include integrated aquaculture systems, constructed wetlands, and periphyton based systems (Sutherland and Craggs, 2017). These systems differ from others in that the focus is not to "get rid" of waste nutrients in the effluent, but to use it for the system. Integrated multi-trophic aquaculture systems (IMTA) make use of organisms from different trophic levels to re-use nutrients in effluent. Typically, IMTA involve finfish culture in conjunction with an inorganic extractive species such as seaweed, and an organic extractive species such as bivalves (Edwards, 2015). In this system, three products are generated rather than just one while taking care of waste nutrient loads. Constructed wetlands perform both mechanical and biological filtration. As water flows through the wetland, particulates are trapped by vegetation and deposited, while excess nutrients, such as nitrogen and phosphorous, are assimilated by plants or microorganisms (Edwards, 2015). Periphyton based systems include algal turf scrubbers (Sutherland and Craggs, 2017). Periphyton systems involve providing structure or substrate to promote the growth of a periphyton community. Water is pumped over the substrate, where the periphyton grow, and the periphyton assimilate nitrogen and phosphorous. Harvesting the periphyton biomass is a necessary step in removing nutrients from the system permanently as well as stimulating further growth (Sutherland and Craggs, 2017).

Super-intensive shrimp aquaculture systems typically operate under minimal or zero water exchange and as a result water quality needs to be carefully monitored and controlled. As nitrogen levels increase a dense microbial community develops in the system known as biofloc,

and is responsible for much of the nutrient cycling in the system (Holl et al., 2011; Ray et al., 2011; Xu et al., 2018). This becomes especially important with respect to nitrogen containing compounds such as ammonia, nitrite, and nitrate. Biofloc is popular method of controlling nitrogen containing species among those using super-intensive systems, but does not come without drawbacks. Nitrification by biofloc can require up to 22% of the dissolved oxygen in recirculating systems (Holl et al., 2011) and the respiration of the bacteria can cause pH to drop. This requires a need for sodium bicarbonate ($NaHCO_3$) to be added to the system to combat pH problems. Additionally, carbon sources separate from feed are often added to assist with the development of the microbial community until it is mature enough to operate without excess carbon input (Xu et al., 2018). Further, despite being able to cycle the nitrogen in the system the bacteria cannot completely remove it. In order to permanently remove the nitrogen from the system, the bacteria itself needs to be removed (Ray et al., 2011). Periphyton based systems have shown to successfully treat water from semi-intensive shrimp systems (Kumar et al., 2017) and as such may also be able to successfully treat effluent from super-intensive systems. The advantage of using periphyton based systems, such as an ATS, is that harvesting biomass is a necessary operational step. Through harvests nitrogen species are permanently removed from the system.

Algal turf scrubber (ATS) systems, a type of periphyton based system, were invented in the 1980's by Adey and Steneck (1985) after conducting research on coral reefs around St. Croix, Virgin Islands. Adey and Steneck (1985) determined that primary productivity on coral reefs in St. Croix was 5-10 times higher than that of most terrestrial forests, with the limiting factor being the amount of light. Upon further investigation, they found that the source of the increased

productivity values was the filamentous turf algae that covered the hard surfaces of the reef. Screens were then deployed across the reef to promote algal growth in order to facilitate measurement of productivity (Adey and Steneck, 1985). They found that the oscillating water motion caused by winds was the main factor driving increased productivity. These pieces of information were used to create the ATS.

Algal turf scrubbers are a simple system comprised of a downward sloping flow-way onto which a substrate is attached. Nutrient rich water is pulsed over the system, stimulating algal growth. As the algae grows, nutrients, such as nitrogen and phosphorous, are removed from the water thus improving the water quality by the time it reaches the end of the flow-way. The ability of ATS systems to improve water quality (i.e., assimilate excess soluble nitrogen and phosphorous) from various sources has been investigated and include agricultural runoff (D'Aiuto et al., 2015; Kangas and Mulbry, 2014), aquaculture effluent (Ray et al., 2015; Valeta and Verdegem, 2015, 2012) and nutrient-polluted natural water-ways (Adey et al., 2013, 2011; Mulbry et al., 2010; Sindelar et al., 2015). Kangas and Mulbry (2014) found that under continuous flow, productivty of the system was roughly 5 grams dry weight/m^2/day and an average of 100 mg of nitrogen and 11 mg of phosphorous were removed per square meter per day. Working with a smaller system, Ray et al. (2015) found their ATS system produced an average of 88.8 g dry weight/m^2/day of algae biomass and removed 12300 mg N/m^2/day and 250 mg P/m^2/day. ATS systems set up along the Patuxent River averaged 17.9 g dry weight/m^2/day and removed on average 250 mg N/m^2/day and 45 mg P/m^2/day from May to October (Mulbry et al., 2010). ATS systems have been successfully scaled-up, patented (US 4333263 A), and utilized commercially by HydroMentia Inc (Adey et al., 2013). Although various means of remediating water quality are

available to aquaculturists (e.g., biological and mechanical filtration), ATS has several advantages over these more tradtional treatment methods. ATS systems operate at low cost (Adey et al., 2013, 2011) because the flow-way utilizes gravity and the system is solar driven. Therefore, the only cost accrued for its operation involves pumping water to the top of the flow-way. Addtionally, for "single-pass" systems operating outdoors there is no need for supplemental nutrients for algal growth (Adey et al., 2013, 2011). There are often sufficient nitrogen and phosphorus levels in the source water to eliminate the need for supplemental nutrients. Algae can also utilize CO_2 already in the source water or from the atmosphere itself. Finally, ATS systems produce a potentially usable by-product in the form of algal biomass, which could have use as an animal feed supplement, fertilizer, or as a source of biofuel (Adey et al., 2011; D'Aiuto et al., 2015; Mulbry et al., 2010).

In order to effectively remove nutrients and be useful as a source of biomass for biofuel production, ATS systems need to produce a large amount of biomass. The production of biomass is dependent on many variables, such as substrate geometry (e.g., surface area for growth), water flow, retention rate within the flow-way, input nutrient load, and harvest rate. Adey et al. (2013) showed that a flat "2-D" mesh substrate produced 20-30 g dry weight/m^2/day of dry biomass whereas a "3-D" mesh substrate produced 2-3 times more (i.e., 60-70 g dry weight /m^2/day). Aside from Adey et al. (2013), the literature is lacking similar experiments that report on testing different substrate materials. Adey et al. (2013), however, did not study the effectiveness of different substrates with true replication nor did they rigorously determine ash content of the biomass. In their study only two flow-ways were set up on which small patches of substrate were laid at various points. Personal observations have indicated that slower flow rates

(i.e., longer retention time) result in lower biomass production. This observation is consistant with that of Kangas and Mulbry (2014), who examined data from different ATS studies and found that lower flow rates resulted generally in lower productivity. Nutrient loads also affect productivity. As the amount of available nutrients in the source water increases, algal growth increases (Adey et al., 2011). This was described by Mulbry et al. (2008) who found a 10-fold difference in productivity (2.5 g dry weight/m^2/day versus 25 g dry weight/m^2/day) between the lowest and highest nutrient loading rate. Productivity is also dependent on local environmental conditions (e.g., cloud cover, temperature, source water quality) because ATS systems are set up outdoors and typically operate with source water from surface water bodies available in the local environment. Lastly, the harvest rate of biomass from the system influences productivity. Harvesting has been indicated to be essential for ATS operation as it appears to stimulate continued and increased algal growth (Adey et al., 2011; Sutherland and Craggs, 2017). This effect has been observed in practice; however, no published literature indicates research that has been specifically conducted to demonstrate this relationship.

Since their invention in the 1980s, little research has been done with respect to optimizing ATS systems in terms of algal productivity; much of the focus has been instead on the efficacy of nutrient removal (Adey et al., 2013, 2011; D'Aiuto et al., 2015; Kangas and Mulbry, 2014; Mulbry et al., 2010; Ray et al., 2015; Sindelar et al., 2015; Valeta and Verdegem, 2012, 2015). The need for optimization of ATS for productivity is two-fold; increased algal biomass would lead to increased nutrient removal capabilities as well as greater amounts of potentially usable biomass. The need for more research on the relation between substrate and harvest interval on productivity is evident based upon the lack of information available in the literature. The

objective of the present study was to examine and quantify different substrates on ATS productivity and then, using the substrate that yielded the highest amount of biomass, examine the influence of harvest interval on ATS productivity.

MATERIALS AND METHODS

Experimental Design

The research took place at the Texas A&M AgriLife Research Mariculture Laboratory in Corpus Christi, Texas (Figure 1). The facility has direct access to seawater from the Laguna Madre. The substrate trial and data collection took place throughout the winter and early spring (i.e., November 2016 to March 2017). The harvest interval trial and data collection took place throughout the summer (i.e., May to mid-August 2017).

Fig 1. Aerial view of AgriLife Research Center and inlet of the Laguna Madre from Google Earth. The location of pump station from which raw seawater was drawn is circled in white.

Three, four-lane (1 foot-wide/lane, 40 feet-long) algal turf scrubber (ATS) systems (Fig. 2) were utilized to examine the effect of three different mesh substrates (Fig. 3, herein referred to as 2-D, 3-D, and "advanced" 3-D) on biomass production. A control (no-mesh added, gel-coated plywood substrate) lane was included in each system. The 2-D mesh substrate was a flat polyethylene material (InterNet Inc., Minneapolis, MN). The 3-D mesh substrate material has small fiber coils (HydroMentia, Inc., Ocala, FL) that increases the surface area. The "advanced"

3-D mesh material was an Astroturf™ with greater surface area than the 3-D mesh. Each lane in one of the three ATS systems was randomly assigned and outfitted with a mesh substrate (i.e., treatment) or designated as a control. Raw seawater was pumped from a channel connected to the Laguna Madre (Fig. 1) and used as the water source for the duration of the experiment. Water was pumped to each system at a rate of approximately 50 gallons per minute (gpm) or approximately 189 L/min with a dump bucket pulse repetition rate of approximately 20 seconds.

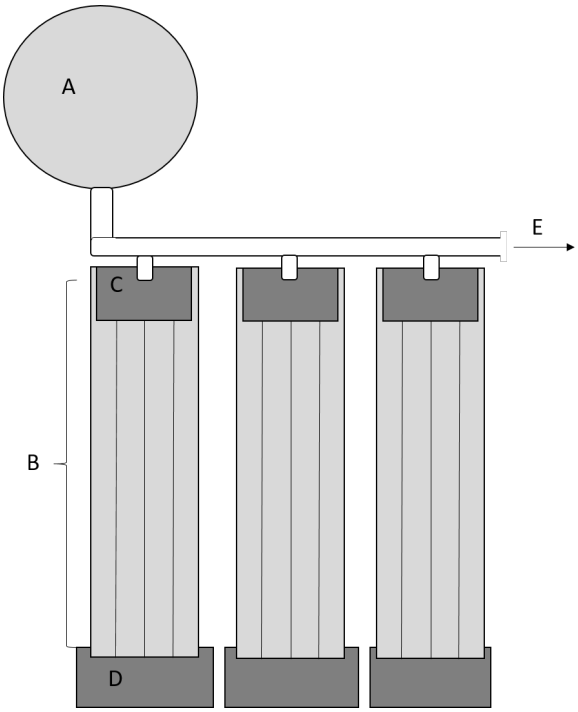

Fig 2. Schematic (not to scale) of the three 4-lane ATS systems showing the head tank (A), ATS lanes (B), dump bucket (C), sump (D), and excess water discharge (E).

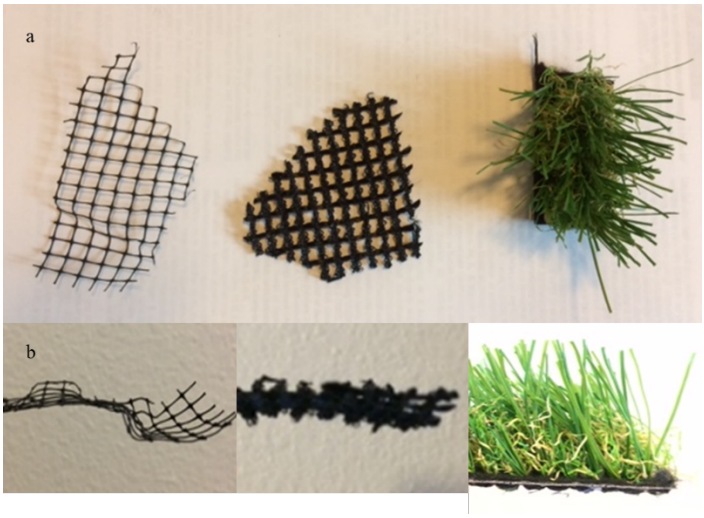

Fig 3. Overhead view of substrates used: (a) (from left to right): 2-D, 3-D, and advanced 3-D. Side view of substrates: (b) (form left to right): 2-D, 3-D, and advanced 3-D.

On a bi-weekly basis, biomass was scraped from the substrate of each lane using a squeegee (Mallory WS1524A Black Window Washer and Squeegee, 15"). Water flow was halted on the system for the duration of harvests (~1.5hours). A felt-lined fish harvest basket (Pentair Aquatic Ecosystems FBK3) was placed at the end of each lane where biomass was collected. Biomass was allowed to de-water in the felt-lined fish harvest basket for approximately six hours before being transferred to a tray and dried in an oven (Model Binder ED720, Tuttlingen, DE) at 75^{o}C until constant weight was achieved. Because biomass was not homogeneous, dried samples were ground into a fine powder using a hammer mill (Model CF198, Chippewa Falls, WI) and mixed to ensure that samples were homogeneous for analysis. Total suspended solids (TSS) and volatile suspended solids (VSS) were determined using APHA (1995) methods #2540D and #2540E, respectively. From this data, moisture (%) and ash (%) were calculated. Biomass productivity was then calculated (Eq. 1) as AFDW[g]/m^2/day

Eq 1. $$(Wet\ harvested\ material\ [g]) * \left(1 - \frac{\%moisture}{100}\right) * (1 - \frac{\%ash}{100}) * \frac{1}{A[m^2]} * \frac{1}{harvest\ interval\ [days]},$$

where A is surface area of harvested flow-way in meters squared. Samples of biomass collected were sent to the New Jersey Feed Lab (1686 5th St, Ewing Township, NJ 08638) for analysis of protein, fat, and fiber to determine its potential as an aquaculture feed ingredient or bioenergy source. Carbohydrate was determined by difference (FAO, 2003). Dry matter energy was calculated in kcals/g (FAO, 2003).

For the harvest interval trial all four lanes of each of the three ATS systems were outfitted with the advanced 3-D substrate material as it was found to have the highest productivity. The head tank and lanes were seeded with periphyton from an existing ATS system located on site in order to promote initial periphyton and algal growth. Each lane in each system was randomly assigned a different harvest interval of either 4, 7, 10, or 14 days between harvests (n=3/harvest treatment). Water source, flow rate (~50gpm, ~189L min^{-1}), and dump bucket pulse repetition rate (20 seconds), were the same as that of the preceding substrate trial. Biomass productivity of each treatment was determined as AFDW[g]/m^2/day using the method described above. Total suspended solids (TSS) and volatile suspended solids (VSS) were also determined using the same methods as described above. From this data, moisture (%) and ash (%) were calculated. It was not possible to dry all the biomass from each treatment due to the amount of biomass produced. After the initial dewatering step, subsamples were collected and analyzed for moisture (%) and ash (%). Biomass collected was sent to New Jersey Feed Lab (1686 5th St, Ewing Township, NJ 08638) for analysis of protein, fat, and fiber to determine its potential as an aquaculture feed ingredient or bioenergy source. Ash composition was not analyzed in detail or over time during either study. Carbohydrate was determined by difference (FAO, 2003). Dry

matter energy was calculated in kcals/g (FAO, 2003). Wet biomass subsamples were also stored in RNALater (Thermo Fischer Scientific) to assist with determining species composition of the ATS biomass, however, these samples were not analyzed as part of the scope of the present study.

Atmospheric and Water Quality Data

Dissolved oxygen, salinity, temperature, and pH of ATS water was measured and recorded with portable meters (YSI EcoSense EC300A and YSI EcoSense pH100A, Xylem, Inc., Yellow Springs, OH) twice daily (a.m. and p.m.). Measurement occurred at the head tank as well as the sump at the end of each replicate system. Continuous on-site atmospheric measurement (air temperature, wind speed, and precipitation) data was obtained from Weather Underground (NAS-Traux Station). Water samples (10mL) were taken every third day from the end of each lane of each system and analyzed for ammonia-nitrogen using flow-injection analysis (FIALab 2600, FIALab Instruments Inc., Bellevue, WA) to determine the efficacy of nutrient removal by the ATS.

Statistical Analysis

Differences among mean AFDW[g]/m^2/day with respect to each treatment (i.e., substrate type and harvest interval) were determined by ANOVA using R software (version 3.4.1). If ANOVA indicated a significant effect (i.e., $P<0.05$), means were separated using Tukey's Honestly Significant Difference (HSD) test (Tukey, 1949) using R software. Statistical differences among ammonia-nitrogen levels as well as proximate composition of biomass samples were also determined by ANOVA and Tukey's HSD test.

Differences in calculated variables (e.g., biomass productivity) among harvest intervals were also determined by ANOVA using R software. If ANOVA indicated an effect (i.e., $P<0.05$), means were separated using Shaffer's method (1986). This method was used as opposed to Tukey's Honestly Significant Difference (HSD) because sample sizes for each treatment were not the same (i.e., unbalanced, Shaffer, 1986). Differences among ammonia-nitrogen levels as well as proximate composition of biomass samples were also determined by ANOVA and upon indication of a significant difference analyzed by Shaffer's method.

RESULTS

Substrate Experiment

Mean (±s.d., n=3) productivity based on a bi-weekly harvest interval for the control, 2-D, 3-D, and advanced 3-D substrates were 3.5±2.4, 4.5±1.9, 4.9±1.8, and 6.1±2.5 g AFDW/m^2/day, respectively (Fig 4). Productivity of the advanced 3-D substrate was significantly higher than both the control and the 2-D substrate ($P < 0.001$, $P= 0.033$, respectively), but not the 3-D substrate ($P > 0.05$). It was noted that due to water resistance, the advanced 3-D treatment experienced less water flow than the other treatments. Measurements showed that lanes with advanced 3-D material had a flow that was only 25% of that of the other treatment lanes. The other 75% of the water was shunted into the other lanes evenly. Correcting for the difference in flow (i.e., about 4x), the advanced 3-D would be expected to produce about 24.4 g AFDW/m^2/day of biomass assuming biomass increase is directly proportional to flow rate.

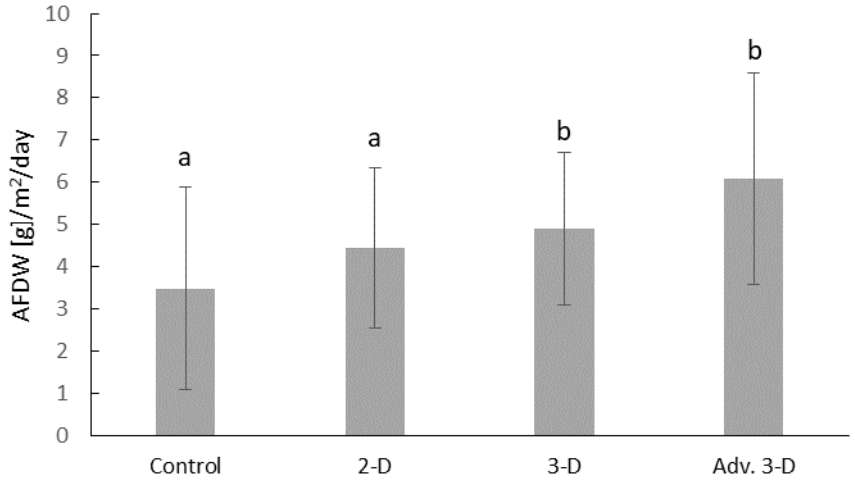

Fig 4. Mean (±s.d., n=3) AFDW[g]/m^2/day of triplicate ATS systems outfitted with different substrate using a bi-weekly harvest interval. Superscripts above bars that differ indicate significance difference ($P < 0.05$).

During the substrate experiment, December and January were significantly (P< 1e-4) colder than November, February, or March (Table 1). December and January were not significantly different from one another nor were November, February, or March (P > 0.05). There was no significant difference (P > 0.05) in wind speed or precipitation among the months of the experiment.

Table 1. Mean (± s.d.) monthly atmospheric data throughout the course of the substrate experiment. For January, precipitation was so minimal it was effectively zero. Values in a column with different superscripts indicate significant difference (P < 0.05).

Date	Temperature (˚C)	Wind (mph)	Precipitation (in.)
November '16 n=30	22.5±3.3[a]	11.4±4.1	0.1±0.1
December '16 n=31	16.5±4.9[b]	11.7±5.8	0.0±0.1
January '17 n=31	16.6±5.1[b]	12.5±4.9	0.0±0
February '17 n=28	20.6±2.9[a]	13.0±3.7	0.0±0.1
March '17 n=31	21.6±3[a]	14.1±3.7	0.1±0.3

Water quality parameters were taken from the head tank (HT) and from the sumps at the end of the three ATS systems to ensure that the three systems were, in fact, replicates. There was no significant difference (P > 0.05) among the head tank or any of the three ATS systems for salinity or temperature (Table 2). The pH of the head tank was significantly lower (P < 1e-05) than that of the three systems (Table 2), however, there was no significant difference (P > 0.05) in pH among the systems themselves. The dissolved oxygen levels were significantly higher (P < 1e-07) at the end of each system than at the head tank, but were not significantly different (P > 0.05) among the systems themselves (Table 2).

Table 2. Mean (± s.d., n=113) water quality parameters for the head tank (HT) and experimental ATS systems during the substrate experiment (November 2016 – March 2017). Values in a column with different superscripts indicate significant difference (P < 0.05).

	Salinity (ppt)	Temperature (˚C)	pH	D.O. (mg/L)
HT	29.6±2.6	19.0±3.8	8.1±0.1^{a}	5.2±1.2^{a}
System 1	29.6±2.7	19.0±3.8	8.2±0.1^{b}	6.2±1.0^{b}
System 2	29.6±2.6	19.1±3.8	8.2±0.1^{b}	6.2±1.0^{b}
System 3	29.6±2.8	19.1±3.8	8.2±0.1^{b}	6.2±1.0^{b}

Water samples collected from the head tank and from the end of each ATS system lane were analyzed for ammonia–nitrogen to determine the nitrogen removal rate. There was no significant difference (P > 0.05) among any of the treatments or the head tank (Fig. 5).

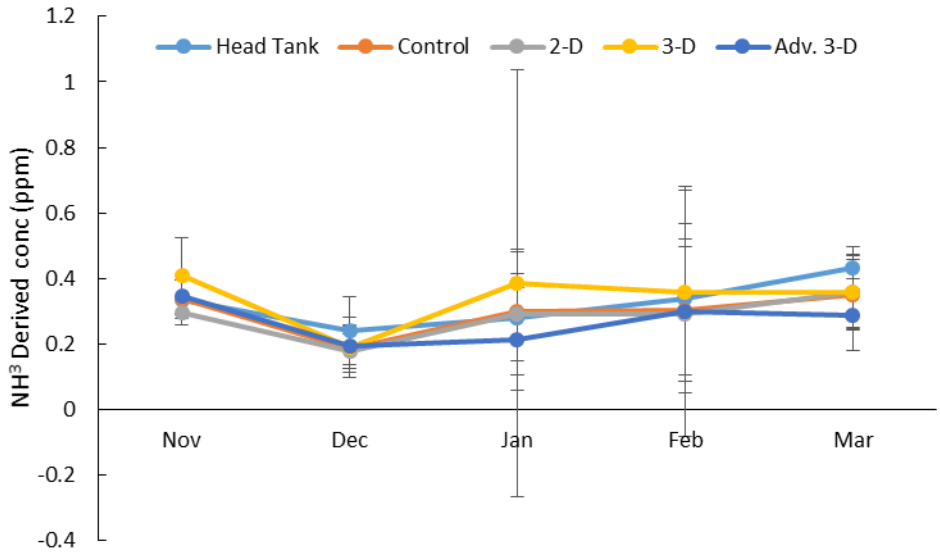

Fig 5. Mean (±sd, n =3) derived ammonia-nitrogen concentration (ppm) for each treatment by month.

There was no significant difference (P>0.05) in biomass composition among the treatments (Table 3). Ash comprised a majority of the biomass for each of the treatment samples at an average of 77.3% (Table 3). Carbohydrate was the next most abundant at approximately 21.3%, followed by protein at 8.1%, fiber at 1.7%, and fat at 0.09% (Table 3).

Table 3. Mean (±sd, n = 9) percent dry weight biomass composition of ATS samples from substrate experiment.

	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Carbohydrate (%)	Kcals/g
Control	8.82±2.9	0.16±0.2	1.76±0.5	77.46±6.4	20.98±5.8	1.1±0.9
2-D	8.04±2.2	0.08±0.1	1.52±0.3	76.12±3.9	22.51±3.5	0.82±0.2
3-D	7.72±1.8	0.09±0.1	1.65±0.6	77.89±3.2	20.78±2.9	0.76±0.1
Adv. 3-D	7.83±1.3	0.05±0.1	1.73±0.5	77.74±3.1	20.96±2.9	0.76±0.1
Average	8.1±2.1	0.09±0.1	1.66±0.5	77.3±4.3	21.31±3.8	0.86±0.3

Harvest Interval Experiment

Mean (±s.d.) productivity (g AFDW/m^2/day) for the 4, 7, 10, and 14 day harvest schedules were 21.9 ± 7.0, 17.7 ± 7.1, 13.0 ± 5.0, and 10.3 ± 4.6, respectively (Fig. 6). The 4 day harvest schedule was significantly ($P < 0.004$) more productive than the other three treatments (Fig. 6). The 7 day harvest schedule was significantly ($P < 0.004$) more productive than both the 10 and 14 day treatments. There was no significant difference ($P > 0.05$) in productivity between the 10 and 14 day harvest schedules.

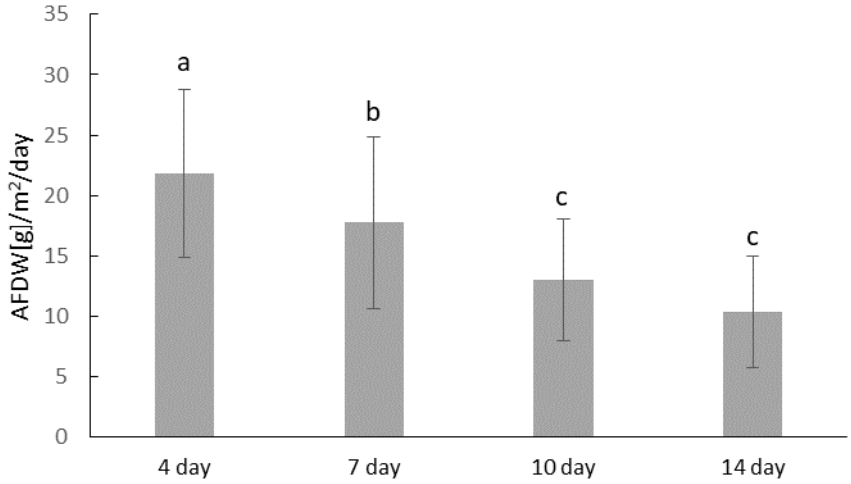

Fig 6. Mean (±s.d., n=3) grams AFDW/m^2/day of biomass from ATS harvested at different intervals: 4 day treatment, 7 day, 10 day, and 14 day. Superscripts above bars that differ indicate significance difference ($P < 0.05$).

June, July, and August were significantly warmer (P < 1e-04) than May with temperatures reaching the mid to upper 80's (Fahrenheit), but they were not significantly different from one another (Table 4). There was no significant difference (P > 0.05) in wind speed throughout the duration of the experiment (Table 4). June experienced significantly more rainfall than July (P=0.021) and August (P= 0.018), which were not different from one another (Table 4), however, the amount of rain throughout the experiment was minimal (Table 4).

Table 4. Mean (±s.d.) monthly atmospheric data throughout the harvest interval experiment. Precipitation in July and August was effectively zero. Values in a column with different superscripts indicate significant difference (P < 0.05).

	Temperature (˚F)	Wind (mph)	Precipitation (in.)
May '17 n=31	26.7±2.0[a]	14.5±4.2	0.1±0.1[a]
June '17 n=30	29.6±1.2[b]	12.2±3.8	0.2±0.4[a]
July '17 n=31	30.3±0.8[b]	11.7±3.1	0±0[b]
August '17 n=31	30.2±1.6[b]	12.9±6.2	0±0[b]

Water quality parameters were monitored for the head tank (HT) and at the end of each lane in a system to ensure replication. There was no significant difference (P > 0.05) in salinity, pH, or temperature among the head tank and the three ATS systems (Table 5). Dissolved oxygen levels were significantly higher (P < 1e-09) at the end of each system as compared to the head tank, however there was no difference (P > 0.05) among the three systems themselves (Table 5).

Table 5. Mean (±s.d) Water quality parameters for ATS, monitored from May – August 2017. Values in a column with different superscripts indicate significant difference (P < 0.05).

	Salinity (ppt)	Temperature (˚C)	pH	D.O. (mg/L)
HT	43.2±3.5	29.4±1.5	8.5±0.1	4.3±0.9[a]
System 1	43.2±3.6	29.8±1.7	8.6±0.2	6.1±0.9[b]
System 2	43.3±3.5	29.7±1.7	8.6±0.2	6.1±0.9[b]
System 3	43.2±3.4	29.8±1.7	8.6±0.2	6.1±1.0[b]

Water samples collected from the head tank of the ATS system and from the end of each flow-way were analyzed for ammonia–nitrogen concentration to determine the nitrogen removal rate of the ATS. There was no significant ($P > 0.05$) difference among any of the treatments nor between the head tank and each of the harvest interval treatments (Fig. 7).

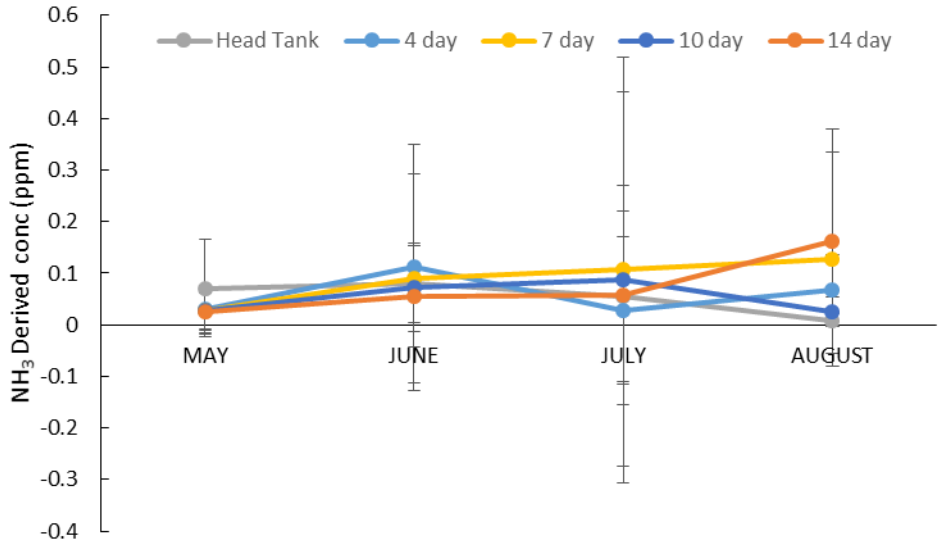

Fig 7. Mean (±s.d., n = 3) derived ammonia-nitrogen concentration (ppm) for each treatment by month.

There was no significant difference in biomass composition with respect to harvest interval (Table 6). Mean ash content was highest (77%) of all measured parameters (Table 6). Protein levels averaged 7.03% while fat and fiber averaged 0.06% and 1.34%, respectively (Table 6).

Table 6. Mean (±s.d.) biomass composition of ATS samples harvested at different intervals. Due to the nature of the experiment, n for each harvest differed: 4 day (n=22) 7 day (n=13) 10 day (n=9) 14 day (n=6).

	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Carbohydrate (%)	Kcals/g
4 day	6.96±0.6	0.07±0.1	1.27±0.3	77.52±1.6	21.29±1.6	0.61±0.1
7 day	7.27±0.4	0.06±0.1	1.43±0.3	76.40±1.5	22.40±1.5	0.63±0.1
10 day	6.94±0.6	0.03±0.1	1.32±0.4	76.66±2.5	22.20±2.5	0.61±0.1

14 day	6.90±0.6	0.03±0.1	1.34±0.4	76.65±2.6	22.59±2.5	0.58±0.1
Average	7.02±0.6	0.06±0.1	1.34±0.4	76.71±2.0	22.12±2.0	0.61±0.1

DISCUSSION

The primary usage of the ATS is to remediate water quality and much of the literature has focused on the effectiveness of ATS to do such. To meet this end, enough biomass needs to be produced to remove excess nutrients, however, the production of biomass is a subject that has received little attention. Prior to this study only one experiment, Adey et al. (2013), addressed the effectiveness of different substrate materials on the productivity of ATS systems. In their experiment, Adey et al (2013) found that substrates with increased surface area exhibited higher productivity than those with less surface area, results that are consistent with the findings of the present study. Both the 3-D and advanced 3-D materials exhibited higher productivities than the 2-D and control materials. However, it should be noted that due to the increased surface area of the advanced 3-D material it also exhibited increased resistance. The water flow down the lanes with the advanced 3-D material was measured to be ~25% of the other lanes. Assuming productivity was directly porportional to the flow rate, if the flow been equal to that of the other lanes, then productivity would be ~24 g AFDW/m^2/day. This was what was seen for productivity during the second experiment when all lanes were outfitted with the advanced 3-D material and flow was equal. The reason the advanced 3-D material outperformed the other substrates is likely due to its structure. The advanced 3-D material is essentially two layers of substrate interwoven together. Not only did this create increased surface area but likely also created turbulence by disrupting laminar flow. An increase in productivity as a result of increased turbulence is consistent with other ATS studies (Blersch et al., 2013).

The ATS harvest interval experiment is the first of its kind to my knowledge. Similar studies have been done with microalgae comparing batch to semi-continuous cultivation methods with similar results. Typically, semi-continuous microalgae culture methods yield higher productivity as opposed to batch culture (Benvenuti et al., 2016; Hewes, 2016; Hewes, 2015). Semi-continuous culture methods involve harvesting a set amount of culture at a set interval and replenishing the volume with nutrient rich media. This affords two advantages: the culture can be maintained indefinitely (as long as it remains free of contaminants) and the culture remains in the exponential growth phase (Hewes, 2016). Alternatively, microalgae grown using batch culture is subject to the lag phase for each batch being brought up and after several days comes out of the exponential growth phase and enters a stationary phase followed by a senescence phase. It is possible that a similar phenomenon occurred during the ATS harvest interval experiment of the present study. The 4- and 7- day harvest intervals were significantly more productive than both the 10- and 14- day intervals. It is reasonable to conclude that the 10- and 14- day lanes may have reached their stationary and senescence phases before their harvest dates while the 4- and 7- day lanes were held in a state of growth with steady productivity. It was noticed that during harvests of the 10- and 14- day treatment lanes, biomass typically exhibited white areas in a state of decay. The smell noticed from the biomass of these lanes suggested anaerobic, anoxic conditions. Some of the patches of decaying algae were present underneath layers of diatom growth suggesting that perhaps the decay was a result of overgrowth and subsequent lack of light as opposed to general senescence of the algae.

Investigating productivity as it relates to seasonality could not be done between these two experiments because of the change in experimental variables. However, comparing the

productivity data from the advanced 3-D treatment in the substrate experiment during the winter/spring (bi-weekly harvests) and the 14 day treatment from the harvest interval experiment in the late spring/summer there is an increase in productivity from ~6 g AFDW/m^2/day to ~10 g AFDW/m^2/day. It is possible that the increased sunlight and warmer temperatures of summer helped to increase productivity. It is impossible to say conclusively that seasonality was the cause of the increase until an experiment designed to test the effect of seasonality on productivity is performed. However, the difference seen may be, as stated earlier, because advanced 3-D lanes from the substrate experiment were experiencing a lower flow rate than the other lanes whereas in the harvest interval experiment flow was even across lanes. Additionally, ammonia levels in the source water in the winter (Fig. 5) were, on average, about half that of those in the summer months (Fig. 7). The combined effect of difference in flow rate and nutrient load could have influenced the increased productivity seen in summer versus winter just as much as changes in environmental conditions.

The composition of the biomass for each of the trials was primarily ash, approximately 77% (dry weight), while protein, fiber, and fat only made up a small percentage. The feasibility of using algae biomass as a source of biofuels lies in the high lipid content of the algae (Man and Keat, 2012; Rodionova et al., 2017; Vassilev and Vassileva, 2016). The lipid content of the biomass from each of the trials was always under 1%. Given the low levels of lipid in the ATS biomass it is unlikely that it would be useful as a source of biofuels through traditional methods such as lipid extraction and esterification processes. Low lipid levels (<0.5%) for ATS algae biomass were also reported by Adey et al., (2013, 2011) and Mulbry et al., (2010) who suggested fermentation processes to generate biofuels rather than extracting oils. Another approach to

generating bio-oils is through hydrothermal liquefaction (HTL). Through this process feedstock (in this case algal biomass) reacts with, or without, a catalyst in water at high temperature (200-370^{o}C) and high pressure (2-20MPa, Gollakota et al., 2018; Raikova et al., 2017; Saber et al., 2016). HTL is attractive as a means of generating bio-oils with respect to algae because unlike pyrolysis and lipid extraction/esterification reactions, biomass does not need to be dried (Gollakota et al., 2018; Raikova et al., 2017). HTL has already been used to generate bio-oil from a number of different macroalgal species across all three classes—Chlorophyceae (green), Heterokontopyceae (brown), and Rhodophyceae (red) (Raikova et al., 2017). Yang et al. (2015) reported that proteins and polysaccharides contribute to increase bio-oil yields from algae liquefaction. Because macro- and micro- algae are typically high in polysaccharides and protein, respectively, this means usable oil can still be generated without manipulating culture conditions to increase lipid levels.

Until production of biofuels and bio-oils from algae becomes economically feasible, the biomass generated from systems such as ATS can be put to other uses. Algae as a biofertilizer has its origins in Asia as an integral part of rice agriculture (Painter, 1993), significantly increasing product yields (Kantachote et al., 2016; Tripathi et al., 2008; Wuang et al., 2016). In addition to rice, other food items such as pak choy, arugula, bayam red, Chinese cabbage, kai lan, and white crown have shown increased yields when using algae as a biofertilizer as compared to those grown with no soil enhancement (Wuang et al., 2016). The yield of these food items were comparable to that of plants grown with chemical fertilizers, showing further promise of the potential for ATS biomass to be utilized for agriculture.

Another potential use of ATS biomass is bioplastics. Bioplastics are plastics that have been derived from renewable biological sources (Puppala et al., 2012). Polyhydroxybutyrate (PHB) is a naturally occurring molecule that exhibits thermoplastic properties, making it useful in the development of biodegradable bioplastics (Hempel et al., 2011; Maheswari and Ahilandeswari, 2011). PHB is found in bacteria as well as microalgae such as *Spirulina* spp. (Maheswari and Ahilandeswari, 2011). However, macroalgae has more potential than microalgae to be used in the bioplastics industry because of its high biomass yield, polysaccharide content, ease of harvest, and ability to be grown in a wide range of environments (Puppala et al., 2012). Rather than PHB, the large amounts of polysaccharides found in macroalgae would be utilized for bioplastic production (Puppala et al., 2012).

Utilizing ATS biomass for these purposes as opposed to microalgae offers two major advantages: the lack of culture crash as well as ease of harvest. Typically for large scale microalgae culture, the goal is to grow specific strains of algae. These strains are scaled up and transferred to commercial sized raceways outdoors where risk of contamination is high. As a result, cultures "crash" becoming unusable; the time and energy taken to scaling up the culture has been wasted. ATS, on the other hand, does not experience such crashes. Because the system grows species that naturally occur in the source water being used, rather than being grown from axenic laboratory cultures, the resulting biomass has an increased resistance to natural contaminants. The implication then is that, for whatever purpose ATS biomass is destined, production can be continual. One of the other issues facing microalgae culture is the difficulty of harvest, especially on a commercial scale. The process typically involves dewatering the algae either through centrifugation or a similar means which is neither time nor cost efficient (DOE,

2016). Harvest of biomass from the ATS used in the present study was simply done with a squeegee and dewatered using gravity through felt-lined baskets. The entire system (40' x 12') took only ~1.5 hours per harvest. Despite its advantages over microalgae, ATS biomass does not come without drawbacks. One of the biggest hurdles facing ATS is generating large amounts of biomass while keeping ash content low. As seen in this study, ash content can become quite high, up to 70-80% which can impede downstream processing. The high ash content could be a result of sediment and saltwater inevitably harvested along with the biomass. Studies done by Sandia National Labs have shown that washing biomass with freshwater can greatly reduce ash content. However, the more processing that goes into collecting and harvesting biomass, the more costly ATS biomass can potentially become.

Water samples taken from the head tank and from the end of each lane were analyzed for ammonia-nitrogen in order to determine the nitrogen removal rate of the system. The ammonia concentration in the head tank was not significantly different than at the end of the systems likely due to the short residence time of water on the raceways and low levels of incoming ammonia-nitrogen. This, coupled with the large variation of ammonia found in the samples made it difficult to calculate an accurate nitrogen removal rate. Mulbry et al. (2008) reported productivity at ~25 g dry weight/m^2/day under a loading rate of 2.5 g total nitrogen (TN)/m^2/day and reported a nitrogen recovery rate in the biomass of 57% (±13%). For the 4 day harvest treatment using the advanced 3-D material, biomass productivity reached an average of 98 (±34) g dry weight/m^2/day. Assuming nitrogen uptake is directly proportional to the amount of biomass, the nitrogen removal rate would be approximately four times higher than that reported by Mulbry et al. (2008).

However, in order to determine the actual N recovery rate of the 12-lane system further experimentation will be necessary. Once this is accomplished it will be possible to model the appropriate size ATS for use on a super intensive aquaculture system.

Until that is done it is possible to approximate the size ATS that could be utilized on a super intensive shrimp system. To do that we will make some assumptions—the following numbers are based on super intensive systems operated at the Texas A&M AgriLife Maiculture lab (Dr. Siccardi, personal communication, April 2018). We assume we are operating in $40m^3$ raceways, stocked at a density of 500 shrimp/m^3. We use a 35% crude protein diet, assume a Food Conversion Rate (FCR) of 1.2, and growth of 2.0g/week. Given that FCR= feed intake/ average daily growth, we can calculate that approximately 5.6kgfeed/day is needed for this system. Of this feed, given the percentage of crude protein (35%), 1.96kg is protein. Assuming ~50% of the protein from feed is excreted as waste that leaves 0.98kg NH_3/day in the system. Because Ray et al. (2015) achieved productivity values most similar to what was achieved using the advanced 3-D, 4-day harvest treatment (88.8 DW[g]/m^2/day versus 98 DW[g]/m^2/day) we will use the nitrogen removal rate they reported from their system: 12.3g N/m^2/day. For a system outlined above producing 0.98kg (980g) N/day an ATS with a removal rate of 12.3gN/m^2/day would need to be roughly $80m^2$. As stated, because of the assumptions made about the system and because productivity and nitrogen removal rate are influenced by a host of factors this is an imperfect approximation. But nonetheless, it demonstrates that ATS has the potential to be used for such a system while remaining a manageable size.

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